AMR in Gram-negatives

**P553** Prevalence and molecular epidemiology of infections caused by extended-spectrum and plasmid-mediated AmpC β-lactamase producing Gram-negative bacteria in hospital and community settings in Bosnia and Herzegovina

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**Objectives:** To investigate the prevalence of extended-spectrum β-lactamase (ESBL) in Gram-negative bacteria obtained from various clinical samples of inpatients (from various departments, n = 247) and outpatients (n = 265) in Cantonal Hospital Zenica, Bosnia and Herzegovina during 2009.

**Methods:** Double-disc synergy test was used to detect ESBLs. Minimum inhibitory concentrations (MICs) were determined by broth microdilution method according to CLSI. The transferability of cefazidime resistance was tested by conjugation (broth mating method). PCR was used to detect alleles encoding ESBL enzymes.

**Results:** ESBL was detected in 168 (68%) and 246 (93%) in- and outpatient samples, respectively. ESBL prevalence was highest in non-surgical departments, 59 (35%, p = 0.000). Both in- and outpatient ESBLs were mostly isolated from urine and surgical wounds, 29% and 78%, 27% and 14%, respectively. ESBL/non-ESBL prevalence was highest in an inpatient Klebsiella spp. and in outpatient Escherichia coli isolates, 36%/41% and 34%/42%, respectively. Inpatient urinary isolates were more resistant to all antibiotic tested than outpatient ones. Inpatient surgical wound isolates were more resistant to all cephaplosporins than outpatient ones, but lower to other antibiotics.

Cefuroxime, cefazidime and cefotaxime were the least potent antibiotics with MIC90 of ≥256 mg/L. Forty nine (33.6%) of all isolates had plasmid-mediated AmpC ESBL by phenylboronic acid phenotypic test. Conjugation frequency was in the range 10^-4−10^-7. Resistance to cefotaxime and ceftriaxone. High resistance rates of resistance to cefotaxime and ceftriaxone. High resistance rates observed for gentamicin and ciprofloxacin probably due to the fact that plasmids encoding ESBLs also contain resistance genes for non-β-lactam antibiotics.

**Conclusions:** The study demonstrated high prevalence of CTX-M β-lactamases and the presence of intrinsic SHV-1 ESBL. Twenty-one strains had both, plasmids encoding ESBLs also contain resistance genes for non-β-lactam antibiotics. Twenty-one strains had both, plasmids encoding ESBLs also contain resistance genes for non-β-lactam antibiotics. Twenty-one strains had both, plasmids encoding ESBLs also contain resistance genes for non-β-lactam antibiotics. Twenty-one strains had both, plasmids encoding ESBLs also contain resistance genes for non-β-lactam antibiotics.

**P554** High rates of multidrug resistance among Gram-negative pathogens in Europe. Results from GLOBAL 6 Surveillance (2009–2010)

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**Objective:** The emergence and spread of β-lactamases and the prevalence of multi-drug resistance (MDR) among Gram-negative organisms has complicated selection of an appropriate empiric therapy. Data from surveillance is useful for monitoring the activity of current agents, and for any change in activity patterns as a result of emergence or spread of resistance. Given the clinical impact of resistance among Gram-negatives, this study evaluates resistance among common agents for Enterobacteriaceae (EB), P. aeruginosa (PA), and Acinetobacter spp. (AS) collected across Europe as part of the GLOBAL surveillance initiative, both current resistance (2009–2010).

**Methods:** During GLOBAL 6 (2009–2010), clinical isolates of EB, PA and AS from Belgium, France, Italy, Germany, Spain, and the United Kingdom, were centrally tested for susceptibility by broth microdilution (CLSI M7/M100). MDR was defined as resistance to ≥3 different classes of antibiotic.

**Results:** The current activity profile from GLOBAL 6 is presented in the table below. Of all evaluated agents, sitafloxacin had the lowest MIC90s, while MIC90s of 16 mg/L and higher were commonly observed for other evaluated agents. MDR rates were 8.7% among EB, 20.1% among PA, and 39.6% among AS. 1.4% of evaluated P. aeruginosa were imipenem resistant based on new CLSI breakpoints. Rates of MDR and resistance was highest in single agents varied across evaluated countries, with the highest degree of resistance observed in Italy.

**Conclusions:** MDR was common among PA and AS in Europe. Based on the degree of resistance among Gram-negatives, it is critical to understand current levels resistance and continue to monitor for changes in these patterns on a regional and local level when considering an appropriate antimicrobial chemotherapy. This data also highlights the need for agents with strong activity against resistant Gram-negative isolates.

**Global Surveillance 6**

| Organism | Antibiotic | Resistance | Sensitivity | Intermediate | MDR | β-L | AmpC |
|----------|------------|------------|-------------|--------------|-----|-----|------|
| EB       | Ceftriaxone| 57%        | 31%         | 12%          | 1%  | 2%  | 0%   |
| PA       | Gentamicin | 78%        | 3%          | 19%          | 1%  | 0%  | 0%   |
| AS       | Ciprofloxacin | 99%       | 0%          | 1%           | 0%  | 0%  | 0%   |

**P555** Antimicrobial resistance of Citrobacter spp., Enterobacter spp. and Klebsiella spp. strains isolated from meat samples

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**Objectives:** The aim of the present study was to assess the antimicrobial resistance of Citrobacter spp., Enterobacter spp. and Klebsiella spp. isolated from raw and processed meat products.

**Methods:** During a 5 years period (2004 and 2009) there were examined 642 samples of different types of raw meat (pork, beef, goat and lamb) and thermally processed meat products. The samples were collected from restaurants, supermarkets and abattoirs of the greater area of Ioannina, in Northwestern Greece. The antimicrobial resistance of the isolated bacterial strains was tested using the disk diffusion method according to Bauer-Kirby.

**Results:** From the raw meat samples, there were isolated 122 Citrobacter spp. strains, 19 Klebsiella spp strains and 76 Enterobacter spp strains. No pathogenic bacteria were isolated from the thermally processed meat samples. The Citrobacter spp. strains were resistant to ampicillin (51%), cefuroxime (47%), nitrofurantoin (40%) and tetracycline (35%). The Enterobacter spp. strains exhibited resistance to ampicillin (67%), carbenicilline (54%) and tetracycline (41%) and the Klebsiella spp strains were resistant to ampicillin (84%). The most tetracycline resistant strains were isolated from pork meat samples and the most cefaluoxime resistant strains were isolated from ruminant’s meat samples (beef, goat, lamb). Resistance to imipenem was always intermediate for all bacterial strains.
Conclusion: The results indicate that meat can be a source of resistant bacteria which could be potentially transmitted to humans via the food chain. The high frequency of tetracycline resistance of the pork strains is related to the extended use of this antibiotic in pig breeding and the high frequency of ceftriaxone resistance of the resistant strains is probably due to the extended use of cephalosporins in the ruminant breeding farms. The low occurrences of intermediate imipenem-resistance strains is attributed to the no use of this antibiotic in the current veterinary practice, emphasizing the fact that the prudent use of antibiotics in farm animals is essential to limit the spread of antibiotic resistant bacteria and to protect public health.

P556 Comparative antibacterial activity against E. coli from pediatric patients in North America and Europe: 2006–2009
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Background: E. coli (Ec) remains a frequent cause of both community and hospital infections. Increasingly these isolates are multi-drug resistant and pose therapeutic challenges especially in pediatric populations where fluoroquinolones are generally contraindicated. The Tigecycline Evaluation Surveillance Trial (TEST) has monitored trends in susceptibility of tigecycline and comparators globally since 2004. This report evaluates the in vitro activity of tigecycline and comparators to E. coli isolated from pediatric patients in North America (NA) and Europe (EU) utilizing continent-specific breakpoints (NA: CLSI; EU: EUCAST).

Methods: In 2006-2010 1550 Ec (NA: 702; EU: 848) were collected in NA (Mexico, USA, Canada) and EU (26 countries). MICs were determined using supplied microdilution panels and interpreted according to CLSI (NA) or EUCAST (EU) breakpoints (BPs).

Results: Summary data for tigecycline and comparators (% Susceptible) are shown by year and continent.

Conclusions: Decreased susceptibility of Ec to ceftepime, ceftriaxone, minocycline and pip-tazic in EU vs NA in some study years may reflect lower susceptible BPs in EU vs NA. Detailed analysis of resistant isolates on both continents to confirm isolate distributions is needed.

P557 Increase of polymicrobial infections and multi-resistant pathogens causing prosthetic joint infections
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Objectives: Prosthetic joint infections (PJI) are a serious complication after total joint arthroplasty. Earlier descriptions of microorganisms causing PJI revealed Gram-positive cocci in the majority of cases and therapy guidelines were designed accordingly. Little information is available about changes in distribution of pathogens causing PJI in an era of increasing antibiotic resistance.

Methods: We conducted a retrospective analysis of all patients treated for PJI at a center for revision total hip and knee arthroplasty between 2006 and 2008 according to a modified “Listeriat treatment algorithm”. Diagnosis of PJI was based on operative findings, subsequent microbiological culture and histological examination. Pathogens were identified by intraoperative and synovial culture. Superficial wound swaps or swaps from sinus tracts were not taken into account.

Results: 147 patients with PJI (total hip arthroplasty: n=89 (60.5%), total knee arthroplasty n=58 (39.5%)) were included in the study. 19% (n=28) presented with acute PJI, 81% (n=119) with chronic PJI. 26 patients (17.7%) were treated with debridement and retention, 121 (82.3%) with two-stage exchange. No pathogen could be detected in 39 patients (26.5%). One or more pathogen could be identified in 108 patients (73.5%). 55 patients (50.9%) comprised monomicrobial, 53 patients (49.1%) polymicrobial PJI. In 32 patients two pathogens were detected, three pathogens were found in 11 patients and four or more pathogens in 10 patients respectively. 21 polymicrobial PJI included only Gram-positive pathogens, 1 PJI only Gram-negative pathogens, 24 PJI consisted of Gram-positive and Gram-negative pathogens, 7 included fungi additionally. CoNS (n=63) were the most commonly isolated pathogen (MRSE: 59%, MSSE: 41%) followed by S. aureus (n=45) with 64% MSSA and 36% MRSA, and by Gram-negative rods (n=44). Infections caused by multiresistant pathogens (n=50) were found significantly more often in polymicrobial (n=36; 67.9%) than in monomicrobial PJI (n=14; 25%) (p < 0.05).

Conclusion: Distribution of pathogen causing PJI varies with time and location. Earlier descriptions of pathogens causing PJI cannot be inferred to centers specialized in revision arthroplasty. Our data demonstrate a much more widespread range of pathogens, including all kinds of multiresistant bacteria and a high number of polymicrobial infections. Existing therapy regimens need to be adapted to this development.

P558 Emerging quinolone resistance in typhoid outbreaks caused by haplotype H58 in Kenya
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Objectives: To conduct surveillance for antimicrobial susceptibility and determine molecular epidemiology of typhoid outbreaks in Kenya.

Methods: We characterised a total of 323 S. Typhi isolated from outbreaks in Kenya over the period 1988-2008 for antimicrobial susceptibility, and phylogenetic relationship using single nucleotide polymorphism (SNP) analysis.

Results: Only 17.9% were fully sensitive while the majority (60.5%) were multiply-resistant (MDR) to most commonly available drugs – ampicillin, chloramphenicol, tetracycline (MICs >256µg/ml) and co-trimoxazole (MIC >32µg/ml). Resistance to these antibiotics was encoded on self-transferrable IncHI1 plasmids of the ST6 sequence type. For MDR S. Typhi MICs for nalidixic acid and ciprofloxacin were respectively 5- and 10-fold higher than for sensitive strains and the proportion of MDR strains also resistant to nalidixic acid rose by a factor of 20 between 1994 and 2005 to make up 25% of all strains. Genotyping by pulsed-field gel electrophoresis (PFGE) showed that the MDR S. Typhi strains were closely related and were different from the fully-susceptible strains. Of the 94 representative S. Typhi selected for genome-wide haplotype analysis sensitive isolates fell into several phylogenetically different groups whereas MDR isolates all belonged to a single haplotype, H58, associated with MDR and fluoroquinolone resistance which is also dominant in many parts of SE Asia.

Conclusion: With the emergence of nalidixic acid resistant S. Typhi with elevated MICs for ciprofloxacin more expensive and infrequently available third generation cephalosporins may be required to effectively treat most typhoid outbreaks in Kenya.

P559 Multilocus sequence typing of IncN plasmids
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Objectives: IncN plasmids have been associated to the wide dissemination of antimicrobial resistance, including relevant β-lactamases genes such as the blaVIM-1, blaCTX-M-1 and blaKPC. A plasmid Multilocus Sequence Typing (pMLST) was developed on the DNA sequences of 12
fully sequenced IncN plasmids available in the Genbank and applied to a collection of 57 IncN plasmids from 6 European countries of human, animal and environmental origin.

**Methods:** Fully sequenced IncN plasmids available in the Genbank were analyzed in silico and the repA, traJ and korA genes were selected as loci for the IncN specific pMLST. 57 plasmids of our collections were all assigned to the IncN group by the Plasmid-Based Replicon Typing (PBRT) method, transferred by conjugation in a recipient *E. coli* strain, analyzed for β-lactamase genes and classified by DNA sequencing of the amplicons obtained for the repA, traJ and korA loci.

**Results:** The repA, traJ and korA loci sequences were analyzed for 69 IncN plasmids, 12 available in Genbank and 57 from our collections. Eleven Sequence Types (STs) were defined on the basis of the loci sequences and allele assortment, setting up a new pMLST scheme for typing this plasmid family. ST1 and ST2 were included in the same clonal complex (CC-1), since plasmids belonging to these STs were highly related differing only for one mismatch in one allele. Similarly, ST10 and ST11 were included in the clonal complex CC-10. Plasmids carrying the blaCTX-M-1 gene, isolated in different countries from food producing animals and humans were all but one assigned to the clonal complex CC-1, suggesting the wide spread of an epidemic plasmid carrying this ESBL through the food chain. Plasmids carrying blaVIM-1 from *Klebsiella pneumoniae* and *Escherichia coli*, isolated along a five years period in Greece were all assigned to the clonal complex CC-10, suggesting the spread and persistence of this particular IncN lineages in this country.

**Conclusions:** The IncN plasmid family is successfully disseminating in different environments linked to relevant resistance genes. This study proposes the pMLST as a suitable and rapid method for identification of IncN epidemic plasmids.

**P560** The ecology of major resistance plasmids in *E. coli*

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**Objectives:** We wished to understand whether incoming globally dominant resistance plasmids were displacing a native population of similar type or integrating into it upon arrival by recombination.

**Methods:** At two geographically separated sites, we compared InIC1 plasmids carrying blaCMY-2 with those carrying no resistance phenotype at all (“sensitive”) and those carrying only ampicillin resistance, in terms of plasmid size (S1 nuclease digestion) and RFLP and in terms of published plasmid MLST alleles. We also compared IncFIB plasmids carrying blaCTX-M-15 with IncFIB plasmids carrying no resistance phenotype at all (“sensitive”) and those carrying only ampicillin resistance, using the same approach except for an MLST. Host strains were compared by PFGE and MLST as needed to establish strain-plasmid associations.

**Results:** The local ‘sensitive’ plasmids were relatively diverse, and had plasmid MLST alleles that had not previously been described. They were quite different from each in the two sites, while established ‘resistance’ plasmids (eg InIC1/blaCMY-2 and IncFIB/blaCTX-M-15) were similar to each other and at each site as well as to published plasmids from around the world.

**Conclusion:** Whether this picture is unique to, say, IncIC1/blaCMY-2 remains to be seen. However, the data suggest that *E. coli* plasmid populations in the local microflora are displaced by invading resistance plasmids. Known associations between plasmids and antibiotic resistance genes and host strains (eg IncF blaCTXM15 ST131) implies the possibility of a long-term ecological risk which has not been considered in detail and is deserving of closer scrutiny in these and other plasmid populations. A more complete toolkit for characterisation of plasmid populations and a clearer picture of the natural plasmid ecology of the Enterobacteriaceae is needed.
including PCR detection of PMQR determinants and of β-lactamase-encoding genes (for putative producing isolates with diminished susceptibility to β-lactams), the respective coding proteins were identified through nucleotide sequencing. Class 1 integrons (int1 gene) were searched by PCR.

**Results:** Overall, we detected 19/369 PMQR determinants, namely 11 QnrS1 in *E. coli* isolates from poultry. Two of these strains also carried TEM-1 β-lactamase and one of them carried TEM-135 plus SHV-108 (ESBLs). Among the 6 QnrB detected, we identified 3 QnrB19: one in an *E. coli* (from a pig) and 2 in Salmonella spp isolates (from poultry), and 3 QnrB2 in Salmonella spp isolates (from embryonated eggs). We also have detected 2 Aac(3′)-Ib-cr in *E. coli* and Salmonella spp isolates, from broiler and bovine origin, respectively. One isolate carrying Aac(3′)-Ib-cr was from consumable meat. Among PMQR-negative isolates, we detected one DHA-type (plasmid-mediated AmpC β-lactamases) and one TEM-135 (ESBLs) *E. coli*-producing isolates. Class 1 integrons were detected among 8 out of 19 PMQR-positive isolates, but were not always associated to multidrug-resistance (MDR).

**Conclusions:** This investigation has demonstrated that PMQR may act additively with other plasmid-encoded resistance mechanisms in 16% of studied isolates and in 42% may be disseminated by plasmids carrying class 1 integrons, contributing to the scenario of MDR in animal production facilities.

**P563 Colistin therapy for multidrug-resistant Gram-negative infection: clinical outcome and risk factors**

Y. Jun*, H.J Choi (Seoul, KR)

**Objectives:** As most antibiotics used in current Gram-negative bacterial infection are ineffective, colistin has re-introduced in MDR treatment strategy. Many questions regarding the clinical utility of colistin remain unclear. The aim of this study is to evaluate the efficacy of intravenous colistin treatment for MDR Gram-negative bacteria and understand the risk factors for its therapy.

**Methods:** We retrospectively reviewed the medical records of patients who had history of intravenous colistin for MDR *Acinetobacter* or *Pseudomonas* which were sensitive to colistin. Patients were excluded if they received <72h of colistin treatment. We analyzed the risk factors of poor clinical response to colistin. The type of infection, characteristics of patients including age, sex, underlying diseases, causative organism, co-infection, antibiotics history before and after colistin treatment, duration of colistin therapy, and treatment outcomes including 30-day mortality were analyzed.

**Results:** Total 107 patient records were analyzed, 104 patients (97%) were treated in the Intensive Care Unit. The good clinical responder was 60 (56%) and poor clinical responder was 47 (44%). Average age was 63.8±15.8 and average hospital stay was 124.5 days. Total duration of colistin use was 14.7±12.1. In univariate analysis, Age >64, Hospital day >125days, ICU stay <7, Bacteremia, Diabetes Mellitus, Malignancy, MDR pseudomonas infection, History of carbapenem or Glycopeptides use were statistically meaningful. Antibiotic factors were not significantly meaningful. In logistic regression analysis, Hospital day >125 (OR: 1.12 CI= 1.090–8.555), Bacteremia (OR=1.22, CI 0.066–1.438), Diabetes Mellitus (OR: 1.07, CI: 0.198–2.043) were independent risk factors for the outcome of colistin therapy.

**Conclusion:** Long term hospital patients, bacteremia, and Diabetes Mellitus suggested poor outcome of the colistin therapy. The large controlled prospective study on colistin should be considered in treatment for MDR Gram-negative bacterial infection.
Comparison of antimicrobial susceptibility of Serotypes, antibiotic resistance, and class 1 integrons in Salmonella: 2002-2010

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(Schaumburg, US; Epalinges, CH)

Objectives: The Study for Monitoring Antimicrobial Resistance Trends (SMART) has tracked susceptibility levels and incidence of extended-spectrum β-lactamases producing (ESBL+) E. coli causing intra-abdominal infections (IAI) since 2002. In late 2009 pathogens from inpatient urinary tract infection (UTI) were added to the study. This report compares susceptibility and ESBL+ rates of E. coli from IAI and UTI in Europe in 2009–2010.

Methods: 21 hospitals in 12 European countries each collected up to 100 consecutive non-selected isolates from IAI and 50 from UTI. All isolates were sent to a central laboratory (HMIA, Inc., USA) for confirmation of identification, susceptibility testing, and ESBL determination; the latter two were done following CLSI guidelines. Percent susceptibility was determined using EUCAST breakpoints. Fisher’s exact test was used to determine statistical significance of differences between IAI and UTI susceptibility levels.

Results: 2,726 isolates of E. coli were tested (IAI 2190, UTI 536). ESBL+ rates were 11.1% in IAI and 18.8% in UTI. The following table summarizes susceptibility of E. coli and ESBL+ E. coli from IAI and UTI:

| Source Group | Elg | Imp | Cep | CRO | CAX | ACC | ABT | PL | CP | Lum |
|--------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| UTI E. coli (IAI) | 98 | 2300 | 79 | 79 | 81 | 79 | 84 | 87 | 89 | 68 | 67 |
| UTI E. coli (UTI) | 95 | 2300 | 79 | 79 | 81 | 79 | 84 | 87 | 89 | 68 | 67 |

Conclusions: Although differences in susceptibility percentages between IAI and UTI E. coli were generally small (all <10%), 7/11 drugs showed significantly lower susceptibility in UTI than in IAI (P <0.05). This is at least partially explained by the much higher ESBL+ rate seen in UTI isolates (18.8%) than in IAI (11.1%). Imipenem, ertapenem, amikacin, and piperacillin-tazobactam were the most active in vitro among the drugs studied in SMART, but only ertapenem and imipenem inhibited >90% of E. coli from both IAI and UTI. ESBL+ isolates were >92% susceptible to both carbapenems, but <88% susceptible to all other study drugs. The relatively high rate of ESBL+ E. coli in UTI in Europe is concerning, as further increases may eventually render many drugs commonly used to treat UTI ineffective.

A. Stylianakis*, S. Tsipakou, V. Papasavvou, P. Thomaidis, A. Panagopoulos, C. Karra, A. Koukoutsou (Athens, GR)

Objectives: The purpose of this work was to study the Stenotrophomonas maltophilia isolates derived from clinical samples and their resistance profile to the antibiotics used.

Methods: During a three year period (11/2007–10/2010) we examined n=123 non duplicated samples corresponding to n=103 patients hospitalized in all the wards of our hospital. The examined specimen sources were pus (n=64), bronchoalveolar excretions (n=22), catheters (n=17), blood (n=13), pleural liquid drainages (n=5), and urine (n=2). The isolates identification was performed using the automated system VITEK 2 (Biomerieux, Marcy L’Etoile, France). The MIC values of ticarcillin/clavulanic acid, sulfamethoxazole/trimethoprim, cefazidine, minocycline and levofloxacin were determined by using the E-test system following the manufacturer’s instructions (ABI Biodisk, Solna, Sweden), and interpreted according to the CLSI guidelines (Vol. 28, No 1, M100 – S18).

Results: The number and the resistance rate of the examined isolates were as follows: cefazidime (85/69,1%), ticarcillin/clavulanic acid (54/68,3%), levofloxacin (14/11,4%), sulfamethoxazole/trimethoprim (6/4,9%). The sulfamethoxazole/trimethoprim-resistant isolates corresponded to n=4 patients hospitalized in the medical wards. These isolates were detected during the last year period. No resistant isolates were found to minocycline.

Conclusion: The examined Stenotrophomonas maltophilia clinical isolates possess a high resistance rate to cefazidime and ticarcillin/clavulanic in contrast to minocycline, sulfamethoxazole/trimethoprim and levofloxacin. The last three drugs remain in vitro, active antimicrobial agents against Stenotrophomonas maltophilia isolates. The emergence of sulfamethoxazole/trimethoprim resistance poses the necessity strategies to be encouraged to prevent Stenotrophomonas maltophilia infections and to restrict the dissemination of this resistance.

A. Stylianakis*, G.M. Giammanco, S. Farshad, O. Aleo, N. Jonaidi, M. Izadi, N. Sadeghifard, C. Mammina (Tehran, IR; Palermo, IT; Shiraz, IR; Ilam, IR)

Objectives: The present study was conducted to investigate serotype distribution, antimicrobial resistance patterns, carriage of class 1 integron, and clonality of Salmonella strains isolated from patients aged 0–12 years in Tehran, Iran, during 2007–2008.

Methods: The study included all Salmonella isolates recovered from all cases of enteritis in patients aged less than 12 years admitted to a major children hospital in Tehran, Iran, during 2007–2008. Serotyping, drug susceptibility testing, and analysis of class 1 integron were carried out on 139 Salmonella isolates. Pulsed field gel electrophoresis (PFGE) was used to investigate genetic relatedness among the isolates.

Results: Salmonella serotypes Enteritidis, Infantis, and Typhimurium included 84.9% of isolates, Enteritidis accounting for 41.7%. The most prevalent resistances were to doxycycline (64.7%), nalidixic acid (61.2%), tetracycline (51.8%), and streptomycin (42.8%). Fifty-three (38.1%) isolates contained class 1 integron. Eight different gene cassettes
were identified, aadA1 being the most frequently encountered. Pulsed-field gel electrophoresis showed that integron-positive Salmonella strains belonging to serotypes Infantis, Enteritidis, and Typhimurium were attributed to two, three, and five different pulsotypes, respectively. **Conclusions:** The findings indicated that the distribution and drug resistance pattern of most prevalent Salmonella serotypes were broadly similar to that reported globally from human isolates. Presence of class 1 integrons was common among Salmonella serotypes in Tehran, Iran. Concurrent clonal expansion and horizontal transmission events seem to contribute to increase in drug resistance prevalence among Salmonella serotypes.

**P569** AAC(3)-II is the major aminoglycoside-modification enzyme in China, which leading to the obvious partial cross-resistance in *Escherichia coli* to agents

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**Objectives:** To study the aminoglycoside resistance and epidemiology of aminoglycoside-modifying enzymes in *Escherichia coli* in China, evaluate the clinical value of different aminoglycoside agents.

**Methods:** National wide clinical isolates of *E. coli* being resistant to gentamicin or kanamycin, followed by tobramycin, netilmicin and eticmin, but less than 15% of the strains were resistant to amikacin and isepamicin. 88.2% of gentamycin-resistant strains were susceptible to isepamicin. 5 antibiotic modifying enzyme genes were detected in 191 bacterial strains. AAC(3)-II was the most common enzyme, which was positive in 162 strains, the following enzymes were AAC(6\(^\prime\))-I (28 strains), AAC(3)-II (28 strains), AAC(3)-II/AAC(3)-II (20 strains), ANT(2\(^\prime\))-I (20 strains); the most common enzyme combinations were AAC(3)-II/AAC(3)-II/AAC(3)-II (28 strains), AAC(3)-II/AAC(3)-II/ANT(2\(^\prime\))-I (11 strains) and AAC(6\(^\prime\))-I/10 strains). The bacterial resistance and antibiotic enzyme distribution in different cities were minor, but the enzyme combination in Guangdong, Junan and Dalian had some difference with other cities, from which aminicin and isepamicin-resistant strain were isolated mainly.

**Conclusions:** The major aminoglycoside-resistance in *E. coli* in China is to gentamycin and kanamycin, rather than amikacin and isepamicin. The aminoglycoside-resistant phenotype in *E. coli* closely connects with the antibiotic modifying enzymes. Amikacin and isepamicin keep high antibacterial potency against *E. coli*, which is worthy for clinical application.

**P570** Surveillance of nosocomial and community-acquired enteric pathogens shed in equine faeces and analysis of risk factors associated with antibiotic resistant *E. coli*

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Antimicrobial-resistant bacteria represent a significant threat to human and animal health. The emergence of such resilient pathogens is often associated with hospital settings, and faeces from horses in this environment can be a source of community outbreaks. We compared equine faecal samples (*n=264*) from 138 horses from hospital and non-hospital (livery stable and riding school) premises in North West England to determine the prevalence of *Escherichia coli*, *Salmonella*, and *Campylobacter* and the presence of antimicrobial-resistant strains. All faecal samples were found to contain *E. coli*, while *Campylobacter jejuni* was detected only in hospitalised horses (1.1%). *Salmonella* was identified. A total of 296 resistant *E. coli* strains were isolated. We evaluated data of the horses’ management and veterinary treatment to identify the risk factors associated with faecal shedding of antimicrobial-resistant *E. coli*. Statistical analysis using multi-level logistic regression revealed that the hospital was the major source of both resistant and multiple drug resistant (MDR) *E. coli*. Moreover, shedding of antimicrobial-resistant *E. coli* was correlated with hospitalization for a gastrointestinal problem (odds ratio: +95%CI = 8.50: 1.79–40.32), receipt of oral antimicrobial (odds ratio = 3.52; 1.11–11.10 95% CI), receipt of more than one antimicrobial in hospital (OR = 1.05: 1.01–1.09), or gelding (OR = 4.62: 1.23–17.46). Interestingly, intravenous antimicrobial appeared to be negatively correlated with faecal shedding of antimicrobial-resistant *E. coli* (OR = 0.18: 0.04–0.76). The prevalence of MDR *E. coli* was positively correlated with hospitalization, antimicrobial in hospital (OR/Tx = 3.65: 1.54–8.68), and increased age (odds ratio: +95% CI = 1.11: 1.03–1.19 per year). Thus, equine hospitals appear to be an important source of zoonotic antimicrobial-resistant and MDR *E. coli* strains, but are unlike reservoirs of zoonotic *Salmonella* or *Campylobacter*, in this geographic location. It is important to control the amount of antimicrobial *Tx* given to horses in hospital to lessen the chance of zoonotic exposure in areas proximal to these facilities.

**P571** Prevalence of carbapenem-resistant Enterobacteriaceae and carbapenem-resistant *Pseudomonas aeruginosa* and Acinetobacter baumannii in routine ESBL surveillance samples

M. Pirsa*, A. Andlovic, T. Cerar, T. Furlan, J. Gazej, K. Seme (Ljubljana, SI)

**Objective:** The aim of our study was to determine the prevalence of carbapenem-resistant (CR) Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in routine ESBL surveillance samples taken from patients in a tertiary hospital. Direct and enrichment protocol were evaluated.

**Methods:** Samples were vortexed in tryptic soy broth (TSB), aliquots were inoculated onto ChromID ESBL agar (bioMérieux), MacConkey agar (MAC) onto which 10 μg carbapenem discs were placed, and TSB. TSB was subcultured following 24h incubation onto MAC agar onto which carbapenem discs were placed. Reduced susceptibility to carbapenems was suspected in any colony growing within the 23 mm inhibition zone for Enterobacteriaceae or 16 mm for non-fermentative gramnegative bacilli. Those colonies were subcultured followed by antimicrobial susceptibility testing. Strains with reduced susceptibility to carbapenems were identified and MICs were determined using Etest. Phenotypical tests for detection of carbapenemases (modified Hodge test (MHT) and inhibition tests) were performed as well as molecular detection of blaKPC, blaVIM, blaIMP, blatraXA-48 and blabdMDM-1.

**Results:** 343 surveillance samples from 269 patients were screened (83.7% rectal swabs). CR Enterobacteriaceae were detected in 15 patients (5.6%) (Table 1). Eight (44.4%) of CR isolates were not detected on ESBL chromogenic, 2 (16.7%) of those were detected by enrichment protocol only. Phenotypical tests for detection of carbapenemases were negative. CR *P. aeruginosa* was detected in 22 patients (28 isolates); 63.6% of isolates had isolated resistance to carbapemems, 40.9% were MDR strains; 10.7% of MDR CR *P. aeruginosa* isolates were detected by enrichment protocol only (Table 1). Among MDR strains one had positive inhibition test for class A and 2 for class B carbapenemases. CR *A. baumannii* was detected in one patient by positive MHT and inhibition test for class B carbapenemase (Table 1). Molecular screening for 5 carbapenemase genes was negative in all isolates.

**Conclusions:** CR Enterobacteriaceae, CR *P. aeruginosa* and CR *A. baumannii* were detected in 5.6%, 8.2% and 0.4% of routine
ESBL surveillance samples taken from patients in a tertiary hospital, respectively. We were not able to detect carbapenemase genes in our CR isolates. Our study supports the use of MAC onto which carbapenem discs are placed in combination with nonselective enrichment step for detection of CR bacteria in surveillance samples.

**P572 Evaluation of a new gradient-diffusion system for MIC determination with Gram-negative pathogens**

V. Conte, M.M. D’Andrea, T. Giani, G.M. Rossolini* (Siena, IT)

**Objectives:** Minimum Inhibitory Concentration (MIC) remains the cornerstone parameter for evaluation of antimicrobial susceptibility. MIC determination is crucial with some antimicrobial agents–pathogens combinations, when disk diffusion testing is not reliable or when the MIC value correlates with outcome. MIC determination has become important also when dealing with multiresistant pathogens and treatment options are seriously limited. However, most clinical laboratories cannot afford the expensive and labor-intensive reference MIC testing or complex automated systems for precise MIC determination, and a number of products based on gradient-diffusion has been developed for easy determination of MIC values. In this work we evaluated the performance in MIC determination of a new gradient-diffusion system against a panel of Gram-negative pathogens including several multiresistant strains with emerging resistance mechanisms.

**Methods:** 100 bacterial isolates were studied, (70% Enterobacteriaceae and 30% Gram negative non fermenters), including 30 reference strains with known resistance mechanisms (acquired ampC, MBL and ESBL producing Enterobacteriaceae, OXA-carbapenemase producing Acinetobacter baumannii, MBL producing Pseudomonas aeruginosa), determined by molecular methods, and 70 routine isolates. MIC determinations were performed in parallel by MIC Test Strips (Liofilchem srl, Italy) and Etest (Biomerieux, France) on MHA (Becton-Dickinson, USA) medium, according to EUCAST methods. The following strips were evaluated: Ceftazime (CAZ), Cefotaxime (CTX), Colistin (COL), Piperacillin/Tazobactam (PTZ), Imipenem (IMI), Meropenem (MEM). Paper-strips containing CAZ or CTX, alone and in combination with clavulanic acid, were used for the detection of ESBL. Comparator MICs were obtained by broth microdilution (BMD), following EUCAST guidelines.

**Results:** MIC determinations were considered concordant when obtained results fell in the experimental error (+1 log2 dilution). Overall agreement of MIC values between MIC test strips and BDM was 79.4% (n = 467), with a specific agreement ranging from 89.5% of CAZ to 74.6% of CTX. Combination strips correctly detected ESBL in 24 cases without any false positive. Similar values were obtained using the Etest method.

**Conclusions:** The evaluated product gave overall good agreement with BMD method, similar to that obtained with Etest. MIC test strips appear therefore to be a valid alternatives to the Etest.

**P574 Surveillance of antimicrobial susceptibility of Pseudomonas aeruginosa isolates from a central hospital in central Portugal: 7 years of evolution or involution?**

S.G. Pereira*, H. Oliveira, L. Albuquerque, R. Leitão, O. Cardoso on behalf of the Center for Pharmaceutical Studies

**Objectives:** Pseudomonas aeruginosa (PA) is an important nosocomial pathogen causing a wide variety of infections, being at present the fourth most common nosocomial pathogen. In order to apply optimal therapeutic guidelines, physicians must be aware of recent resistance surveillance and epidemiological data. The aim of this study was to determine antimicrobial susceptibility of PA isolates in order to assist the guidelines for empirical antibiotic prescription regimens and to evaluate the infection control measures implemented in the hospital surveyed.

**Methods:** From April 2003 to April 2010, 2719 PA isolates were collected in Centro Hospitalar de Coimbra: 1719 were nosocomial isolates and 1000 from ambulatory. Isolates were obtained from sputum (40.9%), urine (25.3%), exudates (12.4%), blood (4.7%) and other sources (16.6%). Identification and susceptibility patterns of the isolates were performed with API32GN (BioMérieux) and MicroScan WalkAway (DadeBehring). Susceptibilities to Piperacillin (PIP), Piperacillin plus Tazobactam (TZP), Aztreonam (AZT), Cefazidime (CAZ), Imipenem (IP), Meropenem (MP), Amikacin (AMK), Gentamicin (GN), and Ciprofloxacine (CIP) were guideline by CLSI.

**Results:** Considering all isolates, AMK was the best agent (86.5%), and CIP was the worst (61.0%). The same result was observed in nosocomial isolates, with AMK as the best agent (82.8%) and CIP the worst (54.4%). In ambulatory isolates, MEM was the best agent (95.3%). Since the beginning of the study, susceptibility rates have progressively diminished in nosocomial and ambulatory isolates. In the year 2007-2008, it was observed a small increase in the susceptibility rate of several antimicrobials in all isolates (AZT, CAZ, GN, IP, MP, AMK, CIP), but in the following years, it has decreased, being at present in the lowest
level since the beginning of the study. Susceptibility rates of the most important β-lactams IP, MP and CAZ have diminished in 16.5%, 21% and 13.8%, respectively, in nosocomial isolates, and 10.9%, 8.9% and 9.9%, respectively, in ambulatory ones. Contrary, the susceptibility rate of TZP has increased (9.8%) since 2007–2008.

Conclusion: Appropriate empirical treatments based on knowledge of particular resistance patterns are important determinants to the success of therapeutics. Studies of surveillance can be helpful in fighting the development and spread of resistance among pathogenic agents.

**P575** Resistance rates of *Pseudomonas aeruginosa* clinical isolates in a tertiary hospital

S. Tsipidakou, A. Stylianakis, V. Papatoanou, P. Thomaidis, E. Chatziandreou, C. Karra, A. Koutsoukou* (Athens, GR)

Objectives: The aim of the present study was to assess resistance rates to various antimicrobials of *Pseudomonas aeruginosa* clinical isolates for a period of three years in a tertiary hospital.

Methods: During a 3 year period from 2007 to 2009, a total of 1975 *P. aeruginosa* isolates, one per patient, were collected from patients hospitalized in ICUs (730 isolates) and non-ICU wards (1245 isolates) of our hospital. *P. aeruginosa* was isolated from 736 pus, 464 urine, 324 respiratory, 238 catheter, 193 blood, and 20 pleural fluid cultures. Identification and susceptibility testing were performed using the Vitek 2 automated system (bioMérieux, Marcy l’Etoile, France) and MICs were determined by E-test (AB Biodisk, Solna, Sweden) when necessary, according to CLSI guidelines. The antimicrobials tested were aztreonam (AZT), cefepime (FEP), ceftazidime (CAZ), imipenem (IMP), meropenem (MER), piperacillin (PIP), piperacillin/tazobactam (TZP), amikasin (AN), gentamicin (GM), tobramycin (TM), ciprofloxacin (CIP) and colistin (CS).

Results: Higher resistance levels were observed for isolates that derived from ICU patients, reaching up to 69% for AZT, 68% for FEP, 74% for CAZ and CIP, 76% for IMP and 74% for MER, 60% for PIP, 50% for TZP. Resistance rates to aminoglycosides varied from 68% for AN, to 70% for GM and TM, and to 71% for NET. *P. aeruginosa* isolates from non-ICU patients exhibited lower levels of resistance up to 50% for AZT, 40% for FEP, 43% for CAZ, 47% for CIP, 42% for IMP and 38% for MER, 33% for PIP, 20% for TZP. As for aminoglycosides resistance was 38% for AN, 40% for GM and TM, and 44% for NET. Colistin was the most active antimicrobial with resistance that reached 2.7% and 2.4% for isolates from ICU and non-ICU patients respectively. During the 3 years of the study there was a gradual decrease of resistance to gentamicin of 9% and to ceftazidime of 10%, which was not observed for the rest of the antimicrobials tested.

Conclusion: *Pseudomonas aeruginosa* isolates from ICU patients exhibited higher levels of resistance in contrast to those from non-ICU patients. With most isolates being multidrug resistant the therapeutic choices for these critically ill patients are restricted therefore the prudent use of antibiotics along with the use of effective infection control measures are necessary, in order to limit the development and spread of resistance.

**P576** Activity of colistin against European *P. aeruginosa* from the TEST Study: 2009–2010

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Background: Successful treatment of infections due to *Pseudomonas aeruginosa* has become problematical due to widespread resistance to currently available therapies. As many isolates exhibit resistance to multiple antimicrobial agents, older agents, such as colistin (polymyxin E), are being reexamined for their treatment potential. This study investigated the activity of colistin against European clinical isolates of *P. aeruginosa* collected during 2009–2010.

Methods: A subset of 504 isolates collected from 25 European countries during the 2009–2010 TEST program (Tigecycline European Surveillance Trial) from various infection sources were included in this study. MICs were performed following CLSI guidelines and interpreted according to EUCAST breakpoints.

Results: See the Table.

Conclusions: Colistin was the most active agent in vitro, with 80.6% of the *P. aeruginosa* isolates, including multi-drug resistant strains, susceptible by EUCAST criteria. Colistin represents a potential addition to the treatment of drug-resistant *P. aeruginosa*. As usage of this agent increases, careful monitoring of the incidence of resistance is warranted.

**P577** Population structure of clinical and environmental *Pseudo- monas aeruginosa* isolates from five Mediterranean countries

M. Maatallah, A. Bakhrouf, C. Giske* (Monastir, TN; Stockholm, SE)

Objectives: Based on various approaches, several studies in the last years provided evidence that *Pseudomonas aeruginosa* has a non-clonal population structure punctuated by highly successful epidemic clones or clonal complexes. However, few studies have been conducted with multilocus sequence typing (MLST). The role of recombination in the diversification of *P. aeruginosa* clones has been suggested, but too a little extent demonstrated by using MLST-data.

Methods: Clinical (n=124) and environmental (n=19) isolates of *P. aeruginosa* were subjected to pulsed-field gel electrophoresis (PFGE), MLST (n=106, mostly with different Spel macrorestriction profiles), serotyping and PCR targeting the virulence genes exoS and exoU. Susceptibility testing was performed with disk diffusion according to EUCAST. The occurrence of multi-resistance (>3 antipseudomonal drugs) was analyzed. MLST data were analyzed with BioNumerics 6.0, using minimal spanning tree analysis as well as eBURST. The inference of clonal relationship was assessed with Clonal frame. The Neighbor net algorithm was used to generate a phylogenetic network and to test for the presence of recombination. Genetic diversity and linkage disequilibrium was measured with the index of association with LIAN 3.5.

Results: Among the 143 isolates we identified 95 PFGE-types. The MST connected 63 sequence types, among which ST235 was by far the most common. ST235 was very frequently associated with the O11 serotype (21/23 O11), and very frequently displayed multi-resistance and the virulence genotype exoS/exoU+. The eBURST analysis revealed a relatively high diversity among the isolates with some related STs. The Clonal frame linked several groups previously identified by eBURST and provided insight to the evolutionary events occurring in the population. A Neighbor net analysis based on the concatenated sequences revealed a complex network, providing evidence of frequent recombination. The index of association 0.551 when all the strains were considered indicated a freely recombining population. The mean genetic diversity at all loci was relatively high (0.8345±0.0245).

Conclusion: *P. aeruginosa* isolates from the Mediterranean countries display an epidemic population structure, particularly dominated by ST235-O11, which has earlier also been coupled to the spread of blaVIM in many countries. ST235 was also frequently associated with exoU and with multi-resistance. We found evidence of frequent recombination.
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**[P578] Can Helicobacter pylori reside in the human oral cavity?**
A. Al-Ahmad*, A. Kürschner, A. Wittmer, E. Hellwig, M. Kist, B. Waidner (Freiburg, DE)

Objectives: Helicobacter pylori is a Gram-negative, highly motile, microaerophilic, spiral-shaped organism, which colonizes the stomachs of at least half of the world's population. Infestation of humans results in persistent gastritis, which can develop into peptic ulcer disease and adenocarcinoma. The role of the oral cavity for the infection cycle of this germ is still unclear. Published data are very contradictory and up to now, there are no clear data demonstrating comprehensible isolation of **H. pylori** from any of the different compartments of the oral cavity.

The aim of this study was to evaluate the prevalence of **H. pylori** in the oral cavity of 15 H. pylori positive tested patients (stool antigen ELISA). Mini-DNA was competitive. All patients were asked to attend an experienced dentist. Thus survival and proliferation of those species which were frequently isolated from the oral cavity may cause can not be detected in any sample taken (161 oral samples). In one patient with bad periodontal status and oral hygiene as well as with high gingival inflammation, **H. pylori** positive PCR signals were obtained in two samples (periapical exudates and tongue swabs). The PCR inhibition controls conducted in parallel confirmed the validity of the PCR reactions. **H. pylori** could not be cultivated from these two PCR positive samples.

**Conclusions:** The results of this comprehensive study challenge the depiction that **H. pylori** resides in the human oral cavity.

**Cytokine levels in serum of patients with gastric pathologic infection**

**[P579]**
A. Al-Ahmad*, A. Kürschner, A. Wittmer, E. Hellwig, M. Kist, B. Waidner (Freiburg, DE)

Objectives: Helicobacter pylori is a Gram-negative, highly motile, microaerophilic, spiral-shaped organism, which colonizes the stomachs of at least half of the world's population. Infestation of humans results in persistent gastritis, which can develop into peptic ulcer disease and adenocarcinoma. The role of the oral cavity for the infection cycle of this germ is still unclear. Published data are very contradictory and up to now, there are no clear data demonstrating comprehensible isolation of Helicobacter pylori from any of the different compartments of the oral cavity.

The aim of this study was to evaluate the prevalence of Helicobacter pylori in the oral cavity of 15 Helicobacter pylori positive tested patients (stool antigen ELISA). Mini-DNA was competitive. All patients were asked to attend an experienced dentist. Thus survival and proliferation of those species which were frequently isolated from the oral cavity may cause can not be detected in any sample taken (161 oral samples). In one patient with bad periodontal status and oral hygiene as well as with high gingival inflammation, Helicobacter pylori positive PCR signals were obtained in two samples (periapical exudates and tongue swabs). The PCR inhibition controls conducted in parallel confirmed the validity of the PCR reactions. Helicobacter pylori could not be cultivated from these two PCR positive samples.

**Conclusions:** The results of this comprehensive study challenge the depiction that Helicobacter pylori resides in the human oral cavity.

**Assessment of peroxisome proliferator-activated receptor-γ polymorphisms in Helicobacter pylori infected patients**

**[P580]**
H. Goudarzi* (Tehran, IR)

Peroxisome proliferator-activated receptor-γ (PPAR-γ), is a member of superfamily of nuclear hormones receptor which involved in cell regulation. As demonstrated previously, two main function i.e inhibition of growth of cancerous cells as well as induction of apoptosis are attributed to PPAR-γ. In the other hand, Helicobacter pylori can induce peptic ulcer and cancerous. This study aimed to determine the PPAR-γ polymorphism in Helicobacter pylori infected patients. A total of 200 Helicobacter pylori infected patients were included. The history of Helicobacter pylori infection was confirmed by detection of IgG using commercial test. DNA extraction was followed by PCR-RFLP of PPAR-γ gene. This study showed the polymorphic types as follows: 5% GG (Ala12Ala), 35% GC, and 60% CC. A statistical association was found between GC polymorphism and duodenal ulcer (P=0.03). In addition, GG polymorphism associated with gastric cancer (P=0.004). Our study demonstrated that GG and GC polymorphism in PPAR-γ gene can be used as predictive factor for detection of high risk cases of Helicobacter pylori infected patients.

**Detection of H. pylori and clarithromycin and/or fluoroquinolone resistance in gastric biopsies by Genotype® HelicoDR**

**[P581]**
T. Alarcon*, A. Somodevilla, S. Agudo, P. Urrozuno, M.J. Martinez, M. Lopez-Brea (Madrid, ES)

Clarithromycin susceptibility is an important factor to predict Helicobacter pylori eradication failure. In addition, the resistance to this antibiotic is high in Spain. Therefore, early detection of resistance is important in treatment of Helicobacter pylori infection. The aim of this study was to evaluate a commercially available kit Genotype® HelicoDR (Hain, Diagnostika, Nehren, Germany) for detection of Helicobacter pylori strains that are clarithromycin and/or fluoroquinolone resistant and compare it with conventional protocols.

A total of 146 biopsies were obtained from patients with gastric symptoms. Standard procedures were used for Helicobacter pylori culture. Clarithromycin and ciprofloxacin resistance was determined by E-test as described before. DNA extraction was carried out by the NucliSens easyMAG platform (bioMérieux), and Genotype® HelicoDR was performed as manufacturer recommended.

**Results:** 121 of the 146 biopsies were positive for Helicobacter pylori, 57 (47.1%) of them were clarithromycin susceptible and 64 (52.9%) clarithromycin resistant: 57 (89%) showed A2143G, 4 (6.2%) showed A2142G mutation and 3 (4.7%) double mutations A2142C-A2143G. 17 of them showed heteroresistance (wild type and mutation simultaneously). 114 (95%) of the biopsies were susceptible to fluoroquinolones and 6 (5%) were resistant. The result in one biopsy was too light and was not possible to define the presence of wild type or resistant genotype.

**Conclusions:** A high prevalence of clarithromycin resistance in Helicobacter pylori from gastric biopsies from Spain was found. The use of a commercially available kit is useful for rapid detection of both clarithromycin and fluoroquinolone resistance.

**Cytokine levels in serum of patients with gastric pathologic infected with Helicobacter pylori cagA positive and cagA positive**

**[P582]**
D. Ortiz Prinz*, O. Rodríguez, M. Cucuzza (Caracas, VE)

Helicobacter pylori is recognized as a human-specific gastric pathogen and carcinogen type I that colonizes the mucus layer in the stomachs of at least half of the world's population. In some subjects, the infection causes acute or chronic gastritis or peptic ulceration and plays an important role in the development of peptic ulcer, gastric adenocarcinoma and lymphoma MALT. The cagA gene is part of the cag pathogenicity island, code for a putative Helicobacter pylori secretion system that is associated with export of virulence factors to the extracellular compartment. Gastric mucosal levels of proinflammatory cytokines have been reported to be increased in Helicobacter pylori infected subjects. In Venezuela there is a high prevalence of Helicobacter pylori cagA positive and also a high incidence of gastric cancer. The aim of this study was determine serum cytokine levels in patients infected with Helicobacter pylori cagA positive and negative.

**Methods:** 42 adults male and female were evaluated in the Gastroenterology Service Jose Maria Vargas Hospital, Caracas, Venezuela. They were carried out clinical evaluation, histopathological, determination of serum cytokine levels for flow cytometry, identification and typing of Helicobacter pylori by PCR. The serum cytokine levels (INFγ, TNF, IL-10, IL-6, IL-4, and IL-2) were simultaneously quantified by cytometric bead array. The procedure was carried out according to the manufacturer's instruction (CBA™, BD Biosciences), finally samples were analyzed on FACS DIVA using the supplied cytometer setup beads. DNA from Helicobacter pylori was extracted from culture obtained from gastric biopsies of patients evaluated. The cagA gene was amplified by PCR using the primers CAG31 and CAG2 (Yamaoka, 1998) were used to amplify the 3′ region of the cagA gene.

**Results:** 78% of patients tested were cagA positive. We found that the values (pg/ml) of IFNγ, TNF and IL-6 were significantly higher.
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(p < 0.001) in patients infected with of H. pylori cagA positive strains than in those who were infected with H. pylori cagA negative.

Conclusion: These results can help us better understand the mechanism by which H. pylori prevent a severe immune response and thereby facilitates its stay in the stomach. The balance in the production of anti-and pro-inflammatory cytokines may play an important role in the development of severe gastric pathologies and the pathological mechanisms of H. pylori infection in individuals at risk.

Prevalence and distribution of virulence factor jhp0562 and jhp0563 genes among Spanish Helicobacter pylori clinical isolates

P. Somodevilla*, T. Alarcón, E. Aznar, M.J. Martínez, M. López-Brea (Madrid, ES)

Objective: The aim of this study was to determine the prevalence and distribution of two glicosiltransferases encoded by jhp0562 and jhp0563 genes. These glicosiltransferases are involved in the lipopolysaccharide (LPS) synthesis. The presence of jhp0562 has been recently related with peptic ulcer disease.

Methods: Helicobacter pylori strains were obtained from biopsies of symptomatic patients between 2008 and 2009. Biopsies were cultured in Blood and Pylori Agar plates incubated at 37°C in a 5% CO₂ atmosphere. DNA extraction of each strain was performed by using the automatic system EasyMag (BioMérieux).

After conventional PCR with previously described primers, fragments of 301 bp (jhp0562) and/or 602 bp (jhp0563) were detected by electrophoresis with 1.2% agarose gel.

Results: 63 strains from symptomatic Spanish patients were studied. Patients included 27 children (42,86%) and 36 adults (51,14%). The prevalence of jhp0562 gene in the whole population was 53,9% (34 out of 63) whereas for jhp0563 was 88,88% (56 out of 63).

Among pediatric patients results showed a 51,85% of positivity for jhp0562 (14 out of 27), while the prevalence for adult patients was 58,33% (21 out of 36). Differences were not statistically significant. For jhp0563 results showed 96,3% of prevalence in children (26 out of 27) and 86,11% (31 out of 36) in adults. Differences were not statistically significant.

Distribution of CagA and Vacs1 genes were: 20 CagA (31,74%) and 20 vacs1 (31,74) positive strains respectively.

Relationship between jhp0562 and jhp0563 with these two virulence factors is presented in the table.

Conclusions: Jhp0562 was present in more than 50% of the population studied whereas jhp0563 prevalence was almost 90%. Age seemed not to be an important factor for the prevalence of any genes studied. However, jhp0562 was statistically related with the presence of other important virulence factor as Vacs1 and CagA.

|               | Jhp0562 | Jhp0563 |
|---------------|---------|---------|
| CagA positive | 90% (18 out of 20) | 79% (15 out of 20) |
| CagA negative | 35,35% (17 out of 48) | 97,67% (42 out of 43) |
| Vac s1        | 90% (18 out of 20) | 75% (15 out of 20) |
| Vac s2        | 35,35% (17 out of 48) | 97,67% (42 out of 42) |

P < 0.01.

Prevalence of virulence factor HomB among Spanish Helicobacter pylori clinical isolates

P. Somodevilla*, T. Alarcón, M. Espinola, P. Urruzuno, M. López-Brea (Madrid, ES)

Objective: The aim of this study was to determine the prevalence of Helicobacter pylori (H. pylori) infection in our region and to define the genotype diversity of identified strains and their association with clinical presentation.

Methods: A prospective and randomized study has been conducted in the gastroenterology department of university hospital Hassan II of Fez from May 2009 to September 2010. A personal interview and a clinical examination were conducted for all consenting patients enrolled in the study. They had undergone endoscopy for diagnosis of abdominal pain or discomfort and to collect gastric biopsies. For each patient, two biopsies were examined by Polymerase Chain Reaction (PCR) to detect ure C gene of H. pylori. The genotype characterization consists on the Cag A status and Vac A sub-types determination, directly from biopsies. The statistic analysis has been done using Epi-info.

Results: During the 16 months of the study, 330 patients have been recruited. The mean age of the participants was 49 years. Clinical data shows that 65.7%, 22% and 8% of patients have gastritis, peptic ulcer disease and gastric cancer respectively. However, 4.3% of participants present normal mucosa at endoscopy. PCR results showed that H. pylori infection occurs in 68.4% of the cases. Genotypic analysis of 226 positives PCR showed that cag A gene has been found in 26.10%. Vac A signal sequence genotypes s1 and s2 were detected in 35% and 24.3% of the studied specimens respectively. The vac A middle region m1 has been detected in 31.4% of the cases versus 55.9% for m2. All vac A subtypes combination were founded with predominance of vaca m2-s2 (33.2%) whereas, vaca m1-s2 was present with rang of 1.3%. A multiple infection was detected in 9.4% of H. pylori positive cases. It is also noticed that sub-type m1-s1 was the most related genotype to cag A (31%). In the 14 patients with a normal endoscopy, 8 were H. pylori positives. Determined genotypes of those specimens were vac A m2-s2 (37.5%) or one allele type of m or s region (62.5%). No significant association has been established between genotype and gastric pathology.

Conclusions: The primary results of our study showed that the subtype vaca m2-s2 is the most predominant in our series. No association between H. pylori genotypes and clinical outcomes has been determined in our region.

Molecular characterisation of Helicobacter pylori associated to different gastric pathology in adult Moroccan population: prospective study

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Objective: The aim of our study is to determine the prevalence of Helicobacter pylori (H. pylori) infection in our region and to define the genotype diversity of identified strains and their association with clinical presentation.

Methods: A prospective and randomized study has been conducted in the gastroenterology department of university hospital Hassan II of Fez from May 2009 to September 2010. A personal interview and a clinical examination were conducted for all consenting patients enrolled in the study. They had undergone endoscopy for diagnosis of abdominal pain or discomfort and to collect gastric biopsies. For each patient, two biopsies were examined by Polymerase Chain Reaction (PCR) to detect ure C gene of H. pylori. The genotype characterization consists on the Cag A status and Vac A sub-types determination, directly from biopsies. The statistic analysis has been done using Epi-info.

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Conclusions: The primary results of our study showed that the sub-type vaca m2-s2 is the most predominant in our series. No association between H. pylori genotypes and clinical outcomes has been determined in our region.
Among pediatric patients results showed a 55.69% of positivity for HomB (15 out of 26), while the prevalence for adult patients was 47.05% (16 out of 34). Differences were not statistically significant. Differences were not statistically significant for both virulence factors. Relationship between HomB and HomA with the two virulence factors is presented in the table. Differences were not statistically significant for both virulence factors.

Conclusions: HomB was present in more than a half of the population studied. It seemed to follow an age pattern being more prevalent among pediatric patients, although results in the population studied are not statistically significant. Differences in HomB distribution among different genotypes had not been found in this study.

|          | HomB | HomA | Total |
|----------|------|------|-------|
| CagA+     | 11   | 15   | 18    |
| CagA−     | 22   | 39   | 42    |
| VacA+     | 15   | 15   | 30    |
| VacA−     | 22   | 44   | 66    |

**PS85** Mice immune responses to UreB332-HpaA, a recombinant fusion protein from Helicobacter pylori

B. Hajikham*, S. Najir Peerayeh, H. Soleimanjahahi, Z. Hassan (Tehran, IR)

Objectives: Helicobacter pylori (H. pylori) is a spiral-shaped, microaerophilic bacterium that colonizes the human gastric and duodenal mucosa, where it induces chronic gastritis and peptic ulcer. H. pylori infection is also associated with gastric MALT lymphoma and gastric cancer. Current antibiotic therapies together with a proton-pump inhibitor entail problems such as patient compliance, increasing antibiotic resistance, recurrence, re-infection and high cost. Vaccination is an attractive strategy, either as an alternative or a complementary to antibiotic treatment, for clearance of H. pylori. In this way many of H. pylori virulence factors have been tested to investigate their role in different immune response induction.

Methods: At the current study a recombinant fusion protein from H. pylori consisting of a fragment of B subunit from H. pylori urease (UreB332) and helicobacter pylori adhesion A (HpaA) named UreB332-HpaA have been produced and then the immune responses to this fusion protein were tested in BALB/c mice.

Results: Subcutaneous administration of the fusion protein significantly induced immune responses in mice which were associated with H. pylori-specific IgG1, IgG2a and sIgA antibodies production as well as IFN-γ and IL-4 cytokines which proposed a Th1/Th2 immune responses. The Fusion antigens elicited stronger immune responses compared to each single antigen (p < 0.001).

Conclusion: The results show that this bivalent fusion protein efficiently elicited different types of immune responses and that an antigenic fragment of the protein can be used instead of the whole protein to construct a multivalent vaccine in further studies.

**PS86** Prospective application of the Lumixn xTAG®-GPP multiplex PCR in diagnosing infectious gastroenteritis

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Objectives: Infectious gastro-enteritis (GE) is a major diagnostic challenge as it can be caused by parasites, bacteria and viruses. The Lumixn Gastro-enteritis Pathogen Panel (xTAG®-GPP) detects 18 most common GE causing pathogens including viruses (norovirus GI and GII, adenovirus, and rotavirus), bacteria (Campylobacter, Salmonella, Shigella, E. coli O157, Vibrio, and Jerinia), toxins (Clostridium toxins A/B, Shiga toxins 1 and 2, and ETEC toxins) and parasites (Giardia, Cryptosporidium and Entamoeba histolytica). This study shows the first prospective application of this assay.

Methods: From June to August 2010, 200 consecutive samples were subjected to routine diagnostic procedures, i.e. culture for bacterial pathogens and multiplex real-time PCR for viruses and parasites. The samples were also analyzed by the xTAG®-GPP assay. In addition 77 positive (and 23 negative) samples were selected to include more different pathogens in the analysis.

Results: 70 out of the 200 samples were positive in the xTAG®-GPP assay. A total of 55/200 (27.5%) samples were positive for Campylobacter sp., 4 for Shigella sp., 1 for Salmonella, 6 for Clostridium difficile, and 4 contained the ETEC ST toxin. Only six of these positives were detected by bacterial culture. The diagnostic positive results for parasites (4 Giardia lamblia, 1 Cryptosporidium parvum) and viruses (4 Norovirus GI) were all detected by the xTAG®-GPP. Unresolved inhibition was observed in 11 samples.

In the selected group, 39 Campylobacter sp. positive samples were detected of which 17 were culture positive. Out of 37 virus positive samples, 33 were confirmed by the xTAG®-GPP assay and 4 with high Ct values were not detected. Of 23 parasite positives, 17 were detected by the xTAG®-GPP but the additional Giardia positive samples were detected. Added value of the xTAG®-GPP assay is detection of additional positive results, undetected in routine diagnostics mainly because the analysis had not been requested by the clinician. For instance, in two Cryptosporidium positive samples, E. coli O157 and ETEC-LT were detected.

Conclusion: The 18-target multiplex of the xTAG®-GPP assay results in a slight decrease of sensitivity for some targets. In general, the concordance with current diagnostic molecular methods was good and clearly superior to bacterial culture. Added value of the x-TAG®-GPP is that pathogens not suspected in the infection are being detected, resulting in improved diagnosis of gastro-enteritis.

**PS87** Screening for enteropathogenic Escherichia coli by an eight-plex PCR of nucleic acids extracted directly from human stools using the NorDiag Bullet stool DNA kit

J.K. Møller*, B. Kolmos, M.H. Dahl, L. Pødenphant, D. Ørnskov (Vejle, DK)

Objectives: To evaluate the performance of an in-house eight-plex PCR assay, amplifying eight specific virulence genes and one internal control gene (16S rRNA) in a single reaction to screen for the five main pathotypes of diarrheagenic E. coli. Three different approaches for nucleic acids extraction were applied: (1) directly from stool samples, (2) from a scrape across the primary culture plates after conventional culture of the stool samples on the SSI Enteric Medium (Statens Serum Institut, Copenhagen), (3) from E. coli representing different colony morphology subcultured on chromID™ CPS® plates (bioMerieux).

Methods: Nucleic acids were extracted directly from stool specimens using the Bullet stool DNA sample kit and protocol from NorDiag (Oslo, Norway) and from culture plates by boiling and dilution of scrapings or E. coli colonies. Primer sets for the eight virulence genes were used: Stx1, Stx2, and eaeA for enterohemorrhagic E. coli (EHEC), eaeA for enteropathogenic E. coli (EPEC), STib, Sta, and LTI for enterotoxigenic E. coli (ETEC), ipaH for enteroinvasive E. coli (EIEC), and aggR for enterogegregative E. coli (EAEC). Each forward primer was labelled with a fluorochrome and the PCR product was separated by multicolour capillary electrophoresis on an ABI PRISM™3130 Genetic Analyzer (Applied Biosystems). True positive samples were defined as being PCR positive in at least 2 of the 3 comparative assay protocols.

Results: A total of 387 randomly selected stool samples submitted from patients with persistent diarrhoea were included during the study period. Overall 102 (26.4%) stool samples contained one or more of the seven virulence genes. The sensitivity of the eight-plex PCR on direct stool material, culture scrapings, and E. coli colonies was 97.1% (99/102), 91.2% (93/102), 76.5% (78/102), respectively. The
corresponding specificity was 98.2% (280/285), 98.6% (281/285), and 100% (285/285), respectively. The eaeA gene was the most commonly found gene, 61.5% (64/104) of PCR-positive stools, 54.6% (53/97) of PCR-positive scrapings, and 48.2% (38/78) of PCR-positive E. coli colonies. Second most frequent gene was the aagR gene correspondingly seen in 45.4%, 44.2%, and 52.6% of the PCR-positive samples.

**Conclusion:** Screening for virulence genes by PCR of DNA extracted directly from stool samples followed by culture confirmation of PCR-positive samples may provide an effective and rapid alternative strategy for detection of enteropathogenic E. coli.

### P588

**Microarray-based detection of virulence genes in verotoxic Escherichia coli strains isolated from human, cattle and food in Poland**

A. Januszkiewicz*, J. Szych, J. Osieck, W. Rastawicki (Warsaw, Pulawy, PL)

**Objectives:** Shiga toxin-producing *Escherichia coli* (STEC), is one amongst major causative agents of foodborne illness worldwide. That emergence pathogens can cause diarrhea and hemorrhagic colitis with life-threatening complications, such as hemolytic uremic syndrome (HUS). In Poland a few cases of human STEC infections are documented per year among them so far only two patients developed HUS. There is no data about prevalence of potential genetic virulence markers in STEC strain in Poland. The aim of this study was to compare the distribution of known virulence determinants in STEC strains isolated from human, cattle and food in Poland.

**Methods:** We investigated 71 STEC strains from human (n=45), cattle (n=16) and food (n=10) isolated from 1999 to 2010. The isolates were tested for verotoxin production by VTEC-RPLA (Oxoid) and Vero cell cytotoxicity assay. Enterohemolytic phenotype (Ehly+) was determined on blood agar plates containing 5% washed, defibrinated sheep erythrocytes (Oxoid). Presence of stx1, stx2, eae, ehly were performed by conventional PCR. Sub-typing of the verotoxin genes were determined by PCR. Microarray system (Idetibac Ee) containing 92 virulence genes of various *E. coli* pathotypes, was used for detection of virulence determinants.

**Results:** All of STEC strains produced active verotoxins which were cytotoxic for Vero cell. STEC strains were positive for stx1 (n=22), stx2 (n=27) or both (n=22). The following variants of verotoxin were detected: stx1a, stx2a, stx2b, stx2c, stx2d, stx2g. All STEC strains except 3 isolates from cattle (95.8%) were positive for the eae gene. Enterohemolysin production (Ehly+) was detected in almost all STEC strains (94.4%). The STEC strains were assigned to 40 different virulotypes. We have found stable a core set of virulence genes in STEC O26, O111 and O157. The core sets were serotype specific. In addition to the cores there were strain specific determinants found in virulotypes. STEC O157 isolates from human were similar to several STEC O157 isolates from cattle and food. While non-O157 STEC isolates from cattle possessed virulence genes: subAB, ssa, stl, eaeA, not detected in strains from human and food.

**Conclusion:** The virulotyping microarray chip was able to distinguish various virulotypes among STEC strains. STEC strains isolated in Poland harboured serotype specific stable core of virulence genes which in some strains was be extended by additional strain specific determinants.

### P589

**Evaluation of a new multiplex PCR as a tool for the identification of Campylobacter species in bacterial gastroenteritis**

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**Objectives:** Bacteria belonging to the species *Campylobacter* represent the most frequent etiological agent of human bacterial gastroenteritis in the world. Their identification can be made with phenotypic methods which are time consuming, or with genotypic methods which are faster. The aim of this study, was to develop a new PCR strategy to identify *Campylobacter* species and compare the results to those obtained by the phenotypic methods.

**Material and Methods:** We conducted a retrospective cohort study on fecal isolates obtained at CHUL's Hospital from 2007 to 2009. 242 consecutive isolates of campylobacteria that the microbiological lab had identified with the hippurate test were submitted to PCR. Three pairs of primers were combined in a triplex: one targets the tuf gene (this study) of the *Campylobacter jejuni jejuni* subsp. *jejuni* and *Campylobacter jejuni* subsp. *doylei*; and two others target napA and region flanking napA of *C. jejuni jejuni* and *Campylobacter coli* (W.G. Miller et al. 2007). With detection by agarose gel electrophoresis, *C. jejuni jejuni* yields two bands: 836 pb and 1454 pb; *C. jejuni doylei* produces two bands: 836 pb and 1240 pb; *C. coli* yields a single band at 1454 pb. Based on this triplex PCR and confirmation by sequencing, we should be able to improve our ability to identify *Campylobacter* species and subspecies.

**Results:** Among the 201 hippurate-positive isolates, 197 were confirmed by PCR as *C. jejuni jejuni*, and 4 were classified as other species. Within the 41 hippurate-negative isolates, 31 were *C. jejuni jejuni* and 10 other species by PCR. No *C. jejuni doylei* was found.

**Conclusion:** The results of the phenotypic methods have a good positive predictive value when the hippurate test is positive; but when it is negative, it misses a lot of *C. jejuni jejuni*. When the test for hippurate is negative, it is essential to use the tuf-napA triplex PCR to have a better representation of the real distribution of the *Campylobacter* species. This new triplex PCR can help to precise the epidemiology and assist in the choice for antibiotics in campylobacteriosis.

### P590

**Contrast-enhanced sonography for the diagnosis and therapy of liver abscesses**

R. Chiaiaardi*, P. Grimà, L. Gillini (Galatina, IT)

**Objectives:** Liver abscesses are often diagnosed by ultrasound detection of focal liver lesion in patients with flank pain and fever. Ultrasound (US) guided drainage is useful to achieve an etiological diagnosis and to speed up the clinical resolution after antibiotic therapy. Otherwise US often underestimate small hepatic lesion while Computed Tomography (CT) may fail to characterize necrotic lesions mimicking liver malignancy. In our small study we showed how Contrast-Enhanced Sonography (CES) can improve diagnosis and echo-guided therapy of liver focal infections.

**Methods:** In our Department, from January to November 2010, we admitted 10 patients (3 males and 7 females aged 11–65 years) with fever and 29 total US detected focal lesions of the liver. All patients were submitted to CT and CES using 2.4ml of second generation contrast medium (Sonovue − Bracco, Milan, Italy. Every patient were submitted to CT and CES using 2.4ml of second generation Contrast Medium (Sonovue − Bracco, Milan, Italy. Every patient was also submitted to CES-guided drainage, pus and blood-culture, serology for Bartonella henselae, Anoeba bistolytica and Echinococcus granulosus. The diagnosis of liver abscesses was achieved in all patients and they were submitted to iv antibiotic therapy for 10 days. Monthly follow up by CES was performed for a period of 3–9 months.

**Results:** In four out of ten patients pus and blood culture allowed to achieve the final diagnosis of pyogenic hepatic abscess by *E. coli* (2 patients), *K. pneumoniae* (1 patient) and *S. holo* (1 patients). All patients showed to be affected by underlying biliary or colonic diseases. In one patient with a cat scratch disease serology for *B. henselae* allowed to achieve the final diagnosis. US examination detected only 18 out of 29 total liver abscesses while CES and TC were able to detect all 29 hepatic lesions. CT fail to characterize all liver lesions by detecting a rim arterial enhancement in the early phase and a clear wash out in the portal and late phase. Successfully pus-drainage and follow up was performed in all patients by using CES to guide the procedures because of improved detection of anechoic necrotic avascular areas.

**Conclusions:** In our experience CES is a safe and effective tool in the management of liver abscesses. CES may improve and speed up the diagnosis of hepatic focal lesions detected by US.
CES is also useful to characterize suspected liver malignancy after inconclusive CT study and it is safe and effective to guide pus drainage and clinical follow up.

**P591 Distribution of Salmonella enteritidis and Salmonella typhimurium phage types in Slovakia**

L. Maštánová, V. Maštán* (Bratislava, SK)

**Objectives:** The objectives of this study were to identify phage types of both dominant *Salmonella* serovars, Enteritidis, and Typhimurium, and to ascertain their distribution during the period 1995–2009 in Slovakia.

**Methods:** The phage typing of 3900 S. Enteritidis isolates and 1741 S. Typhimurium isolates has been carried out in the period 1995–2009. A total of 3346 (85.8%) S. Enteritidis human isolates collected from outbreaks or sporadic cases were isolated from faeces and 554 (14.2%) strains originated from food. A total number of 5. S. Typhimurium isolates from human faeces was 1722 (98.9%) and only 19 (1.1%) strains were isolated from food. For S. Enteritidis strains the phage typing system described by Ward et al. (1987) has been used. The S. Typhimurium strains were phage typed according to Callow (1959) and Anderson et al. (1977) schemes.

**Results:** A total of 3802 (97.5%) S. Enteritidis isolates were typeable and belonged to most frequent phage types PT8, PT4 and PT6. The most predominant was PT3 over the period of study. In addition of these observations, it was found that 183 S. Enteritidis strains with PT8 (67% of the total PT8 food isolates), 58 strains with PT4 (59% of the total PT4 food isolates) and 44 strains with PT6 (45% of the total PT6 food isolates) were isolated from eggs and egg products. A total of 1343 S. Typhimurium strains (77.1%) were typeable and belonged to different phage types. Using the Callow typing scheme the most frequent phage type was 2h. According to Anderson’s typing scheme the most frequent PTs were DT104, U302, DT193, DT20a, DT68, DT1 and DT2. The most dominant phage type of isolates from food sources was PT U302.

**Conclusion:** Continual surveillance of both dominant *Salmonella* serovars, Enteritidis and Typhimurium phage types monitors their distribution during of large periods. The value of phage type information is important for epidemiologic analysis salmonella infections, and in following the emergence and spread of the important phage types such as PT8, PT4 and DT104, U302 and relevant other.

**P592 Comparison of two different Rappaport broths for the detection of Salmonella in faecal specimens from patients with gastroenteritis**

L. Conter*, R. Vaisari, R. Botrugno, M. Reboldi, S. Missorini, R. Colombo (Brescia, IT)

**Objectives:** In recent years automated instruments are available for the microbiology laboratory that can be used only with samples in liquid format. Copan is the first company that introduced the Liquid Base microbiology (LBM) concept for samples collection followed by the Walk Away Specimens Processor (WASP) to the microbiology laboratory. Several specimens’ collection/transportation devices and enrichment broths in a format suitable for automation are available including a Rappaport Vassiliadis Soya (RVS) broth. The objective of this study was to compare the performance of Copan RVS broth to the Rappaport-T (RT) broth (bioMérieux) for the detection of *Salmonella* in faecal specimens.

**Methods:** Clinical faecal specimens (253), received at the Fleming Labs for the investigation of gastroenteritis, were used for this comparison. Each sample was tested in duplicate, one as per routine method, 10 mg of sample was seeded into the 9 mL RT broth in a glass tube, and a same sample aliquot was seeded into the 2 mL RVS broth in a plastic tube. After 18–24h incubation at 37°C inoculated 10 ul of each broth were inoculated in SS and chromogenic SMID agar plates (bioMérieux). After 24 incubation all positive colonies were tested with a latex poly *Salmonella* antisera (Oxoid) and serotyped with *Salmonella* group specific antisera (DID/BioRad). Dubious culture were sub-cultured in the same agar plates and identified as per above procedure.

**Results:** In the 253 tested 27 were salmonella positive and 224 salmonella negative in both RT and RVS broths. One sample was in RVS was found salmonella positive on the 3rd passage and negative in 2nd passage in the Rappaport-T broth. The 20 Salmonellae positive were identified eight serotypes O:9, 3, five serotypes O:6,7,8, six serotypes O:4,5; eight were not identified.

**Conclusions:** Similar numbers of salmonella positive samples were found in RVS broth compared to the RT broth. The 2 ml RVS in plastic tube demonstrate a good performance for the detection of salmonella in faecal samples and was found as better device because is easy to use and not prone to accidental breakage of during routine laboratory testing.

**P593 Isolation of the emerging food-borne pathogen Arcobacter from human stool**

K. Hoof*, S. Drieghe, A. Van den Abeele (Merelbeke, Ghent, BE)

**Objectives:** Arcobacters are Gram-negative slender curved bacteria closely related to campylobacters. In humans, predominantly *A. butzleri* has been associated with enteritis and occasionally septicaemia, but also *A. cryaerophilus* and *A. skirrowii* have been isolated from stool of diarrheic patients. Infection may occur by the consumption of contaminated water or food or by direct contact. At present, for the isolation of arcobacters from human specimens, *Campylobacter*, *Tersinia* or *Leptospira* media are used. The aim of this study was to evaluate a specific Arcobacter procedure (Houf et al., 2007) for the examination of human stool from patients.

**Methods:** From January 2008 to November 2010, all stool samples from out-patients and from in-patients admitted less than 72 hours to a large secondary care hospital were included. Culture for all common bacterial pathogens was performed. *Arcobacter* isolation was carried out inoculating 1g faeces into 9 ml of *Arcobacter* enrichment broth and overnight incubation at 28°C. Subsequently, 50 μl was streaked onto an Arcobacter selective agar plate. Plates were screened after 72 hours microaerobic incubation at 28°C by dark field microscopy for typical colonies and further identified by an Arcobacter species-specific PCR- assay (Douidah et al., 2010). For patients with *Arcobacter* positive stools, medical records were investigated for the presence of acute or recurrent diarrhoea, abdominal pain and underlying disease.

**Results:** From 4868 eligible samples, 3597 (73.8%) were cultured for arcobacters. In this group, *Campylobacter* spp. were on top of the enteric bacterial pathogen list (5.4%) followed by *Salmonella* (3.0%) and toxicigenic *C. difficile* (2.7%). *Arcobacter* was the fourth most common isolated genus (1.3%), with almost equally isolation of *A. butzleri* and *A. cryaerophilus*. *Arcobacter butzleri* positives tended to be in-patients with diarrhoea and an underlying disease compared to *A. cryaerophilus*. *Arcobacter thereuis*, recently isolated from aborted porcine foetuses (Houf et al., 2009), was isolated for the first time from a human patient.

**Conclusion:** *Arcobacter* was significantly more isolated in this study than in studies with other methods applied. Arcobacters were the fourth most common organism isolated from stool in the study population and *A. thereuis* was isolated for the first time. Using the recently validated veterinary isolation technique, routine recovery of arcobacters in humans becomes feasible.

**ESBL and AmpC in Enterobacteriaceae and A. baumannii**

**P594 Acquisition of carbapenem resistance in multi-resistant Klebsiella pneumoniae strains harbouring blaCTX-m-15, qrsr1 and aac(6)-Ib-cr genes**

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**Objective:** To characterize the acquisition of carbapenem resistance of *K. pneumoniae* strains in a patient treated with carbapenems.
Methods: Three *K. pneumoniae* isolates were recovered from two clinical specimens from that patient. Presence of resistance genes, type 1 and 2 integrons and mutations in *gyrA* and *parC* genes was studied by PCR and sequencing. The presence and mutations in ompK35, ompK36 and ompK37 porin genes were analyzed by gene sequencing and corresponding proteins were visualized by SDS-PAGE. Molecular typing and phylogenetic group were determined by MLST and PCR-RFLP, respectively. Genetic transfer of quinolone resistance genes was carried out and the number and type of plasmids were analyzed by PFGE-S1 and PCR-based rep licon-typing (PBRT). Hybridization experiments were performed with specific probes for blaCTX-M-15, qnrS1 and aac(6′)-Ib-cr genes. The genetic environment of quinolone resistance genes were studied by PCR-mapping and the aac(6′)-Ib gene expression was determined by Real Time-PCR.

Results: The three isolates presented a close related PFGE pattern and belonged to the Kp phylogenetic group and to a new MLST registered as ST433. All of them showed the same resistance phenotype except for the fact that only two of them were resistant to ertapenem, cefoxitin and fosfomycin. The MICs to ertapenem, imipenem, meropenem and doripenem in cabapenem susceptible/resistant isolates were 0.3/≥3, 0.75/3−4, 0.13/8−12, and 0.19/32 g/L, respectively. All the strains harboured qnrS1, aac(6′)-Ib-cr, blaCTX-M-15, blaOXA-1, blaSHV-11, aac(3)-II, qnrA, qnrB and qnrS genes and wild type GyrA and ParC proteins. The sequence of ompK35 and ompK36 genes showed premature stop codons and the porins were not detected in the SDS-PAGE gels of the ertapenem-resistant strains. The OmpK36 porin was present in the SDS-PAGE gels of the ertapenem-susceptible strain. The aac(6′)-Ib gene expression was three fold higher in one of the ertapenem-resistant strains than in the susceptible one. Five plasmids were observed by PFGE-S1 and colE and IncR plasmids were determined by PBRT. Hybridization of blaCTX-M-15, qnrS1 and aac(6′)-Ib-cr probes was detected in a plasmid of 30Kb in all the strains.

Conclusions: The use of carbapenems in the treatment of multiresistant clinical isolates can cause the acquisition of carbapenem resistance due to modifications in porin genes. This fact poses a serious health threat due to the limited remaining options of treatment.

**P596 Genetic characterisation of CTX-M producing Proteus mirabilis and Morganella morganii clinical strains with association of resistance genes in Tunisia**

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Objectives: The aim of this study is to characterise the extended-spectrum β-lactamases (ESBL) genes and their genetic environments and the associated resistance genes in seven *Proteus mirabilis* and six *Morganella morganii* ESBL-positive isolates recovered in the Military Hospital of Tunisia.

Methods: From May 2005 to June 2009, 200 clinical isolates of *Proteus mirabilis* and *Morganella morganii* were isolated from patients with respiratory or urinary tract infections hospitalized at Military hospital in Tunis (Tunisia). Antibiotic susceptibility was tested with the agar disk diffusion method according to CASMF guidelines. ESBLs were detected using a standard double-disk synergy test. Characterisation of ESBLs and their genetic environments as well as integrons and their gene cassette composition were performed by polymerase chain reaction (PCR) and nucleotide sequencing. For the genotyping method, we used pulsed-field gel electrophoresis using SfiI and Spel restriction endonuclease.

Results: 21 of 200 (7%) strains exhibited non susceptibility to third generation cephalosporins and among these strains the double-disk synergy test confirmed the phenotype of ESBLs in 13 isolates (62%). These ESBLs producers were coresistant to chloramphenicol, tetracyclin and ofloxacin, but remained susceptible to ertapenem (MIC <0.25). By PCR and nucleotide sequencing, we detected the presence of bla genes as follows (number of isolates): blaCTX-M-15 (7), blaCTX-M-8 (1), blaTEM-24 (10), blaTEM-1 (4) and blaTEM-2 (4). ISEP1, IS26 and IS903, ORF477 were located upstream and downstream, respectively, of the blaCTX-M-15 gene in 7 strains. The intI1 gene was detected respectively in four and one strains of *M. morganii* and *P. mirabilis* including different gene cassettes: the combination dfrA1, ocrC and aadA1 were found, respectively, in 2 and 3 isolates. All CTX-M-15 producing isolates showed unrelated PFGE patterns.

Conclusion: The emergence of mobile genetic elements like ISEP1 carried blaCTX-M-15 gene in *P. mirabilis* and *M. morganii* was first discovered at the Military hospital in Tunisia. The incidence of these strains invites continuous surveillance of the epidemiologic evolution of these strains to prevent their dissemination.

**P597 Biochemical characterisation of TEM-107 extended-spectrum β-lactamase with an additional Arg164His substitution compared to TEM-52**

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Objectives: TEM-52 was a predominant TEM-type ESBL in Korea. TEM-52 had the unusual feature of conferring decreased susceptibility to moxalactam (MOX), and the enzyme hydrolyzed cefotaxime (CTX) more efficiently than ceftazidine (CAZ). TEM-107 (GenBank accession No, AY101764) is a novel ESBL found in a *K. pneumoniae* clinical isolate. TEM-107 has an additional Arg164His change compared to TEM-52, but the isolate was more resistant to CAZ than to CTX, and susceptible
to MOX. Therefore, we investigated the biochemical characteristics of TEM-107.

**Methods:** MICs were determined by an agar dilution method. The recombinant plasmid (pET9a/blaTEM-107) was used to transform *E. coli* XL1-blue, and BL21(DE3). TEM-107 was produced by culturing *E. coli* BL21(DE3) (pET9a/blaTEM-107) in ZYP-5052 medium. β-lactamase was purified using two anion exchange chromatography steps. The hydrolytic activity was determined at 30°C, and the kinetic data were analyzed using the Hanes-Woolf linearization of the Michaelis-Menten equation.

**Results:** The MICs for *K. pneumoniae* isolate were CAZ 64 µg/mL, and CTX 2 µg/mL. The resistance was transferred by conjugation. Arg164Ser or Arg164His are frequently observed changes in TEM-type ESBLs, which confer a higher level resistance to CAZ than to CTX. The MIC of MOX was 1 µg/mL. The purified TEM-107 protein preparation (purity, >95% by SDS-PAGE analysis) showed an approximate pl of 6.0. The turnover rate (kcat) of TEM-107 was higher for CAZ than for CTX. Hydrolysis of MOX was not determinable. The catalytic efficiencies (kcat/Km) of TEM-107 was slightly higher for CAZ than for CTX. The IC50’s of clavulanic acid was 0.61±0.1 μM.

**Conclusion:** The MIC of CAZ was much higher than that of CTX for a TEM-107-producing clinical isolate and the contransjugant, and the isolate was susceptible to MOX. The kcat value of TEM-107 was much higher for CAZ than for CTX, and the kcat/Km value was slightly higher for CAZ than for CTX.
Mechanisms of carbapenem-resistance in Enterobacteriaceae and *P. aeruginosa*

**Mechanisms of carbapenem-resistance in Enterobacteriaceae and *P. aeruginosa***

**P692** Investigation of carbapenem resistance in *Klebsiella pneumoniae* isolates from St. James's hospital, Dublin, Ireland

C. Roche* (Dublin, IE)

**Objectives:**
- To identify β-lactamases in a collection of *K. pneumoniae* isolates with reduced susceptibility to carbapenems.
- To examine the role of altered permeability as a potential mechanism of carbapenem resistance in *K. pneumoniae*.
- To investigate the contribution of efflux mechanisms to carbapenem resistance.

**Methods:** Phenotypic detection of carbapenemases was performed using the Modified Hodge test. Further phenotypic detection of KPC carbapenemases was performed by measuring ertapenem MIC values in the presence and absence of phenylboronic acid. Detection of metallo-β-lactamases and class A ESBLs was performed using Etest methods. Carbapenem MIC values were measured in the presence and absence of phenyl-arginine β-naphthyl-amine dihydrochloride (PABN) to examine the contribution of efflux to resistance. DNA amplification of MBL genes including blaVIM, blaIMP, blaGIM and blaSPM, serine carbapenemase genes blaKPC, blaSME and blaOXA-48, blaAmpC genes and class A β-lactamase IMP-1 with lack of 37kDa outer membrane protein.

**Results:** Reduced susceptibility to carbapenems shown by nine *K. pneumoniae* isolates in this study found that for eight isolates, this was the result of class A ESBL production, predominantly CTX-M types, in combination with lack of expression of both OmpK35 and OmpK36 porins. Porin loss was shown using SDS-PAGE analysis and the mass spectrometry techniques, MALDI-MS and nanoLC-ESI MSMS. Lack of expression was proposed to result from mutations in the regulatory regions of the porin genes, since these regions could not be amplified by PCR techniques. One isolate produced a KPC-2 carbapenemase. This isolate did not express the OmpK35 porin, which probably contributed to the resistant phenotype.

**Conclusion:** This study found resistance to carbapenems demonstrated by either production of class A ESBLs in combination with a loss of both OmpK35 and OmpK36, or by production of a class A serine carbapenemase in combination with loss of OmpK35. In both cases, carbapenem resistance was seen in conjunction with resistance to other antibiotic classes.

**P693** Lack of 37kDa outer membrane protein in *Klebsiella pneumoniae* associated with decreased susceptibility to imipenem in Japan

T. Sho*, T. Marutani, R. Hamauna, H. Yakashii, M. Honda, T. Kobayashi, N. Fujimoto, T. Matsumoto (Kitakyushu, JP)

**Objectives:** Resistance to carbapenems is uncommon in Enterobacteriaceae except for *Serratia marcescens*. There are some reports of carbapenem-resistant *K. pneumoniae* strains with the production of β-lactamases that are capable of hydrolyzing carbapenems combined with decreased drug permeability through the bacterial outer membrane due to loss or alternation of porins. In 2006, we isolated imipenem-high-resistant *Klebsiella pneumoniae* (imipenem MIC of 32 μg/mL) harbouring metallo-β-lactamase IMP-1 with lack of 37kDa outer membrane protein. DNA amplification ofompK35 and ompK36 genes was performed to examine the contribution of outer membrane impermeability. Expression of OmpK35 and OmpK36 was examined using SDS-PAGE and mass spectrometry techniques.

**Results:** Lack of 37kDa outer membrane protein was found in 9 strains, which showed decreased susceptibility to imipenem in Japan.

**Conclusion:** Lack of 37kDa outer membrane protein is associated with decreased susceptibility to imipenem in Japan.

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**Mechanisms of carbapenem-resistance in Enterobacteriaceae and *P. aeruginosa***

**P691** Role of IS50 in the expression of the blaADC gene in *Acinetobacter baumannii*

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**Objective:** Multidrug-resistant *Acinetobacter baumannii* is a major nosocomial pathogen that is rapidly evolving and developing resistance to all major classes of antibiotics. It is identified by the presence of intronic blaOxa-51-like gene and restriction of 16S-23S rRNA intergenic spacer sequences. The aim of this study was to investigate the role of insertion sequences related to the expression of cephalosporinases present in *A. baumannii*.

**Methods:** A panel of 17 geographically diverse *A. baumannii* isolates was screened for the presence of insertion sequence upstream and downstream of blaADC gene. The isolates were confirmed as *A. baumannii* by blaOxa-51-like PCR and by restriction analysis using the enzymes Aul and Ndel. The MICs of ceftazidime, cefotaxime, cefipime and cefepidine were determined by the BSAC guidelines. The strains were checked for ESBL production by synergy disc assay between cephalosporins and augmentin (co-amoxiclav).

**Results:** The MICs for ceftazidime showed that 4 strains had an MIC of >256mg/L, 7 strains had an MIC of 128mg/L, 1 strain had an MIC of 64mg/L and 5 strains had an MIC of 32mg/L. None of the strains produced ESBLs as tested by synergy between augmentin and cephalosporins such as ceftazidime, cefotaxime, cefpime and cefepidine. PCR results showed that ISAb1 was present upstream of blaADC gene in 12 strains (MICs ranging from 32–128mg/L). Out of the five strains having MIC of 32mg/L one strain did not have ISAb1 present upstream of blaADC gene. The 4 strains with MICs >256mg/L harboured IS30 upstream of blaADC gene. There was no insertion sequence detected downstream for all 17 strains making it a defunct transposon. The IS30 insert was observed 126bp upstream of blaADC gene. Although the region just upstream of blaADC had functional had a functional promoter, the IS30 element also had a promoter for the transposase it carries and this may also increase the expression of the blaADC gene.

**Conclusions:** These data indicate the presence of a novel insertion element IS30 besides ISAb1 that can cause over-expression of blaADC gene. The isolates with IS30 may encode novel ADC variants having significant hydrolysing activity against ceftazidime. The IS30 element may also have a better promoter than ISAb1 to cause blaADC overexpression.
**Methods:** *K. pneumoniae* strains were isolated from clinical material obtained from our hospital in 2008 to 2009. The MICs of various antimicrobials were determined by agar dilution method in accordance with CLSI. The presence of β-lactamases was confirmed by PCR and DNA sequence analysis. Outer membrane proteins (OMPs) were isolated by Sarkosyl extraction and examined by SDS-PAGE.

**Results:** In the 1693 *K. pneumoniae* isolates collected in our hospital, five isolates showed decreased susceptibility to imipenem (MICs of 2 to 4 μg/ml). These five isolates, *K. pneumoniae* Lkp1008, Lkp1012, Lkp1015, Lkp1026, and Lkp1027 had blaDHA-1 or blaSHV, but did not have genes of carbapenemases like IMP-1, IMP-2, VIM-1, VIM-2, NDM-1 or KPC-type β-lactamases. Strains showing imipenem MICs under 2 μg/ml had two OMPs of approximately 36 and 37kDa. Three isolates, *K. pneumoniae* Lkp1012, Lkp1026, and Lkp1027 showing imipenem MICs of 4 μg/ml lacked OMPs of approximately 37kDa.

**Conclusion:** We reported *K. pneumoniae* isolates with lack of 37kDa OMP showing decreased susceptibility to imipenem in Japan. Lack of 37kDa OMP appeared to be one of the mechanisms of imipenem resistance. In our knowledge, this is the first report of carbapenem resistance *Klesbiella pneumoniae* due to lack of outer membrane protein of approximately 37kDa in Japan.

**Objectives:** In the last years, an increasing frequency of carbapenem-resistant *K. pneumoniae* isolates has been reported worldwide. In some of these reports, resistance to carbapenems was caused by the combination of different resistance mechanisms including loss of major porins (OmpK35/36) expression. In this work, we investigated the expression of alternative porins that might compensate the absence of the major porins in the carbapenem resistant isolates.

**Methods:** Analysis of the outer membrane proteins from isogenic pairs of carbapenem-susceptible and resistant strains isolated from different hospitals was performed by SDS-PAGE and MALDI-TOF MS. Mutants deficient in the expression of those porins identified in the resistant isolates and absent in the susceptible ones were constructed from the resistant isolates by insertion-duplication mutagenesis. MICs were determined by agar dilution method in accordance with CLSI. The presence of β-lactamases was confirmed by PCR and DNA sequencing as KPC-2. *K. pneumoniae* Lkp1 also harboured two bla genes encoding TEM-1 and the extended-spectrum β-lactamase (ESBL) SHV-12. The Ps, Pm and Ec strains were found to produce the metallo-β-lactamase (MBL) VIM-1 with an additional ESBL, SHV-5, while both Pa and KP strains had blaVIM-1 alone. Genes encoding VIM-1 and SHV-5 were co-transferred to *E. coli* J53 by in vitro conjugation from Ps, Pm and Ec. Only blaVIM-1 was transferred from KP2. The fingerprints obtained from plasmid DNA digestions of the transconjugants TCPs, TCPm and TCEc were highly similar, while the pattern obtained from TCKp2 was different. In all the MBL producers, the blaVIM-1 gene was integrated in a class I integron. PCR mapping of the variable region of the integron revealed three distinct types of cassette arrays, of which one corresponded to a truncated class-I integron shared by TCPs, TCPm and TCEc. In vivo conjugation in a gnotobiotic mouse model permitted the transfer of the integron carrying blaVIM-1 from the Ps strain to the recipient strain *E. coli* J53.

**Conclusions:** The time sequence of β-lactamase producers detection, the molecular analysis of the genetic support of blaVIM-1 and the conjugation results obtained in vivo in the gnotobiotic mouse model strongly suggest the in-patient transfer between Ps, Pm and Ec of a plasmid carrying a truncated class-I integron harbouring blaVIM-1. This inter-species transfer, together with a probable acquisition of multiple multidrug-resistant bacteria, led to a worrying accumulation of carbapenemase-producing strains in a single patient.
performed by PCR-based replicon typing (PBRT), PFGE-S1, PFGE-Xbal, southern blotting and hybridization (blaIMI-2, IncI1, IncF and CoIETP probes).

Results: E. coli W635 was ascribed to the new sequence type ST1998, and showed resistance to imipenem, MEM, ertapenem, ampicillin, ticarcillin, amoxicillin-clavulanic acid, cefazolin, cefotaxim, cefuroxime, streptomycin, nalidixic acid, ciprofloxacin, sulphonamides, trimethoprim, fosfomycin; and intermediate resistance to aztreonam and chloramphenicol. A class A carbapenemase positive phenotype was found in E. coli W635 that harboured the blaIMI-2 gene with its LysR-type regulator. In addition, this strain presented the blaTEM-1b gene, the repC-sul2-tra-strB-ISC2R structure, the S83L-D87N substitutions in GyrA and the S80I in ParC, and harboured a class 1 integron with intermediate resistance to aztreonam and chloramphenicol. Noteworthy, the blaOXA-181 gene, that latter encoding a carbapenem-hydrolysing class D-β-lactamase, was either plasmid- or chromosome-located in these two isolates. This study evidences a wide diffusion of OXA-48 producers in France, with isolates recovered from patients with an expected link to Turkey but more surprisingly to North African countries. Considering the important size of the North African diaspora in France and cultural relationships with that geographical area, it likely indicates that France will face out a threatening dissemination of those carbapenemase producers that need to be very early recognized in order to prevent their further diffusion. Similar observations shall be observed in Germany, Italy, and Spain in near future.

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Methods: The genetic environment of the blaOXA-48 gene was studied by PCR combination using specific primers of IS1999 and blaOXA-48 followed by sequencing. The plasmid scaffolds were typed by a PCR specific for the RepP replicase featuring the blaOXA-48-positive plasmid known to circulate at least in Turkey. Twenty enterobacterial isolates exhibiting reduced susceptibility or resistance to carbapenems were studied. They had been isolated in our hospital or in other French hospitals.

Results: Six isolates produced the KPC-type or NDM-1 carbapenemases, whereas fourteen were found positive for a blaOXA-48-like gene. Most of these cases corresponded to patients transferred from foreign hospitals, and corresponded to K. pneumoniae, E. coli, and E. cloacae isolates from Turkey, but also E. cloacae from Morocco, E. coli from Egypt, E. coli from Algeria. For some K. pneumoniae isolates, the patients did not travel abroad. Most of these isolates showed only decreased susceptibility to carbapenems according to the updated 2010 CLSI guidelines. In addition, some of them produced extended-spectrum [β-lactamases (ESBLs)] being either of the CTX-M-14 or CTX-M-15 types, whereas others were not ESBL producers and therefore remained susceptible to broad-spectrum cephalosporins. The same 70-kb plasmid, that does not harbor other resistance determinants and possesses the RepP backbone, was always identified in those OXA-48 producers.

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were also observed. The typical tunnel-like entrance of the OXA-24 active site is observed in the complex structure and apparently does not hinder inhibitor binding. As already observed in the OXA-48/NXL104 complex, the active site Lys73 remains carbamylated even in the presence of the inhibitor.

Conclusions: This study provides structural insights on the interaction between the new broad-spectrum non-β-lactam β-lactamase inhibitor NXL104 and the OXA-24 class D carbapenemase. NXL104 is able to inhibit OXA-24 in vitro by forming a stable covalent complex.

**P610 Evaluation of a commercial microarray to detect carbapenemase-producing Enterobacteriaceae**

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Objectives: To evaluate a commercial microarray for detecting genes encoding carbapenemases in clinical isolates of Enterobacteriaceae.

Methods: Sixty-four Enterobacteriaceae isolates were tested on the Check-MDR CT102 ESBL-Carbapenemase Array (Check-Points): 41 were known to have a carbapenemase; 16 were carbapenem-resistant through a combination of ESBL/ AmpC activity plus impermeability (porin loss), and 7 were carbapenem-susceptible controls. Total bacterial DNA was extracted using a QIAGEN Blood and Tissue DNA purification kit and 10 μl of undiluted DNA was used as template for the test. The assays were undertaken according to the manufacturer’s instructions. Image analysis and interpretation of the results used proprietary software (Check-Points). Array results for carbapenemase genes were compared with the carbapenem resistance mechanisms defined previously by PCR and sequencing.

Results: The correct carbapenemase gene was detected by the arrays in all 41 carbapenemase-positive isolates, which included producers of KPC (8), OXA-48 (11), IMP (12), NDM (7) and VIM (3) enzymes. There were no false-negative results. The 16 carbapenem-resistant strains without a carbapenemase genes were all negative by array. Six of the carbapenem-susceptible controls were negative for all carbapenemase genes by the array method, but one gave a false positive for blaVIM, though no such gene was detectable by PCR.

Conclusion: This commercial array was easy to use and allowed accurate detection of selected carbapenemase genes, including those emerging or most prevalent in Europe and the USA. Simultaneous detection of co-resident ESBL genes (not evaluated here) represents added value for this method.

**P611 Identification of OXA-198, a novel carbapenem-hydrolysing class D β-lactamase, in Pseudomonas aeruginosa**

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Objectives: To investigate an unusual enzymatic resistance mechanism to carbapenems observed in a clinical isolate of *P. aeruginosa*.

Methods: Antimicrobial susceptibility was determined by Etests and interpreted as recommended by CLSI. Class 1 integron, bla MBL, ESBL and OXA-carba genes were sought by PCR-sequencing. Plasmid DNA was extracted and electroporated in wild type *P. aeruginosa* PAO1 strain. blaOXA-198 gene was cloned in Escherichia-Clostridium shuttle vector pUCP24 and recombinant plasmid pOXA-198 was transferred in *E. coli* TOP10 and in PAO1 by electroporation. β-lactamases activity was observed by isoelectric focusing of crude sonicated cell extracts. Hydrolysis of imipenem was followed on crude extracts at 300 nm and with a modified Masuda's phenotypical test.

Results: In April 2010, a multidrug carbapenem-resistant *P. aeruginosa* (PA14137) but susceptible to expanded-spectrum cephalosporins was recovered from lower respiratory tract specimen of an hospitalised 79-yr old man. No synergy could be evidenced between imipenem and EDTA, cloxacillin, boronic acid or clavulanic acid while a crude extract clearly enhanced the growth of *E. coli* TOP10 near an imipenem disk (Masuda test). By PCR none of the targeted β-lactamases genes were detected but sequencing of a 3 kb amplicon obtained with primers targeting class 1 integron revealed the presence of a novel blaOXA-198 (HQ634775) in addition to aacA7 (encoding a aminoglycosides modifying enzyme) and cmlA1 (encoding a chloramphenicol efflux pump). OXA-198 shares 35%, 32%, 30% amino acid identity with OXA-48, -58, -143 respectively and 29% with OXA-51, -23 and OXA-40. blaOXA-198 was located on a non typable 50 kb plasmid which, once transferred in *P. aeruginosa* PAO1, eventually led to a reduced susceptibility to carbapenems. Cloning of blaOXA-198 gene in pUCP24 in *E. coli* TOP10 and *P. aeruginosa* PAO1 also led to transformants with reduced susceptibility to carbapenems. A slight imipenem hydrolytic activity of an OXA-198 transformant crude extract was observed by spectrophotometry (=[nmole/mg/min]).

Conclusion: We identified a novel class D carbapenem-hydrolysing β-lactamase (CHDL) in *P. aeruginosa* responsible for a decreased susceptibility to carbapenems both in clinical strains and in transformants. OXA-198 seems to be the first representative of a new subgroup of CHDL and the second CHDL recovered in *P. aeruginosa*.

**P612 Characterisation of OprD in Pseudomonas aeruginosa clinical strains showing high and intermediate levels of resistance to imipenem and meropenem**

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Objective: To evaluate the impact of OprD inactivating mutations (IMs) on the appearance of different resistance levels to imipenem (IMP) and meropenem (MEP) in clinical strains of *P. aeruginosa* (Pa).

Methods: Fifty strains of Pa isolated from bacteremia in a multicenter study and showing decreased susceptibility (MICs: 4–8 mg/l) to IMP and/or MEP (Pa-D, n = 25) or resistance to these agents (MICs >16 mg/l) (Pa-R, n = 25) were studied. MICs of IMP and MEP were determined by microdilution, following the CLSI guidelines. The presence of IMs in oprd was investigated by PCR amplification followed by sequencing of the entire gene. Nucleotide (nt) and amino acid (aa) sequences were compared with those of the reference strain PAO1. OprD expression was confirmed by outer membrane protein (OMP) analysis. OMPs were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). OprD profiles from clinical isolates were compared with those of the reference strain PAO1 and a PAO1-derivative oprd deficient mutant. The presence of genes encoding metallo-β-lactamases (MBLs) was also explored by PCR.

Results: Sequencing of the oprd gene showed that the studied strains had several IMs resulting in the loss of oprd. As confirmed by analysis of their OMP profiles. The most frequent IMs were frame-shift mutations due to nt insertions or deletions (62%, including 12/25 Pa-R and 19/25 Pa-DS), premature stop codon due to nt substitutions (24%; 9/25 Pa-R and 3/25 Pa-DS strains), several polymorphisms (10%, 2/25 Pa-R and 3/25 Pa-DS) and disruption of the coding sequence by an IS-like element (4%; 2/25 Pa-R and 0/25 Pa-DS). A determined IM did not directly correlate with the occurrence of a concrete level of resistance to IMP and MEP. None of the isolates were found to produce MBLs.

Conclusion: IMs in oprd leading to OprD loss have been observed in most Pa strains with either decreased susceptibility or resistance to IMP and/or MEP. The level of resistance was independent of the IM type, supporting the relevance of additional resistance mechanisms in this phenotype.
Methods: Fifteen NDM-1-producing Enterobacteriaceae (Escherichia coli [EC; 6], Enterobacter cloacae [ECL; 3] and Klebsiella pneumoniae [KPN; 6]) displaying resistant to amikacin (AMK), gentamicin (GEN) and tobramycin (TOB; >32, >8, >16 mg/L, respectively) were screened for the presence of armA, rmtA, rmtB, rmtC and npmA by PCR. Amplicons were sequenced on both strands. Isolates were further tested by CLSI reference broth microdilution MIC methods against arbekacin, apramycin, kanamycin, neomycin, streptomycin and extended ranges for AMK, GEN, TOB. Clonality was assessed by PFGE.

Results: Thirty-nine of the 15 (87.0%) NDM-1-producing isolates were positive for 16S rRNA methylase-encoding genes and NDM-1 metallo-beta-lactamase among 15 Enterobacteriaceae isolates collected in India during 2006 and 2007. Isolates were collected in four hospitals located in three cities (New Delhi, Mumbai, Pune). Nine strains carried armA and three harboured additional genes: rmtC (2 EC) and rmtA (1 ECL). Three strains carried rmtC alone (1 each species). Gene sequences showed 99% homology with armA, rmtB and rmtC published sequences. Genetically identical EC and KPN carried armA and ECL possessed rmtC. EC strains with similar PFGE profiles harboured armA, rmtC or both. One ECL carried armA and rmtA. Susceptibility profiles showed that all strains had AMK, GEN, TOB and kanamycin MIC results at ≥1024, ≥512, ≥512, ≥1024 mg/L, respectively. Arbekacin MIC results varied from 256 to >1024 mg/L and most isolates had streptomycin MIC values between 16 and 512 mg/L; however, one isolates had an MIC of only 4 mg/L (rmtC-carrying ECL). Apramycin and neomycin MIC results were lower compared to other aminoglycosides tested (4–16 and 1–32 mg/L, respectively).

Conclusions: A150-Kb plasmid carrying blaIMP-18 to Enterobacteriaceae species (KOX) in a unique integron structure, increasing the genetic diversity of MBLs in Mexican hospitals.

Results: Three (1.6% overall) isolates showed carbapenem-R (one of each: E. coli, K. pneumoniae and KOX). KOX (imipenem and meropenem MIC values, 4 and 0.5 mg/L, respectively) displayed a positive MHT result and PCR followed by sequencing revealed the presence of blaIMP-18. This isolate was recovered from a 2 yo female presenting with severe dysuria, later diagnosed with a bladder Rhabdomyosarcoma. After 13 months of chemotherapy a wound infection developed. Vancomycin and meropenem were utilized after poor initial response to ceftriaxone, amikacin and metronidazole. Patient returned to the hospital with fever and bloodculture grew KOX. Prolonged IV infusion of meropenem was initiated and 15 days later the patient was discharged. blaIMP-18 was located in an integron with the follow cassette arrangement: intI1-blaIMP-18-aacA4-aadA1-like(K201R and 235E236 insertion)-blaOXA-2-like (17 nucleotide insertion)-qacEdelta1/sul1. This MBL gene was located in a 150-Kb plasmid.

Conclusions: A great variety of MBL enzymes have been described in Mexico (VIM-2, VIM-23 in Enterobacteriaceae and IMP-15 and IMP-18 in PSA). Although IMP-18 was detected in several countries, this gene was only reported in PSA. Our results demonstrate the mobilization of blaIMP-18 to Enterobacteriaceae species (KOX) in a unique integron structure, increasing the genetic diversity of MBLs in Mexican hospitals.

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**P616**

Genotypic evaluation of a collection of Cfr-producing staphylococcal clinical isolates from the SENTRY Antimicrobial Surveillance Program

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**Objectives:** To evaluate the genotypic and phenotypic characteristics of Cfr-producing staphylococcal clinical isolates submitted as part of the SENTRY Antimicrobial Surveillance Program. Cfr-encoding gene is often plasmid-located and therefore this recent linezolid (LZD) resistance mechanism has the potential for mobilization.

**Methods:** Staphylococcal strains (76,303) submitted to the SENTRY Program were tested for susceptibility (S) by reference CLSI methods (M07-A8) and interpretations (M100-S20-U). Isolates with elevated LZD MIC results (>4 mg/L) were screened for resistance mechanisms, including cfr and mutations in the 23S rRNA-, L3- and L4-encoding genes. Cfr-positive strains were selected for this study and further evaluated. Clonality was accessed by PFGE, spa (S. aureus [SA] only) and MLST. Strains were identified by Vitek 2 and confirmed by 16S rRNA sequencing.

**Results:** Within 2006–2008, five cfr-positive strains were detected, while nine and five Cfr-producing staphylococci were detected during 2009 and 2010, respectively (Table). Most (73.7%) cfr strains were identified in coagulase-negative staphylococci (CoNS) and the LZD MIC results in SA (4−16 mg/L) were usually lower than those noted in CoNS (8−200 mg/L). All SA were methicillin-resistant (MRSA) and associated to internationally disseminated MRSA lineages, including USA300 (isolate 1848). Among SA, no sequence type (ST) prevailed, whereas ST23 was the commonest ST among CoNS. PFGE results indicated the presence of CoNS strains with indistinguishable profiles in medical centers in Guadalajara (Mexico), Tempe (USA) and Rome (Italy). Overall, mutations in the 23S rRNA were not detected, except in isolates 3147 (C2534T) and 27805 (G2576T), while alterations in the L3 and L4 proteins were commonly observed, especially among CoNS.

**Conclusions:** Cfr-producing SA strains usually exhibited lower LZD rRNA values when compared with CoNS. This finding may be associated with additional LZD resistance mechanisms in CoNS, such as mutations in 23S rRNA, L3 and L4 proteins. Presence of cfr and 23S rRNA alterations seems to be rare. The prevalence of cfr strains appears to be increasing slightly over years in staphylococcal strains submitted to the SENTRY Antimicrobial Surveillance Program. Cfr-encoding gene is a major opportunistic human pathogen and is increasingly multi drug-resistant and variable regions, called pathogenic islands (PI), contribute to their antibiotic resistance and virulence generally. Although, several PI have been identified in *P. aeruginosa*, there has not been a detailed study of these regions and the possible strain specific differences they provide, especially in relation to the antibiotic resistance. We have reported a carbapenem resistant *P. aeruginosa* isolate from Sydney (Australia), which had two class 1 integrons, one associated with a blaVIM-1 gene, and within a new transposon, Tn6060. This transposon was inserted in a previously identified PI, in the cystic fibrosis *P. aeruginosa* strain PACS171b. Here, we further assess the presence of this or related transposons in other *P. aeruginosa* strains.

**Methods:** Isolates were collected from hospitals in Sydney, Australia and Uruguay and screened by Polymerase Chain Reaction (PCR) for int1 and cassette arrays. Fosmid libraries were constructed from a representative positive isolate from each country. These were CH79 (Australia) and U09 (Uruguay). The sequences obtained were assembled and identified using NCBI-BLAST. Pulsed-Field Gel-Electrophoresis (PFGE) was performed to determine clonal relationships.

**Results:** Fosmid libraries from CH-79 and U-09 revealed that both have the same Tn6060-like transposon seen previously but carrying different cassettes which, in both isolates, comprised, adaA6-orfD. For both CH79 and U09 the point of insertion in the pathogenic island PACS171b was the same as seen previously. Both of the isolates found here had a single integron transposon in the transposon location unlike the two seen in Tn6060. However both CH-79 and U-09 had a second integron at a second location in their respective genomes which had arrays that included a blagES in the former and a blaoXa gene in the latter. The location of each of these two additional integrons was in different locations in the chromosome. For CH-79 the integron was in a second PI and for U-09 it was in the chromosomal oprD gene. The two strains were different based on PFGE analysis.

**Conclusion:** These results suggest that insertion of class 1 integrons in PIs may be a global phenomenon with spread occurring via complex mobile regions independent of clonal lines. More detailed analysis of *P. aeruginosa* strains with a broader geographic spread will be needed to confirm this.

**P618**

Provisional report of an association between the carbapenemase KPC and the pandemic clone *E. coli* 025b:ST131

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**Objective:** Escherichia coli 025b:ST131 is an extremely successful pan-demic clone that has been implicated in the international dissemination of CTX-M-15. The increasing emergence and dissemination of carbapenem resistant (CR) Enterobacteriaceae is a major public health concern. The association of carbapenemase enzymes with epidemic clones, such as the recent association of KPC-2 and KPC-3 with the successful *K. pneumoniae* ST258 clone represents a significant public health threat. The aim of this project was to characterise a *E. coli* (U490995.1) identified during a project screening Enterobacteriaceae isolated at the Mid-Western Regional Hospital in Ireland for carbapenemase production.

**Methods:** All isolates of Enterobacteriaceae collected from all specimen types since 25th January 2010 were screened for carbapenemase production in accordance with Clinical Laboratory Standards Institute (CLSI) screening and confirmatory procedures. A single isolate of carbapenemase-resistant *E. coli* was isolated from urine from a patient co-infected with a *K. pneumoniae*. This isolate was examined for susceptibility to 13 antimicrobial agents by disk diffusion and screened for the presence of blaVIM, blaIMP and blaKPC by PCR as previously described. A duplex PCR targeting the p stabbed a trpA genes was used to determine if the isolate was a member of the 025b:ST131 clone group.

**Results:** The isolate was susceptible to four of the thirteen antimicrobial agents: streptomycin, chloramphenicol, minocycline and kanamycin. PCR confirmed the presence of blakPC and indicated that the isolate was 025b:ST131.

**Conclusions:** The identification of an association between KPC and *E. coli* 025b:ST131 is of major concern. CTXM-15 is now the
most widely reported extended spectrum β-lactamase worldwide. It’s widespread dispersion is considered to be related at least in part to its association with epidemic clones, predominately E. coli 025b; ST131. Given the limited treatment options available for infection with KPC and other carbapenemase producing strains, it’s association with the successful E. coli 025b; ST131 clone is a cause for concern. Further work to confirm MLST type and characterise the plasmid from this isolate is in progress.

**Methods:** We analyzed vanA-Efm isolates belonging to ST78-CC17 (n=13) and CC9 (n=13; 2ST9; 2ST10; 2ST12; 3ST29; 1ST124, 1ST134, 1ST176) isolated from clinical, animal, food and healthy human samples. Clonality was studied by PGFE and MLST. Plasmid analysis included identification of size and content (SI-PGFE), and characterization of rep initiation proteins (rep), relaxases (rel), and toxin-antitoxin systems (TA) by PCR-typing systems, hybridization and sequencing. vanA location was determined by I-Ceu hybridization and diversity of Tn1546 was searched by overlapping PCR.

**Results:** ST78 isolates were classified in 5 related PFGE types, 1 type comprised clinical isolates from Spain and Italy; other corresponded to a food and to a German clinical outbreak strain. CC9 strains were classified in 12 different PFGE patterns, some STs being identified in animal and human reservoirs from different locations. ST78-CC17 isolates harboured higher number of plasmids than CC9 (1–6 vs. 1–3) ranging from 20 to 350kb. All isolates contained plLG1-like megaplasmids (100% repLG1) and similar percentage of RCR (38/23% regF1071). However, differences between CC9/ST78 isolates were found for rep of small theta replicating plasmids (0/85% repCIZ, 38/8% repEFE18), and those of Inc18 (100/62% repIPn18, 92/0% repVEF, 54/0% rep2Inc18) and RepA_N (0/100% repRUM) families. Content in rep and TA was consistent with that observed for rep sequences: 8/100% repCIZ and 23/85% repIS42; 69/100% repEF1 and 62/92% to-p (both linked to Inc18 plasmids), and 0/100% of Axe-Tse (linked to pRUM). Tn1546 was mainly located on chromosome in ST78 isolates. Truncated variants IS1216-Tn1546 were located on particular mosaic Inc18-like plasmids in CC9 isolates.

**Conclusion:** Results show a intra/international spread of Efm ST78 and CC9 clones and/or their plasmids among animals and humans and confirm a possible debilitation of the species bottleneck for transmission and adaptation of host-specific strains, as we recently reported for Efm CC5 and CC17.

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**References:**

1. High incidence of horizontal transfer of ampicillin resistance (pbp5) among CC17 Enterococcus faecium

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**Objectives:** Most CC17 E. faecium (Efm) are resistant to ampicillin (AMPR). Transfer of AMPR has been linked to pbp5 mobilization by conjugative transposons (CTn) Tn5382 or, more rarely, Tn916 and Tn5386. We analyzed the occurrence of pbp5 transfer among Efm from several sources in Portugal.

**Methods:** AMPR Efm (n=80) from clinical samples (C, n=39), Healthy Humans (HH, n=2), Hospital Sewage (S, n=15), Animals (A, n=22) were studied. Transfer of pbp5 was screened by filter mating using receptor strains lacking (EfmGE1) or containing (EfmBM4105RF/Efm64-3) pbp5, and BHI with AMP, fusidic acid and rifampicin. Antibiotic susceptibility was tested by disk diffusion/agar dilution methods (CLSI) and clonality by PFGE/MLST. The stability of the acquired pbp5 (EfmGE1 transconjugants (TC)) was evaluated by serial passages (30x)

**Results:** pbp5 was transferred to EfmGE1 in 19% of the cases (n=15; HH, 2A, 4S, 8C), all belonging to CC17 (8 PFGE types, 8 STs comprising ST18, ST280, ST341, STnew). All isolates presented a chromosomal location for pbp5 but none of them carried any of the screened CTns. TCEfmGE1 were AMPR (MIC=16–64mg/L) even after consecutive inoculations in AMP free BHI. Resistance to glycopeptides, erythromycin and/or high level of resistance to streptomycin was also observed in 6, 6 and 1 of TCEfmGE1, respectively. Transfer of pbp5 to Efm64-3 (2/15) or EfmBM4105RF (6/15) was also observed. EfmBM4105RF and Efm64-3 TC strains increased their MICAMP values from 2mg/L to 8–16mg/L and 64mg/L, respectively. pbp5 hybridized with Smal DNA fragments of 290kb and 250kb in EfmBM4105RF and Efm64-3 recipient strains while no hybridization was seen in EfmGE1. In the TC, pbp5 was located on bands of 180kb (1 TCEfmGE1), 200kb (2 TCEfmBM4105RF), 210kb (32; 13 wild Efm, 13 TCEfmGE1, 5 TCEfmBM4105RF, 1 TCEfm64-3), 180kb +250kb (1 TCEfm64-3) and 210kb+242kb (1 TCEfmBM4105RF).

**Conclusions:** pbp5 may be transferred to EfmGE1 from several origins and associated with pbp5 mobilization by conjugative genetic elements different from those previously described. Further studies to characterize the genetic context and to explain the variable levels of AMPR on distinct genetic backgrounds (pbp5-pbp5+) are in process.

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**References:**

1. Quantitation of bacteria in gastric biopsy specimen from patients with gastrointestinal disorders: relationship between counts and 23S rRNA point mutations

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**Introduction and Objectives:** H. pylori is the only bacteria that infect humans gastric for which endoscopy examination is routinely recommended. The risk of development of gastrointestinal disorders (GIDs) and clinical features in the presence of H. pylori infection depends on a variety of factors including bacterial, host, and environmental ones that mostly relate to the pattern of bacterial load. The aim of this study is the assessment of 4 types of 23S RNA point mutations affect on H. pylori count.

**Methods:** A prospective study of the concentrations of bacteria in the gastric and their relationship to point mutations was conducted with 200 H. pylori suspected patients with GIDs. Initially, RUT and 16SrRNA PCR were performed for identification of H. pylori. Then, gastric biopsy specimens were analyzed for bacterial count by using specific primers to analyze 1ST134, 1ST176 and/or their plasmids among animals and humans and confirm a possible debilitation of the species bottleneck for transmission and adaptation of host-specific strains, as we recently reported for Efm CC5 and CC17.

**Results:** Out of 200 samples, 164 (82%) had confirmed H. pylori positive. For 164 patients, the number of H. pylori CFU in gastric biopsy samples were a value of 10^4 to 10^5 CFU/mml. The results revealed that more relationships between wild types and 10^4 (p=0.026), 10^5 (p=0.00) and 10^6 (p=0.00) CFU. Also, results showed more relationships between 10^6 and A2144G (p=0.033), A2143G (p=0.005), A2143C (p=0.005), and A2142G (p=0.015) peptidyl transferase point mutations of 23S rRNA gene.

**Conclusion:** This study has demonstrated a same relationship between bacterial load and 4 types of 23S rRNA point mutations and wild types. In conclusion, this finding supported the high prevalence of these mutations in H. pylori population.
Prevalence and molecular characterisation of extended-spectrum β-lactamase-producing *Escherichia coli* in Moroccan community

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**Objectives:** The study aimed at the assessment of the prevalence of ESBL-positive isolates of *Escherichia coli* collected from community setting and their molecular biology analysis.

**Methods:** 1598 community *E. coli* strains responsible for urinary tract infections were collected from five geographical areas of Morocco. Antibiotic susceptibility testing and detection of ESBL production were performed as recommended by the CA-SFM. The MICS were determined using E-test method. The detection of the genes ESBL, AmpC, aac(6’)-Ib-cr and class I integrons was performed by PCR amplification. Specific ISS and genes associated with blaCTX-M genetic environment were investigated by PCR and sequencing. Conjugation experiments were done to determine the mobility of blaCTX-M. The epidemiological relationships between ESBL-producing *E. coli* strains were analysed by PFGE.

**Results:** Forty nine isolates (3%) were defined as ESBLs producers. All were resistant to 6 or more antibiotics. ESBLs identified were CTX-M group1 (63.2%), CTX-M group9 (2%), TEM (42.8%) and SHV (28.5%) type. Eighteen strains expressed at least two types of β-lactamase. Genes encoding acquired AmpC enzymes were detected in 7 isolates, AmpC detected were DHA (n=2), CIT (n=1), EBC (n=2) and CMY (n=2) type. The qnrS (n=2) and qnrA (n=1) genes were found in three isolates. The class I integrons were detected in 28 isolates with amplicons ranging from 0.4 to 2 kb in length. The PFGE analysis revealed the spread of the strain in the community.

Ten CTX-M-type genes were sequenced and had amino acid sequences indistinguishable from previously sequenced CTX-M-15 β-lactamases. The blaCTX-M-15 gene presented the following genetic environment: IS26-MICA-blaCTX-M-15-orf477. We have shown that blaCTX-M-15, blaOXA-30, aac(6’)-Ib-cr and qnrS1 were co-transferred and that these genes are carried by a conjugative plasmid of high molecular weight.

**Conclusion:** 3% of *E. coli* isolates produced multiple β-lactamases with predominance of CTX-M-15. Management and treatment of ESBL-producing *E. coli* infections can be challenging. It is therefore essential to include the molecular technique as part of the surveillance to monitor the circulation of these resistance genes in a community setting.

*P623* Mutations on the quinolone-resistant determining regions in *gyrA* or *parC* gene of *Mycoplasma genitalium*

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**Objectives:** *Mycoplasma genitalium* is one of the pathogens of male urethritis. Macrolides as azithromycin are effective against male urethritis. Macrolides as azithromycin are effective against male urethritis. Fluoroquinolones can be alternative agents against *M. genitalium* found. Fluoroquinolones can be alternative agents against *M. genitalium*.

**Methods:** The QRDR on *gyrA* and *parC* gene of *M. genitalium* were sequenced and analysed. DNA samples were purified from 23 *M. genitalium* strains which were determined antimicrobial susceptibilities and *M. genitalium*-positive urine specimens collected before and after the treatment with gatifloxacin.

**Results:** Twenty-one *M. genitalium* gene from patients before the treatment with gatifloxacin and 4 genes from patients with treatment-failure were examined. Regarding the QRDR in *gyrA*, all genes before treatment were wild type. However, mutations including amino-change (Asp-99 to Asn or Asp-99 to Gly) were found on 4 genes from the treatment-failure cases. Regarding the QRDR in *parC*, 4 types of mutations (Ala-69 to Thr, Asp-87 to His, Pro-72 to Ser and Ser-83 to Ile) were found on *M. genitalium* genes from 5 patients before treatment. In these patients, 2 patients with mutated *M. genitalium*-gene (Ala-69 to Thr, Pro-72 to Ser and Ser-83 to Ile) failed to treat. From a patient with treatment-failure, a mutation (Ser-83 to Ile) was found.

From *M. genitalium* strains, one strain has high MICs against moxifloxacin and gatifloxacin. This strain has mutations on *gyrA* (Asp-99 to Asn) and on *parC* (Ser-83 to Ile). Other *M. genitalium* strains have some mutations on *parC* (Pro-62 to Ser, Ala-60 to Thr, Asn-87 to Tyr and Ile-146 to Val), but these strains were sensitive to moxifloxacin and gatifloxacin and other fluoroquinolones.

**Conclusion:** In *M. genitalium*, mutations on QRDR in *gyrA* and *parC* gene are also related to fluoroquinolone-resistance as other bacteria.

*P624* Association of virulence markers and antimicrobial susceptibility in *Escherichia coli* clinical isolates

V. Calhau*, T. Lin, G. Ribeiro, N. Mendonça, G. Da Silva (Coimbra, PT; Ann Arbor, US)

**Objective:** *Escherichia coli* can cause a wide variety of infections and is often resistant to multiple antibiotics. They fall into phylogroups A, B1, B2 and D, with group B2 and D being more virulent and reported to be more susceptible to quinolones. The main objective of the work was to screen for the major pathogenicity islands (PAIs), to determine the phylogeny and to associate the virulence markers with antimicrobial resistance profile.

**Methods:** Between October and December 2007, 97 *E. coli* non-duplicate isolates were collected from diverse wards and samples of inpatients of a Portuguese hospital. Antibiotic susceptibility was performed by Vitek2AES. ESBL production was confirmed by the disk diffusion synergy test. The screen of PAIs and the determination of phylogenetic background were performed by Multiplex PCR. The amplicons were sequenced.

**Results:** The majority of strains were recovered from urine (67%), followed by the blood (17%), exudates (12%) and other samples (4%). All phylogenetic groups were represented in all the strains, but those belonging to group B2 (39%) and A (32%) were prevalent. Urine isolates were assigned mostly in B2 group (43%), while only 11% were from group D. Septicaemic isolates were also predominantly from B2 lineage (38%), but their distribution in the other groups was not significantly different (A, 25%; B1 and D, 19% each). None of the strains of A, B1 and D groups carried more than 3 PAIs, whereas 84% and 16% of B2 isolates showed 2 or 3 PAIs and 4 or 5 PAIs, respectively. *P. J4536* was the most prevalent pathogenicity island in all groups. PAI I536, PAI IJ96 and PAI IJ96 were not detected. The PAI ICTF073 and PAI IICFT073 islands were distributed in all phylogenetic groups but were more prevalent in groups B2 and D. Resistance to ciprofloxacin (CIPR) was observed in 56% of all isolates and 16% were ESBL producers. Among B2 group the combination of PAI IV536, PAI ICTF073 and PAI IICFT073 was detected in 80% CIPR strains and in 75% of ESBL producers. Of these 80% were collected from urines.

**Conclusions:** The phylogroup B2 was prevalent and showed the highest number and variety of PAIs. The so-called communal strains of phylogroup A also carried several PAIs and were associated with urinary infections and septicemia. A considerable number of CIPR strains were also PAI carriers (B2 group), some produced also ESBL, which may indicate the emergence of a new sub-lineage among this phylogroup in this clinical setting.

*P625* Mutations in the dihydropterotate synthase gene in *Pneumocystis jiroveci* isolates from children in Cape Town

S. Kumar, L. Ah Tow, C.M. Samuel, B. Morrow, M. Zampoli, H.J. Zar, A.C. Whitelaw* (Cape Town, ZA)

**Background:** *Pneumocystis jiroveci* is a major cause of morbidity and mortality in HIV infected children. Molecular analysis of the mitochondrial large subunit (mtLSU) rRNA locus and the dihydropterote
Characterisation of the penA mosaic gene in \textit{Neisseria gonorrhoeae} strains with decreased susceptibility to cephalosporins in Amsterdam, Netherlands

R. Heymans*, S.M. Bruisten, H.J. de Vries, A.P. van Dam (Amsterdam, NL)

\textbf{Objectives:} In 2006 to 2008, an increase in prevalence (7.3\%) of multidrug-resistant \textit{Neisseria gonorrhoeae} (NG) strains with decreased susceptibility to the extended-spectrum cephalosporin (ESC) cefotaxime was observed among visitors of the STI clinic in Amsterdam, the Netherlands. To determine whether this was due to the rapid clonal expansion of a NG strain harbouring a mosaic penA gene, polymorphisms in the penA gene were correlated to the susceptibility to cefotaxime, cefixime, and ceftriaxone. Strain clonality and epidemiological concordance of mosaic penA patterns were assessed by genotyping.

\textbf{Methods:} From 2006 to 2008, 74 NG isolates with a cefotaxime MIC of \(>0.125\ \mu\text{g/ml}\) (group A), 54 with a cefotaxime MIC of \(0.125 \text{ to } 1.25 \mu\text{g/ml}\) (group B), and a control group of 74 with a cefotaxime MIC of \(<0.125 \mu\text{g/ml}\) (group C), were included in this study. All samples were characterized using antibiograms, gel electrophoresis of the amplified penA gene, and NG multiple-locus variable-number of tandem repeat analysis (MLVA). Sequencing was used to identify and confirm the mosaic penA positive MLVA types.

\textbf{Results:} The proportion of NG isolates with decreased susceptibility to cefotaxime and ceftriaxone (MIC \(\geq 0.016 \mu\text{g/ml}\)) were higher in group A (68\% and 76\%, respectively) than group B (11\% and 57\%), while group C had slightly elevated ceftriaxone MICs only (5\%). The mosaic penA gene (\(n=53\)) was identified only in group A (64\%) and B (11\%), and showed a correlation with the higher ESC MICs. Hierarchical cluster analysis of the MLVA data assigned all 53 patients with a mosaic penA positive NG strain to the same large cluster (\(>10\) patients; \(n=56\)). The presence of a mosaic penA gene in this NG strain was confirmed by sequencing and was shown to be identical to a previously published sequence (GU723422). This cluster contained Dutch homosexual men (66\%), patients with frequent Chlamydia co-infection (32\%), and commercial sex workers (7\%). A second large cluster (\(n=39\)) was identified that also contained isolates of group A (21\%) and B (67\%), but no mosaic penA positive NG strains.

\textbf{Conclusion:} A correlation was found between the decreased susceptibility to the ESCs and the introduction of a clonal mosaic penA positive NG strain among visitors of the STI clinic Amsterdam. High-risk sexual behaviour might facilitate the rapid spread of this multidrug-resistant NG strain, posing new public health concerns.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
\textbf{Genotype} & \textbf{Number} & \textbf{\%} \\
\hline
Wild-type & 55 & 71.4\% \\
M1 (ES34) & 3 & 5.9\% \\
M2 (Ps55) & 13 & 16.9\% \\
M1 + M2 & 6 & 7.8\% \\
\hline
\end{tabular}
\caption{Distribution of mutations in the DHPS gene of 77 \textit{P. jirovecii} isolates}
\end{table}
is predominantly involved in urinary tract infections and bacteremia and can cause significant morbidity and mortality. We report here on the characteristics of CTX-M isolates from cattle, chickens and turkeys from Great Britain (GB) with respect to their CTX-M sequence type, plasmid Inc group, conjugation rate, plasmid size and presence of antimicrobial resistance and virulence genes.

**Methods:** Isolates from chicken (n=27) and turkeys (n=93) were obtained as part of previous studies whilst isolates from cattle (n=202) were mainly from diagnostic samples submitted to the Veterinary Laboratories Agency (VLA) since 2004. Most isolates were tested for CTX-M sequence type, whilst representative isolates were tested for conjugation rate to a *Salmonella* Typhimurium strain, Inc plasmid type, plasmid size and the presence of antibiotic resistance and virulence genes by micro-array.

**Results:** The predominant CTX-M sequence types from cattle were 15 (37.5%) and 14 (36%), from chickens were 1 (73%) and 15 (17%); and from turkeys were 14 (45.1%) and 1 (36.5%) - Table 1. The mean relative conjugation frequency mean and range were similar for isolates from different animal species. The predominant plasmid Inc group for isolates tested from cattle, chicken and turkeys was II-γ (Table 1), although due to bias in the sample population, this may not be representative of the general population for all these species. Most CTX-M sequence type 14 isolates had an IncK plasmid. Similar size plasmids were present in isolates from all species (Table 1).

Antimicrobial resistance genes found included aadA1, aadA2, aadA4, and anaphylaxis modifying enzymes, AMEs), and fluoroquinolones resistant to fluoroquinolones was conferred by sense mutations in the gyra and parC genes. However sense mutations in the parC were not found in 12 A. genomospecies 3 and 13TU isolates. The **2**-lactam resistant patterns were identified mainly encoded by blaTEM-1, and the third major resistance pattern was exhibited by 19 isolates (21.2%) from cattle, chicken and turkeys. The second major restriction pattern was exhibited by 19 isolates (21.2%) resistant to ACSSuTm. The resistance was encoded by blaTEM-1, strAB, sul2, tetA or tetB and blaOXA-30-adjA1 gene cassette array. The second major resistance pattern was exhibited by 19 isolates (21.2%) resistant to ACSSuTm. The resistance was encoded by blaTEM-1, strAB, sul2, tetA or tetB and blaOXA-30-adjA1 gene cassette array. The second major resistance pattern was exhibited by 19 isolates (21.2%) resistant to ACSSuTm. The resistance was encoded by blaTEM-1, strAB, sul2, tetA or tetB and blaOXA-30-adjA1 gene cassette array. The second major resistance pattern was exhibited by 19 isolates (21.2%) resistant to ACSSuTm. The resistance was encoded by blaTEM-1, strAB, sul2, tetA or tetB and blaOXA-30-adjA1 gene cassette array. The second major resistance pattern was exhibited by 19 isolates (21.2%) resistant to ACSSuTm. The resistance was encoded by blaTEM-1, strAB, sul2, tetA or tetB and blaOXA-30-adjA1 gene cassette array.

**Conclusion:** The results show some diversity and some similarity between isolates from different animal species on the basis of CTX-M sequence type, replicon type and antimicrobial resistance and virulence genes. This suggests that strains may be partly restricted to a particular animal species, but it is possible that there is also some exchange of resistance and virulence genes between different animal species.

**Table 1:** Characteristics of clinical chicken and turkey Acinetobacter spp. isolates with respect to CTX-M sequence type and plasmids.

| Genotype | CTX-M sequence type | plasmid size (kb) | Inc group | conjugation frequency (g) |
|----------|---------------------|-------------------|-----------|----------------------------|
| A. baumannii | 15 | 14.5 | II-γ | 0.017 |
| A. baumannii | 14 | 13.8 | II-γ | 0.019 |
| A. baumannii | 3 | 12.0 | II-γ | 0.022 |

**P629** Comparison of genetic characteristics between *Acinetobacter baumannii* and *Acinetobacter* genomospecies 3 and 13TU isolated from Tae-jeon, Korea

S.H. Koo*, K. Kwon, G. Sung on behalf of the Korean Society of Clinical Microbiologists

**Objectives:** *Acinetobacter* spp. are important opportunistic pathogens and have given rise to significant therapeutic challenges in the treatment of nosocomial infections. For the difference of resistance genes between the *A. baumannii* and A. genomospecies 3 and 13 TU, we investigated integrons and the various genes involved in resistance to carbapenems (blaOXA, blaIMP, blaVIM, and blaSIM), aminoglycosides (armA and aminoglycoside modifying enzymes, AMEs), and fluoroquinolones (gyrA and parC) in 51 imipenem-nonsusceptible *Acinetobacter* spp. isolates.

**Methods:** Thirty-nine imipenem-nonsusceptible *A. baumannii* and 12 A. genomospecies 3 and 13 TU isolated from Tae-jeon, Korea were studied. The minimal inhibitory concentrations (MICs) of 10 antibiotics were determined by the agar dilution method. PCR and DNA sequencing were used to identify the genes that potentially contribute to each resistance phenotype. Repetitive extragenic palindromic-PCR (RE-PCR) was also performed to assess the clonality of the isolates.

**Results:** All *A. baumannii* isolates harbored OXA-51 and 18 (46.2%) isolates co-produced OXA-23. In addition, ISAba1 was identified upstream of the gene encoding OXA-51 and OXA-23. However, blaOXA-1 was not found in 12 A. genomospecies 3 and 13TU isolates, which contained blaIMP-1 or blaVIM-2. Most *A. baumannii* and genomospecies 3 and 13TU (96.1%) showed the resistance to amnoglycoside and harbored class 1 integrons, armA, and/or AMEs (aac(6')-Ib, aph(3')-Ia, and/or aph(3')-VI). armA and aph(3')-Ia were detected only in *A. baumannii*, whereas the frequency of aph(3')-VI was significantly higher in A. genomospecies 3 and 13TU. In all 39 *A. baumannii* isolates, resistance to fluoroquinolones was conferred by sense mutations in the gyrA and parC genes. However sense mutations in the parC were not found in 12 A. genomospecies 3 and 13TU. While *A. baumannii* isolates were clonally spread, clonal spread of A. genomospecies was not found in the studied hospital.

**Conclusion:** Several differences of genes related to carbapenem, aminoglycoside, and fluoroquinolone resistance were detected in *A. baumannii* and A. genomospecies. The co-occurrence of several resistance determinants may also become a significant threat. These differences can give an influence on choosing antibiotics in clinical setting.

**P630** Genes basis of antimicrobial resistance in clinical multidrug-resistant strains of *Salmonella typhimurium*, southern Italy

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**Objectives:** The objective of this study was to assess both the genetic basis of the antimicrobial resistance and the clonal relatedness in 88 multidrug-resistant (MDR) strains of *Salmonella enterica* subspecies enterica serovar Typhimurium isolated in Southern Italy (2006–2008) from clinical cases.

**Methods:** Antimicrobial susceptibility was determined by the disc diffusion method. Detection of SGI, class 1 integrons and antimicrobial resistance genes were performed by PCR. Resistance genes undetected by PCR were identified by plasmid libraries: partial Sau3AI restrictions of genmic DNA were separated by agarose gel electrophoresis, purified and cloned into either plasmid pBluescript II SK(--) or pHSG396 and transformed into JM83 competent cells. Plasmids isolated from each candidate were characterized by restriction profiles and the suitable DNA fragments were sequenced.

Genomic relationship was established by Pulsed-field gel electrophoresis (PFGE) according to the standardized *Salmonella* protocol of the CDC PulseNet.

**Results:** A total of 255 S. Typhimurium strains from clinical cases were investigated. Eight-eight strains were multidrug-resistant (exhibiting resistance to three or more antimicrobials). Two major restriction patterns were detected. The first was identified in 56 isolates (63.6) which exhibited resistance to ACSSuT. Resistance was mainly encoded by SGI detected in 85.7% of isolates. In strains SGI negative resistance was encoded by catA1, sul1, tetB and blaOXA-30-adjA1 gene cassette array. The second major resistance pattern was exhibited by 19 isolates (21.2%) resistant to ACSSuTm. The resistance was encoded by blaTEM-1, strAB, sul2, tetA or tetB and blaOXA-30-adjA1 gene cassette array. Five minor resistance profiles were identified mainly encoded by blaTEM-1, strAB, sul2, tetA or tetB and by the blaOXA-30-adjA2 gene cassette array integrated into an integron of class1.

Strain characterization by PFGE highlighted the prevalent identification of the pulse-type STXMXB.0061.

**Conclusion:** The study has highlighted the widespread of SGI among MDR S. Typhimurium clinical strains isolated in Apulia. Strains positive for SGI1 were largely identified with respect to place and date of isolation; additionally these strains showed an indistinguishable PFGE profile (STXMXB.0061). Taken together, these data argue for a clonal diffusion and persistence of this major group of MDR strains.
Objectives: The incidence of *Pseudomonas aeruginosa* strains resistant to carbapenems and fluoroquinolones (FQ) is important in most of hospitals in Bucharest. In order to examine the contribution of some carbapenem resistance mechanisms in clinical isolates that lack metallo-β-lactamase genes, our study aim was to investigate the prevalence of extended-spectrum AmpC β-lactamaes (ESAC) and of oprD mutants. The same clinical isolates of *P aeruginosa* were examined for alterations in the quinolone resistance-determining regions (QRDR). An additional interest was to search for a putative correlation between Multilocus Sequence Typing (MLST) results and the distribution of QRDR silent mutations and blaAmpC gene polymorphism, respectively.

Methods: Thirty-three imipenem- and FQ-resistant *P aeruginosa* clinical isolates, collected from ten Bucharest hospitals over an 11 year period, previously screened for blaPER-1 and class 1 integrons occurrence, were selected for this study. PCR using blaAmpC, oprD and gyrA, gyrB, parC, parE QRDR specific primers, followed by DNA sequencing was performed. The resulting sequences were compared to that of the reference strain PA01. Clonal relationships were established by MLST.

Results: The determination of the AmpC amino acid sequences of all 33 isolates, allowed us to identify 8 *Pseudomonas*-derived cephalosporinases (PDC) variants. Five of those were new variants, submitted to EMBL/GenBank database. All variants contained Ti05A substitution, consistent with an ESAC activity. For most of the studied isolates, oprD sequence analysis revealed the presence of frame shift mutations produced by one base pair insertions or deletions. Single substitution T83I in GyrA was detected in all FQ-resistant isolates. Twenty-two isolates contained equally a single substitution S87L in ParC. Silent mutations were found in all QRDR, mainly in parF and gyrB. Analysis of these silent mutations and of ESAC-type PDC variants resulted in isolates clusters suggesting clonal relatedness, confirmed by MLST results. A cluster of 16 isolates is characterized by serotype O11, sequence type ST235 and a new PDC-2-derived variant. A cluster of 8 isolates, mainly of serotype O12, belonging to ST111 displays PDC-3 variant.

Conclusion: This is the first report about ESACs occurrence and specific point mutations associated with FQ reduced susceptibility in multidrug resistant *P aeruginosa* clinical isolates from Romania.
Methods: Antimicrobial susceptibility was determined by agar dilution according to CLSI guidelines and the presence of the oqxA and oqxB genes was evaluated by PCR in a total of 290 consecutive clinical isolates of *E. coli* (*n* = 136) and *K. pneumoniae* (*n* = 154), collected at Huashan Hospital in Shanghai, China from March 1st to May 31st, 2010.

Results: Of the *E. coli* strains, 89.1%, 71.3%, 73.5% and 70.6% were nonsusceptible to nalidixic acid, ciprofloxacin, norfloxacin and levofloxacin, while 78.6%, 66.9%, 61.7% and 50.7% of the *K. pneumoniae* strains tested nonsusceptible to the same agents, oqxA and oqxB were present in 9 of 136 (6.6%) *E. coli* and all 154 *K. pneumoniae* strains. The oqxA and oqxB PCR products of 9 *E. coli* and 74 randomly selected *K. pneumoniae* strains were sequenced and showed 99% to 100% similar to the original oqxAB sequence of pOLA52 (EU370913). Eight PCR primer pairs were designed to amplify the full-length of the oqxAB genes from 9 strains of *E. coli* and 21 strains of *K. pneumoniae*. Compared to the sequences encoded by pOLA52, one to two amino acid substitutions were detected in the OqxA or OqxB sequences from two *E. coli* strains and were temporarily designated as oqxA2, oqxB2 and oqxB3. Among 21 OqxA and OqxB sequences from *K. pneumoniae*, 10 strains had 1 to 2 and 1 to 6 amino acid substitutions, respectively, compared with four sequences of OqxA and OqxB from genome sequences from *K. pneumoniae* available in the GenBank (FJ975560, FJ975561, CP000647, AP006725). I-CeuI digestion, transfer and hybridization with specific probes for oqxA and 16S rRNA are being undertaken on *K. pneumoniae* strains and may be locate on the chromosome, thus identifying the genome of *K. pneumoniae* as a possible reservoir of oqxAB.

Conclusion: New oqxAB genes, oqxA2, oqxB2 and oqxB3 were identified in two *E. coli* strains. The oqxAB gene is present in all *K. pneumoniae* strains and may be locate on the chromosome, thus identifying the genome of *K. pneumoniae* as a possible reservoir of oqxAB.

Genetic basis of expanded-spectrum cephalosporin resistance in *Salmonella* from food animals detected by the Italian Veterinary Monitoring System (ITAVARM)

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Objective: The objective of the study is to describe the presence and frequency of genes encoding resistance to Expanded-Spectrum Cephalosporins (ESCs), in *Salmonella* from food animals detected through a monitoring network (ITAVARM) of Veterinary Public Health Institutes (IZSs). ESCs are among Critically Important Antimicrobials for the treatment of invasive infections in humans caused by zoonotic bacteria (WHO, 2008).

Methods: Isolates are collected following the guidelines issued by the EFSA and by the EU Commission (Comm. Dec. 407/2007/EC). Representativeness is obtained by the inclusion of isolates from baseline studies and national control plans (Reg. 2160/2003/EC), along with active monitoring and passive laboratory surveillance in food animals. Isolates are tested by microdilution method (MICs) and interpreted with the EUCAST epidemiologic cut-offs by the National Reference Laboratory for Antimicrobial Resistance, where also characterization of resistance genes is conducted.

Results: ESC-resistance was detected in *Salmonella* from poultry holdings, and was attributed to ESBL, AmpC-like, or TEM conferring ESBL phenotype genes. In broiler chicken holdings, samples from the 2008 EU harmonised baseline Survey (Comm. Dec. 2007/516/EC) and passive surveillance yielded 13.2% (16/121) isolates resistant to Cefotaxime (CTX-R). Among these isolates, 6.6% (8/121) were positive for blaCTX-M group 1 or group 2, 2.5% (3/121) for blaSHV encoding ESBLs, 1.7% (2/121) for blaTEM, and 2.5% (2/121) for blaCMY-2 genes, respectively. In holdings of fattening turkeys, samples from the 2007 EU baseline survey (Comm. Dec. 662/2006/EC), yielded 3.6% (5/139) isolates CTX-R. All isolates were positive for blaCTX-M Group 2. Passive laboratory surveillance yielded 7% (3/43) isolates CTX-R, of which 4.7% (2/43) were positive for blaCTX-M group 2, and 2.3% (1/43) for blaSHV. PCR-based replicon typing of plasmids in these *Salmonella* isolates detected the presence of different replicons such as H12, P, I, N, FIA, A/C.

Conclusions: The veterinary monitoring system in place in Italy has adequate sensitivity to detect emerging resistances and the underlying mechanisms to some important antimicrobial classes such as ESCs in food animals. In Italy ESC-resistance is mediated more frequently by ESBL, otherwise by AmpC-like and blaTEM genes, and it is widespread in *Salmonella* spp. from poultry although there is no medicinal product registered for veterinary use in such animal species.

Identification of Inc plasmids types and its association with plasmid-mediated quinolone resistance and extended-spectrum β-lactamases determinants carried by *Escherichia coli* clinical strains

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Objectives: Replicon typing plasmids is a useful tool to trace the dissemination of plasmids conferring antimicrobial resistance. The aim of the study was to classify and establish the frequency of the Incompatibility (Inc) plasmids types among clinical *Escherichia coli* strains and to identify the extended-spectrum β-lactamases (ESBLs) and plasmid-mediated quinolone resistance (PMQRs).

Methods: During October-December 2007, 97 *E. coli* strains were collected from patients of the University Hospital of Coimbra. The Inc plasmid groups were identified by PCR-based replicon typing, PMQR (qnr, aac(6’)-lb) variant and qepA and ESBL (blaTEM, blaSHV and blaCTX-M) determinants were screened by PCR. Antimicrobial susceptibility profile and the identification of ESBL-producing isolates were determined using the Vitek2AES commercial system.

Results: Ninety nine percent of strains showed at least one plasmid type. Forty three percent of strains presented at least 2 different plasmids, 25% carried only 1 and 24% showed 3 different plasmids. The major Inc group found was IncF (88%), followed by the IncN (60%), FIB, FIA and FIC replicons were present in 57%, 40% and 17% of the strains, respectively. Among the 67% of strains recovered from urine, the Inc plasmid mostly identified were IncF, IncN and IncI (85%, 58% and 23% respectively). We detected 15% of ESBL producers distributed by the clinical samples: urine (n = 12), exudates (n = 2), and blood (n = 1). All ESBL producers expressed a CTX-M-15 enzyme. An IncM plasmid was identified in all ESBL strains and, of these, 20% also carried an IncN plasmid and 7% an IncI plasmid. PMQR were detected in 44% of strains: 3% QnrS, 3% QnrB and 38% aac(6’)-lb-cr. Of these, 64% were resistant to ciprofloxacin, while 33% and 2% showed susceptibility or intermediate level to this antibiotic. PMQR strains showed high plasmid diversity, with 86% carrying an IncF, 43% an IncK and 21% an IncI. All strains expressing simultaneously CTX-M-15 and a PMQR presented an IncI plasmid, while 20% and 7% presented an IncN and IncL, respectively.

Conclusion: IncF was the prevalent group. It was present in all strains expressing an ESBL and/or PMQR, suggesting its high association with the spread of these determinants, namely the widely disseminated CTX-M-15, and its capacity of mobility to other Enterobacteria. The heterogeneity of plasmids seen in PMQR-strains may indicate independent events of acquisition rather lateral transfer.

Molecular detection of antibiotic resistance among Gram-negative bacteria

Characterisation of carbapenemases in multidrug-resistant *Acinetobacter baumannii* isolates from intensive care units

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Objectives: Carbapenem resistance in *Acinetobacter baumannii* is a growing public health concern and represents serious problems in the treatment of related infection. Several carbapenem-hydrolysing β-lactamases have been identified from *A. baumannii* so far. This study
characterise carbapenem resistance in *A. baumannii* strains recovered from intensive care units of Gulhane Military Medical Academy, Turkey.

**Methods:** From June 2006 to January 2010, 138 clinical *A. baumannii* isolates were collected. Antimicrobial susceptibility tests of the isolates were performed by using Phoenix™ 100 Instrument (Becton Dickinson, USA). The MICs of imipenem and meropenem were also determined by E-test method (AB Biodisk, Sweden). The 61 carbapenem resistant *A. baumannii* strains were selected for further study. Firstly, to determine the presence of carbapenemases, Modified Hodge test (MHT) was performed. The presence of Metallo-β-lactamase (MBL) was investigated by using MBL IP/PI E-test strips (AB Biomérieux, France). Detection of the four groups of OXA carbapenemase (OXA-23, OXA-24, OXA-51 and OXA-58) was carried out using a multiplex PCR assay. Sequence analysis was performed by using BigDye Cycle Sequencing Kit V3.1 on ABI 3130 XL Genetic Analyzer.

**Results:** Non-duplicate, multidrug resistant 61 clinical *A. baumannii* isolates were determined resistant to imipenem and meropenem. In the 61 isolates, the MIC50 of imipenem and meropenem were 16 and >32; MIC90 were 192 and >32 respectively. MHT was positive for all 61 *A. baumannii* strains. None of these isolates showed MBL activity. All of 61 isolates had blaOXA-51 genes, 50 isolates had blaOXA-23, and 11 isolated had blaOXA-58 genes. Alleles encoding OXA-24-like enzymes were not detected in any isolates.

**Conclusions:** All of the 61 carbapenem resistant *A. baumannii* strains showed OXA-type carbapenemase activity in this study. Ninety-five percent (n = 58) of the isolates have two carbapenemases, one intrinsic and the other acquired. OXA-51+OXA-23 was the most prevalent (77%).

**P638** Evaluation of a low-density microarray for the identification of ESBL, AmpC and carbapenemase-producing Enterobacteriaceae

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**Objectives:** We describe the validation of commercially available low density microarrays (Check Arrays, Check-Points, Netherlands, Hain Lifescience, Germany) for the reliable and highly specific identification of the most prevalent ESBL, AmpC and Carbapenemase-types.

**Methods:** The Check Arrays use specific DNA markers for the detection of ESBL strains conferring TEM, SHV and the CTX-M types, AmpC and carbapenemases (NDM-1, KPC, VIM, OXA-48, IMP). DNA was extracted from colonies grown on an agar plates using the EasyMAG system (bioMérieux, Germany). Before PCR with biotin-labelled primers, specific oligos containing a artificial 5′ sequence are ligated when they are annealed to a complementary DNA-structure. PCR products of up to three isolates can be pooled and hybridized to a low density microarray (platform based on CLONDIAG chip technologies GmbH). Detection was performed with a CP-Array tube reader and the CP ESBL software. For validation a panel of 36 (19 ESBL-positive, 17 ESBL-negative) and 5 Carbapenemase-positive strains, genotypically (sequencing, PCR) and phenotypically (Phoenix System, [BD Diagnostic Systems, USA]) characterized Enterobacteriaceae-strains were used. In addition to these reference strains 115 routine isolates characterized by VITEK 2 and additional CLSI ESBL disk diffusion test and the AmpC&ESBL Detection set (Mast Diagnostika, UK) were analyzed with the ESBL array.

**Results:** All (100%) of the 19 characterized ESBL-positive isolates (12 TEM, 7 SHV), were correctly identified with the Check-arrays. 3 of the 17 ESBL reference strains were negative with the molecular test and positive with the phenotypic methods. Of the 115 routine isolates tested in total, 111 (96,5%) of the ESBL-positive and -negative strains showed congruent results compared to phenotypic ESBL detection methods. One routine isolate was ESBL-positive with the Check array and negative with the phenotypic methods and one isolate was negative with the molecular method and positive with the phenotypic method. 2 strains were invalid with the Check array. All strains characterized as Carbapenemase-positive (n = 5) were correctly detected by the molecular arrays.

**Conclusions:** The CP ESBL array showed accurate results for the identification of the TEM, SHV and CTX-M ESBL-types, AmpC and Carbapenemases. The test can be performed within one workday (7–8h).

**P639** Diversity of gene cassette promoters in antibiotic-resistant bacteria from wastewater environments

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**Objectives:** To investigate the diversity of the Pc-P2 promoter combinations involved in gene cassette transcription, in class 1 integrons from wastewaters isolates.

**Methods:** Enterobacteriaceae and Aeromonadaceae were isolated from two distinct environments: urban wastewaters, consisting mostly of domestic effluents and slaughterhouse wastewaters, consisting of discharges with animal origin. Class I integrons-containing strains were characterized in terms of phylogenetic affiliation and antimicrobial resistance profiles. The Pc-P2 promoter region was amplified by PCR and sequenced to identify the promoter variants.

**Results:** Among 47 integrons identified, the following 6 Pc-P2 configurations were detected: PcS, PcH2, PcH1, PcWTG-10, PcW and PcW+ PcP. The promoter types were neither species- nor gene cassette array-specific. The diversity of Pc-P2 configurations differed according the origin of the sample. In integrons from urban wastewaters (n = 23), the 6 Pc-P2 configurations were detected: PcH1 (44%), PcW (26%), PcWTG-10 (13%), PcS (9%), PcW-Pc2 (4%) and PcH2 (4%), while in integrons from slaughterhouses' wastewaters (n = 24), only 3 configurations could be detected: PcW (83%), PcH1 (13%) and PcW-Pc2 (4%).

**Conclusions:** Results obtained showed the predominance of the weak PcW and PcH1 variants in both types of effluents. However, the PcW variant largely prevailed in integrons from animal's wastewaters, while PcH1 was prevalent in domestic effluents. Interestingly, these low efficient promoters are known to determine the most efficient integrons integrase for recombination of gene cassettes. Hence, their prevalences provide insights into the gene cassette dynamic in integrons from wastewater environments. Other environments should be investigated in order to determine whether the Pc diversity correlate with some specific environmental conditions.

**P640** Evaluation of a commercial miniaturised DNA microarray for detection of β-lactamase genes in Gram-negative bacteria

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**Objectives:** We evaluated the Identibac AMR-VE Genotyping (Alere GmbH, Germany), a miniaturized DNA array, for high-throughput detection of clinically important β-lactamase (CTX-M, TEM, and SHV) genes in various Gram-negative bacteria.

**Methods:** The array was validated on 30 well-characterized Gram-negative organisms harbouring β-lactamases and belonging to the following species: *Escherichia coli* (n = 15); *Klebsiella pneumoniae* (n = 5); *Klebsiella oxytoca* (n = 1); *Proteus mirabilis* (n = 4); *Serratia marcescens* (n = 2); *Enterobacter cloacae* (n = 2); *Citrobacter freundii* (n = 1). Strains were identified by mass spectrometry. Presence of CTX-M, TEM, and SHV β-lactamase genes was confirmed by PCR and sequencing. For array testing, genomic DNA was extracted with chlorof orm/isoamyl alcohol, treated with RNase, and the required concentration (0.1–0.3 μg/μl) was calibrated spectrophotometrically. Template DNA was biotin-labelled in a linear PCR followed by hybridization (Array Hybridization Kit) to the microtube DNA array system. Data was analyzed on the Array Tube Reader (ATR 03, 2.0) using IconoClust software (Standard version) and normalised using 10 internal control probes. Mean signal intensities of two replicate spots per probe were used for analysis. Intensities of ≥0.4 were considered positive, <0.3 were negative, and 0.3–0.4 were ambiguous (manufacturer recommendations).
Results: Of the 30 strains tested, 19 harboured CTX-Ms and 12 (63%) could be detected on the array. By PCR-sequencing, these were identified as CTX-M-15 (n = 5), CTX-M-3 (n = 3), CTX-M-1, -27 (n = 2 each), and CTX-M-2, -5, -9, -14, -32, -55, and a non-typeable CTX-M (n = 1 each) (Table). Eight of the 12 array-positive CTX-Ms were present in E. coli, 3 in K. pneumoniae, and 1 in E. cloacae. However, of the 8 SHV genes detected by PCR-sequencing, only 2 (25%), SHV-1 present in K. pneumoniae gave positive signals on the array. In contrast, all 23 TEM genes were correctly detected. Array specificities for detection of CTX, SHV and TEM were 100%.

Conclusion: Our data indicate that the Identibac AMR-VE array is a high-throughput, easy-to-use method for detection of multiple β-lactamase genes in Gram-negative bacteria, although iterative modifications to increase its sensitivity for CTX-M and SHV detection are required.

### Table 1: Distribution of β-lactamase genes and subtypes in Gram-negative bacteria and detection on the microarray

| Betalactamase genes | True status of β-lactamase harbouring Gram-negative bacteria | Detection results on Identibac AMR-VE microarray |
|---------------------|-------------------------------------------------------------|-------------------------------------------------|
|                      | Bacteria (n=90) distributed per gene subtype* (no. of strains) | No. positive strains | No. ambiguous strains | No. negative strains |
| CTX-M-1 (n=9) |                                         | 2 | 0 | 0 |
| CTX-M-15 (n=6) | E. coli (n=5), K. pneumoniae (n=1) | 1 | 1 | 3 |
| CTX-M-3 (n=10) | E. coli (n=9), K. pneumoniae (n=1) | 1 | 0 | 0 |
| CTX-M-27 (n=2) | E. coli (n=2) | 1 | 0 | 1 |
| CTX-M-4 (n=1) | E. coli (n=1) | 2 | 0 | 1 |
| CTX-M-32 (n=1) | E. coli (n=1) | 1 | 0 | 0 |
| CTX-M-5 (n=14) | E. coli (n=13), K. pneumoniae (n=1) | 0 | 0 | 1 |
| CTX-M-9 (n=10) | E. coli (n=9) | 0 | 0 | 1 |

*Several bacteria harboured more than one β-lactamase gene

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### P641 | Multiplex polymerase chain reactions for extended-spectrum β-lactamases, AmpC β-lactamases and carbapenemases

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**Objectives:**
- Several multiplex PCRs for the detection of a broad range of clinically important β-lactamase families have been published. However, these PCRs use different amplification protocols and do not cover the wide range of clinically important Extended-Spectrum β-lactamases (ESBLs), AmpC β-lactamases and carbapenemases. The aim of this study was to develop a set of multiplex PCRs for the detection of the majority of genes encoding clinically important β-lactamases causing third-generation cephalosporin or carbapenem resistance using a single amplification protocol.

**Methods:** Twenty-six strains with sequenced β-lactamase genes were used as positive controls. Primers were either described by (Dallene et al., JAC, 2010) or designed in this study and evaluated in the following protocol. DNA was isolated using the NucleoSpin® Tissue kit according to the instructions of the manufacturer. The DNA concentrations of the samples were equalised to approximately 50 ng/μl and 1 μl was added to each multiplex PCR in a 25 μl reaction mixture, containing 1x PCR buffer (10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1% Triton X-100, 0.1% (w/v) stabilizer), 50 μM of each deoxynucleotide triphosphate, 1 U SuperTaq, and a variable concentration of primers and MgCl2. Amplification was carried out as follows: initial denaturation at 94°C for 1 min; 30 cycles of 30 sec at 94°C, 40 sec 60°C, 1 min 72°C, and a final elongation step at 72°C for 1 min. Amplification products were visualized after running at 100 V for 1 h on a 1% agarose gel containing gelred. A 1 kb DNA ladder was used as a size marker.

**Results:** Amplification products of the expected sizes were obtained with all control strains, confirming the specificity of the primers. This extends the possibility to use multiplex PCRs for the detection of the following β-lactamase genes: GIM, IMI, NDM, SIM, SME, SPM β-lactamase families and the OXA-1, OXA-2, OXA-4, OXA-23, OXA-24, OXA-51 and OXA-58 groups. These β-lactamase genes can be detected, along with previously described β-lactamase genes by Dallene et al., using one amplification protocol.

**Conclusion:** We describe a set of multiplex PCRs that enables the detection of the majority of clinically important β-lactamases causing third-generation cephalosporin or carbapenem resistance using one amplification protocol.

### P642 | Development of a real-time multiplex PCR assay for the detection of plasmid-mediated AmpC β-lactamases directly from blood cultures

G.L. Vanstone*, L. Ramzi, B.M. Charalambous, I. Balakrishnan

**Objectives:**
- AmpC B-lactamase producing Enterobacteriaceae have a multi-drug resistant phenotype and represent an infection control challenge. The rapid detection of these organisms is critical to implementing optimal antibiotic therapy and appropriate infection control procedures. Currently, detection is based on phenotypic methods and can take 48 hours to complete.

**Methods:** The conventional PCR (Perez-Perez and Hanson, 2002) was transferred onto a real-time platform (RotorGene 6000) using SYBR Green chemistry. High Resolution Melt (HRM) analysis of the products was evaluated as a method to distinguish between the different AmpC genes. Control organisms were then spiked into negative blood cultures to give concentrations of 10³ to 10⁷ bacteria/ml. Bacterial DNA was
Epidemiology of NDM-1

**P643 Long-term evolution of hyper-mutable strains increases the mutational cost of antibiotic resistance**

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**Objective:** Increased mutation rate (m) should offer more opportunities for antibiotic resistance, but simultaneously bacterial populations with this trait might accumulate mutations affecting their replication rates. These contradictory effects suggest that the overall success of strains with increased m will be higher in the early stages after the emergence of the hyper-mutable status. This work explores how the time-length of established mutators influences the biological cost of newly acquired antibiotic resistance mutations.

**Methods:** Four *E. coli* variants with different mutation rates (m) were studied: i) a non-mutator *E. coli* strain (nA) with m = 1.07x10−9; ii) its isogenic mutator variant (mA) with m = 5.37x10−8 and two evolved variants from (mA) obtained after 1,500 generations in LB; iii) an evolved mutator (mE) with m=6.60x10−8; and iv) an evolved non-mutator (nE) with m=2.47x10−9. Quinolone- or rifampicin-resistant variants of all these four strains were obtained in the lab, all of them carrying the same single changes S83L (NalR) and D516N (RifR), which do not affect to biological cost (W). Pairwise competition experiments in LB-broth were performed between NalR or RifR versus wild-type strains in order to measure W in mutator and non-mutator backgrounds.

**Results:** 1) Competition experiments between strains (nA and nE) with m close to modal value (m=1x10−9) did not yield differences in W independently from the number of generations (W0=0.99±0.06 and W1500=0.97±0.06, respectively). However, in the first generations, the biological cost of nA strain was 15% higher than nA strain (W0=0.85±0.05), significantly rising in to 41% (W1500=0.59±0.09) after 1,500 generations (mE). 2) Exposure to an SOS-inducing agent (nalidixic acid) increased m of non-evolved mutator in 4.3-fold (mA, 2.31x10−7) and only 2.6-fold in the evolved mutator (mE, 1.76x10−7), suggesting weaker activation of SOS system in evolved mutators. When an antibiotic non-inductor of SOS system (rifampicin) was used no significant changes in m were observed.

**Conclusions:** In stable environments, mutator strains might reduce their frequency in bacterial populations, because of the reduction of their replicative capacities imposed by the new mutations. The possible attenuation of SOS system in these strains might act as a compensatory effect to decrease this mutational load, but also reducing their adaptive opportunities.
cases occurred between December 2009 and August 2010. Patient one (NDM-1 K. pneumoniae), a 30-year-old Austrian male was admitted to our University Hospital in November 2009. History revealed that he had suffered multiple open fractures of his upper and lower left leg and rectal laceration due to a motorcycle accident in Pakistan. In patient two the NDM-1 K. pneumoniae was detected in August 2010, the patient being a 14-year-old Kosovarian boy transferred from Kosovo to the Department of Pediatrics, University Hospital Graz, with multiple intraabdominal abscess formations and peritonitis. He had undergone appendectomy in Pristina, Kosovo, in April 2010 and had developed abdominal sepsis consecutively. Patient number three (NDM-1 E. coli), a 56 year old male developed a necrotizing fasciitis when being in India in January 2010, after a short admittance at a hospital in New Delhi the patient was transferred to Austria.

Conclusion: Gram-negative Enterobacteriaciae with the newly described resistance enzyme NDM-1 have been recently shown to pose the potential of growing to a major public health problem worldwide. We report the emergence of NDM-1 among Enterobacteriaciae in Austria. During December 2009 and August 2010 two patients with K. pneumoniae carrying NDM-1 and one patient with NDM-1 E. coli were identified at the Medical University of Graz, Austria.

Characterisation of the first NDM-1 metallo-β-lactamase producing Escherichia coli isolates from an Italian hospital

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Objectives: Metallo-β-lactamases (MBLs) are resistance determinants of great clinical relevance, given their ability to hydrolyze almost all β-lactams, including carbapenems. Among MBLs, NDM-1 is one of the most worrisome due to its propensity to rapid dissemination among Enterobacteriaciae. Here we report on the characterization of the first NDM-1 positive isolates from an Italian hospital.

Methods: A total of 8 NDM-1-positive E. coli isolates obtained during 2009–2010 from stool and wound-exudate samples of two Italian patients were studied. Susceptibility testing were performed using Vitek2 system (Biomerieux). MICs of carbapenems, amikacin, piperacillin-tazobactam, colistin and tigecycline were confirmed by Etest (Biomerieux). Detection of resistance genes was performed using a custom microarray and all positive signals were confirmed by PCR. Clonality was investigated by PFGE and MLST. Localization of blaNDM-1 was investigated by Southern blot hybridizations. Gene transfer experiments were performed both by electroporation and conjugation. The genetic environment of blanDM-1 was investigated by the inverse-PCR (iPCR) technique.

Results: Isolates showed a MDR phenotype being not susceptible to all tested antibiotics, except colistin and, in 7 cases tigecycline. Carbapenem MICs were 4–6 mg/L (imipenem), 8–32 mg/L (meropenem), 2–16 mg/L (doripenem), and >32 mg/L (ertapenem). Microarray, in conjunction with PCR and sequencing, revealed the presence in all isolates of blaNDM-1, blaTEM-1, blaOXA-1, blaOXA-9, blaCTX-M-15, tetA(B), tetA(C), armA, aac(6′)-Ib-cr, aac(3)-II, catB3, and arr3 resistance determinants. PFGE and MLST demonstrated that isolates were clonally related and belonged to ST405. blanDM-1 was not transferred either by electroporation or by conjugation using different E. coli recipients. iPCR experiments revealed an original genetic structure, characterized by an intact IS26 insertion sequence upstream blanDM-1. Results from Southern-Blot experiments revealed the likely presence of 2 copies of blanDM-1 in the investigated isolates, with one plasmid-encoded.

Conclusions: Characterization of the genetic environment of the first NDM-1 positive Italian isolates revealed an original arrangement, underscoring the promiscuity of genetic elements carrying blanDM-1. The presence of a plethora of different clinically relevant resistance determinants in such isolates is a worrisome finding given the scarce number of effective therapeutic options available.

Klebsiella pneumoniae harbouring NDM-1 metallo-β-lactamase isolated in Croatia

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Objectives: A. K. pneumoniae strain, resistant to carbapenems, was isolated in May 2009, from a blood culture of a 40-years-old man, rescued in a surgical intensive care unit of the Clinical Hospital Center in Zagreb, Croatia. The patient had been transferred from another hospital of a neighbouring country after 5 days of hospitalization due to a car accident. The clinical history mentioned antibiotic treatment not including carbapenems (gentamicine, metronidazole and ceftriaxone), and no link to India or Pakistan. We investigated the carbapenem-hydrolyzing metallo-β-lactamase NDM-1 isolated in Croatia.

Materials and Methods: Antimicrobial susceptibility testing was performed by both Vitek2 and microdiluition, and interpreted according to the latest EUCAST documents. The presence of a carbapenemase was investigated by means of an MBL-test, hydrolysis of carbapenems and EDTA inhibition of hydrolysis. PCR and sequencing were carried out by standard procedures. Resistance was transferred by conjugation to E. coli J53.

Results: The strain was resistant to imipenem and meropenem (MICs 16 mg/L and 64 mg/L, respectively), and to all 3G-cephalosporins (MICs 21st ECCMID/27th ICC, Posters

Emergence and epidemiology of New Delhi metallo-β-lactamase 1-producing Klebsiella pneumoniae in a Korean tertiary care hospital

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Objectives: Acquired carbapenemases-producing Enterobacteriaciae has been a legislative notifiable infectious disease in Korea since November 2010. For a month period of November 2010, four cases of a novel acquired carbapenemase, New Delhi metallo-β-lactamase 1 (NDM-1) was first detected in a tertiary care hospital in Korea.

Methods: Species identification and susceptibility testing of Enterobacteriaciae isolated from clinical and surveillance specimens was performed with MicroScan NBC44 (Siemens, UK). Since November 2010, Nonsusceptible Enterobacteriaciae to one of etrapenem, imipenem and meropenem have been tested with modified Hodge test and disk diffusion testing using imipenem disks, combined imipenem and meropenem disks, and boronic acid–combined imipenem and meropenem disks. For the isolates revealing carbapenem-hydrolyzing metallo-β-lactamase were submitted to NDM-1-specific PCR and sequencing. All carbapenem-resistant K. pneumoniae (CRKP) isolated in November 2010 was typed using pulsed field gel electrophoresis with Xba-1 restriction. Electronic medical records of the patients infected or colonized with NDM-1 were reviewed for demographic, clinical, and epidemiological data.

Results: Four NDM-1 producing K. pneumoniae were detected and three of them were resistant to etrapenem, imipenem, and meropenem, but one was susceptible to imipenem and intermediate to meropenem. Only a few antimicrobials were remained active for those; colistin for all, tigecycline and amikacin for three, tobramycin for two, aztreonam for one, and tetracycline for one. Two NDM-1 K. pneumoniae were isolated from urine cultures and the others from stool surveillance. All patients were hospitalized for 1 to 5 months and received meropenem for 8 to 47 days before isolation of NDM-1. One of them admitted and two had been admitted to medical intensive care unit (MICU), but the periods staying at MICU were not overlapped each other. The remaining one stayed at a surgical ward after liver transplantation. There was no history of traveling abroad and epidemiological linkage among the cases found. Total of 8 CRKP including 4 NDM-1 were typed with PFGE, which revealed clonality among two NDM-1 CRKP from MICU and a medical ward, and one CRKP other than NDM-1.

Conclusion: Nosocomial clustering of NDM-1 was emerged in a Korean tertiary-care hospital. Careful surveillance of carbapenemase-producing Enterobacteriaciae are required in Korea.
First description of an Escherichia coli strain producing NDM-1 carbapenemase in Spain

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Objectives: Metallo-beta-lactamases (MBL) are important enzymes that hydrolyze all b-lactam antibiotics except aztreonam. Recently, a Klebsiella pneumoniae isolate carrying a novel carbapenemase, named NDM-1, was identified from a Swedish patient transferred from India. In this study, we report the first description of an E. coli strain producing NDM-1 in Spain.

Methods: Antimicrobial susceptibility testing was performed by BD Phoenix and Etest. Hodge and Imipenem-EDTA Etest synergy tests were used to screen for MBL production. Detection of genes encoding carbapenemases (VIM, IMP, NDM), ESBLs, plasmid-mediated cephalosporinases, armA (to investigate aminoglycoside resistance) as well as genes encoding heat-stable (ST), heat-labile (LT) toxins, verotoxins (VT) and enteroaggregative E. coli virulence factors was performed by PCR and sequence analysis. Conjugative transfer between DVR22 and E. coli J53 was done by conventional biparental mating. Plasmid analysis was performed both to the NDM-1 bearing strain and selected transconjugants. After a four month period another stool specimen from the same patient was screened to detect NDM-1 carriage.

Results: A carbapenem-resistant E. coli (DVR22) was recovered from a stool specimen from a patient with traveller’s diarrhoea who had travelled to India. The DVR22 strain was resistant to all antibiotics tested except tigecycline, fosfomycin and colistin. MICs of carbapenems were as follows: imipenem (8 μg/mL), meropenem (8 μg/mL), ertapenem (24 μg/mL) and doripenem (6 μg/mL). Hodge and Imipenem-EDTA Etest synergy tests were positive. PCR and sequencing identified the presence of genes encoding NDM-1, DHA, CTX-M-15, TEM-1 and armA genes. E. coli transconjugants showed resistance to aminoglycosides and all b-lactam antibiotics and were positive for NDM-1, CTX-M-15, DHA, TEM-1 and armA genes. Plasmid analysis revealed that E. coli transconjugants harboured a circa 180-kb plasmid. PCR screening of genes encoding virulence factors of diarrhoeagenic E. coli was negative. Four months later the DVR22 strain was no longer isolated from the stool of this patient.

Conclusions: This is the first isolation of an NDM-1 metallo-beta-lactamase in Spain. The location of NDM-1 in a conjugative plasmid increases the risk of dissemination and transfer to other bacteria. Our results, however, suggest that this strain does not persist long in the intestinal tract, most likely due to the fitness cost associated with the conjugative plasmid.
NDM-1 in Israel, evidence of travelling and carriage of carbapenem-resistant organisms

M. Castanheira*, L. Deshpande, G. Smoluan, R. Mendes, R. Jones, D. Ben-David, M. Hindiyeh, S. Gefen-Halevi, N. Keller (North Liberty, US; Tel-Hashomer, IL)

Objectives: To investigate the carbapenem (CARB) resistance (R) mechanism in an *Escherichia coli* strain recovered from rectal swab of a patient hospitalized in Israel that received medical care in India during a vacation accident. NDM-1 was detected in several countries harboured by various Gram-negative bacilli species and patient cases were closely linked to receipt of medical care in India or Pakistan.

Methods: Gram-negative isolates from wound cultures were susceptibility (S) tested by CLSI reference broth microdilution methods. Isolates displaying imipenem (IMI) and/or meropenem (MER) MIC at ≥2 mg/L were screened for CARBase production by Modified Hodge test (MHT) and PCR for blaIMP, blaVIM, blaKPC and blaNDM-1. Amplicons were sequenced. S1 nuclease digest were resolved in agarose and hybridized with blaNDM-1 probe. Transformation was performed by electroporation using *E. coli* DH5α and plating in selective media (4 mg/L of ceftazidime). Transformants were confirmed by PCR and S testing.

Results: A 52 year old female was transferred to Chaim Sheba Med Center (CSMC; Tel-Hashomer, Israel) with an infected fracture of the right ankle after a motorcycle accident followed by surgery while vacationing in India. At CSMC, the patient underwent various surgical procedures (five incisions and drainage). Three *Aeromonas hydrophila* species, two *K. pneumoniae* (one each of ESBL-positive and -negative) and a methicillin-S *S. aureus* were recovered from wound cultures. One *K. pneumoniae* was carbapenem-R (MIC, >8 mg/L for IMI and MER) and MHT and CARBase PCR negative. An *E. coli* R to carbapenems (MIC, >8 mg/L for IMI and MER) grew in rectal screen cultures. MHT was positive and initial PCR for blakPC was negative. PCR for and sequencing confirmed the presence of blaNDM-1. This MBL gene was carried on a 150-kb plasmid that was transformed into an *E. coli* host. Transformants displayed elevated MIC values for carbapenem (4 and 8 mg/L for IMI and MER, respectively) and ampicillin-glycylglycine (>32 and >16 mg/L for amikacin and tobramycin, respectively).

Conclusions: The infection control challenges posed by globalization and easy travel are now being highlighted by clinical cases of infections caused by NDM-1-producing Enterobacteriaceae strains. NDM-1 appears to be easily transferred and well expressed in Enterobacteriaceae and elevated carbapenem MIC values were demonstrated in this Israeli clinical isolate and in our laboratory transformant strains.

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ESBL: a growing problem

**P655** Dissemination of *Salmonella enterica* serovar enteritidis sequence type 11 producing CTX-M-15 extended-spectrum β-lactamase in Korea

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**Objectives:** The recent emergence and dissemination of *Salmonella* strains producing CTX-M-type extended-spectrum β-lactamases (ESBLs) is now an important public health concern. This study was performed to investigate the molecular epidemiology of *Salmonella enterica* serovar Enteritidis producing CTX-M-15 in Korea.

**Methods:** A nationwide survey performed in July to September 2009 collected a total of 49 consecutive non-duplicate *Salmonella* isolates from 15 hospitals at 11 cities in Korea. Genes encoding ESBLs were detected by PCR experiments. The genetic organization of blaCTX-M-15 was investigated by PCR and sequencing the regions surrounding the gene. Southern blotting, pulse-field gel electrophoresis (PFGE), and multilocus sequence typing (MLST) were performed to characterize the isolates carrying blaCTX-M-15.

**Results:** Six *S. enterica* serovar Enteritidis isolates resistant to both cefazidime and cefotaxime were found to carry the blaCTX-M-15 gene. The ISecp1 element was identified upstream of the blaCTX-M-15 gene. The isolates shared a same sequence type (ST), ST11, however, they showed two different XbaI-macrorestriction patterns by PFGE. The blaCTX-M-15 gene was located on IncFII plasmids of an identical replicon sequence type (RST), F1:A−:B−, in all six isolates.

**Conclusion:** The present data show that there were two clonal outbreaks by *S. enterica* serovar Enteritidis producing CTX-M-15 in Korea in 2009. Although two clones shared a same ST, ST11, they cannot be considered to be a same clone because they showed a low genetic similarity of <85% by PFGE experiments. Interestingly, they shared IncFII plasmids of an identical RST, F1:A−:B−, suggesting the horizontal dissemination of the plasmid carrying the blaCTX-M-15 gene among *S. enterica* serovar Enteritidis isolates in Korea. Finally, IncFII plasmids carrying the blaCTX-M-15 gene have never been described before.

**P656** Susceptibility and incidence of extended-spectrum β-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* in intra-abdominal infections globally: SMART 2009–2010

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**Objectives:** *E. coli* and *K. pneumoniae* together represent approximately 60–70% of all aerobic Gram-negative bacteria (GNB) from intra-abdominal infections (IAI). It is therefore necessary to take their antimicrobial susceptibility into account when treating IAI. One of the goals of the Study for Monitoring Antimicrobial Resistance Trends (SMART), which tracks susceptibility patterns of IAI GNB globally, is to provide physicians with actionable data with which to tailor empiric therapy of IAI. This report provides the latest (2009–2010) susceptibility data and frequency of extended-spectrum β-lactamase-producing (ESBL+) isolates for these two species.

**Methods:** Approximately 170 hospitals in 39 countries each collected up to 100 consecutive non-selected GNB from IAI; confirmation of identification, susceptibility testing, and phenotypic ESBL determinations were done at a central laboratory (IHMA, Inc.) using custom MicroScan dehydrated MIC panels, following CLSI and manufacturer procedures and quality control guidelines. CLSI M100-S20-U breakpoints were used to determine susceptibility.

**Results:** The following table summarizes ESBL+ rates and susceptibility of *E. coli, K. pneumoniae*, and their respective ESBL+ sub-populations.

**Conclusions:** The most commonly isolated pathogens of IAI, *E. coli* and *K. pneumoniae*, remain very susceptible to ceftriaxone, imipenem, and amikacin; even these drugs, however, showed diminished activity against ESBL+ *K. pneumoniae*. The high proportion (roughly 20%) of each of these species that is ESBL+ renders most of the comparators in this study <90% effective in vitro, with susceptibility to several <80%. In areas where ESBL+ rates exceed the global average (e.g., Asia and Latin America, data not shown), susceptibility rates are even lower, potentially leaving only the carbapenems and possibly amikacin among drugs studied in SMART as options for empiric therapy of IAI.
**P658** Country-wide prevalence of ESBL-producing Enterobacteriaceae in 2009

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**Background:** ESBL is increasing rapidly worldwide also in low antibiotic use countries both in- and outside hospitals. We therefore repeated a national prevalence study of ESBL-producing E. coli, K. pneumoniae and P. mirabilis from 2007, where the overall prevalence was 2.4%, during a one month period in 2009 in all samples from blood and urine from hospitals and the community.

**Methods:** All E. coli, K. pneumoniae and P. mirabilis isolated from blood cultures and urine samples including hospital and community samples were screened for cephalosporin resistance with either cefpodoxime, ceftriaxone, cefotaxime or ceftazidime. Resistant strains were further tested with confirmatory ESBL tests based on synergy with clavulanic acid and sent to the Statens Serum Institute for further confirmatory testing for ESBL or AmpC-pheno- and genotyping.

**Results:** A total of 29,110 urine and 13,353 blood cultures revealed 353 ESBL-producing isolates: 241 E. coli, 110 K. pneumoniae and 2 P. mirabilis, while 126 ESBL-screening positive isolates were confirmatory negative. The strain related ESBL rates were (total/blood isolates): E. coli, 3.3%/7%; K. pneumoniae: 9.9%/14.6%; P. mirabilis, 0.6%/0%, respectively.

**Conclusion:** Prevalence rates of ESBL producing E. coli and K. pneumoniae have doubled since 2007, and this counts for both blood and urine cultures including hospital and community. Prevalence rates for K. pneumoniae in blood are highest in Scandinavia and similar to some Southern European countries. The increase in ESBL is parallel to the increase in broad spectrum antibiotics in Danish hospitals (DANMAP 2009), which is a clear warning to where intervention is needed.

**P659** Clinical, epidemiological and microbiological features of urinary tract infections caused by ESBL-producing Enterobacteriaceae in hospitalised patients

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**Objective:** Extended-spectrum β-lactamase-producing enterobacteriaceae (ESBLE) have been increasingly recognized in the community. Little is known about epidemiology of ESBLE carries in Tunisia. The aim of this study was to describe the epidemiology, clinical and antibiotic susceptibility features of uropathogenic EBSLE at infectious diseases service in a teaching hospital of Tunisia.

**Methods:** Retrospective study included all ESBLE isolated from urine samples of patients admitted for community-acquired urinary tract infections at infectious diseases department in the university hospital of Monastir between January 2008 and August 2010. Clinical and epidemiological features were collected. Urinary tract infection by ESBLE was confirmed, in the Laboratory of Microbiology in the same hospital, by Kass criterias: bacteriuria >10⁶/ml and leukocyte count >10³/ml. Identification of enterobacteria was performed by API20E. The study of antibiotic susceptibility was performed by agar diffusion according to CA-SFM. Detection of ESBL was demonstrated by a synergy test by placing a central disk of amoxicillin-clavulanic acid away from 30 mm disks of cefotaxime and ceftazidime.

**Results:** Twenty six ESBLE were collected. The number of strains was increased from 7 in 2008 (27% of isolates) to 10 in 2010 (38.5%). The median age was 50 years (21-79 years). The odds ratio was 1.36. Factors associated to ESBLE were: previous hospital admission in the last year 73%, previous antibiotics use 50%, recurrent urinary tract infection 46%, diabetes mellitus 38.5%, suprapubic or urinary catheter 23%, benign prostatic hyperplasia 15% and renal lithiasis 11.5%. Pyelonephritis was diagnosed in 92%. Klebsiella pneumoniae was isolated in 65.5% of cases, Escherichia coli in 15.5%. Enterobacter cloacae in 11.5% and Citrobacter freundii in 7.5%. The frequency of ESBL resistance to gentamicin, amikacin, tobramycin, fluoroquinolones, cotrimoxazol and fosfomycin were 65.5%, 11.5%, 77%, 81%, 50% and 15% and respectively. Multiresistance was noted in 11.5% of cases (ESBL, fluoroquinolones resistance and aminoglycosides resistance). No strains were resistant to imipenem.

**Conclusion:** Community-acquired urinary tract infections due to ESBL-producing enterobacteriaceae are increasing in Tunisia. It requires a much better control of antibiotics prescriptions and therefore an important multidisciplinary implication.

**P660** Escherichia coli and Klebsiella pneumoniae ESBL genotyping distribution in Denmark; CTX-M-15 prevails countrywide, also in hospital epidemics

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**Background:** ESBL rates were (total/blood isolates): E. coli: 9.9%/14.6%; P. mirabilis, 0.6%/0%, respectively.

**Objective:** In Denmark, E. coli and K. pneumoniae isolates producing ESBL have increased during the last five years. The aim of this study was to determine possible changes in ESBL genotype prevalence from 2007 to 2009, the distribution of the ESBL genes across the country, as well as the susceptibility pattern of the isolates.

**Methods:** During Sep. 2009 a nationwide search was conducted in which Danish departments of clinical microbiology screened all E. coli, K. pneumoniae, and Proteus mirabilis from blood and urine samples for ESBL enzyme production. Susceptibility testing was performed and ESBL genotyping was conducted by PCR and sequencing.

**Results:** A total of 254 ESBL enzyme-producing isolates were collected; E. coli (blood N= 15; urine N= 158), K. pneumoniae (blood N = 9; urine N = 70), and P. mirabilis (urine N = 2). CTX-M enzymes were the most dominant for E. coli, K. pneumoniae, and P. mirabilis with an occurrence of 89%, 80% and 100%, respectively. The CTX-M-15 enzyme was the most prevalent with 64% in E. coli, 73% in K. pneumoniae and 50% in P. mirabilis. CTX-M-15 and CTX-M-1 were distributed equally around the country, while the CTX-M-15/SHV 28 genotype in K. pneumoniae was found on Zealand only. A combined CTX-M-SSHV genotype was most frequent in K. pneumoniae with 63% compared to only 2% in E. coli.

**Conclusion:** Resistance towards gentamicin was 40% for E. coli and 68% for K. pneumoniae. For ciprofloxacin resistance was >70% overall. E. coli were >90% susceptible to the following antibiotics: amikacin 98%, mecillinam 92%, nitrofurantoin 90%, fosfomycin 99% and colistin 100%. In K. pneumoniae >90% susceptibility was found towards amikacin 95%, fosfomycin 95%, and colistin 100%. Both P. mirabilis isolates were susceptible towards amikacin, tobramycin and colistin.

**Conclusion:** The distribution of ESBL enzymes did not change significantly from 2007 to 2009 in the two Danish prevalence studies. CTX-M-15 continues to be the most prevalent enzyme.

**P661** Prevalence of β-lactamase enzymes in bacteria from inanimate surfaces in Hospital Infante D. Pedro, Aveiro, central Portugal

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**Objective:** The increasing use of β-lactams antibiotics leads to the emergence of β-lactamases enzymes within the hospital environment. We aimed to evaluate the presence of these enzymes in Gram-negative bacteria isolated from inanimate surfaces in a comparative study performed in 2005 and 2008.

**Methods:** Samples from different inanimate surfaces were collected, during 1 month period in 2005 and the same period in 2008. Sterile swabs were rubbed in the surfaces and then placed in rich medium (TSB), and incubated overnight at 37°C. Serial dilutions were plated in MacConkey agar. Phenotypical different colonies were selected and their clonal
relationship was evaluated by rep-PCR. Identification to the species level was determined by 16S amplification. Presence of β-lactamases enzymes (ESBL and metallo-β-lactamases) and their characterization were performed by PCR, using appropriate primers. Nucleotide and deduced aminoacid sequences were analyzed with Blast and ClustalW programs.

**Results:** Escherichia coli, Klebsiella spp., Pseudomonas spp. and Enterobacter cloacae strains were the most frequent microorganisms isolated. In 2005 only 33% of the isolates possessed class 1 integrons. In these isolates the presence of β-lactamases types revealed a high percentage of TEM (59%), VIM (18%) and SHV (14%), and lower percentages of OXA (4%) and IMP (5%). CTX-M was not detected. In 2008, 46% of the Gram-negative bacteria possessed class 1 integrons. Similar percentages of all β-lactamases types were observed: TEM (28%), OXA (28%), SHV (22%), CTX-M (17%) and VIM (5%). IMP was not present. Carbapenemase KPC was described, for the first time in Portugal, in one Klebsiella oxytoca and one Klebsiella pneumoniae.

**Conclusion:** The results show the high prevalence of β-lactamases in bacteria associated with inanimate surfaces within the hospital environment. This aspect is preoccupant since these bacteria can also infect compromised patients. The percentage of class 1 integrons and some β-lactamases enzymes tends to increase due to excessive use of β-lactams antibiotics, thus compromising the effective treatment of the patients.

**P662** Emergence of third-generation cephalosporin-resistant *Escherichia coli* from bloodstream infections in Denmark: this is due to both clonal and non-clonal spread of CTX-M-15

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**Objectives:** Before 2007 the occurrence of third-generation cephalosporin resistance was low among *E. coli* isolated from bloodstream infections in Danish patients. However, the rate of resistance among invasive *E. coli* in Denmark has increased from 2.5% in 2006 to 6.2% in 2009 (EARS-Net report 2009). In the present study the spread of third-generation cephalosporin resistant *E. coli* in Danish hospitals was investigated by molecular characterisation of collected isolates.

**Methods:** In 2009, four Danish departments of clinical microbiology (representing four regions in Denmark) collected all the third-generation cephalosporin resistant *E. coli* isolated from bloodstream infections. ESBL and/or AmpC phenotypes were tested with a combination disk method using Rosco Neosensitabs (Rosco Diagnostica, Taastrup, Denmark). Based upon the obtained phenotypes, PCR amplification and sequencing was performed to identify the ESBL- and plasmid mediated AmpC present. All ESBL and/or AmpC genotype positive isolates were PFGE typed and compared using BioNumerics 6.5 (Applied Maths, Sint-Martens-Latem, Belgium).

**Results:** In total, 104 isolates were investigated, 71 of these were ESBL positive and two isolates were plasmid mediated AmpC positive, exclusively. In total, 58/71 (77%) ESBL positive isolates produced a CTX-M-15 enzyme. In addition to this, six CTX-M-group 1-enzymes, six CTX-M-group 9-enzymes, two (non-TEM-1) TEM-enzymes and one SHV-enzyme were detected. The two AmpC-enzymes produced were both CMY-22. PFGE showed a pronounced degree of diversity with a total of 40 different PFGE types. However, 38% of the isolates (n = 28), belonged to three relatively large clusters, including 12, 9 and 7 isolates, respectively. The isolates in the second-largest cluster (n = 9), all originated from the same hospital, while geographical origin of isolates in the other two clusters appeared more diverse.

**Conclusion:** Third-generation cephalosporin resistant *E. coli* in Denmark is partly clonally unrelated. However, the presence of these large clusters, strongly indicates that clonal spread of ESBL in Danish hospitals does occur, within the same hospital as well as beyond regional borders. CTX-M-15 remains by far the predominant ESBL enzyme-type in *E. coli* in Denmark, representing 77% of all the ESBL-positive *E. coli* detected in this study. The occurrence of plasmid mediated AmpC in bloodstream infections is still low in Denmark.

**P663** Significant increase in antimicrobial co-resistance among extended-spectrum β-lactamase-producing *Escherichia coli* urinary isolates in Spain (2005-2009)

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**Objectives:** The appearance of extended-spectrum β-lactamases (ESBL)- producing *E. coli* in urinary tract infections (UTI) constitutes an important therapeutic challenge that requires the study of its evolution throughout time in order to establish a suitable empirical treatment. Our aim was to determine the percentage of ESBL-producing *E. coli* urinary isolates from 2005 and 2009. We also compared antimicrobial co-resistance to unrelated antimicrobial such as aminoglycosides, claramphenicol, trimethoprim-sufametoxazole, quinolones, fosfomycin and nitrofurantoin, between ESBL-producing *E. coli* from 2005 with that of 2009.

**Methods:** We analyzed 5053 and 6324 *E. coli* isolates obtained from urine cultures in our laboratory in 2005 and 2009 respectively. Duplicate isolates from the same patient were excluded. Antimicrobial susceptibility was determined by the Wider microdilution system (Soria Melguizo S.A.) and the phenotype pattern of resistance that indicated a BLEE-producing *E. coli* was selected. The CLSI breakpoints of 2009 were applied.

**Results:** One hundred and ninety eight strains were ESBL producers (3.9%) in 2005 and 463 (7.3%) in 2009. The resistance percentages of five β-lactamic and nine non β-lactamic agents are shown in the table.

**Conclusions:** Our institution had a higher prevalence of ESBL-producing *E. coli* in 2009. Other additional mechanisms of resistance to β-lactamic agents could explain the increase in the resistance percentage for all the β-lactamic analyzed. Resistance to carbapenems (imipenem, meropenem and etrapenem) was detected in 2009. The increase of resistance was significant for amikacin and fosfomycin (p < 0.01). The high level resistance to quinolones detected in 2005 indicated that they were not suitable for the empirical treatment of uncomplicated UTI and alternative agents like fosfomycin and nitrofurantoin were indicated. That change in therapeutic options could explain the increase in the resistance percentage to these antibiotics.

**P664** Studying the urinary reservoir of extended-spectrum β-lactamase-producing Enterobacteriaceae in a teaching hospital

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**Objectives:** Extended-spectrum β-lactamase producing Enterobacteriaceae (ESBLE) represent an important concern of public health. Our objective was to assess the urinary reservoir of ESBLE by identifying them in colonized urine samples.

**Methods:** The study was conducted during one month in a French teaching hospital with a high prevalence of ESBL. In the laboratory, urine samples are plated in a chromogenic agar medium (UTI, Oxoid, UK). The local usual practice is to not perform antibiograms and bacterial identifications when there are more than two different types of colonies in those plates. Bacterial identifications and strain conservation are performed when two different types of colonies are visible or in the absence of leucocytes. Otherwise, strains are identified and their susceptibility to antibiotics is tested. During the study, all colonies of Enterobacteriaceae visible onto the chromogenic agar plates were
isolated onto a selective agar medium for Gram-negative bacteria resistant to third-generation cephalosporins (BLSE, AES, France). In case of positive subculture on this medium, the production of ESBL was tested by the combined disk method on Mueller-Hinton agar plates. ESBL were identified thereafter with the API 20E system (bioMérieux, France). Uries in which bacteria were studied (identification + antibiogram) according to our usual practice were considered as infected. Uries in which ESBL were sought according to the study protocol were considered as colonized.

Results: During the study, 2312 urine samples were addressed to the laboratory for identifying urinary-tract infections. Among them, 682 (29.5%) were infected and 114 (4.9%) were colonized with at least one Enterobacteriaceae. Overall, 145 colonies were subcultured onto the selective medium. Fourteen ESBL were isolated in 13 colonized urines (11.4%) and 36 were isolated in 35 infected urines (5.1%) (Prevalence ratio = 2.12, P < 0.01). Among the 13 patients for whom an ESBL was identified in colonized urines, 10 had not been recorded as ESBL carriers in other samples. Lastly, the distribution of the three species identified was similar in infected and colonized urines.

Conclusion: The prevalence of ESBL is significantly higher in colonized urines than in infected urines. In hospitals where contact precautions are implemented for ESBL carriers, searching ESBL in urine samples could be a cheaper and less time-consuming alternative to rectal swabbing for identifying carriers.

**P665** Risk factors and outcome of bacteraemia due to cefotaxime-resistant Enterobacteriaceae in a Japanese university hospital

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**Objectives:** Cefotaxime is recommended as first-line empirical therapy for the treatment of bacteraemia due to enterobacteriaceae (En), however, extended-spectrum β-lactamase (ESBL) and plasmid mediated- AmpC β-lactamase (pAmpC) producing En is reported to be increasing. We investigated the epidemiology, risk factors, and outcome of bacteraemia due to cefotaxime-non-susceptible (CTXR)-En.

**Methods:** 249 Escherichia coli, 122 Klebsiella pneumoniae, 43 K. oxytoca, and 2 Proteus mirabilis were consecutively isolated from blood cultures between April 2005 and March 2010. Only the first isolate for each patient was included. MICs were determined by broth microdilution method and interpreted following CLSI (M100-S20). All the isolates were tested by PCR and sequencing to detect CTX-M, TEM, SHV, and pAmpC genes. A case control study was performed. Cases were patients with CTXR-En, control patients with cefotaxime-susceptible (CTXS)-En. Cases and controls were matched 1:2 based on species and isolated year. Children and patients with polymicrobial bacteraemia were excluded. Variables with P-values < 0.05 in univariate analysis were included in stepwise multivariate logistic regression analysis.

**Results:** 58 of 59 CTXR-En had ESBL or pAmpC (41 CTX-M, 7 CMY-2, 4 CMY-2/CTX-M, 3 TEM, 2 SHV, and 1 SHV/CTX-M), while only 1 of 357 CTXS-En had ESBL (TEM). CTXR-En increased from 6% in 2005 to 19% in 2009. Among CTXR-En, susceptibility rate was the highest in imipenem (100%), followed by amikacin (95%). 52 cases and 104 controls were selected. Multivariate analysis identified previous isolation of multidrug-resistant bacteria (OR 6.8, 95% CI 2.5–18.6), neutropenia (OR 3.4, CI 1.3–8.8), and severe sepsis or septic shock (OR 3.8, CI 1.8–7.2) as risk factors for CTXR-En. Cases more frequently received inappropriate therapy over 24 hours (21% vs 4%, p = 0.001) and died within 14 days (13% vs 3%, p = 0.02), but the durations between appropriate therapy and treatment response were similar (median 3 days, p = 0.9). 14-day mortality among patients with En bacteraemia was associated with severe sepsis or inappropriate therapy (OR 9.3, CI 2.0–43.2) and septic shock (OR 7.1, CI 1.3–38.3), irrespective of cefotaxime susceptibility.

**Conclusions:** Bacteraemia due to CTXR-En has been increasing and had higher mortality. Patients suspected of En bacteraemia with risk factors for CTXR-En should be considered for treatment other than cefotaxime, such as imipenem or amikacin.

**P666** Risk factors for and laboratory detection time of ESBLs

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**Background:** Extended-spectrum β-lactamase producing Gram-negative bacilli (ESBL) present challenges in laboratory detection, prevention and treatment. We have recently started to actively monitor for ESBLs to optimise their control.

**Aims:**
1. To assess the incidence of ESBLs in a Dublin tertiary referral hospital which also serves the community
2. To determine risk factors for colonisation or infection
3. To compare reporting time using CLSI versus EUCAST methodologies

**Methods:** All ESBL producing isolates over 7 months, March-May & August-November 2010, were included. Repeat isolates from the same patient were excluded. We recorded: referral source, i.e. general practitioner (GP), long term care facility (LTCF), outpatients and inpatients, patient age, specimen site, i.e. MSU, CSU, blood culture, sputum and swabs, organism, and reporting time, i.e. day of receipt to day of reporting. For all inpatients, prior antibiotic use, the presence of invasive medical devices, recent surgery and contact with healthcare facilities with the previous 3 months were noted.

**Results:** 73 ESBL producing organisms were reported over the 7 months. 68 were E. coli and 5 were K. pneumoniae. 23 (31.5%) originated from GPs, 10 (13.7%) from LTFCs, 15 (20.5%) from outpatients and 25 (34.3%) were from inpatients. The mean patient age was 64.2 years. 52 isolates originated from MSUs, 13 from CSUs, 4 from respiratory specimens, 2 from blood cultures and 2 from swabs. There was no difference in reporting time between CLSI and EUCAST methodologies (3.4 days). All inpatients had been in a LTCF or hospital within the previous 3 months, had invasive medical devices in situ (urinary catheter, central lines, etc.) and all were on antibiotics at the time of the sampling. 75% had also been exposed to antibiotics within the previous 3 months.

**Conclusions:** 34.3% of ESBL producing organisms detected were in inpatients with the remainder originating from the community, LTCF or outpatients. ESBLs were most prevalent in E. coli, most commonly from urine, and were associated with previous antibiotic exposure, having medical devices in situ and in patients with frequent contact with healthcare facilities. The reporting time was the same for both methodologies tested; this is reassuring as this laboratory switched to EUCAST in August 2010.

**P667** Alarming rates of fluoroquinolone resistance in CTX-M-15 harbouring Enterobacteriaceae recovered from patients with hospital-associated diarrhoea

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**Objectives:** We investigated the molecular epidemiology of fluoroquinolone resistance (FQ-R) among ESBL-producing Enterobacteriaceae (ESBL-EN) recovered from 120 hospitalized patients with diarrhea at the Tel Aviv Sourasky Medical Center, Israel.

**Methods:** Seventy-four unique ESBL-EN were recovered from 44 (36.7%) of the 120 stools screened on MacConkey/Drigalski biplate agar with ceftazidime and cefotaxime, respectively, and by the double disk synergy test. ESBL-EN were identified by mass spectrometry, and screened for CTX-M, TEM, and SHV genes by PCR-sequencing and for FQ-R on blood agar with 0.12 μg/ml ciprofloxacin (CIP). Recovered ESBL-EN were tested for CIP-resistance (CIP-R, MIC >2 μg/ml) by agar dilution. Topoisomerase (gyrA, parC) mutations and plasmid mediated quinolone resistance (PMQR) genes (qnrA, qnrB,
Molecular survey of Monomicrobial bacteraemia caused by extended-spectrum β-lactamase-producing Enterobacteriaceae clinical isolates from Vilnius University Hospital Santariskiu Clinics, Lithuania

A. Macioniene, S. Kiveryte, Z. Kucinskiene, J. Gulbinovic, L. Grishevicius, A. Jakubauskas* (Vilnius, LT)

Objective: ESBL producing Enterobacteriaceae is a growing clinical problem. There is only limited information available on the type and prevalence of genes that determine the ESBL phenotype of Enterobacteriaceae in the Baltic region. Over a 2009–2010 year period 230 clinical isolates of Enterobacteriaceae species (132 Klebsiella pneumoniae, 48 Escherichia coli, 32 Enterobacter spp, 8 Serratia marcescens, 5 Citrobacter spp, 3 Klebsiella oxytoca, 2 Proteus mirabilis, 1 Morganella morganii) producing ESBLs were collected at Vilnius University Hospital Santariskiu Clinics, one of the major tertiary multi-profile healthcare institutions in Lithuania. Bacteria were isolated from urine (109), surgical infection sites (61), bronchial aspirates (29), blood (28) and other samples (3). This study was conducted to determine the prevalence of ESBLs that belong to CTX-M and SHV families.

Methods: Real-time PCR technique was used to detect alleles encoding five phylogenetic groups of CTX-M family enzymes and SHV enzymes harbouring mutations G238S, E240K.

Results: Typing for the presence of CTX-M family enzymes revealed three families of blaCTX-M genes: blaCTX-M-group 1 (196 strains, 85.2%), blaCTX-M-group 2 (15 strains, 6.5%), blaCTX-M-group 9 (3 strains, 1.3%). Genes coding for CTX-M-group 8 and CTX-M-group 25 enzymes were not detected. The presence of SHV (G238S, E240K) enzymes was detected in 18 isolates (7.8%): in 8 cases the only gene, blaSHV(G238S, E240K), was detected, and in 10 cases bacteria produced two ESBLs coded by blaCTX-M-group 1 and blaSHV(G238S, E240K). No genes coding for CTX-M and SHV (G238S, E240K) enzymes were detected in 8 samples (3.5%) expressing ESBL phenotype. Therefore, the presence of genes encoding β-lactamases from other families (e.g. OXA, PER, VEB) was presumed.

Conclusion: This work showed high prevalence of CTX-M enzymes that belong to phylogenetic group 1 among Enterobacteriaceae strains expressing ESBL phenotype isolated at Vilnius University Hospital Santariskiu Clinics.

[669] Real-time PCR typing of CTX-M and SHV family genes of extended-spectrum β-lactamase-producing Enterobacteriaceae clinical isolates from Vilnius University Hospital Santariskiu Clinics, Lithuania

A. Macioniene, S. Kiveryte, Z. Kucinskiene, J. Gulbinovic, L. Grishevicius, A. Jakubauskas* (Vilnius, LT)

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Conclusion: This work showed high prevalence of CTX-M enzymes that belong to phylogenetic group 1 among Enterobacteriaceae strains expressing ESBL phenotype isolated at Vilnius University Hospital Santariskiu Clinics.

[670] Monomicrobial bacteraemia caused by extended-spectrum β-lactamase-producing Escherichia coli: predictors of mortality and potential role of ertapenem

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Objectives: Carbapenem are recommended as the drug of choice for infection of extended-spectrum β-lactamase (ESBL)-producing organism. However, clinical studies to demonstrate or to compare the effectiveness of different carbapenems are scarce.

Results: 558 (88%) of 633 isolates (75% E. coli) included carried a plasmid-borne BL: 526 (83%) at least one ESBL. 52 (8%) a plasmid-borne AmpC. The most prevalent ESBLs were CTX-M-15/28 (44%), CTX-M-1 (18%), SHV-12 (8%), CTX-M-14 (8%), and TEM-52 (6%). AmpC’s belonged to the CMY-2 group (52%; all CMY/2), the ACT/MIR group (46%), and DHA-1 (2%). Among the 75 (12%) isolates without a plasmid-borne BL, 3gCeph resistance of 29 (5%) E. coli was caused by AmpC promoter mutations, of 5 (1%) K. oxytoca by OXY hyperproduction and for 22 (4%) E. cloacae likely by chromosomal AmpC. For 19 (3%) isolates no resistance mechanism was detectable. DiversiLab showed no evidence of dissemination of a single strain, nationally or locally. However, 22 E. coli isolates belonged to ST131.

Conclusions: The most prevalent BL genes causing 3gCeph resistance in Enterobacteriaceae in the Netherlands were CTX-M ESBLs, especially CTX-M-15. In comparison with other European surveys a relatively high prevalence of CTX-M-1 and TEM-52 genes was observed. CMV2-55 was the most prevalent AmpC.
Methods: From January 1, 2005 to June 30, 2007, all adult patients with the first episode of monomicrobial ESBL-producing E. coli bacteremia were included in this non-concurrent prospective study. Logistic regression analysis and Cox’s regression model were used to determine the predictors of early and 30-day mortality respectively. Outcomes in patients treated with different carbapenems were compared using propensity score.

Results: Of 97 patients with ESBL-producing E. coli bacteremia reviewed, 71 patients met the study criteria. The early and 30-day mortality rate were 12.7% and 29.6% respectively. Logistic regression analysis identified ICU stay as the only predictor of early mortality. Among 62 patients having received definitive antimicrobial therapy, male gender, ICU stay at bacteremia onset, solid tumor and primary bacteremia were independently associated with 30-day mortality, while definitive antimicrobial therapy using carbapenem was found to be protective by Cox’s regression analysis. Subgroup analysis using propensity score demonstrated that ertapenem was non-inferior to other carbapenems in terms of 30-day mortality.

Conclusions: Patient's co-morbidities rather than initial choice of empirical antimicrobial therapy were major predictors of early and 30-day mortality. Using carbapenem as definitive therapy was a protective factor in 30-day mortality. The choice of ertapenem is reasonable for less severely ill patients who are at risk of ESBL-producing E. coli bacteremia and unlikely to have infection due to Pseudomonas aeruginosa.

Molecular characterisation of Escherichia coli and Klebsiella pneumoniae producing CTX-M-type extended-spectrum β-lactamase in Örebro County, Sweden

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Objectives: During the last decade an increasing prevalence of extended-spectrum β-lactamase (ESBL) producing Enterobacteriaceae has been detected worldwide, mainly due to dissemination of Escherichia coli and Klebsiella pneumoniae producing CTX-M-type ESBL. The most widespread CTX-M-type, and the predominant type in various countries. The blaCTX-M gene is located on mobile genetic elements, such as plasmids, and the dissemination of ESBL is not only caused by clonal spread of epidemic strains, but also by horizontal gene transfer between different strains and different species. The aim of this study was to investigate the molecular epidemiology of CTX-M-producing Escherichia coli and Klebsiella pneumoniae isolated during a ten year period in Örebro County, Sweden.

Methods: 200 clinical ESBLA-producing E. coli and K. pneumoniae from the years 1999–2008 were included in the study. The majority were isolated from urine, and the rest from blood, wounds, and the respiratory tract. The entire genes for CTX-M, TEM, and SHV were amplified by real-time PCR and the amplicons were sequenced in order to determine CTX-M, TEM-, or SHV-type.

Results: Out of the 200 ESBLA-producing isolates 87% were producing CTX-M, belonging to subgroup CTX-M-1 (64%), CTX-M-9 (34%), or CTX-M-2 (2%). The remaining isolates were producing variants of SHV and TEM. Sequencing of the blaCTX-M genes revealed ten different CTX-M-types, with a dominance of CTX-M-15 (E. coli 54%, K. pneumoniae 50%) followed by CTX-M-14 (E. coli 28%, K. pneumoniae 27%).

Conclusion: As in most parts of the world a high proportion of the ESBLA-producing E. coli and K. pneumoniae were producing ESBL of CTX-M-type. In our county this appears to be mainly due to spread of CTX-M-15 and CTX-M-14. Further analysis of the CTX-M-carrying plasmids is ongoing in order to determine if the dissemination of CTX-M-15 and CTX-M-14 is due to frequent horizontal transfer of a few successful plasmids harbouring these genes.

The clinical risk factors and susceptibility to fosfomycin in community-acquired urinary tract infections caused by extended-spectrum β-lactamase producing Escherichia coli

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Objective: The choices of antimicrobial therapy for community-acquired urinary tract infections (CA-UTIs) by extended-spectrum β-lactamase (ESBL)-producing E. coli appear to be limited. Fosfomycin which can be given by oral route could be effective against for this indication. We attempted to evaluate the epidemiological features, resistance rates and effect of fosfomycin in ESBL-producing E. coli isolated from patients with uncomplicated CA-UTIs.

Methods: Single clinical isolates from 139 outpatients between May 2009-July 2010 at Hacettepe University Adult Hospital was included in the study. (E. coli 45% producing ESBL). Several potential clinical risk factors and sociodemographic features of the study group were evaluated. Susceptibility rates of ESBL-producing and non-producing E. coli isolates to fosfomycin and other antibiotics were determined by disc diffusion and Etest methods according to CLSI criteria. A two-sided χ² test was used for statistical analysis.

Results: 75% of patients were female, mean age was 46.5 years. Female gender (p = 0.01), recent hospitalization and/or antimicrobial therapy in the last 3 months (<0.001), any surgical intervention (p = 0.006), urological procedures (p = 0.033), previous UTI (p < 0.001), intravesical disease application (p < 0.001) all within the previous year, malignancy history (p = 0.005), urinary catheter usage (p = 0.014) and presence of renal disease (p = 0.028) were found as significant predisposing factors for CA/ESBL producing E. coli. The frequency of infections were linearly associated with the severity of underlying renal disease. The antimicrobial resistance rates in ESBL-producing and non-producing isolates were as follows: piperacillin tazobactam 48.7% vs 0% (p < 0.001), amikacin 60.5% vs 0% (p < 0.001), ampicillin 98.7% vs 15.9% (p < 0.001), gentamicin 47.4% vs 7.9% (p < 0.001), cepodoxim 100% vs 14.3% (p < 0.001), cefepime 78.9% vs 0% (p < 0.001), ceftazidine 85.5% vs 0% (p < 0.001), nitrofurantoin 37.3% vs 0% (p < 0.001), trimethoprim-sulphamethoxazol 64.5% vs 20.6% (p < 0.001), and fosfomycin 19.4% vs 0% (p = 0.04).

Conclusion: Several risk factors were found to be related with increased incidence of ESBL-producing E. coli for CA-UTIs. Antimicrobial resistance and lack of oral options may compromise treatment in the outpatient. Fosfomycin seems to be a possible oral alternative for this indication.

Healthcare-associated and hospital-acquired infections caused by Enterobacteriaceae producing extended-spectrum β-lactamases in hospitalised patients: are there any significant differences?

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Background: Information is limited on the epidemiology of health care-associated (HCA) and community-acquired (CA) infections due to ESBL+ Enterobacteriaceae (Ent.).

Objectives: To define differences in the epidemiology, treatments and outcome of CA, HCA, and HA-infections due to ESBL+ Ent. in hospitalised patients (pts).

Design: A prospective multicenter cohort study. All adult hospitalised pts with infections due to ESBL+ Ent. were enrolled. Antibiotic therapy, complications and outcome of infections were extracted. Mortality was defined as death occurring during hospitalization. Appropriate antibiotic therapy was defined as the initiation of therapy with activity against the ESBL+ Ent. from the day before to 2 days after the initial positive clinical culture result. HA, HCA and CA infections were defined according to established criteria.
**Results:** Among 312 pts, 223 were diagnosed as having HA-ESBL+ Ent. infections (HAI); of the remaining 92 pts, 90 (98%) met the criteria for HCAI infections and only 2 pts were diagnosed as having “true” CA infections. Mean age (HCA vs HA: 69 vs 66 yrs) and male sex (75% vs 66%) were not statistically different. Pts with HCA-ESBL+ Ent. (HCAI) were more likely to have had at least one hospitalisation in the previous year (49%) and visiting nurse assistance (37%) while pts with HAI were more likely to have urinary catheter (49%). A fourth of pts in both groups received at least one antibiotic in the previous 30 days. E. coli was equally represented in both groups (HCAI vs HAI: 69% vs 63%) while P. mirabilis was more frequent among HCAI (23% vs 16%) than K. pneumoniae (8% vs 18%). For pts with HCAI compared with HAI, UTIs were more frequent (71% vs 53%; RR 2.03, 95% CI: 1.7−3.6; p < 0.01) while PNE and SSI were less frequent (1% vs 20%; RR 0.01; 7% vs 13%, p = 0.08, respectively). BSI were equally distributed (17% vs 20%). Overall mortality was not statistically different (9% vs 7%). Therapy was changed more frequently in HAI than HCAI infections (58% vs 65%). Rate of inappropriate therapy (41% vs 37%) and mortality (17% vs 20%) was not significantly different between HCAI and HAI.

**Conclusions:** HCAI are more frequent than CAI caused by ESBL+ Ent. in hospitalised pts. Epidemiological differences as well as mortality between HCA and HAI are minimum. Health care providers should be aware of the clinical impact of HCA-ESBL+Ent. infections in hospitalized pts.

**P674** Multidrug-resistant chromosomal AmpC-producing Enterobacteriaceae isolates carrying blaESBL, qnrB, aac(6')-Ib-cr, and armA

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**Objectives:** The aim of this study was to investigate the presence of 16S rRNA methylases and plasmid-mediated quinolone resistance (PMQR) determinants (qnr and aac(6')-Ib-cr) in ESBL-producing inducible Enterobacteriaceae isolated from cancer patients in Bulgaria.

**Methods:** A total of 511 clinically relevant enterobacterial isolates with chromosomal AmpC β-lactamases, collected during 2002-2005, were screened for ESBL production by the double-disc synergy method. The β-lactamase genes (blaPER-1, blaTEM, blaSHV, blactXM-3, blavEB-1, blagES-I and blaxoxA-I) were detected by PCR amplification with specific primers. Search for16S rRNA methylase genes (armA, rmtA, rmtB, rmtC, rmtD), qnr and aac(6')-Ib-cr genes was conducted by PCR amplification. Genotypes were determined by direct nucleotide sequence analysis of the amplified products. Pulsed-field gel electrophoresis (PFGE) of SpeI-digested genomic DNA was used to compare isolates.

**Results:** Among the 511 inducible Enterobacteriaceae, 24 (4.7%) were ESBL-producers. Of these, 9 (37.5%) carried armA methylase gene in association with blactXM-3 and blatem-1. Four isolates (16.7%) were positive for aac(6')-Ib-cr. Of these, 2 also carried blactXM-15 and blaxoxA-1. qnrB was detected only in Citrobacter freundii strains (7 of 10). Among them, 4 had qnrB17, 2 had qnrB10 and 1 had qnrB12. None of the isolates carried qnrA and qnrS. Among the qnrB17-carrying isolates, 3 possessed blatem-3 alone and 1 had both blactXM-15 and blaxoxA-1 in association with aac(6')-Ib-cr. The isolates with qnrB10 possessed blatem-3. The qnrB12-carrying isolate had simultaneously blactXM-15, blaxoxA-1, blatem-1 and aac(6')-Ib-cr. Most of the isolates were genetically different according to PFGE.

**Conclusion:** The spread of multidrugresistant isolates expressing chromosomal AmpC β-lactamase together with ESBLs, ArmA methylase aminoglycoside resistance, Qnr and AAC(6')-Ib-cr quinolone resistance is a worrisome development requiring continuous monitoring.

**P675** Amplification of CTX-M-15-B2-ST131 and emergence of A and D widespread clones producing different ESBLs in Portuguese hospitals

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**Objectives:** In a previous survey (2003−05), isolates from a hospital in the centre region of Portugal were mainly identified as Enterobacter aerogenes producing TEM (-10, -24, -116) enzymes. We aim to characterize population structure and epidemiological features of recent E. coli isolates from this institution and a recently opened hospital located nearby.

**Methods:** A total of 52 ESBL-producing E. coli isolates from two Portuguese hospitals located in the Centre Interior region were included (2006−2008). Species identification and antibiotic susceptibility testing were performed by API 20E. ESBL characterization included DDST and searching of blabESBL genes (blatem, blashv and blactXM-15) by PCR and sequencing. Clonal relatedness was investigated by XbaI-PFGE and MLST. E. coli phylogenetic groups were identified by a multiplex PCR.

**Results:** Most isolates were recovered from urines (81%, 63% caused by TEM-producing mostly CTX-M enzymes [91%: CTX-M-1, -14, -15, -32], while TEM 4%: TEM-52 and SHV [6%: SHV-12] types were also detected, and belonged to particular clonal complexes. A B2-ST131 E. coli clone was the most frequently identified in both institutions (n = 42, 81%; 5 PFGE-types), exhibiting resistance to kanamycin (93%), tobramycin (90%), gentamicin (83%), tetracyclines (90%) and ciprofloxacin (88%). It harboured blactXM-15 (n = 41) and blashv-12 (1), frequently associated with blatem-1 and/or blatem-15, and occasionally blatem-10 or blatem-116. E. coli isolates belonging to phylotype D were ST117 (n = 2, encoding CTX-M-1 plus TEM-116 or CTX-M-14) and ST648-like (n = 1, TEM-52). A-E coli belonged to ST10 complex (n = 2, CTX-M-15, CTX-M-32), ST23-like (n = 1, CTX-M-1) and fumC11 (CTX-M-15). On the other hand, phylotype B1 isolates were diverse (fumC4, fumC29, fumC65) and produced TEM-52, CTX-M-14 and SHV-12. A, B1 and D clones were frequently resistant to streptomycin, sulphonamides and tetracycline.

**Conclusion:** We demonstrated current widespread of B2-ST131 in different Portuguese hospitals, and the emergence of widespread A and B1 clones producing particular ESBL variants. The simultaneous detection of additional TEM-10−116 variants might indicate diversification of pre-existing blatem and/or recombination events.

**P676** The occurrence of pandemic serotype ST131 in animal Escherichia coli in Slovakia

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**Objectives:** A rapid dissemination of animal isolates producing CTX-M-type extended-spectrum β-lactamases has recently been reported in European countries (Carattoli, 2008). The occurrence of animal E. coli producing ESBL enzymes is increasing in Slovakia. The aim of the study was to characterize the prevalence of ST131 in E. coli isolated in Slovakia.

**Methods:** During year 2010 129 faecal E. coli from calves and 100 E. coli from broilers representing different Slovakian regions were collected and analysed. Antibiotic resistance was phenotypically determined according to CLSI, M31-A3 (2008). The ST131 (Clermont et al. 2009) and ESBL CTX-M (Woodford et al. 2006) were determined by PCR. Also 9 human, phenotypically CTX-M positive clinical urinary E. coli isolates were analysed. Possible clonal relatedness of studied isolates was determined by Maldi tof biotyper.

Results The ESBL phenotype was present in 11 (11%) of broiler and in 16 (12, 4%) of calf E. coli isolates. The presence of CTX-M1 group genes could be confirmed in ten broilers and in eight calf isolates. The ST131 pandemic clone was detected in one broiler and in three calf isolates and was always accompanied by CTX-M gene. All ST 131 positive animal isolates originated from a single geographical region in Slovakia. We could also confirm ST131 in four of 9 human urinary,
phenotypically ESBL CTX-M positive *E. coli* strains tested. The Maldi biotyping of animal ST 131 *E. coli* could confirm a clear relatedness only between one poultry and one calf isolates. Human ST131 clinical isolates were unrelated to animal ones.

**Conclusions:**
1. ESBL CTX-M1 group genes were detected in animal *E. coli* frequently.
2. The ST131 pandemic *E. coli* clone was confirmed for four animal and also in four clinical human isolates. The ST131 type was always accompanied by the CTX-M gene.
3. Just one poultry and one calf ST131 isolates showed a clonal relatedness (Maldi tof biotyping). Animal ST 131 isolates were unrelated to human clinical *E. coli* ST131 isolates.

**Carbapenem resistance in Enterobacteriaceae**

*P677* Comparative in vitro activity of doripenem, imipenem and lack of ertapenem "MIC creep" in Global evaluation of meropenem non-susceptible pathogens since 2002–2010: The Study for Monitoring Antimicrobial Resistance Trends (SMART) has tracked susceptibility of intra-abdominal infection (IAI) pathogens since 2002. As ertapenem is widely used against *E. coli* in IAI, monitoring its activity over time is crucial. This report analyzes ertapenem susceptibility trends of *E. coli* (including ESBL+ and ESBL− isolates) over 9 years for evidence of “MIC creep”.

**Results:** 626 meropenem non-susceptible Enterobacteriaceae were collected in 2006/07 and 594 isolates in 2009/10. These represented 2.1% and 2.7% of all Enterobacteriaceae, respectively—a significant increase (p < 0.0001). The following table shows the MIC50, geometric mean (GM) MIC, and % susceptible for tigecycline and several comparators, as well as p values comparing both time periods.

**Conclusions:** While the MIC50 for tigecycline did not change between 2006/07 and 2009/10, the geometric mean increased significantly, demonstrating that this measure may offer better discrimination. The increase in GM MIC for minocycline was also highly significant, while the decrease for cefepime was only marginally so. Despite tigecycline's increase in GM MIC and a statistically significant decrease in % susceptible, the agent continued to demonstrate the best in vitro activity against meropenem non-susceptible Enterobacteriaceae. Almost 85% of these usually multi-resistant pathogens remained susceptible, confirming tigecycline as an important therapeutic option, especially since most other antimicrobials exhibited poor in vitro activity.

| MIC50 | GM MIC | % Susceptible |
|-------|--------|---------------|
| Tigecycline | 0.12/32 | 0.03/0.12 | 90 |
| Minocycline | 0.03/0.12 | 0.06/0.25 | 90 |
| Cefepime | 64 | 64 | 90 |
| Tigecycline | 0.12/32 | 0.03/0.12 | 90 |
| Minocycline | 0.03/0.12 | 0.06/0.25 | 90 |

**P678** Global evaluation of meropenem non-susceptible Enterobacteriaceae: 2006/07 versus 2009/10

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**Objective:** Carbapenemase-producing Enterobacteriaceae are spreading globally. They are often resistant to all β-lactam antibiotics and co-resistant to most other antibiotics, leaving very few treatment options. Using surveillance data from the global, comprehensive Tigecycline Evaluation Surveillance Trial (T.E.S.T.), we assessed changes in incidence and susceptibility of meropenem non-susceptible Enterobacteriaceae between the years 2006/07 and 2009/10.

**Methods:** A total of 1,220 meropenem non-susceptible Enterobacteriaceae were isolated in 46 countries from multiple infection sources. MICs were determined by each participating laboratory using commercially-prepared microbroth panels. Results were interpreted according to CLSI breakpoints (including new meropenem breakpoints published in June 2010) or FDA breakpoints (for tigecycline). The difference in % susceptible values between 2006/07 and 2009/10 was tested using the Fisher exact test, while the geometric mean MICs were compared by t test of log-transformed MICs.

**Results:** 626 meropenem non-susceptible Enterobacteriaceae were collected in 2006/07 and 594 isolates in 2009/10. These represented 2.1% and 2.7% of all Enterobacteriaceae, respectively—a significant increase (p < 0.0001). The following table shows the MIC50, geometric mean (GM) MIC, and % susceptible for tigecycline and several comparators, as well as p values comparing both time periods.

**Conclusions:** While the MIC50 for tigecycline did not change between 2006/07 and 2009/10, the geometric mean increased significantly, demonstrating that this measure may offer better discrimination. The increase in GM MIC for minocycline was also highly significant, while the decrease for cefepime was only marginally so. Despite tigecycline's increase in GM MIC and a statistically significant decrease in % susceptible, the agent continued to demonstrate the best in vitro activity against meropenem non-susceptible Enterobacteriaceae. Almost 85% of these usually multi-resistant pathogens remained susceptible, confirming tigecycline as an important therapeutic option, especially since most other antimicrobials exhibited poor in vitro activity.
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is noteworthy that the last three years showed declining MICs, nearly returning to 2002 levels.

| Year | N  | MIC 50 | MIC 90 | %S* | GM MIC |
|------|----|--------|--------|-----|--------|
| 2002 | 1403 | <0.03  | <0.03  | 98  | 0.035  |
| 2003 | 2620 | <0.03  | <0.03  | 98.32 | 0.035  |
| 2004 | 2579 | <0.03  | <0.03  | 97.72 | 0.037  |
| 2005 | 2654 | <0.03  | <0.03  | 97.81 | 0.037  |
| 2006 | 3173 | <0.03  | 0.06   | 96.91 | 0.039  |
| 2007 | 3659 | <0.03  | 0.12   | 95.14 | 0.044  |
| 2008 | 3719 | <0.03  | 0.06   | 97.58 | 0.040  |
| 2009 | 5833 | <0.03  | 0.06   | 97.69 | 0.039  |
| 2010 | 2647 | <0.03  | 0.06   | 98.22 | 0.037  |

*Susceptible based on CLSI M100-S20-U

P681 Surveillance of extended-spectrum cephalosporin- and carbapenem-resistance in Escherichia coli from the Greater Toronto Area, Ontario, Canada

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Objective: The goal of this study is to investigate the prevalence of extended-spectrum cephalosporin- (ESC) and carbapenem-resistance in E. coli from the Greater Toronto Area (population ~13 million), as well as the mechanisms involved in these phenotypes.

Methods: 526 non-duplicate E. coli clinical isolates were collected during March 1–5, 2010 from 13 hospitals (TIBDN) in the GTA region. Antimicrobial susceptibility profiles were determined using Vitek2 at each participating laboratory, and agar dilution method (CLSI, 2009) at the Public Health Lab. A sub-collection was selected for molecular studies based in the following criteria: resistance (R) to ESC, R or reduced susceptibility (rS) to carbapenems, and/or R or rS to cefoxitin (FOX, marker for AmpC). Molecular screening of β-lactamase genes (blaTEM, blaSHV, blaOXA-1-like, blaCTX-M groups 1, 2 and 9, blaVEB, blaPER, blaGES, blaOXA-48-like, blaIMP, blaVIM, blaKPC, blaNDM-1 and 6 groups of blaAmpC genes) was performed by PCR. The 110 base pair-ampl promoter was sequenced and analyzed in the chosen sub-collection. Their sequence types (ST) was determined by Multi-Locus Sequence Typing (MLST).

Results: ESCR was detected in 64 isolates. Carbapenem rS or R was not detected. rS or R to FOX was found in 11 strains, which were added to make a final sub-collection of 75 isolates. The most common extended-spectrum β-lactamase (ESBL) found in that subset belonged to the blaCTX-M family, groups 1 and 9 (n = 38, 51%). Other β-lactamasen detected were blaTEM, blaOXA-1-like and blaCMY-2. Some strains harboured only one of the β-lactamases genes tested (30.7%) while other strains had different combinations of them (42.6%). Twenty isolates (26.7%) were negative for all β-lactamase genes tested. Analysis of ampC promoter in these isolates revealed mutations in the −10 and −35 boxes and attenuator region linked to AmpC hyperproduction. MLST results revealed ST131 being the most predominant clone (n = 28), detected in 11 of the 13 participating hospitals, harbouring blaCTX-M-15 and blaCTX-M-14 genes.

Conclusion: 12.2% of the bacterial collection displayed ESCR. No carbapenem rS or R had been detected. blaCTX-M genes (mainly blaCTX-M-15) was the predominant ESBL detected in this surveillance, generally harboured by a ST131 strain. Hyperproduction of the chromosomal AmpC due to promoter mutations is playing a role in the expression of ESCR in the GTA region.

P682 Resistance of Enterobacteriaceae to carbapenems in the University Hospital Olomouc, Czech Republic

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Objective: Carbapenems are the drugs of choice for the treatment of serious infections caused by ESBL- and AmpC-positive Enterobacteriaceae. An increasing trend of resistance to carbapenems has been observed. The aim of the study was to determine resistance to carbapenems in clinical isolates of the Enterobacteriaceae family and its mechanism.

Methods: Between 1 April 2009 and 31 August 2010, Enterobacteriaceae were isolated from clinical samples obtained from patients hospitalized in the University Hospital Olomouc, Czech Republic (1,406 beds incl. 155 ICU beds). The strains were identified using the Phoenix automated system (Becton Dickinson) and their susceptibility to meropenem was determined by the microdilution method and E-test. The isolates were tested for carbapenemase production using the modified Hodge test (mHodge test), a combined test with 3-aminophenylboronic acid (3-APB) and EDTA, the combined-disk test (CD test) and modified DDST for MBL (mDDST for MBL). ESBL and AmpC production was determined by the mDDST and modified AmpC test, respectively. Genes encoding production of serine carbapenemases, MBL, blaOXA-23, blaOXA-48, ESBL and AmpC enzymes were detected with a set of specific primers. TEM- and SHV-positive PCR products were characterized by restriction analysis.

Results: From a total of 12,60S Enterobacteriaceae, 9 strains (7 Klebsiella pneumoniae and 2 Enterobacter cloacae) were isolates with the minimum inhibitory concentration (MIC) of meropenem ≥2 mg/L. The MIC of meropenem for these strains ranged from 2 to 16 mg/L. The mHodge test, the combined test with 3-APB and EDTA, CD test, mDDST for MBL and a series of PCR analyses did not detect production of serine carbapenemases, MBL, OXA-23 or OXA-48 enzymes in any of the tested strains. In 6 Klebsiella pneumoniae strains and 1 Enterobacter cloacae strain, the mDDST test revealed ESBL production, and in 1 Klebsiella pneumoniae and 1 Enterobacter cloacae, AmpC production was detected. Genetic analysis confirmed the presence of CTX-M and SHV types in ESBL strains and DHA and EBC types in AmpC strains.

Conclusion: The prevalence of the Enterobacteriaceae with the MIC of meropenem >2 mg/L in the University Hospital Olomouc was 0.07%. None of the strains produced either serine carbapenemases or MBL. Borderline resistance of the strains to carbapenems was determined by the ESBL and AmpC production with another associated mechanism of resistance.

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P683 Prevalence and molecular characterisation of carbapenem-resistant Enterobacteriaceae clinical isolates from a teaching hospital in Shanghai, China

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Objective: To investigate the prevalence and molecular characteristics of carbapenem-resistant Enterobacteriaceae clinical isolates.

Methods: Seventy-seven isolates resistance to at least one carbapenem from 8254 Enterobacteriaceae clinical isolates were collected from Jan. 2002 to Apr. 2009. Antimicrobial susceptibility testing and molecular typing were performed by agar dilution and pulse-field gel electrophoresis (PFGE), respectively. Carbapenemases productions were detected by modified Hodge test (MHT) according to the CLSI method. β-lactamase genes were identified by PCR and DNA sequencing. Outer membrane porin proteins (OmpK35, OmpK36 and OmpK37) were investigated by SDS-PAGE.

Results: None of the Enterobacteriaceae clinical isolates were susceptible to ertapenem, and the susceptibility rates for imipenem and meropenem were 6.5%, 1.3%, respectively. PFGE analysis revealed four and 10 unrelated genotypes of Citrobacter freundii and Klebsiella pneumoniae, respectively. Of the 77 isolates, 93.5% were found by PCR
A simple phenotypic algorithm for direct and specific detection of KPC and MBL carbapenemase-producing Enterobacteriaceae in rectal screening

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Objectives: Carbapenemase-producing Enterobacteriaceae are rapidly spreading. Early detection of carriers by faecal screening is crucial for their restriction; it is performed by various non-specific tests using carbapenem discs or selective agar plates supplemented with carbapenems. We evaluated a simple phenotypic algorithm for specific detection of MBL and KPC producers directly from rectal swabs.

Methods: Rectal swabs obtained from 55 hospitalized patients during September-December 2010 were suspended in 1ml saline and subsequently cultured onto two MacConkey agar plates (MC). A MC was streaked for colony isolation and two etapenem (ERT) discs were added at the end of first and second quadrant; an inhibition zone of ≤27 mm was applied for detection of carbapenemase producer. A second MC was streaked for confluent growth and four meropenem (MER) discs were placed containing: i) MER alone, ii) MER plus 20 micro-L of 20 mg/ml phenylboronic acid (PBA, KPC inhibitor), iii) MER plus 10 micro-L of 0.1M EDTA (MBL inhibitor), and iv) MER plus both PBA and EDTA. Inhibition zone around MER disc alone of <23 mm was considered positive result for carbapenemase production. A difference of ≥5 mm in the inhibition zone between discs containing MER without and with inhibitors (PBA, EDTA or both) was considered a positive result for detection of KPC, MBL or both carbapenemases, respectively. Rectal suspensions were further tested for carbapenemase genes by PCR. Enterobacterial colonies grown at the edge of the inhibition halo around MER plus inhibitors and ERT discs were identified by phenotypic tests and PCR.

Results: 21 of the 55 screened patient-samples were PCR-positive for carbapenemase genes (9 KPC, 3 VIM, 9 KPC and VIM). By ERT disc test, 20 of the 21 PCR-positive samples (sensitivity 95%) were carbapenemase-positive. By the combined MER disc test, 19/21 PCR-positive samples (sensitivity 90%) were positive for carbapenemases, while all 34 PCR-negative samples were negative (specificity 100%). In all 19 cases the combined MER disc tests correctly differentiated KPC producers from those producing MBL or both enzymes.

Conclusion: This simple combined disc algorithm enables direct and specific detection of carbapenemase production from the first day of rectal screening without further testing, supporting the timely and costly implementation of infection control measures. It could be effectively used in hospitals with high rates of carbapenemase producers.

A study to identify risk factors for the acquisition of carbapenemase-producing Gram-negative organisms in a tertiary hospital serving a major international airport

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Objectives: To identify risk factors for acquisition of Carbapenemase Producing Gram-Negative (CPGN) organisms in patients admitted to the University Hospital South Manchester (UHSMS), a tertiary referral centre providing specialist care for areas such as Burns, Cystic Fibrosis and serving a major international airport.

Methods: CPGNs were identified using automated sensitivity testing, MIC confirmation, a Modified Hodge Test for carbapenemase activity and reference laboratory referral for PCR. Clinical data on all patients with confirmed CPGNs was collected from December 2009 to November 2010, by prospective case note analysis.

Results: 15 CPGNs were isolated from 14 patients from various sample sites including urine (7, 50%) and blood (2, 14%). Various carbapenemases were detected from a spectrum of Enterobacteriaceae. 1 patient had multiple carbapenemases. KPC-production was the most common. 12 patients (86%) had prolonged hospital contact (>5days), 3 cases (22%) had contact with a known outbreak of plasmid-mediated KPC in a neighbouring hospital and 1 case was cross-contamination with a VIM pseudomonas within UHSMS Burns unit. 3 patients (21%) were direct transfers following hospitalisation abroad (Egypt, India, Cyprus). Exposure to broad-spectrum antibiotics was seen in 7 (50%). 1 patient isolated a KPC which clustered with the neighbouring outbreak despite no previous contact. 2 patients (14%) had no discernable risk factors.

Conclusions: Risk factors appear poorly defined. Higher rates of CPGNs in certain countries make contact with medical facilities abroad a risk, as does contact with known outbreaks. Exposure to broad spectrum antimicrobials appears significant. Combinations of risk factors may be important, e.g. acquisition of a resistant organism abroad combined with exposure to broad spectrum agents, creating a selection pressure. A lack of identifiable risk factors in 2 patients raises concern that CPGNs are endemic in the community, impacting on efficacy of
Carbapenem resistance in Enterobacteriaceae

empiric antimicrobial choices and creating significant infection control challenges. Whilst numbers are small, risk factors may differ depending on the type of carbapenemases. KPC appears more common in those with prolonged hospital contact in the UK as well as contact with known outbreaks. NDM-1 is associated with medical contact abroad. Further work around community surveillance and awareness amongst health-care workers is needed to accurately define the current situation and limit spread.

| Resistance Mechanism | Risk factors determined | Proportion of patients multiple risk factors were required | 1 patient multiple risk factors were identified |
|----------------------|-------------------------|----------------------------------------------------------|-----------------------------------------------|
| Serine               | KPC                     | 4 / 3 / 0                                                | 3 / 0                                         |
| Serine               | OXA                     | 0 / 2 / 3                                                | 0 / 1                                         |
| Metallic             | NDM-1                   | 1 / 1 / 0                                                | 0 / 0                                         |
| Metallic             | VIM                     | 2 / 2 / 0                                                | 1 / 0                                         |
| Metallic             | IMP                     | 0 / 0 / 0                                                | 0 / 1                                         |
| Totals               |                         | 7 / 8 / 4                                                | 4 / 2                                         |

**P687** Enterobacteriaceae producing VIM-1 metallo-β-lactamases in England – a project for a EUPHEM fellow

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Objectives: The EUPHEM (European Public Health Microbiology Training Programme) fellowship was aimed to undertake the formation of a microbiological-epidemiological network, this most readily being achieved through the investigation of a clinical problem linking these two disciplines. This study investigates the epidemiology of Enterobacteriaceae metallo-β-lactamase (MBL) producers from the North West of England, which were submitted to the Antibiotic Resistance Monitoring and Reference Laboratory (ARML).

Methods: Patient-isolates of Enterobacteriaceae with carbapenem-resistance were initially submitted by a centre in North West England to ARML for investigation of resistance mechanisms. MICs were obtained for a wide range of cephalosporins and carbapenems by agar dilution (BSAC method); isolates with EDTA potentiation of imipenem were screened for the class B metallo-β-lactamases by PCR. Patient and clinical data (including the date of sample collection) were collected retrospectively for patients infected with these isolates. Based on these findings, a questionnaire was developed to address wider epidemiological questions.

Results: From March 2010 until November 2010, 18 patient-isolates were identified as pan-cephalosporin and carbapenem-resistant, with EDTA potentiation of imipenem. All were VIM-1-positive. Whilst 14 patients were inpatients, 4 were from the community. All Carbapenem-producers were Klebsiella spp. and 11 (61%) cultures were isolated from urine. Mean age was 72 years with nine (50%) of cases being female. Cases were not obviously linked and appeared scattered in time, at intervals of 1–10 weeks. Patients were not associated with any one hospital ward or GP practice.

Conclusion: Klebsiella spp. with VIM metallo-carbapenemase have become scattered across the region, both in terms of time and location, with no clear connection between cases. More active community surveillance is required to elucidate the links between cases and establish routes of strain or plasmid transmission between hospitals and the community.

**P688** Risk factors for carbapenem-resistant Enterobacteriaceae carriage on admission to a surgical unit and acquisition rate during hospitalisation

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Objectives: Carbapenem Resistant Enterobacteriaceae (CRE) are endemic in our geographic region and cause life threatening infections in hospitalized patients. This study was conducted to identify the risk factors for CRE colonization on admission to a surgical unit and the acquisition rate during hospitalization.

Methods: A prospective study was conducted in a tertiary-care hospital located in Athens, Greece, between May 2009 and June 2010. Surveillance cultures (pharynx, rectal, other sites when clinically indicated) were obtained from patients admitted to a surgical unit within 48 hours upon admission and every 7 days afterwards. The samples were inoculated on McConkey agar plates containing 0.5mg/L of meropenem and incubated at 37°C for 48h. All isolated Enterobacteriaceae were examined for production of carbapenemases by combined disk synergy test (meropenem-EDTA/boronic acid) and Hodge test. The presence of blaVIM, KPC genes were detected by PCR. Pertinent information to identify risk factors for CRE colonization on admission was collected in a pre-designed form.

Results: A total of 860 admissions of 750 patients were recorded in the surgical unit during the study period. The median duration of hospitalization was 8 days. The prevalence [95% confidence interval (CI)] of CRE colonization on admission was 3.02% (1.98%, 4.40%). Prior stay in intensive care unit (odds ratio (OR): 8.5; 95% CI: 1.6, 44.9; p = 0.012) or in another hospital ward (OR: 8.0; 95% CI: 2.2, 29.4; p = 0.002) and antibiotic use in preceding three months (OR: 5.3; 95% CI: 1.3, 21.0; p = 0.019) were independently associated with CRE colonization on admission. The incidence rate (95% CI) of CRE acquisition in the surgical unit was 9.4 (6.9, 12.8) per 1,000 patient-days. The probability of a patient to be colonized or infected in the first week of hospitalization was 1.3%.

Conclusion: Significant transmission of CRE occurs in our hospital setting. Enhanced infection control measures are urgently needed to contain this transmission.

**P689** Evaluation of the Dutch surveillance on carbapenemase producing Enterobacteriaceae

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Objectives: There is a world-wide concern about the emergence of carbapenemase producing Enterobacteriaceae. Local and national surveillance is considered an important component of the strategy to control these strains. Since March 2010 the surveillance on carbapenemase producing Enterobacteriaceae (CPE) in the Netherlands consists of three pillars: 1) a national guideline on the phenotypic detection of CPE in the routine clinical setting, which recommends to confirm a non-wild-type carbapenemase MIC by Etest (IJA 2010), 2) the national antimicrobial resistance surveillance system (ISIS-AR) providing feedback alerts on isolates carbapenem I/R reported by the participating labs but not yet confirmed by Etest/disc, and 3) for this study UMCU was available as reference laboratory for confirming the presence of carbapenemase genes in screen positive isolates. The aim of this study was to evaluate this strategy and to estimate the possible underreporting of CPE.

Methods: All E. coli (ECO), Klebsiella pneumoniae (KPN) and Enterobacter spp (ENT) present in the ISIS-AR database from 1–12–2009 to 11–12–2010 were included. These were submitted by 22 labs, serving general practitioners, long-term care facilities and 44% of the Dutch hospital beds. Using this database we calculated: 1) the yearly prevalence of I/R ECO, KPN and ENT, 2) the yearly number of cases of screen positive ECO, KPN and ENB. Screen positive was defined as meropenem MIC $\geq 0.5$ mg/L or disk diameter $\leq 23$ mm or imipenem.
Carbapenemases in Enterobacteriaceae

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Objective: Increasing numbers of carbapenemase producing Enterobacteriaceae (CPEB) are reported from all over Europe. Following London's Health Protection Agency NDM-1 alert in summer 2010, the Austrian ministry of health asked all microbiological laboratories to send suspicious isolates to the NRZ for confirmation. The presented data are from Enterobacteriaceae isolates received over the whole study period, 31 CPEB isolates were detected in 27 patients from 12 hospitals, carrying VIM-1 carbapenemases as well as CTX-M, TEM and SHV derived extended spectrum β-lactamases (ESBL).

Results: Fifteen of 30 strains (50%) were carbapenemase negative. Thereof 8 were wildtypes, 4 carried an ESBL gene (CTX-M1 or M9) and 3 were AmpC hyperproducers combined with reduced permeability and/or efflux pumps. The other 15 (50%) were positive for blaVIM (n = 8), blaKPC (n = 3), blaNDM-1 (n = 3) and blaOXA-48 (n = 1). Using screening breakpoints for ertapenem (E: ≥0.5 mg/L), meropenem, (M: ≥0.5 mg/L), and imipenem (I: ≥2 mg/L), E, M and I detected 13, 12, and 6 of these 15 strains, respectively. The modified Hodge test detected all blaKPC carrying strains, whereas only 2/12 of Ambler class B and D enzymes. This was slightly improved when the Hodge test was done on McConkey agar (5/12). The MBL Etest detected only 3 cases. Compared with the Check-Points NAAT the KPC + MBL Confirm ID kit revealed excellent concordance (14/15 detected). Conclusions: In Austria CPEB of heterogeneous origin are present in low numbers. Half of all Isolates referred to the NRZ did not carry carbapenemase genes, stressing the importance of a confirmation lab. Although the number of strains analyzed is small, we suggest M and E for screening in daily routine followed by the KPC + MBL Confirm ID kit. The Check-MDR CTI02 is a promising tool allowing for detection of several resistance mechanisms simultaneously.
Molecular epidemiology and mechanism of carbapenem-resistant Enterobacteriaceae in a West China hospital

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Objectives: Carbenapenem have a broad spectrum of activity against the drug-resistant Enterobacteriaceae. However, an increasing number of Enterobacteriaceae producing Klebsiella pneumoniae carbapenemases (KPC) were reported in the worldwide. In our study, we aimed to investigate the molecular epidemiology and mechanism of carbapenem-resistant Enterobacteriaceae isolates collected from West China Hospital, Sichuan University.

Methods: 45 Enterobacteriaceae strains, resistant or with reduced susceptibility to carbenapenem were isolated from patients in West China Hospital, and antimicrobial susceptibility were determined by the agar dilution method. The modified Hodge test (MHT) and Boronic acid disk tests were carried to screen the phenotype of carbapenemase-producing bacteria. Specific PCR and DNA sequencing were performed to confirm the carbapenemase genotype.

Results: The resistant rates of the 45 isolates for imipenem, meropenem and ertapenem were 20.0%, 15.6% and 75.6%, respectively. 35 of them were positive in the MHT (77.8%, 35/45). BlaIMP, blaTEM, blaSHV and blaCTX-M were detected in 48.9%, 60.0%, 53.3% and 15.6% of these reduced susceptibility isolates. BlaKPC was identified in 4 (8.9%, 4/45) high-level resistant strains, of which one also carried blaCTX-M, another one carried blaTEM, blaSHV and blaIMP simultaneously. DNA sequencing revealed that the 4 isolates encoded carbapenemase gene, KPC-2. In the boronic acid disk test, by use of disks of imipenem, meropenem, or cefepime, either alone or in combination with 400μg of boronic acid, all 4 KPC producers gave positive results.

Conclusion: Production of KPC-2 carbapenemase contributes to reduced susceptibility of carbapenem in Enterobacteriaceae, but the KPC-producing isolates were still rare in southwestern China. The possessing of MBLs and ESBLs, and possible alterations in outer membrane proteins (OMPs) may be a main factor for carbapenem-resistance or reduced susceptibility in Enterobacteriaceae. KPC-2 carbapenemase gene were found being located on plasmids. Vertical and horizontal transmission plasmid-mediated are probably the primary epidemiical mechanism. Further studies on plasmid are necessary to reveal the perplexing mechanism of how the clone transmitted.

Detection of β-lactamases in Gram-negative rods

P693 Eradication of carbapenem-resistant Enterobacteriaceae colonisation with non-absorbable oral antibiotic treatment

I. Oren*, H. Sprecher, R. Finkelstein, S. Hadad, N. Krivyov, T. Zuckerman (Haifa, IL)

Objectives: Following a continuous outbreak of carbapenem resistant Enterobacteriaceae (CRE) colonization and infection among inpatients in our hospital, we conducted a randomized prospective trial aimed at eradicating gastrointestinal tract CRE colonization, using oral antibiotic administration of gentamicin (GM), colistin (COL), or both.

Methods: Consecutive hospitalized adult patients identified as CRE carriers by rectal surveillance cultures, were included in the study. Rectal isolates were tested for GM and COL susceptibility using E test. Patients who did not consent, or whose isolates were resistant to both drugs, were followed with repeated rectal swabs to assess spontaneous eradication rate (control group). Patients whose rectal isolates were GM-S but COL-R were treated with oral gentamicin sulphate 80mg qid. Patients whose isolates were COL-S but GM-R were treated with oral colistin sulphate 100mg qid. Patients whose isolates were sensitive to both drugs were randomized to three groups of oral antibiotic treatment: GM, COL, or both. Oral treatment was given until eradication, or for a maximum of 60 days. Eradication was defined by the presence of three consecutive negative rectal swabs for CRE including PCR testing of the third specimen. Failure was defined when: 1) rectal swabs still positive at the fifth day of treatment or following; 2) relapse occurred after apparent eradication; 3) rectal isolates turned resistant to the administered drug.

Results: 83 patients were included in the study. 48 were followed for spontaneous eradication (controls) for median of 87 days (range: 60–450). 35 patients received one of the 3 drug regimens: 21-GM; 9-COL; 5–both drugs. Eradication rates in these 3 groups were 38%, 33% and 40%, respectively, each of them significantly higher than the 4% spontaneous eradication rate in the control group (p<0.001, 0.02 and 0.04, respectively) with no difference between the regimens. Also, eradication rate among the 35 patients on any treatment – 37%, was significantly higher than 4% spontaneous eradication rate (p<0.001; OR 0.07; 95%CI, 0.01–0.39). No significant side effects were observed, to any of the treatment regimens.

Conclusion: Oral antibiotic treatment, with non-absorbable drugs that CRE is susceptible to, appears to be an effective and safe measure for eradication of CRE carrier state, and by that, may reduce patient to patient transmission and the incidence of clinical infection with this difficult to treat organism.

Detection of β-lactamases in Gram-negative rods

P694 Evaluation of a confirmatory test for phenotypic detection of acquired AmpC β-lactamases

F. Freitas*, R. Silva, A. Nocas, T.M. Coque, R. Cantón, L. Peixe, E. Machado on behalf of The Portuguese Resistance Study Group

Objectives: Detection of acquired AmpC β-lactamases (qAmpC) should be routinely performed in clinical laboratories, as this resistance mechanism has been associated with false cephalosporin susceptibility reports and therapeutic failures. Some inhibitors could be used in their detection, but a standard method has not yet been recommended. We evaluate the accuracy of a recently described test for phenotypic detection of qAmpC producers based on the inhibitory activity of phenylboronic acid (PBA) on these enzymes in a well characterized collection of AmpC-producing Enterobacteriacae.

Methods: A total of 89 clinical isolates [29 E. coli (EC), 50 K. pneumoniae (KP), 9 K. oxytoca (KO), 1 P. mirabilis] resistant to cefotixin and intermediate/resistant to amoxicillin-clavulanic acid and at least one oxyimino-cephalosporin (disk diffusion) were selected for confirmatory tests of qAmpC production. Isolates were recovered during a 7 year period (2002–08) and included DHA-1 (n=7), DHA-1+ESBL (n=40) and CMY-2 (n=3) producers, as well as qAmpC (–)ESBL(–) (n=6) and qAmpC(–)ESBL(+) (n=33) isolates. The PBA disk test was performed in Mueller-Hinton agar using disks containing only cefotetan (C TT) (30μg) and CTT plus PBA (30μg/400μg). A DHA-1-producing clinical isolate and EC ATCC 25922 were used as positive and negative controls, respectively. A positive test for the presence of qAmpC was considered if an increase of ≥5 mm in the inhibition halo occurred after the addition of PBA to CTT. Identification of known blqAmpC genes was carried out by PCR and sequencing.
Results: The PBA test was positive in 59 isolates (44 KP, 10 EC, 5 K0). DHA-1-producing KP (n=41) and K0 (n=4), and CMY-2-producing EC were correctly detected, but the test failed for DHA-1-EC (n=2). PBA test was positive in 11 isolates (7 EC, 3 KP, 1 K0) which yield a negative result in PCR assays for blaAmpC, those from EC being attributable to natural AmpC hyperproduction. Sensitivity and specificity of this method for this collection of isolates were 96% and 71.8%, respectively. Positive and negative predictive values (NPV) were 81.4% and 93.3%, respectively.

Conclusions: The PBA test combined with our screening method of qAmpC phenotypes displayed high sensitivity in a good NPV, but only moderate specificity for detection of qAmpC producers. Other cephalosporin/boronic acid combinations should be included in the confirmatory tests, especially to rule out EC natural AmpC hyperproduction.

**P695 Detection and differentiation of broad-spectrum β-lactamas from carbapenem-resistant Klebsiella pneumoniae clinical isolates**

A. Paoulou*, F. Markou, S. Pournaras, E. Voulgari, K. Ranellou, G. Vrioni, A. Tsakris (Serres, Larissa, Athens, GR)

Objectives: The emergence of carbapenemase-producing Klebsiella pneumoniae represents a major public health and infection control issue. We present the use of a simple phenotypic algorithm for the early detection and differentiation of broad-spectrum β-lactamas from carbapenem-resistant clinical isolates.

Methods: During Jan 2008-Sep 2010, 74 K. pneumoniae clinical isolates with reduced susceptibility to imipenem and/or meropenem (MIC >1 mg/l) were isolated from hospitalized patients. Identification and susceptibility testing were performed using Microscan (Siemens) and E-testing. β-lactamas were characterized by PCR and sequencing analysis using specific primers for blaVIM, blbIMP, blbKPC, blbOXA-48, blbTEM, blbSHV and blbCTX-M. For the detection of KPC, MBL, or both KPC and MBL, genes 4 disks of meropenem 10 mg were employed without and with 10 μl of 40 mg/ml phenyl boronic acid (PBA), 10 μl of 0.1 M EDTA or both PBA and EDTA. The interpretation of the results was performed according to a previously proposed algorithm (Tsakris et al., JAC 2010; 65: 1664–71). Additionally, a modified CLSI confirmatory test was used for the detection of ESBLs using disks of ceftazidime (CAZ), CAZ plus clavulanic acid (CAZ), cefotaxime (CTX) and CTX plus CA with the addition of PBA and EDTA.

Results: Molecular testing identified 41 KPC producers, 24 VIM producers and 9 producers of both carbapenemases. ESBL production was genotypically documented for 27, 13 and 3 of the KPC, VIM and both KPC and VIM producers, respectively. Phenotypic tests succeeded to detect and differentiate all KPC and VIM producers as well all isolates that produced both KPC and VIM enzymes. The CLSI modified test, using both PBA and EDTA, successfully detected ESBL production among isolates possessing KPC, VIM or both carbapenemases. All ESBL-negative isolates were also successfully documented as non-ESBL producers.

Conclusion: Phenotypic tests using PBA, EDTA or both inhibitors showed excellent sensitivity and specificity for the detection and differentiation of carbapenemases. The newly modified CLSI test also successfully detected isolates that co-produced ESBL and AmpC genes, which is very important for epidemiological purposes. The use of accurate and low cost methods for the detection of various carbapenemases and other broad-spectrum β-lactamas is essential for the early implementation of infection control measures and adequate treatment.

**P696 Rapid detection of ESBL-producing Enterobacteriaceae with a new chromogenic medium: Colorex Orientation/Colorex ESBL**

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Introduction: 1979, first patent filed by Alain Rambach for its chromogenic medium for the detection of Escherichia coli. 2009, a chromogenic medium for the detection of ESBL-producing Enterobacteriaceae (E) is available at the time when the emergence ESBL in the community becomes a major health concern. A study by OnERBA in 2006 found that among a total of 6771 strains of (E)isolated in the community, 72 (1.1%) were ESBL-producers, from which 67% of the species E. coli.

Objective: Evaluation of the sensitivity and specificity of this new medium for detection of ESBL from clinical specimens, as well as from a collection of 65 well-characterised strains.

Methods: ColorexOrientation/ColorexEUBLSE (COCE) is a bi-plate for the isolation and presumptive identification of ESBL. Sensitivity test: a total of 65 isolates (from our collection and/or isolated during the evaluation were plated in parallel on blood agar and on COCE medium and incubated at 36°C, and read after overnight incubation (18h-24h). E. coli (n=55), Proteus mirabilis (n=5), Klebsiella pneumoniae (n=5), Enterobacter cloacae (n=2).

Specificity: During 2 months, 500 samples from various specimen origins (including urine, sputum, gastric, pus) were seeded in parallel with the media used in our laboratory routine (REMIC recommendations). The identification and susceptibility testing were carried (i) with the MICROSCAN Walkaway (SIEMENS) and (ii) additional tests were also carried as recommended by the CASFM-(France-2009).

Conclusion: 50/650 clinical samples lead positive cultures: 135 on the non-selective compartment only (0 ESBL found), 24 on the 2 compartments. 10/24 were not part of Enterobacteriaceae: Pseudomonas spp. (8), Alcaligenes spp. (1) and Stenotrophomonas maltophilia (1), which grew as colourless colonies. The specificity provided by the chromogenic colour makes easy to differentiate the ESBL from other Gram(-) Oxidase (+) bacteria, known to be frequently multi-drug resistant (MDR). Among the Enterobacteriaceae 10/12 were confirmed ESBL.

Conclusion: COCE is particularly interesting for the screening of ESBL, particularly for E. coli ESBL+ (se = 100%) in clinical analysis (urinalysis, colonization or carriage). This new medium, that we would suggest for the use by hospital hygiene teams, could become a necessary tool also for microbiologist dealing with the threat of community acquired ESBL.

**P697 Phenotypic method for the detection of metallo-β-lactamas and KPC carbapenemases in the same isolate of Enterobacteriaceae**

J.B. Casals*, M. Pandrau Duer-Jensen (Roskilde, Rosco, DK)

Objectives: Grundmann and a group of European National Experts describe in Eurosorveillance, Nov 18th 2010 an algorithm for interpretation of synergy tests using combined discs to detect carbapenem-non-susceptible Enterobacteriaceae isolates. They recommend the use of meropenem (MER) and their combinations with clavulanic acid (CLOX), boronic acid (BOR) and dipicolionic acid (DPA), permitting the differentiation of KPC from AmpC variants loss, from metallo-β-lactamas (MBL). The appearance of isolates containing several carbapenemases (in particular KPC+MBL)in Greece and elsewere, may complicate the performance of the mentioned algorithm. The phenotypic traits of one carbapenemase may mask the other. The development of a disc/tablet containing: MER+BOR+DPA could probably detect KPC and MBL in the same isolate.

Methods: 25 K. pneumoniae isolates comprising strains possessing KPC-2 or VIM-1 and KPC-2+VIM-1 or AmpC-2 isolates were tested against disc (tablets) containing MER, MER-BOR, MER+CLOX, MER+DPA and MER+DPA+BOR on Mueller Hinton agar using McFarland 0.5 inoculum. Synergism indicated by an inhibition zone >5 mm larger around the combined disc/tablet compared to MER alone, was considered a positive reaction. In the case of the triple combination (MER+DPA+BOR) the zone was compared to MER+DPA and MER-BOR respectively. An isolate possessing both KPC and MBL would produce a zone around the triple combination tablet >5 mm than both around MER+BOR and MER+DPA.
Results: Isolates with KPC-2 carbapenemase showed synergy with MER+BOR, but not with MER+CLOX. Isolates with AmpC porin loss showed synergy with both MER+BOR and MER+CLOX. 10 isolates with KPC-2+VIM-1 carbapenemases, showed average zones of 11 mm around MER, 14 mm around MER+BOR, 16 mm around MER+DPA and 21 mm around the triple disc combination (MER+DPA+BOR). 

Conclusions: The triple combination tablet containing MER+DPA+BOR detected both KPC-2 and VIM-1 carbapenemases in the same strain of Klebsiella pneumoniae.

P698 Evaluation of Colorex C3Gr agar for the detection of resistant Escherichia coli and Klebsiella sp. rectal colonisation

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Objective: Evaluate the performance of Colorex C3Gr agar (Daylinn, Canada) and MacConkey with 2 mg/L cephalodoxime (CPD, Oxoid) for detecting Class A extended-spectrum β-lactamase (ESBL), AmpC-type β-lactamase (AmpC) and carbapenemase (CPM) producing Escherichia coli (EC) and Klebsiella spp. (KS) rectal colonization. C3Gr agar is a selective chromogenic agar allowing presumptive identification of ESBL, AmpC and CPM producing Enterobacteriaceae; EC colonies are expected to appear red and KS, Enterobacter sp. (ES) and Citrobacter sp. (CS) blue.

Method: 477 consecutive rectal swabs were inoculated to both media. Oxidase negative colonies on CPD, and red or blue colonies on C3Gr were investigated to confirm species identification and ESBL/AmpC/CPM phenotype by standard methods. A true positive, for sensitivity purposes, was defined as ESBL/AmpC/CPM-producing EC or KS detected on either medium.

Results: Growth was observed on 114 (24%) and 153 (32%) specimens for CPD and C3Gr, respectively. 144 isolates grew on CPD and 181 on C3Gr. Of the isolates growing on C3Gr, 70 appeared red with 69 (99%) identified as EC and 60 (87%) confirmed ESBL/AmpC producers. 111 isolates appeared blue on C3Gr, 101 (91%) were ES or CS, while 8 (7%) were KS. Of the 8 KS, 5 (63%) were confirmed ESBL producers. Of the 144 isolates recovered on CPD, 88 (61%) were either EC or KS and 82 (93%) of these were confirmed ESBL/AmpC producers. Overall, the sensitivity was 94% and 90% for CPD and C3Gr, respectively, for detecting EC or KS with one of the phenotypes of interest. On C3Gr, the positive predictive value (PPV) of a red colony being EC was 99%. The PPV of a red colony being an EC producing ESBL-A, AmpC or CPM was 86%. The PPV of a blue colony being KS, ES or CS was 99%. The PPV of a blue colony being an ESBL/AmpC/CPM producing KS was only 5%.

Conclusion: C3Gr performs according to the manufacturer’s specifications. The ability to differentiate EC from other Enterobacteriaceae has the potential to reduce turn-around time for detection of resistant EC phenotypes. The poor PPV of a blue colony for very resistant KS phenotypes, however, may limit the utility in some settings.

P709 Rapid strategy to detect colonisation by KPC-producing Klebsiella pneumoniae during an outbreak

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Objectives: We aimed at developing a new direct screening method (DSM), for rapid detection of Klebsiella pneumoniae carbapenemase-producing Klebsiella pneumoniae (KPC-KP) colonization to promptly establish infection control measures.

Methods: Patients admitted in rehabilitation units and intensive care units of the north-western area of Tuscany where cases of infection due to KPC-KP had been observed, were screened for KPC-KP colonization, from August to November 2010. Rectal swabs were inoculated on MacConkey agar and two paper disks, one containing meropenem (MER, 10 μg) and the other containing MER plus phenylboronic acid (PB, 600 μg), were placed on the seeded medium. DSM was considered positive in presence of an inhibition zone lower than 16 mm around MER alone or larger than ≥5 mm around the MER-PB disk with respect to MER alone.

Results: On the first 82 samples a comparison between MER disk and imipenem disk (IMP 10 μg) was performed. For confirmation, all the isolates suspected to be KPC-KP were also tested with the same method on Mueller-Hinton (MH) agar on a single colony. On 63 samples detection of blaKPC genes was carried out by direct polymerase chain reaction (PCR). Genotyping of KPC-KP by pulsed field gel electrophoresis (PFGE) of chromosomal DNA and multilocus sequence typing (MLST) were also carried out.

Results: A total of 123 patients were screened and 27 tested positive. The mean inhibition zone around MER±standard deviation (DS) was 7.6±7.5 mm, significantly lower (p<0.001) of the mean inhibition zone around MER±SD that resulted 19.7±4.1 mm. KPC production was confirmed by the phenotypic method on MH agar in all the strains with a positive screening test. All these isolates were identified as K. pneumoniae strains. MER resulted more effective than IMP (p=0.002) in screening KPC-KP: the mean inhibition zone around MER±SD was 7.6±7.5 mm while the mean inhibition zone around IMP±SD was 13.3±6.8 mm. Compared results of DSM and PCR are reported in table 1. The sensitivity and specificity of DSM, compared to PCR, were 81% and 90%, respectively. All isolates were closely related by PFGE typing, belonged to ST258 by MLST analysis, and were
generally susceptible to gentamicin, fosfomycin and colistin; tigecycline MIC ranged between 1 and 2 mg/L.

**Conclusion:** DSM is a simple, rapid, cheap, sensitive and specific method for the detection of KPC-KP colonization or infection.

### TABLE 1: Detection of KPC-KP in clinical specimens by MEER-PR method and direct PCR

| No of specimens | McGonigle agar MER-PB | Direct PCR (w/rab) |
|-----------------|------------------------|-------------------|
| 50              | Negative               | Positive          |
| 24              | Positive               | Positive          |
| 3               | Positive               | Negative          |
| 7               | Negative               | Positive          |

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**Acinetobacter baumannii**

**P701 Acinetobacter baumannii in Italian hospitals: results from a retrospective data analysis from the Micronet network laboratories**

F D Ancona*, A. Sisto, A. Raglio, M. Meledandri, A. Rocchetti and the Micronet Network participants

*Acinetobacter baumannii* is a pathogen frequently isolated in hospital that can survive for long periods with different environmental conditions. This Gram negative pathogen can cause outbreaks with clinical pictures of sepsis, pneumonias, urinary and wound infections. Rarely it can cause meningitis. It can be responsible of colonization in hospitalised patients. Patients in intensive care units and surgeries are often affected by this infection with an increased mortality.

In order to evaluate the circulation of this pathogen and describe the phenotypic resistance to antimicrobial drugs, we conducted a retrospective descriptive analysis of the infections caused by *A. baumannii, A. calcoaceticus* complex and *Acinetobacter spp* from 1st January 2010 to 30th June 2010 in 22 Italian hospitals participating in the study. The total number of ordinary hospitalization days in participating hospitals was 2,012,521. The total number of beds in the hospitals included in the study was 14066.

586 *A. baumannii* (considering *A. calcoaceticus* complex, *Acinetobacter spp* and *A. baumannii*) were isolated by culture (isolates from the same patients before 30 days were excluded).

The range by hospital was 0 to 80 isolates (mean 25, median 20). The most frequent specimen types of first isolation were not protected respiratory specimens (n: 254, 43.3%), Urine (n: 92, 15.7%), protected respiratory specimens (n:63, 10.8%), blood culture (n:38, 6.5%).

272 were isolated from Intensive Care Units (46.4%), 57 (9.7%) from surgeries, 180 (30.7%) from medicine ward.

Resistance to imipenem (539 samplings), meropenem (421), ampicillin (521), cefazidime (574) were respectively 73.5%, 72.9%, 30.9%, 77.7%.

307 (79.3%) of 387 isolates were non-susceptible both to imipenem and meropenem. Considering the percentage of circulating multiresistant *A. baumannii* circulating in hospital, it is a priority to activate control measure in the hospitals to avoid outbreaks that, especially in ICU where it can cause an excess of mortality. Surveillance of this events and monitoring of the antimicrobial resistance should be taken in consideration at national and local level.

### P702 Epidemiology of multidrug- and carbapenem-resistant *Acinetobacter baumannii* in Belgium: an emerging threat?

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**Objectives:** To analyze the susceptibility, the genetic basis of resistance and the molecular epidemiology of MDR *Acinetobacter baumannii* (Ab) strains recently isolated in Belgian hospitals.

**Methods:** All Ab isolates referred on a voluntary basis to the National Reference Centre from 09/2004 until 11/2010, were included in the study.

Identification was performed by VITEK 2 and PCR for blaOXA-51 gene. Antimicrobial susceptibility testing was performed by disk diffusion and results were interpreted according to CLSI breakpoints. PCR-sequencing targeting blaOXA-23,24,58-like, blaVIM,IMP, blagES,PER,VEB and blaADC were performed. Isolates were typed by PFGE (SpeI).

**Results:** Overall, 306 isolates (246 clinical, 12 environmental isolates and 48 of unknown origin) were obtained from 30 Belgian hospitals. Respiratory (34%), wound (18%) and urine (13%) were the most common sites of isolation. 202 strains were found carbapenem (imipenem or meropenem) resistant and among these, 158 isolates were associated with 10 major nosocomial outbreaks. Carbapenem-resistant Ab expressing the blaOXA-23 gene were at the source of 7 outbreaks whereas the remaining outbreaks were caused by an Ab expressing an OXA-24 group enzyme, an OXA-58 group enzyme or a GES extended-spectrum b-lactamase (1 epidemic each). Index cases could be identified in 80% (30 outbreaks) as initiated by medical transfers from North Africa (3), France (2), Greece (1) and Thailand (1). Outbreak scales ranged from 3 to more than 50 patients, mainly affecting intensive care and burn unit patients, and lasted up to several months in some cases.

Further, carbapenem-susceptible Ab strains were also associated with outbreaks including 2 with OXA-58 group, 1 with PER-1 extended-spectrum b-lactamase and 1 with overexpressed chromosomal ADC cephalosporinase.

PFGE analysis performed on Ab strains from 8 outbreaks evidenced 2 major clones (PFGE type 12 and 39) genotypically related to European clone (EC) I, and 3 unrelated to classical EC I, II and III (PFGE type 10, 42 and 44).

**Conclusion:** Ab is an emerging nosocomial pathogen in Belgium associated with the occurrence of major nosocomial outbreaks. A systematic screening, pre-emptive isolation and reinforced barrier precaution measures should be adopted for patients undergoing sanitary transfer from hospital in foreign countries. An active surveillance programme is clearly needed.

### P703 Strain diversity and antibiotic resistance including carbapenem resistance in *Acinetobacter baumannii* in Bulgarian hospitals

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**Objectives:** Since 2002, carbapenem resistant *A. baumannii* has emerged in Bulgarian hospitals. The aim of our study was to assess type diversity and antibiotic resistance including carbapenem resistance in multidrug-resistant *A. baumannii* (MDRAB) isolates from nine Bulgarian hospitals obtained between 2004 and 2008.

**Methods:** Investigated were 106 non- replicable MDRAB isolates including 58 prospective isolates from the Military Medical Academy (MMA) (2004–2006), 15 from 3 other Bulgarian hospitals from 2005–2006, and 35 from 5 non-medical transfers from North Africa, France, Greece, and Thailand (1). Outbreak scales ranged from 3 to more than 50 patients, mainly affecting intensive care and burn unit patients, and lasted up to several months in some cases.

Further, carbapenem-susceptible Ab strains were associated with outbreaks including 2 in OXA-58 group, 1 with PER-1 extended-spectrum b-lactamase and 1 with overexpressed chromosomal ADC cephalosporinase.

**Results:** Overall, 306 isolates (246 clinical, 12 environmental isolates and 48 of unknown origin) were obtained from 30 Belgian hospitals. Respiratory (34%), wound (18%) and urine (13%) were the most common sites of isolation. 202 strains were found carbapenem (imipenem or meropenem) resistant and among these, 158 isolates were associated with 10 major nosocomial outbreaks. Carbapenem-resistant Ab expressing the blaOXA-23 gene were at the source of 7 outbreaks whereas the remaining outbreaks were caused by an Ab expressing an OXA-24 group enzyme, an OXA-58 group enzyme or a GES extended-spectrum b-lactamase (1 epidemic each). Index cases could be identified in 80% (30 outbreaks) as initiated by medical transfers from North Africa (3), France (2), Greece (1) and Thailand (1). Outbreak scales ranged from 3 to more than 50 patients, mainly affecting intensive care and burn unit patients, and lasted up to several months in some cases.

Further, carbapenem-susceptible Ab strains were also associated with outbreaks including 2 with OXA-58 group, 1 with PER-1 extended-spectrum b-lactamase and 1 with overexpressed chromosomal ADC cephalosporinase.

PFGE analysis performed on Ab strains from 8 outbreaks evidenced 2 major clones (PFGE type 12 and 39) genotypically related to European clone (EC) I, and 3 unrelated to classical EC I, II and III (PFGE type 10, 42 and 44).

**Conclusion:** Ab is an emerging nosocomial pathogen in Belgium associated with the occurrence of major nosocomial outbreaks. A systematic screening, pre-emptive isolation and reinforced barrier precaution measures should be adopted for patients undergoing sanitary transfer from hospital in foreign countries. An active surveillance programme is clearly needed.
Conclusions: Results indicated a wide endemic or epidemic presence of different strains in the hospitals. Furthermore, there were indications for interspreader spread. The high degree of resistance to antibiotics including carbapenems is worrying.

Objectives: The aim of the study was to characterize the mechanisms of carbapenem resistance in *A. baumannii* isolates collected in the frames of a multicenter study and to study their molecular epidemiology.

Material and Methods: In total 115 *A. baumannii* isolates were collected in 13 diagnostic laboratories located in Northern part of Croatia in 2009. Antibiotic susceptibilities were determined by broth microdilution method according to CLSI. E-test MBL strips were used for detection of metallo-β-lactamases (MBLs). PCR was used to detect the presence of oxacillinases (OXA-51, OXA-23, OXA-24 and OXA-58) and MBLs of VIM, IMP and SIM series. The genetic context of blaOXA-51 and blaOXA-58 genes was determined by PCR mapping with the primers for ISab1 and ISAbaIII combined with forward and reverse primers for blaOXA-51 and blaOXA-58. Genotyping of the isolates was performed by PFGE.

Results: There were 97% of the strains resistant to piperacillin, 94% to ceftriaxone, cefotaxime and gentamicin, 91% to ciprofloxacin, 89% to cephalotin, 86% to amikacin and 85% to colistin. No resistance to colistin was observed. Colistin resistance was observed in two isolates. From one patient, two clonally related isolates were identified of which only one was colistin resistant. The patient had been treated with colistin, suggesting that colistin resistance has emerged during treatment. All isolates were resistant to at least one aminoglycoside. Two isolates showed high-level pan-aminoglycoside resistance. However, no 16S rRNA methylase gene was detected. Six isolates belonged to international clone I, including 4 clonally related isolates. No isolates belonged to international clone II. The class 1 integrase gene (intI1) was detected in 7/14 isolates.

Conclusions: MDR- and carbapenem-resistant *A. baumannii* isolates from Sweden are mostly associated with import of OXA-carbapenemase producing strains with some belonging to international clone II. MLST and sequencing of integrons are ongoing.

**OXA-carbapenemase-producing multidrug-resistant Acinetobacter baumannii isolates from Sweden**

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Objectives: Multidrug- and carbapenem-resistant *Acinetobacter baumannii* has increasingly emerged as a problematic pathogen for hospital-acquired infections worldwide. The aim of this project was to characterise carbapenem-resistant *A. baumannii* isolates identified in Sweden in 2004 and 2007.

Materials and Methods: The study included 14 carbapenem-resistant *A. baumannii* isolates identified in Sweden from 11 patients, including 2 patients transferred to Sweden after the 2005 tsunami in Thailand. Susceptibility testing was performed by Etest. PCR assays were performed for detection of OXA-carbapenemase genes, 16S rRNA methylase genes, ISAba1, intI1 and for determination of epidemic clonal lineages. PFGE was performed using Abal digested total genomic DNA.

Results: Molecular typing was performed by PFGE-Apal and identification of European clonal lineages I-III (EC I-III) was carried out by multiplex-PCR.

Conclusions: Of 154 isolates, 137 (89%) were resistant to five or more antibiotics. Majority of isolates were resistant to ciprofloxacin, ceftazidime and piperacillin (136/154 90%, 138/154 90%, 137/154 89%, respectively) while resistance to cefoperazone/sulbactam, meropenem and imipenem was lower (51/154 33%, 48/154 31%, 45/154 29%, respectively). All except three carbapenem resistant isolates conferred resistance to both carbapenems – meropenem and imipenem.
from ICUs (44/84 52%) as well as among isolates from TUs/SUs (24/59 62%). Identification of EC-III showed that all isolates of Cluster I and Cluster II belonged to EC I and EC II, respectively.

Conclusions: This study shows the high incidence of MDR phenotype among A. baumannii isolates from tertiary care university hospital in Lithuania. A close clonal relatedness was observed among these isolates which were assigned to globally disseminated European clonal lineages I and II.

P707 OXA-carbapenemase producing Acinetobacter baumannii in tertiary care university hospital in Lithuania

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Objectives: To perform first phenotypic, genotyping and molecular characterization of carbapenem-resistant clinical A. baumannii isolates in Lithuania.

Methods: 44 nonduplicate clinical isolates resistant to carbapenem were collected in the largest tertiary care medical center in Lithuania (Clinics of Kaunas University of Medicine) during year 2010. Their antimicrobial susceptibility was determined by disc diffusion. The molecular characterization of carbapenem resistant isolates was performed by using PFGE, PCR assays and sequencing.

Results: All isolates were resistant to meropenem, imipenem and ciprofloxacin, 98% to piperacillin and ceftazidime, 95% to tazobactam/piperacillin, 64% to amikacin, 43% to gentamicin, 20% to sulbactam/ampicillin and 11% to sulbactam/cefoperazone. According to the PFGE-Apal macrorestriction pattern analysis, carbapenem-resistant isolates showed 10 distinct genotype groups, which belonged to European clone II. Carbapenem resistance was related to OXA-carbapenemases with OXA-40-like the dominant enzyme OXA-72, found in 43 isolates.

Conclusions: OXA-40-positive isolates showed resistance to both carbapenems. (8.5%) were resistant to meropenem but susceptible to imipenem, while OXA-40-positive isolates showed resistance to both carbapenems.

P708 Resistance to antibiotics of Acinetobacter baumannii in Saint Petersburg, Russia

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Objectives: Resistance to antimicrobial preparations in Acinetobacter spp., isolated from patients with nosocomial pyoseptic infections in Saint-Petersburg, Russia, was studied.

Methods: Acinetobacter spp. were isolated from patients with nosocomial pyoseptic infections in 4 major hospitals of Saint-Petersburg, Russia. Identification was performed by routine methods and/or sequencing of 16s rRNA gene (ABI Prism 3130, MicroSeq ID v2.0 Software, MicroSeq ID 16s (DNA500 Library v2.0). Susceptibility of Acinetobacter spp. to 10 antibiotics was tested by dilution techniques in Muller-Hinton agar (Oxoid, GB). The antibacterial preparations included ampicillin (Amp), ceftazidime (Cz), imipenem (Im), meropenem (Mer), ampicillin-sulbactam (Arns), cefoperazone-sulbactam (Cs), piperacillin-tazobactam (Ppt), ciprofloxacin (Cip), amikacin (Amk), tigecycline (Tig).

Results: 83 strains of Acinetobacter spp. were isolated from bronchoalveolar lavage, blood, wounds and pleural fluid, including 79 strains of A. baumannii, 2 – A. genospecies 3, 2 – A. genospecies 14. The majority of strains were resistant to antibiotics (98.8%). Resistance to Amp was observed in 98.8%, to Cz in 90.4%, to Ppt in 81.9%, to Cip in 73.5%, to Im in 65.1%, to Mer in 65.1%, to Amk in 44.6%, to Amr 36.1% and Cs in 32.63% strains. All strains of Acinetobacter spp. were susceptible to Tig. Only 1 strain of A. baumannii was susceptible to all antibiotics tested. Multiple drug resistance was revealed in 81.9% of the tested strains. The majority of Acinetobacter spp. were resistant to 6 (31.3%) and 7 (28.9%) antibacterial preparations.

Conclusions: 1. Multiple drug resistance prevailed in Acinetobacter spp., isolated from patients with nosocomial pyoseptic infections in Saint-Petersburg, Russia.
2. Resistance to antibiotics, claimed to be highly effective in the treatment of nosocomial Acinetobacter infections, was revealed: strains, resistant to carbapenems, comprised a major part (65.1%) of the Acinetobacter spp.; resistance to cefoperazone-sulbactam was registered in 1/3 of the tested strains.
3. All strains of Acinetobacter spp. in the present study were susceptible to tigecycline.
Antibiotic resistance profile in hospital-acquired multidrug-resistant Acinetobacter baumannii.

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Objectives: Acinetobacter spp strains are most often responsible for serious nosocomial infections, especially in the intensive care units. Recent studies indicated a great increase in the antimicrobial resistance against old and new aminoglycosides, fluoroquinolones, carbapenems and colistin (polymyxin E). Today, researches on the resistance profile of multidrug-resistant MDR Acinetobacter spp have become important. There are several phenotypic and molecular typing methods which used for investigating the origin of infection, route of spread and prevalence of the resistant strains. The aim of this study was to determine the resistance profile and antibiotyping of MDR Acinetobacter spp isolates.

Methods: Between 2009 and 2010, 79 MDR Acinetobacter spp isolates were collected from different clinical specimens at the Clinical Microbiology Laboratory of Izmir Atatükr Training and Research Hospital. Antibiotypes of Acinetobacter spp isolates were determined by using the minimum inhibitory concentration-MIC values of amikacin, ciprofloxacin, meropenem, amikacin, rifampicin, moxifloxacin, colistin, respectively. All the resistant and intermediate isolates were evaluated as resistant. Thus, it was obtained 15 different antibiotic resistance profiles. Only one isolate was found as resistant against all the antibiotics tested, on the other hand 5 isolates were found susceptible. When compared with the other antibiotics, colistin was more influential. It was found clearly that moxifloxacin, the newer fluoroquinolone, was more effective than the other antibiotics, colistin was more influential. It was found clearly that moxifloxacin, the newer fluoroquinolone, was more effective than ciprofloxacin.

Conclusion: Acinetobacter spp are significant problem worldwide and increasingly responsible for numerous outbreaks and nosocomial infections. It is known that several typing methods are in use for the epidemiological investigation. Antibiotyping is one of the basic and useful typing methods, however, it should be supported by molecular techniques such as plasmid typing, ribotyping, etc. for the reliable classification.

Risk factors for acquisition of imipenem-resistant Acinetobacter baumannii in Taiwan

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Objectives: To elucidate risk factors for acquiring imipenem-resistant A. baumannii (IRAB), clinical data of case group (patients with IRAB) and control group (patients with imipenem-sensitive A. baumannii, ISAB) were compared.

Methods: Clinical presentations of patients infected with A. baumannii from Chang Gung Memorial Hospital and Saint Paul's Hospital in December of 2009 were all collected and analyzed. Susceptibility of the bacterial isolates to imipenem were determined by the standard disk diffusion method and E-test analysis. Risk factors for acquiring IRAB were analyzed by comparing data of 42 patients infected with IRAB and 20 patients with ISAB.

Results: The most common diagnosis was pneumonia (79% IRAB and 65% ISAB), followed by sepsis (11.3% IRAB and 10% ISAB), urinary tract infection (4.8% IRAB and 10% ISAB) and soft tissue infection (4.8% IRAB and 15% ISAB), such as cellulitis or abscess. There was no significant difference between case group and control group in their sources, diagnosis, age, male gender, number of underlying diseases, white cell count (WBC) or C-reactive protein (CRP) (all P < 0.05).

Patients infected with IRAB had significantly higher mortality rate
(P = 0.009), longer admitted days (P < 0.001), and longer treated days (P = 0.027) than those with ISAB. Comparing data of 21 mortal patients and 41 alive ones, there was no significant difference between these 2 groups in WBC, CRP or age (all P > 0.05). Results of the univariate analysis revealed significant differences between the patients with IRAB or ISAB in terms of previous ICU stay at least 5 days, use of mechanical ventilation at least 5 days, given carbapenems or extended-spectrum cephalosporines for at least 5 days, and given carbapenem (Table 1). However, only given carbapenems or extended-spectrum cephalosporines for at least 5 days was the only independent risk factor for acquiring imipenem resistance by multivariate logistic regression analysis (Odds Ratio: 342.07, 95% CI: 2.07–56514.17). Any of other risk factors was correlated with this independent risk factor (P < 0.001).

**Conclusion:** Patients infected with IRAB had higher mortality rate and longer hospital stay than those with ISAB. Prior use of carbapenems or extended-spectrum cephalosporines for at least 5 days was the only independent risk factor significantly correlated with IRAB infection. Therefore, restricted use of carbapenems and extended-spectrum cephalosporines is crucial to reduce acquisition of IRAB.

### Table 1: Univariate analysis of risk factors for acquiring imipenem-resistant A. baumannii (IRAB)

| Characteristic* | IRAB (NC=42) | ISAB (NC=20) | P-value | Odds ratio (95% CI) |
|----------------|-------------|-------------|---------|---------------------|
| Mean age, years | 66.2±18.1   | 63.1±18.7   | 0.549   | 1.01 (0.98-1.04)    |
| Male gender     | 29 (69%)    | 10 (50%)    | 0.199   | 2.23 (0.75-6.66)    |
| Number of underlying diseases | 2.3±1.4     | 1.9±1.1     | 0.177   | 1.34 (0.88-2.06)    |
| Hospital size > 1000 beds | 19 (45.2%) | 7 (35%)     | 0.446   | 1.53 (0.51-4.62)    |
| Previous ICU stays ≥ 25 days | 39 (92.9%) | 4 (20%)     | <0.001  | 2.00 (0.14-29.10)   |
| Mechanical ventilation ≥ 25 days | 39 (92.9%) | 3 (15%)     | <0.001  | 29.00 (2.42-124.89) |
| Use of extended-spectrum antibiotics ≥ 25 days | 42 (100%) | 3 (15%)     | <0.001  | 796.54               |
| Carbapenem    | 24 (57.1%)  | 5 (25%)     | 0.003   | 25.33 (3.00-207.23) |
| Extended-spectrum cephalosporin | 25 (59.5%) | 12 (60%)    | 0.971   | 0.080 (0.03-2.91)   |
| Piperacillin-tazo-bacitracin | 8 (19%)    | 0           | 0.132   | 10.34 (0.50-215.39) |
| Amoxicillin     | 6 (14.3%)   | 0           | 0.204   | 7.32 (0.33-186.57)  |
| Aminoglycosides | 4 (9.5%)    | 0           | 0.342   | 4.61 (0.20-107.56)  |
| Fluoroquinolones | 14 (33.3%) | 3 (15%)     | 0.223   | 2.93 (0.71-11.32)   |

*Unless otherwise indicated, data are the number (%) of patients with each characteristic.

IRAB: imipenem-resistant Acinetobacter baumannii; ISAB: imipenem-susceptible A. baumannii; ICU, intensive care unit.

**P714** Molecular epidemiology of clinical Acinetobacter baumannii isolates in a Korean hospital

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**Objectives:** Acinetobacter baumannii are commonly associated with nosocomial infections, and usually multidrug-resistant. We investigated the characteristics of 35 A. baumannii isolates by multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE), PCR of the antimicrobial resistance determinants, and antimicrobial susceptibilities.

**Methods:** This study included 16 carbapenem-resistant A. baumannii (CRAB) and 19 carbapenem-susceptible A. baumannii (CSAB) from a secondary hospital in Daejeon, Korea between January and July 2009. Samples were suspended in BHI. Isolates were selected for 24h. Micro g/ml yielded small red colonies on this medium after incubation for 18h. MDRP blaVIM-2 did not grow on the medium, permeability was plated on this medium and incubated for 35°C for 18–72 h. In the clinical trial, 5,740 specimens from the pharyngeal swabs, urine and rectal swabs, and 6,617 swab specimens from environmental materials were plated on this medium and incubated for 35°C for 18–72 h.

**Results:** In the trials of stock strains, three genotypes of MRAB showed red and large colonies after cultivation for 18 h at 35°C. ESBL-producing enteric bacilli did not grow on the medium. However, K. pneumoniae blakPC and E. cloacae blalPM-1 grew as small bluecolonies after 18 h of cultivation. MDRP blalPM-2 did not grow on the medium, permeability decreasing MDRP showed small red colonies after 24 h of cultivation and MDRP blalPM-1 yielded small red colonies after 48 h of cultivation. Twenty-one MRAB were detected from clinical and environmental specimens. Clinical and environmental isolates with carbapenems MIC ≥ 2 micro g/ml (P. aeruginosa, P. fluorescens, S. maltophilia, C. indologenes and A. xylosoxidans) grew as small red colonies on this medium for after cultivation for 24–48 h. P. putida isolates with carbapenems MIC <1 micro g/ml yielded small red colonies on this medium after incubation for 24 h. Among red colonies suspected Acinetobacter were easily discriminated from other genus by oxidase test and Gram staining.

**Conclusion:** The novel selective medium CHROMagar Acinetobacter supplemented with KPC was useful for detecting our cases with MDRB blaoX-51-like. In addition, it was especially valuable for active surveillance of specimens containing multiple bacteria, such as those from the pharynx, urine, faeces and the environment.

**P715** Evaluation of a novel selective medium, CHROMagar acinetobacter with KPC supplement, for detection of multidrug-resistant Acinetobacter baumannii from clinical specimens in Japan

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**Objectives:** Multidrug-resistant A. baumannii (MRAB) has recently been reported in both western countries and in China. However, cases of such infections are very rare in Japan. Here, we report hospital-acquired infection by A. baumannii blaoX-51-like resistant to the carbapenems imipenem or meropenem, the aminoglycoside amikacin and the fluoroquinolones levofloxacin or ciprofloxacin. We also evaluated the novel chromogenic medium, CHROMagar Acinetobacter (CHROMagar, France) supplemented with KPC to detect MRAB.

**Methods:** KPC-supplemented CHROMagar Acinetobacter was used for isolation of drug-resistant strains, such as E. coli blalCTX-M-2, P mirabilis blalCTX-M-2, K. pneumoniae blalMP-1, E. cloacae blalM-1, Multidrug-resistant P. aeruginosa (MDRP) blalMP-1, MDRP blalVIM-2, permeability decreasing MDRP, MRA blalMP-1, MRA blaoX-23, MRA blaoX-51-like and S. maltophila. There were incubated at 35°C for 18–72h. For the clinical trial, 5,740 specimens from the pharyngeal swabs, urine and rectal swabs, and 6,617 swab specimens from environmental materials were plated on this medium and incubated for 35°C for 18–72 h.

**Results:** In the trials of stock strains, three genotypes of MRAB showed red and large colonies after cultivation for 18 h at 35°C. ESBL-producing enteric bacilli did not grow on the medium. However, K. pneumoniae blakPC and E. cloacae blalPM-1 grew as small bluecolonies after 18 h of cultivation. MDRP blalPM-2 did not grow on the medium, permeability decreasing MDRP showed small red colonies after 24 h of cultivation and MDRP blalPM-1 yielded small red colonies after 48 h of cultivation. Twenty-one MRAB were detected from clinical and environmental specimens. Clinical and environmental isolates with carbapenems MIC ≥ 2 micro g/ml (P. aeruginosa, P. fluorescens, S. maltophilia, C. indologenes and A. xylosoxidans) grew as small red colonies on this medium for after cultivation for 24 h. Among red colonies suspected Acinetobacter were easily discriminated from other genus by oxidase test and Gram staining.

**Conclusion:** The novel selective medium CHROMagar Acinetobacter supplemented with KPC was useful for detecting our cases with MDRB blaoX-51-like. In addition, it was especially valuable for active surveillance of specimens containing multiple bacteria, such as those from the pharynx, urine, faeces and the environment.

**P716** MDR Acinetobacter baumannii faecal colonisation of nursing home residents of northern Portugal

D. Gonçalves*, H. Ferreira (Porto, PT)

**Objectives:** Our work, in fecal colonization with ESBL producers in nursing home (NH) residents, showed that we could also find carbapenem-resistant isolates. The aim of our work was the detection of carbapenem-resistant Gram negatives, in the fecal flora of NH residents, in the North of Portugal, including Porto metropolitan area.

**Methods:** Faecal samples of NH residents from two NH of Porto Metropolitan area and one in the North of Portugal, were collected during 2008 and 2009. Samples were suspended in BHI. Isolates were selected except one belonged to ST92. The resistance rates of CRAB isolates to all antimicrobial agents tested were higher than those of CSAB isolates.

**Conclusions:** ST92 was dominant sequence-type in a Korean hospital, and it closely correlated with carbapenem resistance and the presence of blaoX-23.
in MacConkey agar with ceftazidime (2mg/l), cefotaxime (2mg/l), aztreonam (2mg/l) and imipenem (1mg/l). Colonies were randomly selected and susceptibility to antimicrobial agents was determined by agar diffusion methods according to the CLSI guidelines. Carbenapenem-resistant isolates were obtained from imipenem containing plates and also from other antibiotic selective media. Phenotypic identification of the selected isolates, was achieved by ID 32 GN. β-lactamases were characterized by isoelectric focusing. MICs were determined by the Etest methodology in Mueller-Hinton agar. Hodgetest, was carried out according to the CLSI guidelines, bioassays and inhibition studies were done according to the literature.

**Results:** Our work showed the presence of 7 non fermenter isolates, identified as Acinetobacter baumannii by ID 32 GN, from three different nursing homes in the same region but geographically apart. Susceptibility testing showed a multi-resistant phenotype including carbenapenem and fluoroquinolones and susceptibility to amikacin. MICs to imipenem were over 16mg/l to meropenem were over 8mg/l and to cefotaxime were over 32mg/l. Bioassays and Hodge test, showed carbenapenem degradation in representative isolates.

**Conclusions:** Hospitals of Porto urban area, have already experienced the installation of outbreaks of MDR Acinetobacter baumannii of difficult resolution, by patients transferred from hospitals in the same metropolitan area. NH of this area interchanging patients with local hospitals, might function as reservoirs of MDRGN contributing for outbreaks in particularly important hospital wards. This question should be addressed as a relevant public health threat, once it might also contribute for community spread of MDRGN via domiciliary care of dependent people and dispersion to the healthy population. Our work seems to suggest the utility of pre-admission screening of MDRGN in the fecal flora of nursing home residents.

**Porins and efflux pumps are good friends**

**Effect of Sub-MIC concentrations of biocides on the expression of genes coding for efflux pumps and porins in Acinetobacter baumannii ATCC 19606**

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**Objective:** We analyzed the ability of sub-MICs concentrations of five biocides commonly used in hospitals to affect the expression of genes coding for efflux systems and porins related with antimicrobial resistance and/or virulence in Acinetobacter baumannii ATCC 19606 (Ab).

**Methods:** The biocides evaluated were domestic bleach (DB), Sterillium (ST; propan-2-ol, propan-1-ol, mecectronium ethyl sulfate), Bdine (BT; povidone iodine), chlorhexidine digluconate (CHX) and benzalkonium chloride (BKC). The Ab was grown in i) Mueller-Hinton broth containing concentrations of biocides equivalent to the respective 0.25x MIC and ii) Mueller-Hinton broth without biocides (control). Expression of genes coding for adeB, adeJ, abeM, OmpA, CarO, OprD-like and oprA was measured by qRT-PCR when RNaseOUT was inoculated in the medium. When RNaseOUT treated heteroresistant population was analyzed by SDS-PAGE, a band of approx. 35 to 40kD was present and was identified as OmpA by MS/MS analysis.

**Conclusions:** RNase-mediated post-transcriptional regulation of gene ompA seems to be the major mechanism of carbapenem heteroresistance in A. baumannii.
2. For porin genes, there was also an increased expression, although it was lower (except for oprD-like using BCK), than that observed with the efflux genes.

3. Some biocides, like BKC, were stronger inducers of the expression of efflux and porin genes than others.

**P719** Effect of environmental factors on outer membrane proteins expression and antimicrobial susceptibility of multi-resistant clinical isolates of *Escherichia coli*

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Objectives: OmpF and OmpC porins represent a relevant component of resistance in *E. coli*. Previous studies in *E. coli* K12 have shown that the expression of these two porins is regulated in response to environmental factors. We have studied several environmental factors on both porin expression and antimicrobial susceptibility of clinical isolates of *E. coli*.

Methods: Twelve isolates, representative of the more frequent porin patterns expressed by 112 clonally-unrelated (defined by Rep-PCR) multidrug-resistant *E. coli* isolated from different patients were studied. OMP expression and susceptibility to amoxicillin (Amx), cefazidime (Caz), ertapenem (Ert), gentamicin (Gm) and ciprofloxacin (Cip) were evaluated in different conditions of osmolarity [Mueller-Hinton broth (MH), Nutrient broth (NB) alone or NB plus 20% sorbitol], temperature (MH or NB at 25°C, 30°C, 37°C or 41°C) and pH (MH or NB adjusted to pH 8.5, 7.2, 5.5 or 5.0). OMPs were obtained from sonicated cells treated with sarcosyl and separated in SDS-PAGE. MICs were determined by microdilution (CLSI). In addition, RT-PCR was performed for measuring the level of the efflux pump AcrB.

Results: None of the 12 isolates overexpressed AcrB. All of them expressed both OmpC and OmpF when grown in standard conditions (MH, 37°C pH 7.2). None of the three factors we studied influenced the MICs of Ert or Amx. No relevant changes in porin patterns were observed when the organisms were grown in NB with or without sorbitol, although the MICs of Gm were at least 4 times lower in NB alone than in MH. At 41°C, some isolates tested in MH lost OmpC and presented increased Gm MICs. Variation in the expression of OmpF was also observed when organisms were grown in media with pH of 5.5 or 5.0, and in these pH conditions increased CMIs of Caz, Cip and Gm were observed.

Conclusions: In the clinical isolates we have studied, the effects of osmolarity, temperature or pH on porin expression are different from those described for the laboratory strain *E. coli* K12. High temperature or low pH affect porin expression and have a moderate effect on the activity of Caz, Cip or Gm. The increased activity of Gm in low-osmolarity medium is unrelated to porin changes.

**P720** Genome analysis of human multidrug-resistant strain of *Enterobacter aerogenes*

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Objective: *Enterobacter aerogenes* is a common and important human opportunistic pathogen that may cause a wide variety of nosocomial infections. In this study we report the complete genome sequence of an *E. aerogenes* strain isolated in our institution which showed successful resistance to all available antibiotics including imipenem and colistin in a patient who died from this infection despite antibiotic treatment. The aim of our study was to identify and decipher all resistance determinants of this pan-resistance isolate using genome sequencing approach.

Method: Whole shotgun sequencing was used to fully sequence this genome. The shotgun libraries of 4 and 9 kb generated were analyzed and assembled using Phred_Phrap and Consed software suite. Genomic annotation was performed using online bioserver RAST server (Rapid Annotations using Subsystems Technology). Standard PCR amplification and sequencing were used to improve sequence regions of low quality on genomic contigs and to characterize putative mutations on candidate genes involved in antibiotic resistance.

Principal findings: The genome of *E. aerogenes* comprises a chromosomal DNA estimated at 5.37 Mb with a G+C content of 55% and one circular plasmid of 162.182 bp in size. The functional annotation of the chromosome gave a total of 5113 predicted coding sequences (CDS) with 3197 (62.53%) being assigned to a biological function including as many as 30 antibiotic resistance encoding genes, 49 antisepsis and heavy-metal resistance encoding genes and more than 100 efflux pump systems. Surprisingly, as compared to other available complete genomes, *E. aerogenes* genome appears to be more closely related to Klebsiella pneumoniae and not to *E. cloacae* species. Interestingly, the *E. aerogenes* conjugative plasmid carries many mobile genetic elements including antibiotic, antisepsis, and heavy metal resistance encoding genes. Finally, we report here the potential molecular mechanism of colistin resistance in this clinical isolate mediated by a single chromosome mutation (G157A) on pmrA gene as compared to a parental susceptible isolate in the PmrAB two-component regulatory system known to be involved in polymyxin resistance.

Conclusion: Our study confirms that whole genome sequence is a critical approach to quickly decipher the resistome of any bacterium of clinical relevance. To the best of our knowledge this is the first complete genome sequence of the *E. aerogenes* species.

**P721** Exposure of *Salmonella enterica* serovar typhimurium to high-level biocide challenge can select for novel-mechanism, multidrug-resistant mutants in a single step

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Objectives: Biocides are used in a wide range of domestic and industrial products to control microbial growth. There are concerns that biocide exposure is helping drive selection of antibiotic resistant bacteria due to common mechanisms of resistance. The aim of this study was to expose *S. enterica* to working concentrations of biocides to identify if this single step exposure can select for multidrug resistant (MDR) mutants.

Methods: *Salmonella enterica* serovar Typhimurium was challenged with 4 biocides of differing modes of action at recommended-use concentration. Flow cytometry was used to investigate the physiological state of the cells after biocide challenge. After 5 hours in biocide, live, viable cells were sorted and recovered after exposure to 2 of the biocides. The recovered cells were tested for their drug resistance profile, real-time investigation of efflux activity was studied by the uptake and accumulation of marker substrate dye and the expression of genes responsible for efflux and stress response was assessed by QRT-PCR and dHPLC analysis.

Results: Flow cytometry identified sub-populations of *S. Typhimurium* capable of surviving working-level biocide challenge. The recovered cells were multi-drug resistant and showed a marked increase in efflux activity. The cells were found to over-express the efflux pump AcrEF and the regulator protein MarA. However, compared to the parent strain, they repressed the expression of AcrAB and RamA.

Conclusions: The data shows that high level biocide exposure can select for MDR efflux mutants. Interestingly, it appears to be the AcrEF efflux system not the AcrAB-TolC system that is facilitating this survival. These data demonstrate the redundancy in MDR regulatory pathways in *Salmonella* and show that *Salmonella* can survive extreme biocide exposure if de-repression of MDR pumps has occurred.

**P722** Evaluation of quinolone resistance determining region mutations and efflux pump expression in *Neisseria meningitidis* resistant to fluoroquinolones

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Objectives: To evaluate additional fluoroquinolone resistance (R) mechanisms in *N. meningitidis* (NMEN) strains carrying a GyrA T91I alteration and displaying elevated ciprofloxacin (CIP) MIC values (0.06 and 0.25 mg/L). CIP-R in NMEN is rare in North America, and to date
only three isolates with this phenotype have been described in the United States (USA).

Methods: Strain B NMM strains (3; courtesy of Dr. Henry M. Wu, CDC-USA) collected from USA hospitals (North Dakota [1 strain] and Minnesota [2 strains]) displaying elevated CIP MICs and gyrA mutation (T91I) were susceptibility tested by CLSI (M07-A8, 2009) reference broth microdilution method. Quinolone R determining region (QDRR) sequencing analysis of gyrA, gyrB, parC and parE was performed. Mapping of the mtrCDE efflux system was carried out using primers anchoring in the components of the pump, coverage included intergenic regions. mRNA expression for pump components was evaluated by quantitative reverse-transcriptase real-time PCR (qRT-PCR) and comparing to NMEN ATCC 13102 control.

Results: Two strains showed CIP and levofloxacin MIC values at 0.25 mg/mL and one strain had a CIP MIC at 0.06 mg/L. parC mutations causing alterations H141N and P186S were detected in all strains. In addition to T91I, the two strains displaying higher MIC values also possessed a T73A alteration on GyrA. All components of the efflux pump mtrCDE (also associated to rifampin-R) were intact and had the correct amplicon size, excluding the presence of insertions/deletions and the Correia element within the pump operon. The promoter region (mtrR) was fully sequenced and was distinct from the susceptible control. Isolates 7782J and 7783J had identical promoter region, whereas isolate 7784J showing lower ciprofloxacin MIC values possessed a different sequence. Expression experiments showed discrepancies in the mRNA in pump components. The most remarkable difference was for the outer membrane protein (OMP) encoded by mtrE that was hyperexpressed on strain 7784J showing a 0.06 mg/L CIP MIC (3600X elevated compared to control).

Conclusions: Our results indicate that T91I alteration on GyrA had an important role in elevated CIP MIC values observed in these NMEN strains. Additional R determinants were present, including other gyrA and parC QDRR mutations. Alterations in expression of mtrCDE pump appear to have minimal contribution to CIP-R. This finding was supported by low rifampicin susceptibility results (MIC, 0.03 mg/L).

Results: All the strains exposed to INH became resistant to INH after 3 weeks of exposure. The INH resistance was reversed or reduced by EPIs in all strains. RT-qPCR analysis indicated over-expression of all efflux pump genes tested. Increased efflux activity was demonstrated in all the INH exposed strains which was inhibited in the presence of EPIs. The strains initially susceptible to INH became monoresistant to this antibiotic. When the RIF resistant strains were exposed to INH, they became MDR. Throughout the INH exposure process different alterations in the katG gene were detected and characterized, namely point mutations and deletions in the katG gene. No phenotypic or genotypic alterations were detected during the exposure to RIF.

Conclusion: The results demonstrate the relevance of efflux as a mechanism of resistance to INH in M. tuberculosis strengthening the hypothesis that the activity of efflux pumps allows the maintenance of an INH resistant population in a patient under a sub-optimal therapeutic level, from which INH genetically resistant mutants emerge. These results illustrate different strategies by which M. tuberculosis strains respond when exposed to clinically relevant concentrations of the same antibiotic, which may result in the emergence of MDR-TB.
Differential recognition of quinolones by efflux pumps in Gram-positive (Staphylococcus aureus, Streptococcus pneumoniae) and Gram-negative (Pseudomonas aeruginosa) bacteria

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Objectives: Active efflux may confer resistance to quinolones in both Gram(+) and Gram(-) bacteria, but structural features determining their recognition by transporters remains largely unknown. We have examined the impact of typical representatives of 3 superfamilies of transporters, each active in a main pathogen (NorA [MFS; S.a.], PatA/B and PmrA [ABC and MFS, respectively; S.p.], and MexAB-OprM, MexCD-OprJ, MexEF-OprN, MexJK-OprM, and MexXY-OprM (RND; Pa.) on the activity of 14 quinolones (see legend of the figure).

Methods: MICs were measured following CLSI recommendations using isogenic strains differing in the level of expression of the corresponding transporter(s) (S.a.: ATCC25923 vs. SA-1 overexpressing norA [Ba et al. AAC 2006, 50:1931–6]; S.p.: SP295 overexpressing patA/B and pmrA vs. its corresponding disruptants [El Garch et al. JAC 2010, 65:2076–82]; Pa.: PA01 with basal expression of Mex efflux systems vs. PA509 [disruptant for 5 RND transporters; Mima et al. J Bacteriol. 2007, 189:7600–9]). The impact of efflux was expressed as the ratio of MIC of the corresponding transporter(+)/transporter(−) isogenic strains (with addition of 10 mg/L reserpine to inhibit NorA in ATCC25923).

Results: The figure shows the correlation between the changes in MIC observed between S.a. and S.p. (NorA vs PatA) and between S.a. and Pa. (NorA vs Mex). There was a high degree of correlation between efflux mediated by the two types of transporters in Gram(+) bacteria (R² = 0.8289 for PatA vs NorA and 0.7943 for PatB vs NorA [not shown]) with PEF, SPX, GAR, DIF, and MXF being almost not affected, and CIP and NOR (more hydrophilic ones) being most affected. Disrupting pmrA was without effect on all quinolones (not shown). Conversely, all quinolones were affected almost to the same level by Mex transporters, making the correlation between NorA (or PatA [not shown]) and Mex largely irrelevant.

Conclusion: Although belonging to very different superfamilies, NorA and PatA/PatB show similar substrate specificities with respect to quinolones, but mainly affect those that are least recommended today for treating infections caused by these organisms. Of note, PmrA, although also an MFS transporter, seems not to efflux any quinolone. Conversely, Mex efflux systems transport all quinolones, including those that are currently used in therapy, which contributes to reduce susceptibility, even in wild-type strains.

P726 Rapid mutation of Staphylococcus aureus norA efflux pump promoter after induction with ciprofloxacin is associated with an increased norA expression and an increased ciprofloxacin MIC

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Objectives: NorA is a chromosomally encoded efflux pump of S. aureus, which is known to be involved in a.o. the efflux of fluoroquinolones, such as ciprofloxacin. In literature as well as in our previous work we showed that mutations in the norA gene and promoter were associated with an increased expression of norA. However, in several cases, sequences that were associated with increased expression differed only in one single base mutation from sequences associated with a wildtype (wt) expression. The goal of this study was to observe whether mutations in the norA gene or its promoter would occur in strains with a norA wt expression after exposure to ciprofloxacin.

Methods: Three clinical S. aureus isolates with a ciprofloxacin MIC of 0.125-0.25 mg/l and wt expression of norA were in duplicate subjected to increasing concentrations of ciprofloxacin (starting at 0.5 MIC) during two weeks. Antibiotic susceptibility profiles were determined by microbroth dilution. The norA promoter and part of the norA gene as well as gyrA and grlA were sequenced to determine the presence of mutations, and relative gene expression experiments were performed using 16S rRNA as a housekeeping gene.

Results: All three S. aureus strains (tested in duplicate) showed an increase of the ciprofloxacin MIC to 8–32 mg/l. Results showed that 3 out of 6 replicates had obtained mutations in gyrA and grlA, of which 2 had acquired a mutation in the norA promoter. Gene expression experiments showed that the norA mutation was associated with an increase in norA expression of >4-fold. The implications of this norA overexpression were seen in one strain, of which one replicate showed only the mutations in gyrA and grlA whereas the other showed additionally the mutation in norA: the latter showed a 2 to 4-fold increase in ciprofloxacin MIC. Retrospective analysis showed that norA mutation was obtained as rapidly as within 5 days of the start of the experiment. All the acquired mutations remained stable during daily subculturing for two more weeks in the absence of ciprofloxacin.

Conclusion: In 2 S. aureus strains mutations in the norA promoter were acquired after induction with ciprofloxacin. These mutations were associated with an increase in norA expression and a 2 to 4-fold increase in ciprofloxacin MIC. Future further studies will have to show which mechanism was behind the increase in ciprofloxacin MIC in the strains where no mutations were observed in either gyrA, grlA or norA.

P727 Total genome sequencing of multidrug and efflux pump inhibitor resistant Streptococcus pneumoniae reveals new roles for genes in PatAB-mediated antibiotic resistance

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Objectives: Over-expression of the ABC transporter genes patA and patB has been shown to be associated with efflux-mediated multidrug resistance in laboratory strains and clinical isolates of Streptococcus pneumoniae. However, the cause of the over-expression of these genes is unclear. The aim of this study was to use next-generation sequencing techniques to identify candidate genes that affect expression of patA and patB.

Methods: The complete genome sequences of a resistant laboratory mutant, M184, which over-expresses patA and patB, and its parent strain, R6, were determined by Illumina Solexa sequencing and compared to each other using the Xbase next-generation pipeline to identify mutations. Genes containing mutations that affected their coding sequences were counted as candidates for affecting expression of patA and patB. The S. pneumoniae cluster analysis tool, ClusterSP, was used to determine which of the candidate genes were part of the pneumococcal core genome and therefore most likely to be involved in resistance. To determine whether any of the observed mutations were natural
polymorphisms, gene sequences from 17 published pneumococcal genomes were aligned using ClustalW.

Results: Fourteen genes in M184 were found to contain mutations that altered their protein coding sequence. By excluding natural polymorphisms and only considering genes that are part of the core genome of S. pneumoniae, the number of candidate genes was reduced to ten. These ten genes belong to various functional groups including metabolism (three genes), transcription (three genes), translation (one gene), membrane transport (one gene) and regulation (one gene). One candidate gene of unknown function was also identified. One gene encoding a regulatory protein was truncated in M184, and this mutation was confirmed by PCR and sequencing. The same gene was also found to contain a mutation in its putative DNA binding region in another reserpine-resistant mutant strain, M168, which also over-expresses patA and patB.

Conclusion: These data suggest that the regulatory protein identified in this study could control patA and patB expression, and hence susceptibility to antibiotics. This is the most promising candidate gene in our set as regulatory proteins are known to regulate expression of efflux pumps in other bacteria. This would constitute a novel role for this protein in pneumococcal antibiotic resistance.

**P728** Variability and inducibility of the mef(A)-msr(D) region in erythromycin-resistant Streptococcus pyogenes

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Objectives: In streptococci, resistance towards 14- and 15-membered macrolides may be due to efflux pumps belonging to the major facilitator superfamily, encoded by mef(A) genes. mef(A) is associated with efflux-mediated macrolide resistance in Streptococcus pyogenes this gene cluster is inserted in and carried by prophages or transposons. We studied the variations in the sequence of the mef(A)-msr(D) region, analysed their correlation with the level of macrolide resistance, and we investigated the inducible expression of the efflux system.

Methods: The 3,200 bp long mef(A)-msr(D) region of 28 S. pyogenes strains with an M-phenotype of macrolide resistance was amplified and sequenced. MICs for erythromycin were determined by the broth microdilution method. Six out of 29 strains plus a reference strain (m46) were cultured with or without sub-inhibitory inducing concentration of erythromycin. After induction, the cultures were challenged with erythromycin at different concentrations and the growth monitored. Total RNA was extracted from induced and non induced cells of the reference strain and analysed by northern blot and RT-PCR.

Results: The strains showed MICs for erythromycin ranging from 4 to 32 mg/L. Cluster analysis showed an essential correlation between the sequence of the mef(A)-msr(D) region and the MIC. In addition, multivariate analysis indicated that the acquisition of the macrolide efflux genes occurred prior to the divergence of the prophages carrying them. The growth curves showed a pattern of induction of the macrolide resistance that was more evident at the sub-inhibitory challenge concentrations. In non-inducing conditions the amount of mef(A)-msr(D) was reduced by 50%. In inducing conditions the mef(A)-msr(D) region was amplified in all strains and sequenced.

Conclusion: In S. pyogenes, the increase in MICs for erythromycin could be overall explained by the accumulation of mutation within the sequence of the mef(A)-msr(D) region. Similarly to the case of pneumococci, efflux-mediated macrolide resistance in S. pyogenes is inducible. In agreement with previous genetic data suggesting that the product of both genes contributes to the overall resistance to macrolides, mef(A) and msr(D) are co-transcribed as a single bicistronic mRNA.

**P729** Impact of inoculum on the antibacterial effect of levofloxacin and moxifloxacin against S. pneumoniae strains with defined resistance mechanisms studied in an in vitro model of infection

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Objectives: S. pneumoniae (Sp) bacterial loads may be very high in pneumonia. Fluoroquinolones (FQ) are thought to be unaffected by bacterial inoculum, however, the long term impact on antibacterial effect of high bacterial loads is rarely studied in pre-clinical models as it is technically difficult to sustain Sp growth over a period of days. We used an in vitro pharmacokinetic model of infection to study the ABE of two FQs at inocula of 10^6 CFU/ml (standard) and 10^8 CFU/ml (high) on a range of Sp with defined FQ resistance mechanisms over a 96h period.

Methods: Five Sp strains: a wild type levofloxacin/moxifloxacin (levo/moxi) MIC 1.5/0.38mg/L; efflux phenotype levo/moxi MIC 0.75/0.38mg/L; parC+ gyrA mutation levo/moxi MIC 1.5/1.5mg/L; parC + gyrA mutation levo/moxi MIC 6/2mg/L; parC + gyrA mutation levo/moxi MIC 6/4mg/L. Free drug concentrations associated with levo 750mg 24hly, levo 500mg 12hly and moxi 400mg 24hly over 96h were simulated. A standard (10^6 CFU/ml) and high inoculum (10^8 CFU/ml) were used.

Results: The log changes after 24h and 96h drug exposure are shown in the table. As 10^6 CFU/ml is the maximum sustainable bacterial density growth in the in vitro model above this inoculum does not occur. High inoculum resulted in slower clearance of wild type Sp from the model and regrowth occurred with levo. With strains with mutation in the QRDR, little or no killing occurred at high inocula, while at standard inocula early reduction in count was noted with the parC strain and one parC + gyrA strain.

Conclusion: Inoculum impacts on moxi and levo antibacterial effect. Levo is more subject to inoculum effects against wild type and efflux strains. Both moxi and levo show an inoculum effect with strains with QRDR mutations.

| Strain | Inoculum | M 24h | 96h | L750 24hly | 96h | L500 12hly | 96h |
|--------|-----------|-------|-----|----------|-----|----------|-----|
| Wild type (WT) | 10^6 | 4.0 | 4.0 | 4.2 | 4.2 | 4.1 | 4.1 |
| Efflux | 10^6 | - | - | 4.1 | 4.1 | 4.1 | 4.1 |
| parC | 10^6 | - | - | - | - | - | - |
| parC + gyrA | 10^6 | - | - | - | - | - | - |

K. pneumoniae: the worst threat amongst Enterobacteriaceae?

**P730** Closely related carbapenem-resistant K. pneumoniae from hospital inpatients and community in Mid-West of Ireland

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Objectives: The worldwide emergence of carbapenem resistant Enterobacteriaceae is a major public health problem. Of particular concern is the rapid international dissemination of KPC producing Klebsiella pneumoniae. The aim of this project was to screen all Enterobacteriaceae isolated at the Mid-Western Regional Hospital for carbapenemase production and to characterise carbapenem resistant Enterobacteriaceae (CRE) by phenotypic and molecular methods.

Methods: All Enterobacteriaceae collected from all specimen types since January 25th 2010 were screened for carbapenemase production in accordance with Clinical Laboratory Standards Institute (CLSI)
Antimicrobial resistance in K. pneumoniae isolates from hospitalised and outpatients in Germany, 2009

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Objectives: A major finding of the European Antimicrobial Resistance Surveillance Network (EARS-Net) Report 2009 is the high prevalence of resistance in invasive K. pneumoniae isolates from hospitalised patients to third-generation cephalosporins (3GC), fluoroquinolones (FQ) and aminoglycosides (AG) and the high level of combined resistance to these classes in most European countries. Data from the German Antimicrobial Resistance Surveillance (ARS) System are used to extend the study of resistance to isolates from urine and respiratory samples as well as to outpatients.

Methods: Analysis is based on non-duplicate isolates of K. pneumoniae collected in 2009 by nine laboratories covering 160 hospitals and 865 practices. Species identification and antimicrobial susceptibility testing is performed by VITEK 2, results are evaluated according to CLSI guidelines. Isolates are classified as resistant to an antibiotic class if they show resistance to one of its agents: 3GC: ceftazidime or cefotaxime, FQ: ciprofloxacin or levofloxacin AG: gentamicin or tobramycin. The distinct class resistances are combined to show resistance to isolates from urine and respiratory samples as well as to outpatients.

Results: The sample is composed of 5,548 non-duplicate K. pneumoniae isolates from inpatients (blood: 299; respiratory samples: 1,118; urine: 4,131) and 2,666 isolates from outpatients (respiratory samples: 173; urine: 2,493). Results are displayed in table 1. Proportions of resistance are highest against FQ in all subsets ranging from 21.1% in blood cultures from inpatients to 4.6% in respiratory samples from outpatients followed by 3GC (16.4% to 3.5%) and AG (13.4% to 2.3%). Regarding resistance against the three antibiotic classes simultaneously reveals that triple resistance is the most frequent pattern in all inpatient subsets reaching 10% in blood cultures, 9.3% in respiratory samples and 6.8% in urine. In outpatient samples, single resistance against FQ is the most frequent pattern followed by triple resistance in second place accounting for 3.0% in urine samples and 1.2% in respiratory samples.

Conclusions: Surveillance limited to invasive isolates from hospitalised patients like EARS-Net captures the sector with highest levels of antimicrobial resistance in K. pneumoniae; the extended approach of ARS reveals an emerging problem in outpatient care that physicians should be aware of, even if resistance proportions might be overestimated as samples are more likely to be taken from pre-treated patients.

Table 1: Resistance to antimicrobial classes in invasive K. pneumoniae isolates from hospitalised and outpatients in Germany 2009: proportions of resistance against fluoroquinolones, third-generation cephalosporins and aminoglycosides and frequencies of combined resistance to these antibiotic classes stratified by origin, blood culture, respiratory and urine samples

| Resistance pattern | Blood | Respiratory | Ure | Resistance to antibiotic class |
|--------------------|-------|-------------|-----|-------------------------------|
|                      |       |             |     | Fluoroquinolones (FQ)         |
| Single resistance    | 75.3  | 82.4        | 95.6| FQ                            |
| 3GC                 | 5.0   | 4.5         | 3.0 | 3GC                           |
| AG                  | 1.7   | 1.5         | 1.2 | AG                            |
|                     | 0.0   | 0.1         | 0.4 | AG                            |
|                     | 7.9   | 4.2         | 6.1 | AG                            |
| Double resistance    | 3.5   | 0.9         | 2.2 | 3GC and AG                    |
|                     | 3.7   | 2.7         | 2.3 | 3GC and AG                    |
|                     | 2.3   | 0.3         | 0.6 | 3GC and AG                    |
| Triple resistance    | 1.0   | 1.0         | 0.9 | 3GC, AG and FQ                |

P732 Antimicrobial susceptibility patterns associated with European KPC producers

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Background: In recent years carbapenem resistance has emerged among Gram-negative isolates due to the acquisition of carbapenemases, which usually belong to Ambler class B metallo-β-lactamases (MBLs) or to KPC-type enzymes. Klebsiella pneumoniae carbapenemase (KPC) is an Ambler class A β-lactamase that confers resistance to all β-lactam agents, including carbapenems, cephalosporins, penicillins, and the monobactam aztreonam. Although this enzyme has been found primarily in K. pneumoniae, it has also been identified in several other Gram-negative bacilli. Because the blaKPC gene is carried on a plasmid, the ease of mobility of this resistance mechanism is of concern and represents a major threat to the antimicrobial treatment of infections with Gram-negative organisms. As part of the Tigecycline European Surveillance Trial (TEST), this study investigated the incidence and susceptibility profiles of KPC producing Gram-negative isolates from Europe during 2009–10.

Methods: 11,316 Enterobacteriaceae isolated in Europe in 2009–10 had minimum inhibitory concentrations (MICs) determined using broth microdilution following CLSI guidelines and interpreted according to EUCAST breakpoints where available. A total of 148 isolates with meropenem MICs of ≥2 mg/L were screened for the presence of KPC genes using multiplex-PCR.

Results: Of 11,316 Enterobacteriaceae isolated in Europe in 2009–10, 148 (1.3%) had meropenem MICs of ≥2 mg/L. 52 (35%) of these 148 meropenem non-susceptible isolates were positive for KPC genes.
All KPC positive isolates were \emph{K. pneumoniae}. Susceptibility of these isolates is shown in the table.

**Conclusions:** The meropenem non-susceptible rate in European 
Enterobacteriaceae from the TEST study in 2009–10 was 1.3%, with 35% of these (0.5%) positive for KPC. The most active antimicrobial against these isolates in vitro was tigecycline with an MIC90 of 1 mg/L, followed by minocycline, with an MIC90 of 8 mg/L. All other antimicrobials exhibited susceptibilities of <20%.

**Objective:** We noted increasing ertapenem-resistant (ETP-R) extended-spectrum \(\beta\)-lactamase (ESBL) positive \emph{Escherichia coli} (EC) and \emph{Klebsiella pneumoniae} (KP) (ETP-R EC/KP) at our hospital. The objective of this study is to assess risk factors for and treatment outcome of ETP-R EC/KP.

**Method:** We conducted a retrospective case-control (1 to 3 ratio) from 1 January 2006 to 30 June 2009. Minimum inhibitory concentration (MIC) by E-test, multiplex polymerase chain reaction for CTX-M, SHV, TEM and AmpC, and genotyping for metallo-\(\beta\)-lactamase and KPC were performed.

**Results:** Of 12 cases, 10 were KP and 2 were EC. All 12 isolates produced CTX-M-13 ESBL (91% group 1); 41% carried SHV-11 and 33% carried TEM-1 genes. Carbapenemases and clonality were not detected. Compared with the 36 controls, the 12 cases were younger (median age, 64 vs 75 years, \(P < 0.03\)) than controls but had similar \emph{P. aeruginosa} bacteraemia (2.8 vs 3.2), Charlson's (6.3 vs 6.6) and APACHE II (13.9 vs 14.9) scores (\(P > 0.05\)). On univariate analysis, ETP-R cases were significantly associated with IPM (\(P = 0.005\)), MEM (\(P = 0.032\)) and ETP (\(P < 0.001\)) exposure, and a history of multidrug-resistant organisms (\(P = 0.001\)) in the preceding 3 months. On multivariate analysis, meropenem was the only independent predictor of ETP-R EC/KP (adjusted OR 29.1, 95%CI 1.8–465.0, \(P = 0.017\)). There was no significant difference in mortality (0% vs 13.5%), mean days to defervescence (3.9 days vs 3.0 days) or median length of hospital stay (3.9 days vs 3.0 days) between ETP-R and ETP susceptible EC/KP isolates (\(P > 0.05\)). Of the cases, 41.7% were treated with IPM or MEM only and 25% were treated with a combination of IPM or MEM with amikacin or polymyxin B. Clinical response was 100% and mortality was 0% with IPM or MEM monotherapy with MIC <1.

**Conclusion:** Meropenem use within 3 months was an independent predictor for ETP-R EC/KP. Ertapenem resistance in KP/EC was mediated by a combination of ESBL and AmpC enzymes. IPM or MEM monotherapy remained effective for ETP-R KP/EC with MIC <1.

**Objective:** The aim of the study was to evaluate the epidemiology of carbapenem resistant \emph{Klebsiella} isolates in our hospital.

**Methods:** Consecutive \emph{Klebsiella} isolates recovered between January 2009 and September 2010 were studied. \emph{Klebsiella} isolates were tested by cefotaxime and cefotaxime-clavulanat combined disk diffusion method for ESBL detection. Ertapenem disks were used for screening carbapenem resistance. Ertapenem E-test was used for confirmation of carbapenem resistance. A modified Hodge was performed in all resistant isolates. MBL E-test (IPM/IPM-EDTA) was used to detect metallo-\(\beta\)-lactamase production, and imipenem – boronic acid double disk synergy method was used to detect KPC carbapenemase production.

**Results:** Totally 283 \emph{Klebsiella} spp. isolated from nosocomial infections were enrolled in the study. ESBL positivity was detected in 61 (21.5%) strains. Overall, 31 of them (10.95%) were resistant to ertapenem. Only two isolates were carrying both carbapenemase and ESBL type resistance together. ESBL positivity was statistically higher among the ICU isolates, but carbapenem resistance did not differ between the units. Among the ertapenem resistant isolates, MBL production was determined in 15/31 (48.3%) isolates, and KPC carbapenemase in only one isolate (3.2%). There was no statistical difference in carbapenem resistance among the isolates regarding to units, clinical samples and type of \emph{Klebsiella} isolates. Carbapenem-resistant \emph{Klebsiella} isolates were also resistant to 3rd generation cephalosporins (96%), ciprofloxacin (96%) and piperacillin–tazobactam (100%). Tigecycline resistance was detected in 32% of the carbapenem resistant isolates. Colistin resistance was not detected in the study.

**Conclusions:** Our results show that carbapenem resistance among \emph{Klebsiella} isolates is an important problem in our hospital, and requires urgent application of proper infection control measures. Major carbapenem resistance mechanism among \emph{Klebsiella} isolates was the production of MBL in our study.
Mortality of 8.3% and infection mortality 23.5%. Co-production of both MBL and KPC was detected in two cases. Susceptibility testing showed that 44 (91.7%) KPC-KP isolates were susceptible to colistin, 37 (77.1%) to tigecycline and 41 (85.4%) to gentamicin.

Conclusion: Urinary acquisition of KPC-KP was observed in patients with prior hospitalizations, urinary tract comorbidities and indwelling catheters. Regarding that it usually represents colonization, thorough evaluation of clinical data is required before administration of antimicrobial therapy.

**P737** Spread of a plasmid-mediated carbapenem hydrolysing OXA-48 β-lactamase in *Klebsiella pneumoniae* in a tertiary teaching hospital, Spain

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**Objectives:** The most common mechanism for carbapenem resistance in *Klebsiella pneumoniae* (KPN) is the production of carbapenemases belonging to Ambler class A, B or D. The oxacillinase OXA-48 was first identified in a KPN isolate in Turkey. In this study we describe the spread of a single clone of KPN producing OXA-48 in Spain.

**Methods:** Twenty non-repetitive KPN isolates with reduced susceptibility to carbapenems were studied. Antimicrobial susceptibility testing was performed by BD Phoenix and Etest. The Hodge test was used to screen for carbapenemase production. PCR and sequence analysis for carbapenemases (blaOXA-48 and blaKPC), ESBLs and plasmidic AmpC cephalosporinases was performed. Clonality was evaluated by REP-PCR. Conjugation experiments with *E. coli* J53 and plasmid analysis were carried out. Outer membrane proteins (OMPs) profiles were analyzed to study their contribution to carbapenem resistance.

**Results:** All strains were Hodge test positive. Eleven strains were resistant to all antibiotics except cefotixin, fosfomycin, tigecyclin and colistin, and showed low level resistance to carbapenems: Imipenem (IMI) (0.75−4 mcg/ml), meropenem (MER) (0.75−4 mcg/ml), ertapenem (ETP) (3−32 mcg/ml) and doripenem (DOR) (1−2 mcg/ml). Nine strains were resistant to cefotixin, and showed high level resistance to carbapenems: IMI (0.75−4 mcg/ml), meropenem (MER) (0.75−4 mcg/ml), ertapenem (ETP) (3−32 mcg/ml). PCR and sequencing identified the presence of OXA-48, CTX-M-15 and SHV-1. *E. coli* transconjugant was susceptible to all antibiotics except ampicillin, amoxicillin-clavulanate and piperacillin-tazobactam and showed higher MIC of carbapenems: IMI 0.5 mcg/ml, MER 0.125 mcg/ml, ETP 0.125 mcg/ml and DOR 0.125 mcg/ml compared to the recipient strain. Plasmid analysis revealed that *E. coli* transconjugant harboured a circa 50-kb plasmid. Indistinguishable REP-PCR patterns were found suggesting that all strains belong to the same clone. OMP analysis revealed that low level resistant carbapenem strains produced OmpK36, whereas high level resistant strains did not.

**Conclusions:** Our study identified the first outbreak of OXA-48-producing KPN in Spain. This identification of KPN isolates carrying the worldwide spread CTX-M-15 ESBL as well as the OXA-48 carbapenemase (and more importantly OmpK36 deficient strains) is worrisome since carbapenems are often the last resort for treating infections caused by ESBL producing strains.

**P739** Clinical and epidemiological study of an outbreak of KPC-producing *Klebsiella pneumoniae* infection in Buenos Aires, Argentina

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**Objectives:** To describe an outbreak of KPC-producing *Klebsiella pneumoniae* (Kpn–KPC) at the “Cosme Argerich” Hospital, Buenos Aires, Argentina.

**Methods:** Prospective and descriptive study of an outbreak of Kpn–KPC infection. Susceptibility tests were performed by disk diffusion (CLSI) and MIC (Vitek 2-Biomerieux). Preliminary phenotypic detection of KPC was based on the boronic acid disk synergy test. Presence of the

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KPC-producing *Klebsiella pneumoniae* strains (CRKP) isolated in a tertiary hospital from January to October 2010 and investigate the epidemiology and the additional resistance mechanisms present.

**Material and Methods:** From January to September 2010, fourteen CRKP were isolated in our hospital, from different patients with hospital-acquired infection, whose records were retrospectively investigated. Bacterial identification and antibiotic susceptibility testing were initially performed using the Vitek2 automated system (BioMerieux). MICs of colistin were carried out by Etest (AB, Biodisk). Resistance to colistin was confirmed by PCR. REP-PCR was performed to investigate the clonal spread of isolates.

**Results:** All patients (n = 14) had received colistin iv, for at least 7 days before CRKP isolation. Twelve were ICU patients (86%), whereas the remaining two patients referred previous ICU hospitalization. Six CRKP were isolated from blood culture, 4 from iv catheter, 2 from urine, 1 from bronchial secretions and 1 from pleuritic fluid. All isolates showed additional resistance to β-lactams and carbapenems. Forty-three percent were susceptible to amikacin, 29% to gentamicin and 50% to tigecycline. All strains were carbapenemase producers, with two isolates harboring the blaKPC and one both genes. Four of the KPC-positive strains were also ESBL-producers. REP-PCR proved the clonal profile of CRKP isolates. However the four KPC (+) ESBL (+) CRKP strains were of the same clone.

**Conclusion:** Colistin resistance proves to be most common among KPC producing *K. pneumoniae* than in VIM (+) strains. The emergence of colistin-resistant *K. pneumoniae* is most possibly the result of antibiotic selective pressure. The excessive use of colistin, in addition to the endemic spread of MDR *K. pneumoniae*, results to a serious threat of infections. Measures should be taken to avoid further dissemination.

**P738*** Klebsiella pneumoniae* in a cohort of urinary tract infections: clinical features, molecular epidemiology and outcomes

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**Objectives:** *Klebsiella pneumoniae* isolates producing KPC enzymes (KPC-KP) have become increasingly prevalent in Greek hospitals. These organisms, usually causing systemic infections, have been also implicated in urinary tract infections. We present the results of a cohort study regarding urinary acquisition of KPC-KP.

**Methods:** During a 2.5-year period (May 2008–October 2010) all single-patient *K. pneumoniae* isolates recovered from urine cultures and exhibiting elevated carbapenems’ MICs (>1 mg/L) were screened for carbapenemase production by phenotypic assays (EDTA and phenylboronic acid combined-disc tests) and PCR. Clinical and laboratory records were reviewed.

**Results:** A total of 48 patients (29 females, mean age 70.7±19.2 years) harbouring KPC-KP urinary isolates were identified. The majority of patients were hospitalized at medical wards (31/48, 64.6%). The mean time from admission to KPC-KP isolation was 9.7±13 days; half of the patients (24/48) yielded KPC-KP within 48 hours of hospitalization, indicating that KPC-KP was not acquired intrahospitalily. Of those patients, 20 had hospitalizations within the past 3 months, three were hospitalized 4−9 months ago and one had no hospital admissions but received prolonged quinolone treatment. Prior surgery were noted for 26/48 (54.2%) patients. Prior rectal colonization was detected in 17 (35.4%) patients. Among comorbidities, urinary tract diseases and diabetes mellitus prevailed (31/48 and 17/48, respectively). Prior antibiotic exposure analysis revealed that most of the patients had received β-lactams/β-lactamase inhibitors combinations (18/48) and fluoroquinolones (17/48). Most patients (41/48, 85.4%) had indwelling catheters. Evidence of clinical infection was documented for 17 (35.4%) patients and 4 of them developed secondary bacteraemia. Tigecycline was mostly used as treatment regimen, either alone or in combinations. Overall mortality was 8.3% and infection mortality 23.5%. Co-production of both MBL and KPC was detected in two cases. Susceptibility testing showed that 44 (91.7%) KPC-KP isolates were susceptible to colistin, 37 (77.1%) to tigecycline and 41 (85.4%) to gentamicin.

**Conclusion:** Urinary acquisition of KPC-KP was observed in patients with prior hospitalizations, urinary tract comorbidities and indwelling catheters. Regarding that it usually represents colonization, thorough evaluation of clinical data is required before administration of antimicrobial therapy.
K. pneumoniae: the worst threat amongst Enterobacteriaceae?

blakPC was confirmed by PCR and DNA sequencing. Molecular typing was performed by PFGE.

**Results:** From August 2009 through July 2010, 27 patients were infected by KPC-2-producing \textit{K. pneumoniae} (surgical care unit: n = 8, medical care unit: n = 6, intensive care unit: n = 5, emergency care unit: n = 4, and other: n = 4). All Kpn-KPC isolates belongs to a single clonal type. Age (median): 67 years (range: 25–91 years). Female sex: 70%. All the cases except the index one had a previous contact with a patient with a Kpn-KPC infection or colonization. All infections except one were nosocomially acquired, with the patients being hospitalized for a median of 31 days (range 6–114 days) prior to isolation of the organism. Site of infection: urinary tract (59%), respiratory tract (14%), intraabdominal (14%), bloodstream (7%), bone (3%) and central venous catheter (3%). Main comorbidities: diabetes mellitus (30%), malignancy disease (23%), cardiopathy (23%) and malnutrition (19%). Most frequent risk factors for Kpn-KPC infection: Urinary catheter (93%), chronic \(\beta\)-lactaemia (52%) and mechanical respiratory assistance (30%). All the patients had prior exposure to antibiotics (median of 30 days; range 11–54 days), being piperacillin-tazobactam (62%), cephalosporins (48%) and carbapenems (33%) the most frequently antibiotics prescribed. All the isolates were only susceptible to colistin and tigecycline. Inappropriate empirical treatment was initiated in a 63%. Specific antibiotics prescribed. All the isolates were only susceptible to colistin and tigecycline. Inappropriate empirical treatment was initiated in a 63%. Specific treatment for Kpn-KPC infection: colistin (74%), tigecycline (4%), tigecycline + colistin (22%). Global mortality: 59% (attributable mortality: 26%).

**Conclusions:** A wide nosocomial spread of Kpn-KPC with a high mortality rate was observed. A previous contact with a patient with a Kpn-KPC infection or colonization, prior exposure to antibiotics and the presence of comorbidities was seen in almost all cases. Kpn-KPC infection creates an important challenge for clinicians to identify risk factors in order to initiate an appropriated empirical treatment.

**Analysis of \textit{Klebsiella pneumoniae} strains with non-enzymatic carbapenem resistance isolated in the Czech Republic, Poland and Russia**

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**Objectives:** The objectives of this study was to investigate the molecular basis of carbapenem resistance in a set of carbapenemase-negative \textit{Klebsiella pneumoniae} strains collected in the Czech Republic, Poland and Russia, and to assess their possible clonality in order to check for eventual clone-associated risk factors of the development of this resistance type.

**Methods:** The study was carried out on 30 \textit{K. pneumoniae} strains from the Czech Republic (n = 12), Poland (n = 7) and Russia (n = 11). MICs of 24 antimicrobials were determined according to the EUCAST methodology. Imipenem hydrolysis activity was assayed by spectrophotometry in crude protein extracts in order to exclude carbapenemase activity. Isoelectric focusing and bioassay were performed to determine \(\beta\)-lactaemia (46%), central venous catheter (33%) and mechanical respiratory assistance (30%). All the patients had prior exposure to antibiotics (median of 30 days; range 11–54 days), being piperacillin-tazobactam (62%), cephalosporins (48%) and carbapenems (33%) the most frequently antibiotics prescribed. All the isolates were only susceptible to colistin and tigecycline. Inappropriate empirical treatment was initiated in a 63%. Specific treatment for Kpn-KPC infection: colistin (74%), tigecycline (4%), tigecycline + colistin (22%). Global mortality: 59% (attributable mortality: 26%).

**Conclusions:** A wide nosocomial spread of Kpn-KPC with a high mortality rate was observed. A previous contact with a patient with a Kpn-KPC infection or colonization, prior exposure to antibiotics and the presence of comorbidities was seen in almost all cases. Kpn-KPC infection creates an important challenge for clinicians to identify risk factors in order to initiate an appropriated empirical treatment.
**Early stage of dissemination of KPC-producing Enterobacteriaceae in Poland (2008–2009)**
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**Objectives:** To characterize Enterobacteriaceae isolates producing KPC-type carbapenemases identified in Poland in 2008–2009.

**Methods:** 119 non-repetitive KPC isolates of *K. pneumoniae* (n = 115), *K. oxytoca* (n = 2) and *E. coli* (n = 2) from 2008–2009 were analyzed. These were identified in 18 hospitals, including 12 in Warsaw. The isolates were derived from infections, mainly UTIs (n = 61), invasive infections (n = 22) and RTIs (n = 8), or from colonization (n = 22). KPCs were identified by PCR and sequencing. Typing was performed by PFGE and MLST. Plasmid profiles of 46 isolates and was conjugative. Another transferable plasmid, the blaKPC-2 gene in the Tn4401a element; one isolate had blaKPC-3 gene. The remaining 70kb plasmid was conjugative. AblaKPC-2 gene was associated with Tn4401a isofrom and located on a ca. 75-kb self-conjugative plasmid. The three isolates were genetically related by PFGE and belonged to ST-258.

**Conclusion:** This study confirms the widespread spread of ST-258 clone that has predominantly been identified among KPC-2 and KPC-3 KP-producers. It underlines that even with strict hygiene precautions silent spread of KP-KPC producing isolates may occur and lead to hospital-acquired infections.

**Results:** In August 2010, an 85-year-old woman was transferred from an Italian hospital to the cardiology ward of a South Paris hospital. After the 1st report in May 2008, by the end of 2008 converted into endemic situations, being a source of transmission to other wards. PCR and sequencing identified blaKPC-3 and blaTEM-1 genes. In vitro transfer of Oxa-48 was tested by transformation of plasmid DNA into E. coli.

**Conclusions:** In a short time Poland has become one of the most KPC-affected European countries, with Warsaw being the epicenter of dissemination. Numerous actions were undertaken, including education, and issuing guidelines for detection and infection control, aimed at controlling the situation.
Antimicrobial susceptibility testing

Conclusion: The present study identified the first outbreak of K. pneumoniae isolates producing the carbapenemase OXA-48 in France. Isolates identified here were not clonally related to the previously identified OXA-48-positive K. pneumoniae strains, but a very similar 70-kb plasmid harboring blaOXA-48 was identified, underlying that spread of the blaOXA-48 gene may be associated to spread of a single plasmid type. Outbreaks of OXA-48 K. pneumoniae producers are now identified in Western Europe after those producing other types of carbapenemases (KPC-2, VIM-1 and NDM).

Antimicrobial susceptibility testing

Correlation between EUCAST disk diffusion and the standardised broth microdilution method

S. Bengtsson*, C. Bjelkenbrant, G. Kahlmeter (Växjö, SE)

Objectives: To evaluate the correlation between zone diameters and MIC values using EUCAST disk diffusion and broth microdilution for clinical relevant species.

Methods: A collection of stored clinical strains comprising of 185 Gram negatives and 177 Gram positive isolates (see table) were tested in parallel with EUCAST disk diffusion and broth microdilution (TREK). Seven to 14 antibiotics were evaluated for Enterobacteriaceae (n = 14), Pseudomonas (n = 8), Staphylococci (n = 12) and Enterococci (n = 7). Special attention was given to MRSA, ESBL, VRE and HLAB. Category (SIR) interpretations of zone diameters and MIC were done according to EUCAST clinical breakpoints (version 1.2, December 21, 2010). Reference strains S. aureus ATCC 29213, ATCC 43300, E. faecalis ATCC 29212, E. coli ATCC 25922, K. pneumoniae ATCC 700603 and P. aeruginosa ATCC 27853 were included as appropriate on each day of testing.

Results: The results are summarised in the table. Overall category agreement was 97.5% for 1641 tests of Gram positives and 95.5% for 2374 tests of Gram negatives. Cefoxitin correctly identified all S. aureus MRSA and MSSA. In Staphylococci one very major error (VME) was seen for moxifloxacin, ten VME for clindamycin (all were inducible clindamycin resistance not detected by the MIC-test). For E. faecium one VME was seen for gentamicin and five major error (ME) for nitrofurantoin (all because the zone diameter breakpoint bisects the natural distribution) and two ME for ampicillin. Among Gram negatives one VME was obtained for piperacillin/tazobactam mE were predominantly for aztreonam (n = 9) and piperacillin/tazobactam (n = 5).

Conclusions: The EUCAST disk diffusion method could accurately predict S, I and R-categorization based on standardized microbroth dilution MIC-values and EUCAST clinical MIC-breakpoints. Minor problems were mostly due to inducible clindamycin resistance in Staphylococci not being detected by the MIC method and the fact that the breakpoint for nitrofurantoin in E. faecium bisects the wild type distribution of this species.

EUCAST MIC breakpoints and inhibition zone diameter correlates for staphylococci

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Objectives: During 2009 and 2010, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) developed a disk diffusion method calibrated to the harmonized EUCAST MIC breakpoints. Zone diameter breakpoints were published at EUCAST website in December 2009, and have been tentative during 2010. The objective of this study was to present correlates between MIC values and inhibition zone diameters in Staphylococcus spp., and to present new breakpoints for mupirocin and S. aureus.

Methods: For S. aureus, a total of 310 isolates with MIC values close to EUCAST clinical MIC breakpoints were selected from the SENTRY collection (MI Laboratories, USA). For coagulase-negative staphylococci (CoNS), 155 isolates were collected from several sites in Europe and the USA, including S. epidermidis (68), S. capitis (30), S. hominis (21), S. haemolyticus (16), S. cohnii (7), S. lugdunensis (5), S. simulans (4) and S. warneri (4). Disk diffusion was performed according to EUCAST methodology. MIC values were determined by broth microdilution with custom panels from TREK Diagnostics for S. aureus and with Etest (bioMérieux) for CoNS. All antibiotic agents with clinical breakpoints in EUCAST tables were investigated (cefoxitin is reported elsewhere). Mupirocin was tested using 5, 20 and 200 μg disks.

Table 1 EUCAST Clinical Breakpoints for Staphylococcus spp.

| Antibiotic agent | MIC breakpoint (μg) | Disk diameter breakpoint (mm) |
|------------------|---------------------|-----------------------------|
| S. aureus        |                     |                             |
| 5                | 10                  |                            |
| 10               | 20                  |                            |
| 20               | 30                  |                            |
| 30               | 40                  |                            |
| 40               | 50                  |                            |
| 50               | 60                  |                            |
| 60               | 70                  |                            |
| 70               | 80                  |                            |
| 80               | 90                  |                            |
| 90               | 100                 |                            |

Results: The correlation between inhibition zone diameters and MIC values was excellent. Isolates categorized as wild type by MIC were categorized as wild type by inhibition zone diameters. The median values of the wild type zone distributions were 0–6 mm lower for S. aureus than for CoNS, but MIC correlates supported common zone diameter breakpoints for most antibiotics (Table 1). However, for aminoglycosides,
the zone/MIC correlates indicated a need for separate zone diameter breakpoints for *S. aureus* and CoNS. Disk diffusion interpretation of >2000 *S. aureus* zone/MIC determinations resulted in 4 very major errors (mainly fusidic acid) and 10 major errors (mainly trimethylproprin). For >150 CoNS correlates, there were 2 very major errors (tetracycline and trimethoprin). For mupirocin, the 200 ug disk most reliably predicted resistance in *S. aureus* (MIC >256 mg/L).

**Conclusion:** For staphylococci, a review of the correlation between MIC values and inhibition zone diameters resulted in minor revisions of zone diameter breakpoints for several antibiotics. The EUCAST zone diameter breakpoints are now well calibrated to the current EUCAST MIC breakpoints for both *S. aureus* and CoNS.

### P748 Screening for β-lactam resistance in Streptococcus viridans group with the EUCAST disk diffusion method

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**Objectives:** Viridans (non *H. influenzae*) streptococci are usually considered weak pathogens but can cause invasive disease, in which case β-lactam antibiotics, often in combination with an aminoglycoside, are important therapeutic agents. The objective of this study was to develop inhibition zone diameter breakpoints for viridans streptococci and β-lactam antibiotics correlated to EUCAST harmonized MIC breakpoints, and to evaluate the benzylpenicillin 1 unit disk as screen for β-lactam resistance.

**Methods:** Antimicrobial susceptibility testing was performed on a collection of 89 viridans streptococci, including *S. anginosus* (S), *S. bovis* (1), *S. gordoni* (2), *S. intermedius* (1), *S. mitis* (30), *S. mutans* (1), *S. oralis* (20), *S. salivarius* (7), *S. sanginis* (7), *S. vestibulitis* (1) and 14 non-speciated isolates. Disk diffusion was performed on Mueller-Hinton agar with 5% defibrinated horse blood and 20 mg/L β-lactam (MH-F) according to EUCAST methodology for benzylpenicillin (PCG) 1 unit, ampicillin (AMP) 2 ug, cefotaxime (CTX) 5 ug, cefuroxime (CMX) 30 ug and mepenoxin (MER) 10 ug. MIC determination was performed with Etest (bioMérieux) on Mueller-Hinton agar with 5% defibrinated horse blood.

**Results:** A PCG disk diffusion breakpoint of S ≥18 mm and R <12 mm reported isolates with PCG MIC 2–8 mg/L as resistant; S; *S. oralis* (3), *S. gordoni* (2) and *S. vestibulitis* (1). Of these, four had elevated MIC values for CTX and CMX and all five had MIC values above the ECOFF for MER. For CTX and MER, all susceptible isolates were reported susceptible with the PCG 1 unit disk. One isolate resistant to CMX (MIC 1 mg/L) was reported as susceptible with both PCG and CMX disks, and two isolates intermediate to PCG (MIC 0.5 mg/L) were reported as susceptible with the PCG disk.

**Conclusion:** EUCAST zone diameter breakpoints for *Streptococcus viridans* group and β-lactam antibiotics are well calibrated to the MIC breakpoints and can be used to predict susceptibility to β-lactam agents. Benzylpenicillin 1 unit is a sensitive screening disk for the detection of β-lactam resistance in viridans streptococci.

### P749 EUCAST proposed new MIC breakpoints for Moraxella catarrhalis and corresponding zone diameter breakpoints

J. Åhman*, E. Matuschek, G. Kahlmeter (Växjö, SE)

**Objectives:** *Moraxella catarrhalis* was long considered a harmless commensal of the upper respiratory tract, but has been recognized as a true pathogen in the last decades. The increasing prevalence of β-lactamases has also renewed interest in this species. Two types of β-lactamases have been identified, BRO-1 (90–95% of isolates) and BRO-2. The BRO-1 enzyme is associated with a higher level of penicillin resistance and higher MICs. Breakpoints for *M. catarrhalis* were originally derived from Haemophilus influenzae, but EUCAST has proposed separate breakpoints for *M. catarrhalis* in 2011. The objective of this study was to compare current and suggested new MIC breakpoints and to establish corresponding zone diameter breakpoints for *M. catarrhalis*.

**Methods:** A total of 106 consecutive clinical isolates of *M. catarrhalis* were collected from Kronoberg County, Sweden. Species identification was confirmed with MALDI-TOF analysis. All isolates were examined for β-lactamase by using the nitrocefin disk. Disk diffusion was performed on Mueller-Hinton agar supplemented with 5% horse blood and 20 mg/L β-lactam (MH-F) according to EUCAST methodology. MIC determination using E-test (bioMérieux) was performed on isolates with inhibition zones at the lower end of the wild-type distribution. All antibiotic agents with clinical breakpoints in EUCAST tables and benzylpenicillin and nalidixic acid were tested.

**Results:** Of the 106 *M. catarrhalis* isolates, 103 (97%) were β-lactamase positive. Zone diameter distributions were bimodal for benzylpenicillin, ampicillin, amoxicillin-sulbactam, amoxicillin, amoxicillin-clavulanate, cefixime, cefotaxime, cefibuten and ceftriaxone. For several cephalosporins (cefalexin, cefepime, cefpodoxime and cefuroxime), the current MIC breakpoints divided the wild-type distributions. An analysis of all MIC breakpoints and wild-type distributions clearly showed that species-specific breakpoints for *M. catarrhalis* are required. EUCAST current and proposed breakpoints for *M. catarrhalis* are presented in Table 1.

**Table 1. EUCAST proposed new clinical breakpoints for Moraxella catarrhalis.**

Proposed breakpoints highlighted in grey, with current breakpoints in parenthesis.

| Antimicrobial agent | MIC breakpoint (mg/L) | Disk diffusion breakpoint | Zone diameter breakpoint (mm) |
|---------------------|-----------------------|--------------------------|-----------------------------|
| Ampicillin          | ≤0.12 ≤0.06          | ≤0.12 ≤0.06              | ≤12 ≤6                      |
| Amoxicillin-sulbactam| 1 ± 1                | ≤2                       | ≤14 ≤7                      |
| Cefixime            | 1 ± 0.5              | ≤2                       | ≤14 ≤7                      |
| Ceftriaxone         | 2 ± 1                | ≤4                       | ≤18 ≤11                     |
| Cephalosporins      | 1 ± 0.5              | ≤2.5                     | ≤14 ≤11                     |
| Cefotaxime          | 1 ± 0.5              | ≤2.5                     | ≤14 ≤11                     |
| Cefuroxime          | 2 ± 0.5              | ≤4                       | ≤18 ≤11                     |
| Cefuroxime-sulbactam| 3 ± 0.5              | ≤6                       | ≤24 ≤15                     |
| Cefuroxime-sulbactam| 4 ± 0.5              | ≤8                       | ≤28 ≤17                     |
| Cefuroxime-sulbactam| 5 ± 0.5              | ≤10                      | ≤30 ≤19                     |
| Cefuroxime-sulbactam| 6 ± 0.5              | ≤12                      | ≤32 ≤21                     |
| Cefuroxime-sulbactam| 7 ± 0.5              | ≤14                      | ≤34 ≤22                     |
| Cefuroxime-sulbactam| 8 ± 0.5              | ≤16                      | ≤36 ≤23                     |
| Cefuroxime-sulbactam| 9 ± 0.5              | ≤18                      | ≤38 ≤24                     |
| Cefuroxime-sulbactam| 10 ± 0.5             | ≤20                     | ≤40 ≤25                     |
| Cefuroxime-sulbactam| 11 ± 0.5             | ≤22                     | ≤42 ≤26                     |
| Cefuroxime-sulbactam| 12 ± 0.5             | ≤24                     | ≤44 ≤27                     |
| Cefuroxime-sulbactam| 13 ± 0.5             | ≤26                     | ≤46 ≤28                     |
| Cefuroxime-sulbactam| 14 ± 0.5             | ≤28                     | ≤48 ≤29                     |
| Cefuroxime-sulbactam| 15 ± 0.5             | ≤30                     | ≤50 ≤31                     |

**Conclusion:** The EUCAST Steering Committee has proposed revised MIC breakpoints for *M. catarrhalis*. These are currently (Dec 2010) under consultation with national breakpoint committees in Europe. We present proposed zone diameter breakpoints, correlated to the new MIC.
Methicillin resistance in Staphylococcus spp. Validation of CLSI and EUCAST disk diffusion screening using cefoxitin
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Objectives: Methicillin resistance in staphylococci is mediated by the mecA gene. For S. aureus, several independent studies have shown cefoxitin to be a reliable predictor of mecA status, and hence the mecA gene. For coagulase-negative staphylococci (CoNS), the cefoxitin disk has been shown to be a better predictor of mecA status than MIC. Both CLSI and EUCAST have published cefoxitin screen breakpoints for S. aureus (R <22 mm or MIC >4 mg/L) and CoNS (R <25 mm). The specificity and sensitivity of the screen for CoNS has been questioned. The objective of this study was to evaluate the cefoxitin disk as a screen for methicillin resistance in Staphylococcus spp. using CLSI and EUCAST zone diameter breakpoints.

Methods: A total of 102 S. aureus with cefoxitin MIC values ≥2 mg/L were selected from the SENTRY collection (JMI Laboratories, USA). For CoNS, 150 species-identified isolates with known mecA status (85 mecA positive and 65 mecA negative) were collected from several sites in Europe and the USA, including S. epidermidis (68), S. capitis (30), S. hominis (21), S. haemolyticus (16), S. cohnii (7), S. simulans (4) and S. warneri (4). Disk diffusion with cefoxitin 30 μg was performed on all isolates according to EUCAST methodology and MIC values were determined with broth microdilution on custom-panels from TREK Diagnostics for S. aureus.

Results: Inhibition zone diameters for S. aureus isolates with MIC values ≥8 mg/L (n = 35) were 6–17 mm and for isolates with MIC values ≤4 mg/L (n = 67) 22–32 mm. For CoNS, 64/65 mecA negative isolates had inhibition zone diameters ≥25 mm and 84/85 mecA positive isolates had zone diameters <25 mm. One mecA negative isolate (S. cohnii) had a cefoxitin zone of 23 mm and one mecA positive isolate (S. epidermidis) had a cefoxitin zone of 27 mm. Repeated testing of isolates close to the breakpoint on Mueller-Hinton agar from different manufacturers showed that misinterpretation was due to either too light inocula or the agar per se. Discrepancies were not related to CoNS subspecies.

Conclusion: Disk diffusion with cefoxitin 30 μg and the use of separate zone diameter breakpoints for S. aureus and CoNS is a reliable screening method for the detection of mecA-mediated methicillin resistance in staphylococci. However, CoNS with inhibition zones of 25–28 mm must be retested if the inoculum is too light.

Table 1: Tentative ECOCFFs for Pasteurella multocida

| Antibiotic (agent) | MIC (mg/L) | Disk strength (μg) | Zone diameter (mm) |
|--------------------|------------|--------------------|--------------------|
| Benzylpenicillin    | 0.25, 1   | 1 unit             | 19                 |
| Amoxicillin         | 0.06, 2   | 2                  | 19                 |
| Amoxicillin-clavulanate | 0.5, 5   | 38                 | 25                 |
| Cefotaxime          | 0.06, 1   | 5                  | 27                 |
| Ciprofloxacin       | 0.06, 1   | 5                  | 27                 |
| Moxifloxacin        | 0.06, 1   | 5                  | 27                 |
| Nalidixic acid      | 0.12, 1   | 36                 | 75                 |
| Tetracycline        | 2         | 30                 | 24                 |
| Trimethoprim-sulfamethoxazole | 0.25 | 25 | 22 |

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Comparison of two disk diffusion-based screening methods for detection and classification of β-lactam resistance in Haemophilus influenzae
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Objectives: β-lactam resistance in Haemophilus influenzae is most commonly caused by β-lactamase and/or altered penicillin-binding protein 3 (PBP3). Isolates with PBP3 alterations (N526K or R517H) are denoted gBLNAR (genetic β-lactamase negative ampicillin resistant) or gBLPACR (genetic β-lactamase positive amoxicillin-clavulanate resistant), depending on β-lactamase status. Isolates without PBP3 alterations are denoted gBLNAS (genetic β-lactamase negative amoxicillin susceptible) or gBLPBR (genetic β-lactamase positive ampicillin resistant).

The objectives of this study were to compare the reliability of two disk diffusion-based screening methods for detection and classification of β-lactam resistant H influenzae according to resistance genotypes and resistance phenotypes: phenoxymethylpenicillin 10 mcg (PCV10) and benzylpenicillin 1 unit (PCG1), respectively, combined with β-lactamase detection and cefaclor 30 mcg (CEC30).

Methods: A collection of 196 well-characterized respiratory tract isolates comprising 109 gBLNAR, seven gBLPACR, nine gBLPBR and 71 gBLNAS isolates was tested by disk diffusion (PCV10, PCG1 and CEC30; EUCAST methodology) and MIC-determination (ampicillin, amoxicillin, piperacillin, cefotaxime, cefotaxime and meropenem; microbroth dilution, CLSI methodology). For the penicillins, MIC determination of β-lactamase positive isolates was performed in combination with sulbactam, clavulananate and tazobactam, respectively.
Disk diffusion results were interpreted for the two methods according to the recommended test algorithms and compared to resistance phenotypes and resistance phenotypes, defining resistant phenotype as resistant to one or more agents according to EUCAST MIC breakpoints.

**Results:** A resistant phenotype was present in 94% of the gBLNAR/gBLPACR isolates and 29% of the gBLNAS/gBLPAP isolates. In general, gBLNAR/gBLPACR isolates with a resistant phenotype expressed higher MICs and were resistant to a wider range of β-lactams than gBLNAS/gBLPAP.

The PCG1-based method was superior to the PCV10-based method with respect to categorization into genotypes (correct categorization 91% and 85%, respectively). Using resistance phenotype as the gold standard, the PCG1- and PCV10-based methods correctly categorized 85% and 83% of the isolates, respectively.

**Conclusion:** Replacement of the PCV10 disk by the PCG1 disk improves performance of the screening method for detection of β-lactam resistance in H. influenzae.

**P753** The EUCAST Mueller-Hinton fastidious agar for antimicrobial susceptibility testing of anaerobic bacteria? U.S. Justesen*, E. Matuschek, G. Kahlmeter (Odense C, DK; Växjö, SE)

**Objectives:** The EUCAST disk diffusion antimicrobial susceptibility testing method for fastidious organisms is based on the use of the Mueller-Hinton fastidious agar, Mueller-Hinton agar with 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F). It is not known in detail to what extent anaerobic bacteria will grow on MH-F. To evaluate this further, the growth characteristics of seven anaerobic ATCC/NCTC strains were tested on MH-F from different manufacturers.

**Methods:** MH-F plates from bioMerieux, the Department of Clinical Microbiology, Växjö (Oxoid MH) and from Statens Serum Institut (SSI), Copenhagen, Denmark (Oxoid MH and BBL II MH) were tested. Supplemented MH-F (hemin and vitamin K) with Oxoid and BBL II MH from SSI were also included. The six agars were compared to the Brucella Blood Agar supplemented with hemin and vitamin K and the anaerobe agar (modified chocolate agar containing hemin and supplemented with vitamin K and cysteine) from SSI. The following ATCC/NCTC strains were used: Bacteroides fragilis ATCC 25285, Bacteroides thetaiotaomicron ATCC 29741, Bacteroides vulgatus ATCC 29327, Clostridium perfringens ATCC 13124, Clostridium difficile ATCC 700057, Fusobacterium necrophorum ATCC 25286 and Peptostreptococcus anaerobius NCTC 11460. A McFarland 1.0 suspension was prepared and diluted for CFU counting from spot test, colony size, expressed as a growth value (0, no growth; 1, haze; 2, <0.5 mm; 3, >0.5 mm; and 4, >1 mm), and summary of growth characteristics (confluent growth or not). All plates were incubated at 37°C in an anaerobe environment for 24 hours.

**Results:** There was no difference in CFU counts between the eight agars. The median growth value for the plates tested as follows: bioMerieux 2, Växjö 2, SSI (Oxoid) 2, SSI (BBL II) 2, SSI (supplemented Oxoid) 2, SSI (supplemented BBL II) 3, supplemented Brucella Blood Agar 4, anaerobe agar 4. Overall confluent growth on MH-F was only achieved with B. fragilis. Confluent growth was achieved with 4/7 strains on the supplemented Brucella Blood Agar and 6/7 strains on the anaerobe agar.

**Conclusion:** The EUCAST MH-F agar is less favourable for antimicrobial susceptibility testing of anaerobic bacteria than the supplemented Brucella Blood Agar or the anaerobe agar. Whether or not the MH-F medium can be calibrated for antimicrobial susceptibility testing of rapidly growing commonly isolated anaerobic bacteria remains to be investigated.

**P754** Feasibility of the E-test for susceptibility testing of doripenem against Gram-negative and Gram-positive bacteria using EUCAST breakpoints

M. Kresken*, B. Körber-Irrgang on behalf of the German Doripenem Study Group

**Objective:** Doripenem (DOR), a member of the group of carbapenems, has been shown to have in vitro activity against a wide range of Gram-positive and Gram-negative pathogens, including P. aeruginosa. The Estet methodology has been proposed as an alternative to the reference broth microdilution (BMD) method (J Clin Microbiol 2010;48:3353–7), but there is no comparative study on DOR using EUCAST interpretive criteria. The aim of this study was to evaluate the Estet as an alternative to the BMD method to establish its feasibility for routine susceptibility testing of DOR.

**Methods:** A total of 1,796 clinical isolates were tested. Isolates were collected from 15 medical microbiology laboratories during two resistance surveillance studies conducted in Germany in 2007 and 2009. Organism groups tested were ceftriaxone (CRO)-susceptible Enterobacteriaceae (n=712), CRO-non-susceptible Enterobacteriaceae (n=539), A. baumannii group (n=139), P. aeruginosa (n=149), MSSA (n=151), MRSA (n=147), CoNs (n=153), E. faecalis (n=148), and S. pneumoniae (n=66). Isolates were sent to a central laboratory where species identifications were confirmed using standard laboratory methods, and tested for susceptibility by BMD according to the standard ISO 20776-1:2006 (ISO BMD)and by Estet. Interpretive criteria were those published by EUCAST. The categorical (breakpoint determination) and essential (within one 2-fold dilution) agreement between the two methods were assessed.

**Results:** As by EUCAST criteria, if available, the percentages of DOR-susceptible isolates were 100% for CRO-susceptible Enterobacteriaceae, 98.3% for CRO-non-susceptible Enterobacteriaceae, 82.7% for A. baumannii group isolates, 77.9% for P. aeruginosa and 100% for S. pneumoniae. For the vast majority of organisms, Estet MICs were 0.5 to 1.5 log2 dilution lower than ISO BMD MICs. The widest difference detected was 2 log2 dilutions. The overall level of essential and categorical agreement of Estet results as compared to BMD MICs was 76.9% and 98.5%, respectively. Categorical discrepancies were observed for 18 organisms, all of which were minor and related either to P. aeruginosa (n=11), the A. baumannii group (n=5) or K. pneumoniae (n=2).

**Conclusion:** Our results suggest that the Estet is an acceptable alternative to the reference BMD method for susceptibility testing of DOR. However, organisms displaying Estet MICs around the clinical breakpoints should be re-tested using the reference BMD method.

**P755** Usefulness of Microscan System panels with EUCAST clinical breakpoints to evaluate the antimicrobial susceptibility of β-lactamase producing Gram-negative isolates

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**Objectives:** Evaluation of the ability of NCBCS, NBCc46 and NB40 Microscan (MS) panels, updated to 2010 EUCAST breakpoints, to identify species level and to correctly define the β-lactams susceptibility of 61 β-lactamases producing Gram-negative isolates.

**Methods:** A collection of 73 fully identified strains was analyzed: 21 Klebsiella spp., 17 E. coli, 15 P. mirabilis, 9 A. baumannii-Ab, 7 P. aeruginosa and 4 Enterobacter spp.. 61/73 were BLs and/or carbapenemases (CBs) producers: 15 CTX-M-1/-2/-14/-15, among them 2 were also VIM-1 positive, 4 TEM-52/-92, 3 PER-1, 2 SHV-12/-18, 6 CMY-16 AmpC acquired, 11 KPC-2/-3, 9 OXA-51/-58/-23, 8 VIM-1 and 2 IMP-13 positive. One K-1 piper producer K. oxytoca, 11 non-BL producers/ATCC control strains and a OprD2 porin lacking P. aeruginosa were also included. All isolates were identified by Api-20E and VITEK-2 Systems and antibiotic susceptibilities were obtained by
broth microdilution method. Resistance genes were identified by PCR and sequencing.

Results: All 73 isolates were correctly identified and a complete agreement for susceptibility patterns was observed for both ATCC control strains and non-BL clinical isolates.

MS failed to detect a BL/Extended-Spectrum-β-Lactamase (ESBL) production in 5/61 cases: any ESBL alert was detected using NBC46 panel for 3/15 CTX-M positive strains and 2 VIM-1/CTX-M-15 producing K. pneumoniae isolates.

Intermediate resistance to cefepine (MIC 16 mg/L), susceptibility to ceftriaxone (MIC ≤1 mg/L) and to piperacillin/tazobactam (MIC ≤8 mg/L) were correctly observed for CMY-16 producers.

KPC producers were always correctly detected, with MIC values of 1–>8 mg/L for ertapenem (ETP), according to previously results. All VIM-1 producers resulted intermediate/resistant to IP and MP; decreased MIC values were observed in 2/8 cases. Carabepenem MICs >8 mg/L were detected for IMP-13 P. aeruginosa producers; 6/9 OXA CBs producing Ab showed IP MIC ≥8 mg/L and 3/6 MER MIC ≥8 mg/L. 3/9 Ab OXA-58/51 producers, tested using NB40 panel, were intermediate or resistant to doripenem and meropenem.

Conclusion: Regarding the detection of BLs overall agreement between MS and reference methods was 91.9%. Carabepenem MIC values required some amendment, and the test performance was modified by the manufacturer to meet EUCAST criteria. The software was capable of identifying resistance to doripenem and meropenem.

**P756 Automating selection of epidemiological cut-off values using a dedicated software tool: MicDat®**

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Objectives: Examination of MIC distributions and the setting of epidemiological cutoff values (ECVs) are now recognised for their contribution to clinical breakpoint setting and as defining wild-type versus non-wild-type phenotypes. We previously developed a statistical method for setting ECVs in an effort to eliminate any subjective contribution to clinical breakpoint setting and as defining wild-type versus non-wild-type phenotypes. We previously developed a statistical method for selecting ECVs in an effort to eliminate any subjective component to their selection. We wished to develop an easy-to-use spreadsheet program that would automate the statistical method and provide measures of dispersion of the ECV estimates.

Methods: We implemented the statistical method in Microsoft Excel® using VBA-encoded macros, standard Excel functions and the Solver add-in, and included a link to the EUCAST wild-type database. The output includes a measure of error of the fit. To validate the software and compare the output to the currently published ECVs (determined by visual inspection and group uniformity), we examined the published EUCAST MIC distributions for ampicillin against 42 bacterial species. Ampicillin was chosen as it was associated with a broad range of distribution and resistance types, including bimodal and trimodal distributions, common and rare “resistances” and intrinsic and acquired resistance mechanisms.

Results: The software easily handled well-behaved MIC distributions, including bimodal populations with small, similar and dominant upper modes, and readily produced reliable estimates of ECVs. The software produced results similar to those obtained by visual inspection when the 97.5% of the modelled wild-type population was used to select the ECV. It also demonstrated that applying group or genus ECVs may not be appropriate. The software was also capable of determining a lower cutoff value, a value of potential use in detecting strain misidentification. Problems estimating ECVs were experienced with M. catarrhalis due to the low prevalence of the wild-type and 4 modes, and N. gonorrhoeae due the lack of distinct wild-type mode. The software analysis also highlighted some unusual MIC distributions, consistent with them including strains with incorrect identifications.

Conclusions: MicDat® simplifies the process of setting ECVs, avoids the subjectivity of selection by visual inspection, and offers potential for on-line automation of ECVs initially and when MIC distribution data are modified. It is also capable of identifying suspect distributions which require review of data sources, susceptibility testing methods and species identification techniques.

**P757 Amoxicillin-clavulanic acid susceptibility testing: fixed ratio versus fixed concentration of clavulanic acid**

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Introduction: In currently available susceptibility testing methods amoxicillin-clavulanic acid (AC) is tested with a fixed ratio of 2:1, while according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) the inhibitor concentration should be fixed at 2 mg/L. In this study, susceptibility testing using a fixed concentration, as advocated by the EUCAST, was examined, and compared to the routine testing with a 2:1 ratio.

Materials and Methods: 100 Escherichia coli clinical strains from routine urinary specimens with known susceptibility, as determined by the agar-dilution method, were included. All were amoxicillin-resistant. For quality control E. coli ATCC 25922 and E. coli ATCC 35218 (for β-lactam-β-lactamase inhibitor combination) were tested. Susceptibility testing was performed by using 3 methods: 1. Mueller Hinton (HM) agar plates containing the routinely used concentrations of AC with a fixed ratio of 4:2, 8:4, 16:8 and 32:16 (mg/L), 2. HM agar plates with the same amoxicillin concentrations but with a fixed clavulanic acid concentration at 2 mg/L, 3. E-test for both amoxicillin and AC. Comparisons were made using the χ² test.

Results: Fixed concentration of clavulanic acid at 2 mg/L resulted in a significantly less inhibition than a fixed amoxicillin/clavulanic acid ratio of 2:1 (P=0.02), regardless of the β-lactam–β-lactamase inhibitor combination tested. Susceptibility testing was performed by using 3 methods: 1. Mueller Hinton (HM) agar plates containing the routine concentrations of AC with a fixed ratio of 4:2, 8:4, 16:8 and 32:16 (mg/L), 2. HM agar plates with the same amoxicillin concentrations but with a fixed clavulanic acid concentration of 2 mg/L (EUCAST breakpoint). 58% of strains were inhibited with the current used methodology (fixed ratio), whereas only 43% would have been inhibited, should a 2 mg/L fixed concentration of clavulanic acid be used.

Conclusion: According to EUCAST the interpretative criteria for amoxicillin-clavulanic acid susceptibility testing is based on a fixed concentration of clavulanic acid at 2 mg/L. However, routinely used susceptibility testing for AC, including Vitek, E-test, agar dilution- and disc diffusion methods, are based on a fixed ratio of 2:1, leading to an underestimation of resistance rates. Therefore, such tests should be modified by the manufacturers to meet EUCAST criteria.

**P758 A rapid and functional assay for detection of resistance against β-lactam antibiotics by MALDI-TOF mass spectrometry**

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Objective: The growing number of antibiotic resistant microorganisms is an increasing health care problem. β-lactam-resistant bacteria express β-lactamases which destroy the β-lactam ring of β-lactam antibiotics by
 Screening and confirmation methods for carbapenemases in Enterobacteriaceae

Methods: Ampicillin and cephalosporins were tested with ESBL E. coli strains and DH5α as negative control. Ertapenem, Imipenem and Meropenem were tested with carbapenem positive Klebsiella pneumoniae strains and a sensitive strain as negative control. Antibiotics were dissolved in water. 10 μL of this solution were inoculated with 5 colonies of the corresponding bacteria and incubated for 3 h at 37°C under agitation. After centrifugation, 1 μL of the supernatant was directly applied to a MALDI sample carrier plate. Dried spots were overlaid with MALDI matrix. MALDI-TOF MS spectra were acquired on a microflex LT.

Results: The MS spectrum corresponding to DH5α revealed the molecular peak of ampicillin at [M+H]+ 350 and the sodium adducts [M+Na]+ at 372 and [M+2Na]+ at 394 Da. In contrast, the ESBL derived spectra revealed clearly decreased peaks for ampicillin and it’s adducts. Additional peaks at 568, 394, 412 and 324 Da appeared corresponding to the hydrolyzed form of ampicillin, its sodium adducts and the hydroxylated, decarboxylated form of ampicillin, respectively. A slight spontaneous hydrolysis of ampicillin was observed for DH5α.

Comparable results were achieved for Klebsiella pneumoniae and carbapenemases. Carbapenemases did not tend to form sodium adducts. Additionally, the hydrolyzed form of carbapenemases is very labile and decarboxylated immediately. A clear difference was observed between the carbapenem sensitive strain and the carbapenamase positive strains. For evaluation, the ratio of the sum of the areas of the non-hydrolyzed forms and the sum of the enzymatically converted forms of the antibiotics was calculated. The quotient is less than 1 for resistant bacteria and exceeds 1 for antibiotics sensitive strains facilitating a simple and easy detection of resistant bacterial strains.

The analysis of cephalosporin resistant bacteria resulted in the hydrolysis of the corresponding hydrolysis products. The developed approach provides a rapid method for the detection of penicillin and carbapenem resistant bacteria with 4 h.

**Conclusions:** The approach offers a rapid method for the detection of penicillin and carbapenem resistant bacteria with 4h.

**P759** Screening and confirmation methods for carbapenemases in Enterobacteriaceae

*J.W. Cohen Stuart*, G. Voets, S. Voskuil, J. Scharringa, A.C. Fluit, M. Leverstein-Van Hall (Utrecht, NL)

**Objectives:** In 2010, our group published a guideline for detection of carbapenemases in Enterobacteriaceae (IJA 2010). This guideline recommends a meropenem screening breakpoint of ≥0.5 mg/L or a zone diameter of ≥23 mm (10 μg disk loading), or, alternatively, the less specific ertapenem (≥0.5 mg/L). Carbapenemase inhibition tests with boronic acid (BA) were recommended for KPC and with EDTA or dipicolinic acid (DPA) for metallo-carbapenemases (MBL). The aim of this study was to evaluate meropenem and ertapenem as screening carbapenemases, and to compare our guideline breakpoints with EUCAST clinical breakpoints. In addition, the inhibition tests with BA and DPA for carbapenemase confirmation were evaluated, and the CICA-B-Test as MBL detection test.

**Methods:** 61 carbapenemase producers were included (44 K. pneumoniae, 7 E. coli, 5 Enterobacter spp., 3 S. marcescens, 2 P. mirabilis of which 30 KPC, 25 MBL (16 VIM, 6 GIM, 3 NDM), 4 KPC/VIM combined, 1 SME, 1 OXA-48) and 136 negative controls (32 AmpC, 84 ESBL, 3 ESBL/plasmid AmpC combined, 8 K1 hyperproducing K. oxytoca, 2 non ESBL TEM/SHV, 7 E. coli without β-lactamases). MICs of meropenem and ertapenem were determined using broth microdilution (Merlin). Carbapenem zone diameters were determined using 10 μg discs (Mast) and tablets (Rosco). The confirmation tests were obtained from Rosco. Cica-B-Tests were obtained from Mast.

**Results:** Results of screening with meropenem and ertapenem are shown in the Table. Confirmation of KPC production using BA + meropenem combination discs had a sensitivity of 85% and a specificity of 100%. Confirmation of MBL production had a sensitivity of 79% and a specificity of 100%. When the KPC/VIM double positive isolates were

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**P760** Discrepancies in susceptibility testing of KPC-producing Klebsiella pneumoniae using Vitek2

*E. Cavassin, E. Sakagami, A. Fogagnoli, C. Santos, G. Von Konell, K. Macedo, S. Sampiao, J. Sampiao* (Sao Paulo, BR)

Carbapenemase producing Klebsiella pneumoniae has become disseminated in Brazilian hospitals during the last two years, restricting the empirical therapeutic options for patients with severe nosocomial infections potentially caused by Gram-negative rods.

Since automation is largely used in Brazilian hospitals we aimed at evaluating the performance of Vitek2 system for detection of KPC producing *K. pneumoniae* and evaluate the susceptibility to carbapenems, tigecycline and colistin.

A total of 22 *K. pneumoniae* strains confirmed to have the blaKPC gene, one per patient, from five different private hospitals located in Sao Paulo, Brazil, were tested with AST-N105 card using Vitek2 software version 4.02. All strain were also tested for polymyxin B, tigecycline, imipenem and meropenem susceptibility using home made broth microdilation panels (BMD) according to EUCAST and CLSI documents.

When tested with Vitek2, all isolates had MICs equal to or higher than 4 mcg/ml for ertapenem and would be classified as resistant by EUCAST or CLSI criteria. Concerning imipenem, 31.8% had MICs equal to or lower than 2 mcg/ml and were susceptible using EUCAST, but 9.9% had MICs equal to or lower than 1 mcg/ml and were susceptible using CLSI criteria. For meropenem, 63.6% of isolates had MICs equal to or lower than 2 mcg/ml and were susceptible using EUCAST while 54.5% were susceptible using CLSI criteria. Contrasting to this findings, all isolates had MICs higher than 2 mcg/ml for imipenem and meropenem when tested by BMD.

Concerning polymyxin B, 77.3% of isolates were susceptible by BMD and there was a 95.4% category agreement with colistin results generated by Vitek2. For tigecycline only 31.8% of isolates had MICs equal to or lower than 1 mcg/ml by the Vitek2 system, contrasting to 95.4% by BMD.

There was a high rate of false sensitivity to meropenem and a high rate of false resistance to tigecycline when KPC producing *K. pneumoniae* were tested with Vitek2. Strains resistant to tigecycline by Vitek2 must be reevaluated by a reference method before results are released to clinicians.
Antimicrobial susceptibility testing

**P761** Evaluation of Alert\textsuperscript{™} PBP2a immunochromatographic assay for detection of methicillin resistance in *Staphylococcus* isolates

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**Objective**: Penicillin-binding protein 2a (PBP2a) mediates methicillin-resistance in *Staphylococcus* spp. The aim of this study was to evaluate the performance of Alert\textsuperscript{™} PBP2a assay; a rapid immunochromatographic assay for the detection of PBP2a in isolates of *Staphylococcus* spp., as a marker for methicillin-resistance.

**Methods**: A total of 175 *Staphylococcus aureus* (75 frozen retrospective isolates, 100 prospective isolates) and 75 coagulase-negative *staphylococcus* (CoNS) isolates (prospective) grown on columbia agar with 5% sheep blood (CA), tryptic soy agar with 5% sheep blood (TSA) or Mueller-Hinton agar (MHA) with cefoxitin and oxacillin disks were tested by the Alert\textsuperscript{™} PBP2a assay and results were assayed and compared with the cefoxitin disk diffusion test (gold standard) per CLSI criteria. *Staphylococcus* spp. was identified by Vitek 2 system.

**Results**: Methicillin-resistance was detected in 86/175 *S. aureus* isolates (48 prospective, 38 retrospective) and 35/75 CoNS isolates by cefoxitin disc testing. The performance of Alert\textsuperscript{™} PBP2a assay tested on *Staphylococcus* isolates grown on different medium was calculated by comparison with cefoxitin results. Overall sensitivity of Alert\textsuperscript{™} PBP2a assay to detect methicillin-resistance was 98% for *S. aureus* and 83% for CoNS. Testing CoNS isolates induced by exposure to oxacillin improved sensitivity to 97%. No false positives were detected with the Alert\textsuperscript{™} PBP2a assay (see table).

**Conclusion**: The Alert\textsuperscript{™} PBP2a assay is a highly sensitive and specific assay for rapid detection of methicillin-resistance in *S. aureus* isolates. For CoNS isolates induction with oxacillin disk is necessary to improve the sensitivity of the Alert\textsuperscript{™} PBP2a assay. Detecting methicillin-resistance in *Staphylococcus* isolates in less than 10 minutes by using the Alert\textsuperscript{™} PBP2a assay provides opportunity to tailor antibiotic therapy.

| Isolates | Sensitivity | Specificity |
|----------|-------------|-------------|
| *S. aureus* (prospective, n=100) | 100% | 100% |
| *S. aureus* (retrospective, n=75) | 95% | 100% |
| *S. aureus* (All isolates, n=175) | 95% | 100% |
| CoNS (prospective, n=75) | 93% | 100% |

*CoNS isolates were picked near the oxacillin disk on the MHA agar (induction)*

**P762** The effects of varying susceptibility test parameters on TP-434 activity in vitro

C. Pillar, D.F. Sahm, T. Grossman*, J. Sutcliffe (Chantilly, Watertown, US)

**Objective**: TP-434 is a novel fluorocyccline entering Phase 2 clinical trials for intra-abdominal infections. To support clinical development, a study was run to evaluate the effects of varying test susceptibility test conditions on the early reading for Enterobacteriaceae. Ampicillin, TMX, and clindamycin are read too high or are difficult to interpret for coagulase negative staphylococci as well as ampicillin and clindamycin for *Staphylococcus aureus*. There were no inconsistencies in susceptibility results between early reading and gold standard for *Escherichia coli*, *Streptococcus pneumoniae* and Group A, B and C streptococci. For the enterococci one major inconsistency for penicillin G was experienced in 1/23 isolates.

**Conclusion**: A practical approach consisting of direct application of Etests on positive blood cultures and Maldi-ToF MS ID deliver both valid antimicrobial susceptibility and ID for 66% (95% CI 60–72) of positive blood cultures within 6 hours with minor workload. Antimicrobials tested for should, however, be carefully chosen and cultures with more than one species should receive special attention.

**P763** A pragmatic approach to blood culture isolate susceptibility testing and ID on the fast track: direct E-test and MALDI-TOF mass spectrometry

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**Objectives**: Both valid susceptibility and identification (ID) of a blood culture isolate are of vital significance for the clinician, guiding treatment from only one of these parameters include serious caveats. We present the evaluation of the application of direct Etest on positive blood cultures and Maldi-ToF MS ID of the isolate when the Etest can be read.

**Methods**: Consecutive Bactec blood culture bottles (BD) positive for bacteria (Bagged positive and positive on Gram/acridine orange microscopy) were included. 2–3 drops were plated onto 140 mm Müller-Hinton plates with 5% sheep blood. Direct susceptibility testing by Etest (bioMerieux) (5–6 Etest strips according to microscopy findings) were read as early as possible (i.e. visible growth), and identified with MALDI TOF MS using the MALDI Biotyper 2.0 software (Bruker Daltonics). Early reading were compared to gold standard Etest and VITEK2 (bioMerieux) susceptibility and ID results.

**Results**: 249 positive blood cultures were included. Mixed cultures were excluded from the present analysis, as well as fastidious and anaerobic isolates. 43% (103/239) had susceptibility from both Vitek2 and Etest “gold standard” in addition to direct Etests. The early reading could be performed at median time 6 hours, range 3–24 hours) and concomitant Maldi-ToF MS ID were obtained on 100% of isolates to genus level and 98% to species level. The MS procedure is completed within 15 min. Meropenem, TMX and gentamicin MIC’s are read too high by the early reading for Enterobacteriaceae. Ampicillin, TMX, and clindamycin are read too high or are difficult to interpret for coagulase negative staphylococci as well as ampicillin and clindamycin for *Staphylococcus aureus*. There were no inconsistencies in susceptibility results between early reading and gold standard Etest for *Escherichia coli*, *Streptococcus pneumoniae* and Group A, B and C streptococci. For the enterococci one major inconsistency for penicillin G was experienced in 1/23 isolates.

**Conclusion**: A practical approach consisting of direct application of Etests on positive blood cultures and Maldi-ToF MS ID deliver both valid antimicrobial susceptibility and ID for 66% (95% CI 60–72) of positive blood cultures within 6 hours with minor workload. Antimicrobials tested for should, however, be carefully chosen and cultures with more than one species should receive special attention.

**P764** Performance of the Dutch national phenotypic ESBL detection guideline in clinical setting

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**Objectives**: In 2008, the ESBL working party of the Dutch Society for Medical Microbiology (NVMM) formulated and implemented a
Vancomycin susceptibility trends in MRSA isolated from blood cultures at the Royal Infirmary of Edinburgh. 2006–2010. Is vancomycin “creep” method dependent?

B. Edwards*, K. Milne, I. Cook, I.M. Gould (Aberdeen, UK)

Objectives: To assess the possibility of a vancomycin ‘creep’ among all MRSA isolates from blood cultures from 2006 to 2010 in Aberdeen Royal Infirmary. Susceptibility testing was performed by E-tests. Complete MICs (see graph), but not in those performed on stored isolates. With the original MICs the highest proportion of isolates at 29% had an MIC of 0.5 in 2006, as opposed to 55.3% with an MIC of 1.5 in 2010. The isolates taken out of storage, the highest proportion of isolates all had an MIC of 0.5, except 2008 where the highest proportion had an MIC of 0.75.

Conclusions: The MRSA isolates from storage were much more susceptible to vancomycin than the samples when tested originally. Raised MICs seem to be an unstable phenomenon. This may explain why the vancomycin ‘creep’ is not consistently reported.
to carbapenems, previously performed by Vitek 2 automated system (BioMérieux, Paris) and additionally by Etest (BioMérieux, Paris). Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 were used as controls. For Flow Cytometry analysis, bacterial cells were incubated in filtered Muller-Hinton broth until exponential phase and then exposed to different concentrations of meropenem, imipenem, doripenem and etrapenem for 1 hour and 2 hours and afterwards stained with Bis-(1,3-dibutylbarbituric acid) trimethine oxonol (DiBAC4(3)), a lipophilic anion able to diffuse across depolarized membranes. From Flow Cytometry suspensions, conventional Colony-Forming Units (CFU) assays were performed in order to establish a correlation between depolarized cells quantified by Flow Cytometry.

**Results:** A clear discrimination between susceptible and resistant strains was possible, soon after 1 hour of treatment with the studied carbapenems. An excellent correlation was obtained between the number of depolarized bacteria quantified by Flow Cytometry and the conventional CFU assays.

**Conclusions:** A novel, simple and fast assay is now available to detect carbapenem-resistant Gram-negative bacteria based upon Flow Cytometry.

**Application of fluorescence in situ hybridisation using peptide nucleic acid probes in gastric samples for detection of Helicobacter pylori clarithromycin resistance**

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**Objectives:** Microorganisms are responsible for several infectious diseases that can cause severe problems to patients and their treatment success is seriously correlated with the fast detection of the infectious agent. Some of the standard methods used, such as culturing methods are fastidious and time-consuming and do not give any information about the antibiotic resistance profile. Therefore, molecular methods have been developed during the last several years in order to overcome these shortcomings. In this work a new genotypic method that permits the identification of the microorganism in clinical samples in a prompt way is proposed. This technique is based on Fluorescence in situ hybridization with PNA probes that are synthetic molecules, complementary to a specific RNA sequence of the microorganism.

**Methods:** A set of PNA probes were designed concerning H. pylori point mutations regarding clarithromycin resistance which is the main problem of gastric diseases treatment failure. An additional probe concerning susceptibility was also designed. After hybridization conditions optimization, probes were applied to H. pylori smears to achieve their practical sensitivity and specificity. At the end they were applied to gastric biopsies in a retrospective study for method validation in real samples. E-test and PCR-sequencing were used to evaluate the results.

**Results:** The probes concerning clarithromycin resistance hybridized only with the resistant strains that had the corresponding point mutations and as such presented 100% sensitivity (95%CI, 79.9–100) and 100% specificity (95%CI, 71.6–100). Results also showed that it is possible to discriminate susceptible from resistant H. pylori strains in gastric biopsy samples since it was presented similar results between the 3 tests used. Overall, the PNA-FISH method was in full agreement with PCR-sequencing although it was a little bit lower when compared to E-test that it was used as gold standard method in this retrospective study (86%).

**Conclusion:** PNA-FISH proved to be an important in situ method for detection of microorganisms in clinical samples in a more prompt way than the standard methods. Due to high H. pylori probes sensitivity and specificity it is proved the applicability of PNA-FISH methodology to clinical material, thus overcoming the need of culturing steps and/or PCR-sequencing procedures and enabling rapid initiation of appropriate antibiotic therapy until culture confirmation several days later.

**Comparison of the PBP2a latex agglutination assay, PBP2a rapid immunochromatographic assay and chromographic medium for identifying methicillin-resistant Staphylococcus aureus directly from positive blood cultures**

S. Hong, B. Son, K. Shin* (Cheongwon, Cheongju, KR)

**Objectives:** Rapid and precise molecular methods (eg., real-time-PCR) have been developed for the detection of methicillin-resistant Staphylococcus aureus (MRSA) in blood cultures. However, available methods for MRSA detection are slow and time-consuming and do not give any information about methicillin resistance. We aimed to compare the sensitivity and specificity of the PBP2a latex agglutination assay, PBP2a rapid immunochromatographic assay and chromographic medium for detecting methicillin-resistant Staphylococcus aureus directly from positive blood cultures.
Staphylococcus aureus (MRSA) from blood cultures; however, molecular testing often requires additional expertise and instrumentation that in many clinical laboratories may not be readily available. This study compared three non-molecular methods for the detection of methicillin resistance directly from blood cultures containing clusters of Gram positive cocci: penicillin-binding protein (PBP) 2a latex agglutination (LA), PBP2a immunochromatographic assay (ICA) and MRSA chromogenic medium (CM).

Methods: In total, 100 S. aureus (50 MRSA and 50 methicillin-susceptible S. aureus (MSSA) confirmed by mecA and nuc PCR) were seeded into blood-culture bottles (Bact/Alert 3D, USA). When the isolates gave a positive signal, 5 mL of blood from the culture broth was added to three serum separator tubes each and the tubes were centrifuged at 1,300 g for 10 min. Two pellets were used as the inoculum for direct PBP2a LA (Denka Seiken, Japan) and MRSA-CM (ChromID MRSA, BioMérieux, France). Subsequently, PBP2a LA and MRSA-CM were tested and interpreted following the manufacturer’s instructions. For a novel PBP2a ICA (PBP2a MRSA Rapid kit, DiNovia, Korea), a pellet was inoculated in BH broth containing 4 μg/mL cefoxitin to induce more much PBP2a for 4 h at 35°C. Then, 0.1 mL of lysis buffer and Tween 20 was added for 10 min and the lysate was tested using the MRSA rapid kit, which was interpreted within 20 min.

Results: For the PBP2a LA, seven isolates of MRSA and five isolates of MSSA showed the ambiguous agglutination and were scored as negative. The respective sensitivities and specificities for the direct detection of MRSA from positive blood culture were 78% and 100% for PBP2a LA, 98% and 100% for PBP2a ICA, and 100% and 100% for MRSA-CM (Table 1).

Conclusions: MRSA-CM and PBP2a ICA gave superior results to PBP2a LA. With PBP2a LA, it is necessary to use an isolated colony rather than a pellet from blood culture broth directly. The accuracies of the PBP2a ICA and MRSA-CM were comparable. MRSA-CM is more effective than PBP2a ICA, based on the simplicity of the procedures and ease of interpretation, but the turnaround time of PBP2a ICA is faster than that of MRSA-CM. Thus, PBP2a ICA is a useful alternative method for the early management of MRSA bacteraemic patients in hospitals that do not have access to molecular methods for detecting MRSA.

**Table 1. Results using three non-molecular methods for identifying MRSA directly from positive blood cultures.**

| Method       | Positive | Negative | Accurate | Specificity |
|--------------|----------|----------|----------|-------------|
| PBP2a LA     | 90%      | 10%      | 100%     | 100%        |
| PBP2a ICA    | 80%      | 20%      | 100%     | 100%        |
| MRSA-CM      | 80%      | 20%      | 100%     | 100%        |

**Conclusion:** Both M.I.C.E. and Etest demonstrated very good categorical agreement (CA) compared to the reference method. EA for both M.I.C.E. and Etest was 83.8%. In some cases where EA was decreased, the antimicrobial agent would not be routinely tested for the organism (e.g. AMC vs. staphylococci). Categorical agreement (CA) was calculated using CLSI breakpoints. Overall CA was 94.7% for M.I.C.E. and 92.3% for Etest. Minor errors for all antimicrobial agents for both strips were <10%, except CIP for Etest, which was 10.2%. Major errors were observed for AMC, IMP and IMP for both strips (2%). Very major errors were observed for AMC for both strips (1.3%). Both M.I.C.E. and Etest results tended to be at least one dilution higher than the reference method for AMC, VA, and LZD. M.I.C.E. P results and Etest results tended to run at least one dilution lower than the reference method.

**Conclusion:** Both M.I.C.E. and Etest demonstrated very good CA compared to the reference method. EA for both M.I.C.E. and Etest was <90% for some organism/strip combinations when compared to a reference method. M.I.C.E. was a useful alternative to Etest.
The emergence of multidrug-resistant (MDR) strains, as KPC possessing Enterobacteriaceae (ENT), has resulted in extremely limited therapeutic options. “Salvage” therapy includes fosfomycin (FOS) i.v., tigecycline (TIG) and colistin. The evaluation of the sensitivity to FOS/TIG usually requires performing a MIC as FOS i.v. breakpoints (BP) are only available for this method or large proportion of errors has been reported with the TIG disk.

**Objectives:** (i) to evaluate the accuracy of FOS and TIG disk diffusion (DD) against MDR strains; (ii) to propose FOS i.v. DD BP; (iii) to optimize TIG DD BP for a more reliable use in routine labs.

**Methods:** A panel of 168 ENT (140 Klebsiella pneumoniae, 20 Enterobacter spp, 5 Serratia marcescens, 2 Citrobacter freundii, 1. K. oxytoca) unique clinical isolates were included. Half of the strains were KPC+ by PCR. MICs for TIG and FOS were obtained by agar dilution (AD) (for FOS, Mueller-Hinton agar – MHA – was supplemented with 25 μg/ml of glucose-6-phosphate – G6P). DD was carried out on MHA with TIG (15ug) and two FOS disks (200ug FOS/50ug G6P and 50ug FOS/50ug G6P). Results were interpreted according to EUCAST BP (FOS: S ≤ 32 μg/ml, R ≥ 64 μg/ml; TIG: S ≤ 1 μg/ml, R ≥ 4 μg/ml and S ≥ 18mm, R ≤ 14mm). Categorical interpretation agreement (CA), very major (VM), major (MA) and minor (MI) errors were calculated.

**Results:** The DD BP obtained for FOS were: (i) S ≥ 17mm, R ≤ 15 mm for the 200ug FOS/50ug G6P disk (CA 94%, MI 6%); (ii) S ≥ 15mm, R ≤ 12 mm for the 50ug FOS/50ug G6P disk (CA 95%, MI 5%). Colonies within FOS halos should not be considered. CA and errors for TIG were: CA 42%, VM 3%, MI 55%. We did not find DD BP that could reduce errors to acceptable levels (VM 1%, MA 1.5%, MI 10%). The best performance was achieved with a TIG BP of S ≥ 21mm and R ≤ 16mm, which eliminated the VM errors (CA 64%, MI 36%).

**Conclusions:** The DD resulted highly reliable for assessing the sensitivity to FOS i.v. using the BP proposed in this study. Both disks (200ug FOS/50ug G6P and 50ug FOS/50ug G6P) tested similar. In contrast, the DD for TIG showed unacceptable levels of errors. Using a tentative BP of S ≥ 21mm and R ≤ 16mm, clinical labs could properly categorize susceptible and resistant strains (VM and MA 0%). But due to the high level of MI errors, strains with intermediate halos (20–17mm) must be confirmed by MIC, since these strains could be susceptible, intermediate or resistant by the reference method. Targeting optimal TIG therapy would require error minimization with the optimized DD BP proposed in this study.

**P774 Study of MIC results obtained with streptococci and pristinamycin using a performance evaluation device MicroScan MicroSTREP® plus panel**

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**Objectives:** Pristinamycin is a streptogramin compound that consists of two components with synergistic antibacterial action against Gram-positive bacteria. This study evaluated pristinamycin microdilution with various streptococci on a Performance Evaluation Device (PED) MicroScan MicroSTREP® plus panel. Panels were read with the MicroScan® WalkAway System and visually. MIC results obtained from both read methods were compared to results obtained with frozen broth microdilution panels prepared according to CLSI methodology.

**Methods:** 217 streptococci, including 69 Streptococcus pneumoniae, 55 group B streptococci, 32 group A streptococci, 22 other β-hemolytic streptococci, and 39 viridans group streptococci, were tested concurrently on a MicroScan PED Dried MicroSTREP® plus Panel and a frozen reference broth microdilution panel prepared according to CLSI methodology. Both panels contained pristinamycin in doubling dilutions from 0.25–8 μg/ml that contained cation-adjusted Mueller-Hinton Broth supplemented with lysed horse blood. Test panels were inoculated using the turbidity standard method and read with the WalkAway System at 20 hours followed by a visual read.

**Results:** The essential agreement for all 217 streptococcal isolates tested was 97.7% (212/217) for the WalkAway System and 96.8% (210/217) for the visual read when compared to the reference panel. The categorical agreement was 100% (217/217) for the WalkAway System and 99.1% (215/217) for the visual read. Categorical errors with the visual read were 1 minor error with a S. pneumoniae isolate and 1 minor error with a viridans group streptococcus isolate.

**Conclusion:** This study shows that testing of pristinamycin with the MicroScan IUO MicroSTREP® plus panel correlates well with a CLSI reference broth microdilution panel when read both with the WalkAway System and visually.

**P775 Performance of the Vitek2 system for detection of inducible clindamycin resistance in staphylococci**

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**Background:** Inducible clindamycin resistance of staphylococci cannot be detected by the conventional antimicrobial susceptibility tests. The Clinical and Laboratory Standards Institute (CLSI) recommends testing for inducible clindamycin resistance in clindamycin non-resistant and erythromycin resistant (CNR-ER) staphylococci by using a D-zone test. Recently, the VITEK2 system was developed to detect inducible clindamycin resistance in staphylococci. We evaluated the performance of the VITEK2 system by comparing it with a D-zone test.

**Methods:** In detecting inducible clindamycin resistance, a total of 142 clinical isolates of staphylococci were tested by using the VITEK2 Antimicrobial Susceptibility Test (AST)-P601 card (bioMérieux, Marcy l’Etoile, France) and the D-zone test. We evaluated the performance of the VITEK2 system by comparing it with a D-zone test.

**Results:** The VITEK2 system shows high concordance with a D-zone test. The inducible clindamycin resistance in staphylococci can be detected easily and conveniently by the VITEK2 system.

**P776 A new reliable screening method for the evaluation of VISA and hVISA strains by “Vancomycin-Teicoplanin MIC Test Strip” (Liofilchem Srl, Italy)**

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**Objectives:** Heteroresistance to glycopeptides, in which subpopulations with reduced susceptibility coexist in a seemingly susceptible phenotype, have been recently associated with clinical failure. Our objective was the validation of an alternative phenotypic test to identify VISA and hVISA strains in parallel with other standardised methods already described and confirmed by population analysis (PAP/AUC) considered the “gold-standard”.

**Methods:** The sample consisted of 23 MRSA strains already characterised as hVISA and VISA by population analysis (PAP/AUC), belonging to the main HA-MRSA and CA-MRSA clones diffused in Italy. Mu50 and NRS403 (VISA), Mu3 and NRS22 (hVISA), ATCC 29213 and 9 clinical VSSA, were also included as control strains.
Vancomycin-Teicoplanin MIC Test Strip (VTMSTS) consists of a double-sided gradient strip of VA 32–0.5 mg/L and TP 32–0.5 mg/L. (Liofilchem srl, Italy). The screening was performed by macro-method (2 McFarland inoculum) in BHI plates (BD, Diagnostic Systems), and tested in parallel with the GRD macro-Etest (AB bioMérieux, France). The interpretive MICS cutoffs used at 24 and 48h were used according to the international guidelines (CLSI 2010; EAS 003).

**Results:** VTMSTS and GRD were able to define hVISA and VISA strains in all cases, confirming that VTMSTS is a reliable test, correlating well with the PAP analysis. All VSSA were also confirmed negative. The test was replicated three times and by two different operators and was found to be highly reproducible.

**Conclusions:** Our results demonstrated that the new VTMSTS method is a good alternative for the evaluation of hVISA and VISA strains for clinical and epidemiological purposes.

**P777** Evaluation of the MicroScan MICroSTREP plus antimicrobial panel for testing β-haemolytic streptococci and viridans group streptococci

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**Objectives:** To determine the clinical usefulness of MicroScan (Siemens Healthcare Diagnostics, Sacramento, CA) MICroSTREP plus antimicrobial panel (MICroSTREP) for the antimicrobial susceptibility test of β-haemolytic streptococci (BHS) and viridans group streptococci (VGS), we compared the MICroSTREP with the Clinical and Laboratory Standard Institute (CLSI) reference method.

**Methods:** Seventy five BHS and fifty nine VGS isolates were tested for antimicrobial susceptibility to penicillin, ampicillin, cefotaxime, meropenem, clindamycin, erythromycin, levofloxacin, and vancomycin with the MICroSTREP and CLSI agar dilution method.

**Results:** Overall essential agreement of MICs (within ±1 doubling dilution) and categorical agreement (CA) determined by the MICroSTREP and CLSI reference method were 98.2% and 96.9%, respectively. For the BHS isolates, the CAs of individual antimicrobial agents revealed 96.0% with erythromycin, and 100% with cefotaxime, meropenem, levofloxacin and vancomycin (ampicillin, penicillin, and clindamycin; 98.7%). For the VGS isolates, the CAs of individual antimicrobial agents were 84.7% with penicillin, and 100% with erythromycin, clindamycin and vancomycin (ampicillin; 86.5%, ampicillin; 88.1%, cefotaxime and levofloxacin; 96.6%). All categorical errors of penicillin and ampicillin in the VGS isolates were minor.

**Conclusions:** The MICroSTREP has an accuracy which is comparable to CLSI reference method, suggesting that this panel can be effective for antimicrobial susceptibility testing for BHS and VGS.

**P778** Comparison of different methods for detection of methicillin resistance in a challenge set of Staphylococcus aureus isolates

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**Objectives:** The detection of methillin-resistant Staphylococcus aureus (MRSA) is still a challenge, as isolates showing low-level (LL) resistance may be misclassified as methicillin susceptible based on phenotypic methods. The aim of this study was to compare disk diffusion, automated system, immunochromatographic assay (ICA) and agglutination for the detection of LL-MRSA.

**Methods:** MRSA isolates (n = 56) referred to the MRSA Reference Laboratory or selected from national surveys were evaluated by the cefoxitin disk diffusion method, the Vitek 2 card AST-P610 (bioMérieux), the MRSA Screen (bioMérieux) and the Clearview Exact PBP2a according to the manufacturer’s instructions. All isolates harboured the mecA gene and showed oxacillin MICs ranging from 0.12 to 16 mg/L.

**Results:** Half (50%) of the LL-MRSA isolates showed oxacillin MICs ranging from 0.12 to 2 mg/L by the agar dilution or the Etest methods and were categorized as susceptible. The SlideX MRSA Screen and the Clearview Exact PBP2a detected all MRSA strains. The disk diffusion method using cefoxitin 30 μg demonstrated a sensitivity of 87.5%. The Vitek 2 system showed various performances: 28 (50%) LL-MRSA isolates had oxacillin MICs above or equal to 4 mg/L and were categorized as resistant, 35 (62.5%) strains were positive by the cefoxitin screen included in the panel. Finally, the Vitek Advanced Expert System (AES) that combined the oxacillin MICs and the cefoxitin screen results detected accurately only 38 (67.9%) LL-MRSA isolates. The difference of sensitivity between the disk diffusion and the Vitek 2 was statistically significant (p = 0.003).

**Conclusions:** Routine phenotypic methods using disk diffusion and automated system failed to detect LL-MRSA isolates. The best performance was obtained by the cefoxitin disk diffusion method. Automated systems need further optimisation for detection of low-level MRSA strains. Detection of the PBP2a by the MRSA Screen or the Clearview Exact PBP2a performed very well, the last test being a very easy and rapid technique (<10 minutes). Accurate detection of LL-MRSA may be increased by combining phenotypic methods.

**Antimicrobial pharmacodynamics**

**P779** Pharmacodynamic and pharmacogenomic profiling of accessory gene regulator (agr) expression in Staphylococcus aureus in response to linezolid

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**Objective:** agr is the master regulator of virulence, exotoxins and resistance in S. aureus. Dysfunction in agr, has been associated with vancomycin heterogeneous resistance and persistence of MRSA in bloodstream infection. Although the relationship between agr and vancomycin has been investigated, there have been no studies on LZD and agr. We evaluated the impact of agr function on LZD killing activity and PD using isogenic knockout strains of S. aureus in time kill experiments, a hollow fiber infection model (HFIM) over and utilized PK-PD approaches to quantify response over a 10 day period.

**Methods:** 6 Strains of S. aureus were evaluated: 3 agr+ and 3 agr− Group I, II, and IV. Time kill studies for were performed for LZD at 0 to 64 mg/L vs. all 6 strains. A hollow fiber infection model was utilized to simulate LZD 600mg q12h vs. agr+ and agr− Group II S. aureus over 240h. PD analysis was completed using a Log Area Ratio approach (AUCCFUdrug/AUCCFUCtrCtrl) vs. drug concentration, fit by a Hill-type maximal effect mathematical model. From the HFIM, total RNA was isolated, mRNA was enriched, reverse transcribed, and RNAIII, the primary transcript of agr was amplified using quantitative real-time PCR (qPCR).

**Results:** LZD displayed similar killing profiles against agr+ and agr− Group I, II, and IV S. aureus, with a partial concordance dependent response and 48h maximal reductions in Log Ratio Area of −3.73 to −3.7, −3.5 to −3.9, and −3.6 to −3.6. PD parameters were similar comparing agr+ vs. agr− Group II: Emax: 3.5/3.4, EC50:1.7/1.8, and Hills Constant: 6.3/4.9. All PD model fits were excellent (R2 > 0.98). In the HFIM, total RNA was isolated, mRNA was enriched, reverse transcribed, and RNAIII, the primary transcript of agr was amplified using quantitative real-time PCR (qPCR).

**Conclusions:** Dysfunction in agr did not alter LZD PD. These data have may implications for the empiric choice of antimicrobial agents in MRSA toxin mediated disease, and support the potential utility of linezolid to impact virulence through alteration in agr expression.

**P780** Intracellular activity of fusidic acid against clinical isolates of Staphylococcus aureus of increasing MIC

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**Background:** Multiresistant S. aureus are of growing concern triggering the reassessment of older antibiotic classes. Fusidic acid is currently
Methods: MSSA ATCC 25923, MRSA ATCC 33591 (susceptible) and 3 clinical isolates of increasing MIC (see Figure) were used for (i) MIC determination (see values in the Figure); (ii) infection of human THP-1 macrophages. For studies with infected cells, phagocytosis was allowed for 1h at 37ºC (after opsonization with human serum), extracellular bacteria were removed by washing and short term exposure to gentamicin, and cells were thereafter exposed for 24h to a wide range of fusidic acid concentrations (from 1/100 to 100× the MIC) to obtain full concentration-effect relationships and determine the static concentrations (Cs), relative potencies (EC50) and maximal relative efficacies (Emax) (see Barcia-Macao et al., AAC 50:841–51).

Results: The Figure shows that for all strains, dose-effect relationships could be modeled using a sigmoidal function (Hill equation; R² > 0.902).

When data are plotted against weight concentrations (mg/L), curves align according to the MIC (increase in EC50 and in Cs). However, when data are plotted against multiples of the MIC, all curves become essentially indistinguishable, with non-significant differences in EC50, Cs and Emax (P > 0.05 [one-way ANOVA]).

Conclusions: Contrary to what we recently observed with moxifloxacin, for which the intracellular activity is markedly decreased (with significant loss of Emax) once the MIC of the target MRSA exceeds 0.125 mg/L (Lemaire et al. JAC in press), the intracellular activity of fusidic acid is directly correlated to its extracellular activity as defined by the MIC. This shows that the intracellular growth of less susceptible intracellular S. aureus can still be controlled by fusidic acid providing its extracellular concentration is raised to reach an MIC/extracellular concentration ratio similar to that of the susceptible ones.
A pharmacokinetic-pharmacodynamic investigation of MUT056399 against Staphylococcus aureus in an in vitro PD model

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Objective: MUT056399 is a novel fatty acid biosynthesis inhibitor which acts on the unexploited target: FabI. The PK-PD profile of MUT056399 against S. aureus using an IVPM simulating human dosing PK and regimens.

Methods: MRSA USA300 was the bacterial isolate studied. MICs were determined by microdilution according to CLSI. A one-compartment IVPM was utilized to simulate the pharmacokinetics of MUT056399, based on phase 1 PK data using a 0.666h terminal elimination half life in plasma and a 5% free fraction, according to %T>MIC = 0.915 for MUT056399.

Results: The MICs for MUT056399 was 0.06mg/L vs. USA300. By 48h, a dose dependent response for all of the MUT056399 dosing regimens was evident, with greater exposure resulting in greater killing activity. MUT056399 q6h regimens were most efficacious compared to other dosage regimens. Simulated regimens of ≥600mg q6h displayed bactericidal killing activity, with rapid and sustainable killing. Complete eradication of MRSA was evident at 900mg q6h and 1200mg q6h.

Conclusions: These data demonstrate similar antimicrobial effects of DOR and CIP, but the greater ability of DOR compared to CIP to restrict two-fold difference in Tms w provided by the dosing regimens with DOR and CIP simulated in this model.

P784 Colistin and doripenem combinations demonstrate synergy and suppression of resistance against Acinetobacter baumannii at multiple inocula in an in vitro PK/PD model

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Background: Colistin (Col) is a last-line therapy for multidrug-resistant (MDR) Acinetobacter baumannii (Ab). However, rapid emergence of Col resistance due to monotherapy is worrying. Our aim was to evaluate the activity of Col & doripenem (D) combinations (combs) against MDR Ab using an in vitro PK/PD model (IVPM).

Methods: A one-compartment IVPM was used to simulate clinically relevant Col & D regimens against Ab at two initial inocula of ~106 & ~108 cfu/mL. Three isolates were examined, one Col-susceptible (ColS) D-resistant (DR) MDR clinical isolate FADDI Ab051 (MICCol 0.5mg/L, MICD 8mg/L) and two Col-heteroresistant isolates ATCC 19606 (MICCol 0.5mg/L, MICD 1mg/L) & FADDI Ab030 (MIC DR clinical isolate, MICCol 0.5mg/L, MICD 16mg/L). Col was given alone as 0.5 or 2mg/L (constant concentration) to simulate Col PK in critically-ill patients, D was dosed alone every 8h (Cmax 2.5 or 25mg/L, t1/2 1.5h) & in combos. Viable counts were determined over 72h & Col population analysis profiles (PAPs) were conducted.

Results: At the 106 inoculum, Col alone (even with 0.5mg/L) resulted in >3.1 log reduction in viable counts against all 3 isolates, followed by extensive regrowth. ColR subpopulations growing at 8mg/L Col increased from <10−6 at baseline to up to ~100% as early as 24h. D alone with Cmax 25mg/L showed initial killing (>1.4 log) against all isolates but rapid & extensive regrowth similar to control values even at 24h (>7.5 log) except for ATCC 19606 (up to 2.2 log less than control); subsequent doses showed no killing. While regrowth occurred with the combo of 0.5mg/L Col and Cmax 2.5mg/L D, all other combos showed significant early killing (>4.8 log) & generally maintained the antibacterial activity over 72h (up to 6.3 log less than control). D was highly active in suppression of ColR subpopulations as evident by comparison of the 72-h PAPs of the combos with that of the control, except in the combo of 0.5mg/L Col and Cmax 2.5mg/L D. Col alone, D alone & the combos displayed a marked inoculum effect. However, substantial synergy (up to 5 log kill within 6h) was observed with the combo of 2mg/L Col and 25mg/L D against all 3 isolates at the 108 inoculum, even with the DR FADDI Ab030 and 051.

Conclusions: Our data highlight that Col/D combos can be highly synergistic against Ab & suppress Col and D resistance. Prospective optimisation of Col and D combinations using a PK/PD approach is essential for clinical utility.
Pharmacodynamic effects of serum on in vitro activities of Mutant prevention concentration values for linezolid against contemporary MRSA clinical isolates with elevated MPC values to vancomycin.

**Objective:** Globally, community and hospital acquired MRSA strains compromises the use of many antimicrobials against this important/ virulent human pathogen. MPC defines antimicrobial drug concentrations preventing growth of bacterial subpopulations, from high density inocula, that have inhibitory values above the susceptibility breakpoint. MPC testing applies to bacterial strains susceptible to the drug by minimum inhibitory susceptibility testing (MIC). We determined MPC values for linezolid against contemporary MRSA clinical isolates with elevated MPC values to vancomycin.

**Methods:** MIC testing was as per the recommended procedure of the Clinical and Laboratory Standards Institute (CLSI) utilizing $10^5$ cfu/ml exposed to doubling drug dilutions by microbroth dilution in Mueller-Hinton Broth. For MPC testing, $>10^{15}$ bacteria were exposed to varying drug concentrations incorporated into agar plates. Following incubation under ambient conditions in O2, the MIC or MPC was the lowest drug concentration preventing growth.

**Results:** A total of 50 MRSA clinical strains were tested. MIC50/90 (μg/ml) values for linezolid and vancomycin respectively were 2/4 and 0.5/1. By MPC testing, MPC50/90 (μg/ml) for linezolid and vancomycin against MRSA respectively were 4/4 and 8/8. No isolates had an MPC value $>4$ to linezolid or $>8$ to vancomycin. For organisms with MPC values to vancomycin of $8$ μg/ml, MPC values to linezolid were $<4$ μg/ml. The Mutant selection window (MSW) is defined by the MIC and MPC values and plotting for each drug against serum drug concentrations showed time above MSW to be $>12$ hours for L and $>6.5$ hours for V. The MIC90/MPC90 ratio for linezolid was 1 and for vancomycin was 8.

**Conclusion:** Limited data exists on linezolid MPC testing against MRSA. MIC/MPC ratios were higher for vancomycin at 8 as compared to 1 for linezolid. Linezolid serum drug concentrations remained in excess of the MSW over the duration of the dose but not for vancomycin. MPC values of $8$ μg/ml for vancomycin is a concern and the mechanism remains unknown. Linezolid had serum drug concentrations in excess of the MSW over the duration of the dose. This observation may have an important therapeutic implication for using linezolid or vancomycin for MRSA infections.

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**P786** Pharmacodynamic effects of serum on in vitro activities of echinocandins against Aspergillus spp.

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**Objectives:** Echinocandins are highly bound to serum proteins which may alter their antifungal activity. In the present study, we investigated the pharmacodynamic effects of human serum on the in vitro activities of caspofungin (CAS), micafungin (MIF) and anidulafungin (ANI) against 5 clinical isolates Aspergillus spp.

**Methods:** Five clinical isolates of each *A. fumigatus* (AFM), *A. flavus* (AFL) and *A. terreus* (AT) were included. The minimal effective concentrations (MEC) of CAS, MIF and ANI against these isolates were 0.5–1, 0.06–0.12 and 0.03 μg/ml, respectively. The in vitro activities of the echinocandins were studied by a broth microdilution method based on the CLSI M38-A2 using standard medium (RPMI 1640, 0.165 M MOPS, pH 7.0) with and without 50% (v/v) pooled human serum. Twofold serial dilutions of each agent ranging from 8 to 0.03 μg/ml were prepared in flat-bottom 96-well microtitration plates and inoculated with $2 \times 10^6$ CFU/ml. The plates were incubated at 37°C for 48h and fungal growth was assessed using the XTT methodology previously described (Meletiadis J. et al JCM 2008).

**Results:** The % of growth inhibition (E) were analyzed using nonlinear regression analysis based on sigmoidal with variable slope. Emax model described by the equation $E = \text{Emax} \times (C/pIC50)$ where Emax is maximal growth inhibition, C the drug concentration and IC50 is the drug concentration corresponding to 50% of growth inhibition and n is the Hillslope. The IC50 and Emax were determined for each drug and isolate in absence of human serum and compared with those values in presence of human serum.

**Conclusion:** The IC50s of all three echinocandins ranged from 0.01–0.06 mg/l without serum and 0.09–0.36 mg/l in presence of serum showing a significant 1–5 two-fold increase. The largest increase was found with AL and ANI. The epsilon max of all three echinocandins was 65 (31–80)% for AFM, 62 (0–72)% for AFL and 67 (42–100)% for AT without serum and 90 (76–98)% for AFM, 94 (78–100)% for AFL and 86 (74–95)% for AT. The addition of human serum increased the Emax of echinocandins by 46 (21–90)% for AFM, 20 (0–37)% for AFL and 16 (6–32)% for AT, resulting complete growth inhibition at concentrations 1–8 mg/l.

**Conclusion:** The activity of all echinocandins was increased at high concentrations and decreased at lower near-MEC concentrations in the presence of human serum.
To date, this topic remains largely unanswered. We tested cefotaxime, ciprofloxacin, meropenem and moxifloxacin against pathogens associated with intra-abdominal infections with and without pancreatic enzymes.

Methods: The following clinical and American Type Culture Collection (ATCC) control strains were tested: Staphylococcus aureus (SA) (ATCC 29213), E. coli (EC) (ATCC 25222), Pseudomonas aeruginosa (PA) (ATCC 27853) and Enterococcus faecalis (EF) (ATCC 29212) and clinical isolates of Klebsiella pneumoniae (KP), Klebsiella oxytoca (KO) and Enterobacter cloacae (ECL). Enzymes tested alone or in combination included: trypsin (porcine), amylase (hog) and lipase (porcine). MIC testing (with and without enzyme(s)) was performed using 10⁵ cfu/ml of organism exposed to doubling drug concentration of each drug in Mueller-Hinton (MH) broth; following incubation under ambient conditions, the MIC was the lowest concentration preventing growth.

Results: MIC values (μg/ml) for cefotaxime respectively against SA, EC, PA, EF, KP, KO, ECL ranged from 2–4, 0.031–0.125, >8, >8.0.016–0.031, 1–2, 0.125–0.25; ciprofloxacin, 0.125–0.5, 0.008–0.016, 0.125–0.5, 0.25–0.5, 0.008–0.031, <0.002–0.004, 0.008–0.031; meropenem, 0.063–0.125, <0.008–0.016, 0.25–1–2–8, 0.016–0.031, 0.016–0.031, 0.016–0.031; moxifloxacin, 0.016–0.031, <0.004–0.031, 1–2, 0.063–0.125, 0.031–0.063, 0.016–0.031, 0.031–0.063. The addition of the enzymes (10,000–1 million units per liter) trypsin, amylase and lipase alone (10,000–1 million units per liter) or in all combinations yielded MIC values that were within 1 doubling dilution of the MIC values without enzyme added.

Conclusion: Against ATCC and clinical isolates of pathogens associated with intra-abdominal infections, the in vitro activity of cefotaxime, ciprofloxacin, meropenem and moxifloxacin was not significantly affected (within 1 doubling dilution) by the addition of trypsin, amylase or lipase (alone or in combinations) as determined by MIC measurements. This data suggests that a mixture of enzymes that may be present during some infections does not appear to impact antimicrobial activity.

Time-kill effect of doripenem against multidrug-resistant pathogens for ventilator-associated pneumonia

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Objectives: Ventilator-associated pneumonia (VAP) by multidrug-resistant (MDR) isolates is a common worldwide situation in Intensive Care Units. Most of these isolates appear resistant to carbapenems. However, doripenem may be active against these isolates at concentrations equal to those achieved in serum after prolonged infusion. This hypothesis was investigated.

Methods: A total of 21 genetically-distinct isolates were tested; nine of Pseudomonas aeruginosa and 12 of Acinetobacter baumannii. All were isolated from different patients at a count greater than 10⁶ cfu/ml from the tracheobronchial secretions within the first 24 hours from advent of diagnosis of VAP; all were MDR to cephalosporins, carbapenems and quinolones. A 5×10⁶ cfu/ml log-phase inoculum of each isolate was exposed over-time to 5, 15 and 30 microg/ml of doripenem and to 18 microg/ml of IPM. Concentrations of doripenem were selected based on published findings for achieved serum concentrations after infusion of 1g within 1 and 4 hours. Mathematical model assessments were validated using various clinically relevant dosing regimens of IPM±MK-7655.

Results: MICs of doripenem against P. aeruginosa isolates ranged within 4 and >256 microg/ml and of meropenem within 32 and >256 microg/ml; those against A. baumannii ranged within 8 and 32 microg/ml and within 16 and 64 microg/ml respectively. Limited killing effect was found by both carbapenems against P. aeruginosa; of doripenem against two isolates and of meropenem against one isolate. However, doripenem had impressive killing effect against all studied A. baumannii isolates mainly when tested at 15 and 30 microg/ml achieving significant decreases of bacterial growth. Meropenem retained a limited killing effect against four isolates (Figure 1).

Conclusions: Doripenem retains considerable time-kill effect against MDR A. baumannii at concentrations equal to 15 and 30 microg/ml. These findings favor the administration of doripenem for infections caused by that species at regimens delivering these concentrations.

Combined activity of MK-7655 and imipenem against carbapenem-resistant Pseudomonas aeruginosa

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Objectives: Carbapenem resistant bacteria pose a significant treatment dilemma given that carbapenems are often the last line of therapy against resistant Gram-negative infections. MK-7655 is a novel β-lactamase inhibitor under clinical development. We investigated the combined killing activity of IPM/MK-7655 against three IPM-resistant P. aeruginosa isolates.

Methods: Time-kill studies (TKS) using approximately 1–5×10⁵ CFU/ml were conducted with IPM and MK-7655 against three strains of AmpC-overproducing, oprD-deleted P. aeruginosa (PA24226, PA24227, PA24228). TKS were performed using 25 clinically achievable concentration combinations in a 5×5 array. Bacterial burden at 24h was determined in triplicate by quantitative culture and mathematically modeled using a three-dimensional response surface. Synergy and antagonism were defined as an interaction index (VUPobserved/VUPexpected) of <1 and >1, respectively. Mathematical model assessments were validated using various clinically relevant dosing regimens of IPM±MK-7655 in a hollow-fiber infection model (HFIM) over 72h.

Results: IPM MICs were reduced at least eightfold in 4 mg/L of MK-7655. PA24226 (32 to 2 mg/L), PA24227 (16 to 2 mg/L), and PA24228 (32 to 4 mg/L). The combination of IPM/MK-7655 was synergistic for all strains investigated. Interaction indices were found as follows: PA24226 = 0.60 (95% CI, 0.58–0.62), PA24227 = 0.70 (95% CI, 0.66–0.74), and PA24228 = 0.55 (95% CI, 0.49–0.61). In HFIM, IPM/MK-7655 considerably reduced bacterial burden at 24h while failure with IPM alone was seen against all 3 isolates. Sustained suppression of bacterial growth was achieved with simulated doses of 500/500mg IPM/MK-7655 in 1 (PA24227) out of 3 strains, and 2 (PA24227, PA24228) out of 3 strains when IPM was increased to 1000mg. Post-HFIM (high-dose) MICs of 3 randomly selected colonies of PA24226 were not significantly elevated.

Conclusions: The combination of IPM/MK-7655 considerably reduced growth of IPM-resistant P. aeruginosa at 24h in HFIM. Suppression at 72h was achieved for 2 of 3 isolates studied. Additional studies are being conducted to explore optimal dosing combinations for maximal killing in P. aeruginosa.
Evaluation of a supratherapeutic dose of intravenous ceftriaxone fosamil on the QTc interval

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Objectives: Ceftriaxone (CPT) fosamil (prodrug of the active component CPT) is a new, parenteral, broad-spectrum cephalosporin. This study assessed the effects of a single supratherapeutic dose of intravenous (IV) CPT fosamil vs placebo (PBO) on the QT interval corrected for heart rate (QTc) using an individual subject correction formula based on the baseline QT-RR slope (QTcIb).

Methods: This was a randomized, double-blind, PBO-controlled, 3-period crossover study of single 1-h IV doses of CPT fosamil (1500 mg), PBO (negative control), and moxifloxacin (MOX; 400 mg; positive control), each separated by a 5-day washout period, in 54 healthy subjects. Continuous digital ECG recording began before study drug administration and continued until 24.5 h after infusion. Three ECG replicates separated by at least 1 min were extracted within the 15-min period before administration (mean of these replicates defined as predose baseline) and at 1, 1.25, 1.5, 2, 4, 8, 12, and 24.5 h after the start of administration. The primary ECG measure was change in QTcIb from predose baseline for each postdose time point. The potential for QTc prolongation was based on comparison of CPT with PBO. Two-sided 90% CIs were calculated for the time-matched difference between CPT and PBO in the mean change in QTcIb using the SE from linear mixed effects repeated measures models. Plasma samples for PK analysis were collected over 24 h after the start of dosing.

Results: The supratherapeutic dose of CPT fosamil resulted in substantially greater systemic exposure to CPT than observed with the standard therapeutic dose (600 mg q12h). The largest between-treatment least-squares difference in change in QTcIb from baseline for CPT vs PBO was 0.66 msec (90% CI: −2.1, 3.4 ms) occurring 1.5 h postdose. The upper 90% CI limit at every time point postdose was <10 msec, indicating that CPT did not cause a clinically meaningful increase in QTc. CPT plasma concentrations were not correlated with time-matched between-treatment differences in change in QTcIb from baseline for CPT vs PBO. The largest difference in change in QTcIb from baseline for MOX vs placebo was 15.7 msec (90% CI: 12.8, 18.5 msec) occurring 1 h postdose. For MOX, the lower 90% CI limit was >5 msec at 5 time points, demonstrating assay sensitivity.

Conclusion: A single supratherapeutic dose of IV CPT fosamil did not cause a clinically meaningful increase in QTcIb at peak plasma concentration or any other time.

| Time from start of infusion (h) | Placebo (n=54) | CPT (n=54) | CPT - placebo (between-treatment difference in change from QTcIb from baseline) |
|--------------------------------|---------------|------------|---------------------------------------------------------------------------------
|                                | Mean QTcIb    | Mean QTcIb | Least-squares mean difference in change from QTcIb |
|                                | change from   | change from | (msec) |
|                                | baseline (msec)| baseline (msec)| (msec) |
|                                | (90% CI)      | (90% CI)   | (90% CI) |
| 1                              | 10.3 (8.50)   | 8.3 (9.89) | -2.01 | -4.8, 0.8 |
| 1.25                           | 7.7 (8.93)    | 6.9 (8.39) | -0.77 | -3.4, 1.9 |
| 1.5                            | 7.4 (9.14)    | 8.0 (9.21) | -0.66 | -2.1, 3.4 |
| 2                              | 6.9 (9.17)    | 4.0 (11.19)| -2.45 | -5.4, 0.5 |
| 4                              | 6.8 (9.17)    | 5.3 (9.07) | -1.43 | -4.0, 1.1 |
| 8                              | -2.1 (9.99)   | -0.4 (10.19)| -1.31 | -4.2, 1.5 |
| 12                             | -0.9 (10.13)  | -1.6 (9.34) | -0.72 | -3.7, 3.4 |
| 24.5                           | -8.4 (14.17)  | -8.0 (13.38)| -0.47 | -3.8, 2.9 |

*Least-squares mean estimates and CI based on QTcIb as an independent variable in a repeated measures linear mixed effects model with treatment regimen, sequence, and period as fixed effects and subject as a random effect.

**SD** = standard deviation.

Data analysis: Differences in mean QTcIb were compared using ANOVA. A two-sided alpha level of 0.05 was considered statistically significant.

Objectives: Ceftriaxone (CRO) and vancomycin (VAN) were comparators.

Methods: Serum-resistant strains of Staphylococcus aureus (including MRSA), Streptococcus pneumoniae, enterococci, Escherichia coli, Klebsiella pneumoniae, and Haemophilus influenzae were selected from a collection based on antibiotic resistance patterns; antibiotic-susceptible strains served as controls. Bacteria were cultured in: 1) cation-adjusted Mueller Hinton Broth (CAMHB); 2) CAMHB plus 50% active human serum; 3) CAMHB plus 50% heat-inactivated serum; or 4) CAMHB plus 45 g/L albumin. MICs were determined using CLSI broth microdilution methods; VAN was tested against S. aureus isolates only. Time-kill experiments were performed; single-point and normalised kill rates and times to reduce the inocula colony-forming unit (CFU) counts by 3log(10) CFU/mL were calculated at up to 8-fold MICs.

Results: CPT MICs for all strains were unaffected by patient status, together with the assessment of trough drug concentrations. Plasma linezolid concentrations in patients developing drug-related side effects with those measured in patients not experiencing linezolid plasma concentrations and length of treatment may be related to the risk of bone marrow toxicity. However, no definitive data are available on the value of linezolid plasma concentration in predicting drug-related adverse events. The primary aim of this prospective observational study was to compare linezolid trough plasma concentrations in patients developing drug-related side effects with those measured in patients not experiencing clinical signs of linezolid toxicity throughout the treatment period.

Methods: On day 3 after starting therapy with linezolid, eligible patients underwent a basal evaluation of renal, hepatic and hematologic status, together with the assessment of trough drug concentrations. These evaluations were repeated at day 7, 14, 21 and periodically till the treatment end. Any relevant information on the clinical status of the patient was collected throughout the study. Plasma linezolid concentrations were determined by a validated HPLC method. The safety outcome was composite and included episodes of anemia.
Pharmacokinetics

**Objective:** To determine the pharmacokinetics of ceftaroline fosamil in healthy volunteers and to compare its bactericidal activity against MRSA and VISA strains.

**Methods:** A dilutional single compartment IVPK model was used to simulate serum concentrations of CPT on a range of MRSA and VISA strains.

**Results:** CPT produced a 2-log reduction in viable count after 6 hr with all strains and a >3.5 log reduction at 12 hr with 5 of the 6 strains. The maximum kill was >4 logs. Bacterial growth suppression was maintained over the 48 hr simulations. There was no relationship between strain CPT MIC and antibacterial effect as measured by AUBKC48.

**Conclusion:** CPT was more bactericidal against VISA strains than MRSA.
in terms of AUBKC48 (p < 0.05) but not AUBKC24. There was no emergence of resistance measured by PAP with any strain.

**Conclusion:** At simulated serum concentrations associated with doses of 600mg 12hly, CPT has a marked bactericidal effect on MRSA strains with MICs in the range 0.25–1.5mg/L. CPT was more active against VISA strains than MRSA strains as assessed by some effect measures.

**P796**

**Interspecies scaling for prediction of human intravenous antimicrobials against Gram-negative bacteria**

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**Objectives:** GSK2251052 (formerly AN3365), is a novel boron-based antimicrobial which specifically targets bacterial leucyl-tRNA synthetase, an essential enzyme in protein synthesis. This study evaluated the interspecies scaling on pharmacokinetics (PK) from four mammalian preclinical species to predict human PK profile.

**Methods:** Pharmacokinetic parameters, including plasma clearance (Clp), volume of distribution at central compartment (Vc) and at steady state (Vss), and mean residence time (MRT), were determined following intravenous (IV) administration of GSK2251052 in female CD-1 mouse, male Sprague-Dawley rat, male Cynomolgus monkey, and male Beagle dog at 30, 10, 10, and 10 mg/kg, respectively. Plasma concentration-time profiles following IV administration were analysed with compartmental analysis using WinNonlin Pro version 5.2, and the bi-exponential decline profile following IV administration was defined by the equation: C = A exp(−α t) + B exp(−β t). Secondary PK parameters, including Clp, Vc, Vss, and MRT, were computed and transformed appropriately, based on total body weights of the species. The interspecies scaling based on the allometric equations describing the relationships between body weight (W), Clp, Vc, Vss, MRT, and primary parameters (A, B, α, β), was determined by fitting variable parameters Y to the standard power equation: Y = a W^b.

**Results:** Applying the interspecies scaling on PK parameters from mouse, rat, monkey and dog, the allometric equations were demonstrated as Clp (mL/h) = 1153.6W^0.74 (r^2 = 0.999), Vc (mL) = 595.8W^0.67 (r^2 = 0.999), Vss (mL) = 367W^0.50 (r^2 = 0.997), MRT (h) = 3.12W^0.21 (r^2 = 0.944). The predicted values of Clp, Vc, Vss, and MRT for a 70-kg human were, 26.7 L/h, 23.7 L, 204 L, and 7.5 h, respectively, which was less than 5% error from actual human PK parameters reported in recent phase-I trial following IV infusion in male subjects.

**Conclusions:** Interspecies scaling successfully described pharmacokinetic parameters of GSK2251052 from four mammalian preclinical species, more importantly, for the first time, which applicability has been proven to predict the pharmacokinetics of a novel boron-based antimicrobial in human. Allometric scaling also allows the simulation of concentration-time profiles following various dose regimens in human.

**P797**

**Pharmacokinetic variability of clarithromycin is due to differences in CYP3A4 activity in patients with cystic fibrosis – a reason for treatment failure?**

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**Objectives:** Chronic lung infections increase the morbidity and mortality in Cystic Fibrosis (CF) patients. Clarithromycin (CLA) is used to treat CF patients and is mainly metabolised in the liver by the enzyme CYP3A4. Multiple SNPs in the gene coding CYP3A4 have been described some of these may lead to increased or decreased enzyme activity, which may lead to therapeutic failure or toxic side effects. The Erythromycin Breath Test (ERMBT) measures CYP3A4 activity in vivo and may be used to identify patients needing larger doses of an antibiotic to avoid therapeutic failure or smaller doses to avoid toxicity. We investigated the correlation between CYP3A4 enzyme activity and the metabolism of CLA and if the activity could be predicted from the CYP3A4 genotype.

**Methods:** We included 22 CF patients (21–53 yr) with chronic P. aeruginosa lung infections. ERMBT was measured by giving 0.15 MBq [14C-N-methyl] erythromycin i.v. Every 10 min for 1 h the patients expired in a glass with 4 ml haemine liquid collecting the exhaled CO₂. The 14C activity was measured by the use of liquid scintillation. 500 mg CLA was given orally and blood samples were collected every half hour for the first 3 h and at 6 and 12 h. The concentration of CLA and the metabolite 14-hydroxyCLA were measured by HPLC, and AUC, Tmax and Cmax were calculated.

**Results:** We found a 10-fold variation in AUC for CLA, median 881 ng/mL × min (range 247–2831), a 12-fold variation in AUC for 14-hydroxyCLA median 366 ng/mL × min (range 84–1041), a 16-fold variation in Cmax for CLA median 3.4 (μg/mL) (range 0.5–7.8) and a 11-fold variation in Cmax for 14-hydroxyCLA median 0.9 (μg/mL) (range 0.2–2.2). We found an 8-fold variation in the CYP3A4 activity (ERMBT %14C/h), median 0.8 (range 0.3–2.0). A linear correlation between the CYP3A4 activity and the metabolism of CLA expressed as the CLA/14-hydroxyCLA ratio was demonstrated (P < 0.05).

Only two patients had another genotype of CYP3A4 than the general population. It was therefore not possible to test for genotype-phenotype correlations.

**Conclusion:** The large variation in the pharmacokinetic profile of CLA in CF patients may course treatment failure. Similar problems may involve other antibiotics which are metabolised by CYP3A4. ERMBT can be used to indentify CF patients who may be in risk of developing therapeutic failure or drug toxicity.

**P798**

**Minimal sampling colistin pharmacokinetics in critically ill patients**

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**Objective:** Colistin is used to treat infections by MDR Gram-negative bacteria. Patients receive colistin methanesulfonate (CMS) that hydrolyses to active colistin A or B. Pharmacokinetic (PK) correlates of activity or toxicity (nephrotoxicity attributed to CMS) are poorly understood because of heterogeneity of pharmaceutical formulations, uncertainties on the concentration- or time-dependence of activity/ nephrotoxicity, limitations of methods to distinguish CMS from colistin, unfeasibility of multiple blood sampling in clinical practice. This study aimed at characterizing minimal sampling colistin PK in critically ill patients.

**Methods:** CMS was given to 7 patients for a minimum of 4 consecutive infusions (3x10⁵ units over 1 h every 8h). Blood was collected at the end and before infusional cycles to determine peak or steady state (SS) drug levels. Plasma was extracted and assayed unmodified (to measure drug levels. Plasma was extracted and assayed unmodified (to measure peak and SS levelscorrelatedinverselywithGFR. This study aimed at characterizing minimal sampling colistin PK in critically ill patients.

**Results:** Plasma peak and SS concentrations of CMS and colistin increased with the number of infusional cycles but in the early about 10 cycles colistin peak and SS averaged below MIC determined in most of patients (0.5–2.0 mg/L). CMS accumulation at SS, but not at peak, correlated inversely with Glomerular Filtration Rate (GFR); for colistin, both peak and SS levels correlated inversely with GFR.

**Conclusion:** Colistin peak and SS levels below MIC over 10 infusional cycles denote that current regimens/schedules are suboptimal for achieving concentration and/or time-dependent antimicrobial activity. Infusional cycle-dependent/GFR-independent accumulation of CMS at peak denotes altered CMS distribution, while GFR-dependent accumulation of CMS at SS denotes impaired renal elimination of CMS. Infusional cycle- and GFR-dependent accumulation of colistin at both peak and SS unravels that in critically ill patients kidney elimination is important for colistin too. Minimal sampling colistin PK in critically ill patients is feasible and informative.
Pharmacokinetics of daptomycin in critically ill patients

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Objectives: Daptomycin dosing recommendations for critically ill patients undergoing continuous renal replacement therapy (CRRT) are scarce. For patients with renal impairment (creatinine-clearance <30ml/min.) the recommended daptomycin dose is 6mg/kg every 48 hrs. At our Intensive Care Unit (ICU) high volume renal replacement therapy (35ml/kg/h) is performed (Multifiltrate, Fresenius Medical Care, Bad Homburg, Germany). Insufficient plasma concentrations were observed when daptomycin was given every 48hrs. Accordingly, the dosing-interval was changed to once-daily and intensified drug monitoring was performed to detect potential overdosing.

Methods: We retrospectively gathered daptomycin peak (Cmax) and trough (Cmin) levels for 14 critically ill patients between 2008-2010 exposed to once daily regimen. Seven of these patients required CRRT. Daptomycin plasma levels were compared with data from healthy volunteers receiving an equivalent daily dose at steady state. Microbiological data, laboratory values and outcome data were collected.

Results: We analyzed 14 patients with sepsis (n = 9), endocarditis (n = 2), wound infection (n = 2) and catheter-related bloodstream infection (n = 1). Causative agent were Enterococcus faecium (n = 9), coagulase negative staphylococcus (n = 2) and staphylococcus aureus (n = 2). Microbiological eradication was successful in 9 of 14 patients. Five patients died during the ICU stay. Daptomycin dose ranged from 3 to 8mg/kg/24h in patients undergoing CRRT and 6 to 10mg/kg/24h in patients without CRRT. Exposure was comparable in CRRT patients to those without CRRT. Cmax and Cmin showed high intra- and interpatient variability in both groups. A possible correlation between dose (mg/kg) and Cmin values but no correlation with Cmax values could be found. Cmin values in ICU patients were comparable with plasma levels in healthy volunteers, whereas Cmax values were substantially lower.

Conclusion: In ICU patients undergoing CRRT, daptomycin once daily led to equivalent Cmin and lower Cmax values compared to healthy volunteers, possibly as a result of the higher volume of distribution in critically ill patients. We conclude that daptomycin clearance in critically ill patients undergoing CRRT is probably equivalent to the clearance in patients with a normal renal function.

Linezolid pharmacokinetics in children: validation of a new method with dried blood spots

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Objectives: Linezolid has been shown to be a valid therapeutic alternative to glycopeptides against multiresistant Gram-positive strains. This antibiotic has time-dependent activity and the T > MIC, is the PK/PD parameter which best predicts its efficacy. However, serum levels fluctuate widely in septic subjects, leading to high intra-individual variability. This may provide the rationale for therapeutic drug monitoring. Up to now linezolid has been mostly assayed by Liquid Chromatography (HPLC). However this approach is time demanding and blood sample volumes must be at least 0.5mL, which may represent a limitation in very young babies. The analysis performed in LC-tandem mass spectrometry (LC-MS/MS) on dried blood spots (DBS) allows a great advantage in terms of costs, affordability, and ease of sampling, especially in infants. Since there are limited results for antimicrobials with these method we evaluated linezolid kinetics in paediatric patients, comparing these two methods in order to validate the DBS technique.

Methods: Linezolid was assayed either in HPLC with UV detection at 254 nm (stationary phase: C18 SODS, 100×4.6mm; mobile phase: 1% orthophosphoric acid, 30% methanol, 2 g/L heptanesulphonic acid, adjusted to pH 5; flow rate 1.0mL/min) using 0.5 mL serum samples, or by LC-MS/MS both in serum and DBS. The calibration curves were obtained by spiking previously analyzed linezolid-free blood and serum samples at 6 concentration levels and the quantitation were performed using a API4000, in multiple reaction monitoring (MRM) mode.

Results: At present 5 hospitalized children with infection due to sensitive strains, aged between 9 mo and 12 yr, were included in the study. Mean±SD peak values were 15.3±1.7mg/L for HPLC and 17.6±3.5mg/L for DBS. Mean±SD trough levels were 0.64±0.39mg/L for HPLC and 0.71±0.39 for DBS, with a variation ranging from 6.4% to 15.9% for single individual samples. These preliminary data demonstrated a high correlation between the two methods (R square 0.97) for both low and high concentrations.

Conclusion: On the basis of our preliminary data the DBS technique was well correlated with a standard analytical method such as HPLC. In conclusion, the minimal sample preparation, with no derivatization steps, high sensitivity/specitivity, high throughput and minimal instrument maintenance make this method a good candidate for a large-scale routine task.

Monitoring plasma voriconazole levels may be necessary to avoid sub-therapeutic levels in lung transplant recipients

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Background: Voriconazole is commonly used to treat fungal infections (in particular, infections caused by Aspergillus spp) after lung transplantation. There are no manufacturers’ recommendations for therapeutic drug monitoring due to its favourable pharmacokinetic profile. However, a correlation between outcome and voriconazole levels has been shown; with a lack of response to voriconazole with low levels (<1mcg/ml).

Method: Pharmacy records were used to identify all lung transplant recipients who had received voriconazole for at least 7 days and had trough plasma voriconazole levels measured between May and December 2010 using validated methods based on high performance liquid chromatography with mass detection (HPLC-MS/MS). Voriconazole was commenced with an initial loading dose of 400mg twice daily orally for 24 hours followed by 200mg every 12 hours thereafter. Trough levels were measured at a minimum of 3–5 days after commencing treatment, a therapeutic level was defined as >1.3mcg/ml (range 1.3–5.7mcg/ml). Cystic fibrosis (CF) lung transplant recipients were compared with non-CF to determine whether this sub-group showed potentially lower absorption of the drug and thus plasma levels.

Results: Twenty-four lung transplant recipients were included in the study (21 bilateral lung transplant, 14 for Cystic Fibrosis, 45.8% male, mean age of 40.3±13 years, at a median of 0.7 months after lung transplantation (range 0.1–262.9). Levels were measured at a median of 10 (range 3–336) days after commencing voriconazole treatment. 12 patients (50%) achieved therapeutic levels with a mean of 2.5±1.0mcg/ml, the remaining 12 patients failed to achieve therapeutic levels with a mean of 0.5±0.3mcg/ml. Of the 14 cystic fibrosis lung transplant recipients only 6 achieved the target level (43%), this was similar to the non-CF group where 6 of 10 (60%) achieved target level (p = 0.68).
Conclusion: This study found that in adult lung transplant recipients receiving standard dose of voriconazole orally, the levels are highly variable between recipients with 50% of patients not achieving the required target level of 1.3 mcg/mL. There was no difference between CF and non-CF patients. We conclude that monitoring plasma voriconazole levels is necessary to avoid subtherapeutic levels in lung transplant recipients. However, further study is required to evaluate clinical outcomes and intra-subject variability in drug levels over time.

**P802** Caspofungin: is standard dosing optimal or inadequate? Pharmacokinetic description in a cohort of patients

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**Objectives:** Caspofungin is an echinocandin used in treatment of *Candida* and *Aspergillus* infections. It has a concentration-dependent fungicidal activity against *Candida* species and concentration dependent fungistatic vs *Aspergillus*. Previous study showed that the Cmax/MIC and AUC0-24h/MIC are the best PK/PD parameters associated with effective therapy. In vivo studies showed that fungicidal activity was not observed until the Cmax/MIC >4 for *Candida* species and AUC0-24h/MIC >250. Aim of the study was to calculate PK parameters in a cohort of patients with different candidemia with MIC of 0.25, 0.5, 1 and 2 mcg/L and to compare them with those in healthy volunteers.

**Methods:** Patients treated with intravenous caspofungin at standard dosages were analysed. Plasma samples were obtained after multiple dosing at steady state conditions immediately before the infusion, at 1, 2, 5, 12±4h and 24 h. Plasma concentrations of total caspofungin were measured using a validated method on ultra-performance liquid chromatography coupled with fluorescence detector system. PK parameters were determined by non compartmental analysis.

**Results:** 17 patients were studied, 9 men and 8 women. Median [IQR] age was 55 [45–67]. Patients in therapy with concomitant cyclopamine, tacrolimus, rifampicin were excluded from the study, Median [IQR] weight and height were 62.5 [55.5–77.5] kg and 170 [160.5–180.0] cm, respectively. Median [IQR] Cmax and Cmin, AUC0–24, Clearance, Distribution Volume and elimination half-life were respectively 7.5 [7.0–8.7] mg/L and 1.7 [1.0–2.4] mg/L; 71.8 [58.2–109.9] mg/Lh/7h, 0.7 [0.4–0.8] L/h; 15.2 [10.5–20.7] L and 13.2 [10.5–15.2] h.

We observed that for MIC 0.25, 0.5 and 1, all patients had Cmax/MIC >4, while for MIC >2 only 16.7% had ratio >4. An AUC0–24h/MIC >250 was achieved in 70.6% and 11.8% of patients with theoretical Candida MIC of 0.25 and 0.5 mcg/L, respectively.

**Conclusion:** Data were similar to those reported in healthy volunteers. These data showed that the dosage achieves adequate PK/PD parameters to treat candidemia in a wide variety of clinical settings, including critically ill patients with septic shock, if the Candida MIC is ≤0.25 mcg/mL, as it is observed in 80% of blood isolates in our hospital.

**P803** Anidulafungin pharmacokinetic evaluation in a cohort of patients

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**Objectives:** Anidulafungin is a new echinocandin that plays an important role in the treatment of invasive candidiasis and candidemia. Anidulafungin is concentration-dependent and fungicidal against *Candida* spp and has peculiar pharmacokinetics with spontaneous degradation and no metabolic interactions. Pharmacokinetic (PK) parameters associated with success include area under the curve (AUC) at steady state >35 mg/L×h and minimum plasma concentration at steady state (Cmin) >1 mg/L. In animal models the best PK/PD parameter associated with success was the AUC/MIC ratio of 250. Aim of the study was to calculate PK parameters in a cohort of critically ill patients undergoing treatment with anidulafungin for candidemia and to describe the AUC compared to different MICs of *Candida*.

**Methods:** Patients treated with intravenous anidulafungin at standard dosages were analysed. Plasma samples were obtained after achieving the steady state conditions immediately before the infusion, at 1.2, 5, 12±4h and 24 h. Plasma concentrations were measured using a validated method on ultra-performance liquid chromatography coupled with PDA detector system. PK parameters were determined by non compartmental analysis.

**Results:** 8 critically ill patients were studied; 6 with severe sepsis and 2 with septic shock. Median [IQR] age was 56 years [46–68]. Median [IQR] weight and height were 65 kg [52.0–68.7] and 170 cm [158–173]. Patients in therapy with concomitant cyclosporine were excluded from the study. Median [IQR] Cmax and Cmin, AUC0–24, Clearance, Distribution Volume and half-life were respectively 6.3 [4.7–9.9] mg/L and 2.4 [1.3–3.18] mg/L; 89.7 [62.2–111.9] mg/L×h, 1.1 [0.9–1.6] L/h, 33.5 [27.7–47.4] L and 20.0 [14.5–27.5] h. All patients had AUC <200 mg/L×h, 1 patient had Cmin <1 mcg/L.

Considering MIC values for *Candida* of 0.25 and 0.5 mcg/L, respectively, 75% and 12.5% of patients had AUC/MIC >250. Considering the MIC 1 and 2 no patients had AUC/MIC >250.

**Conclusion:** Data were similar to those reported in healthy volunteers. Nonetheless, PK parameters may not be satisfied in critically ill patients with *Candida* MIC >0.25 mcg/L. Further studies are needed to better describe PK/PD abnormalities in critically ill patients.

**P804** Population pharmacokinetic study of colistin in combined healthy volunteers and patients with severe Gram-negative multidrug-resistant infections

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**Objectives:** Colistin appears frequently as last line defense therapy against severe Gram(−) MDR infections and it is administered as an inactive prodrug (CMS). Therefore complex pharmacokinetics (PK) alterations in critical care patients must be investigated in order to propose rational dosing guidelines, which was the aim of this study.

**Methods:** PK data in healthy volunteers (n=12) and in critically ill patients (n=32) were combined for analysis. Healthy volunteers had received 1 MIU of CMS as a single dose and plasma and urine samples were collected for CMS and colistin concentrations determinations using a chromatographic assay. Critically ill patients received multiple doses ranging from 0.3 to 4 MIU of CMS and blood samples were drawn after the first dose and at steady-state for CMS and colistin plasma concentrations determinations. Patients under haemodialysis or haemofiltration were excluded for analysis. Population PK analysis was performed with Nonmem VI software.

**Results:** Grouping healthy volunteers and critically ill patients, 29 males (15 females) median (range) age 52 (21–101) yr, weight 73 (40–106) kg, creatinin clearance 94 (30–204) mL/min have been modelled. A PK model with two compartments for CMS and one for colistin, distinguishing between renal (CLR) and non renal (CLnr) clearances for CMS, successfully described the experimental data. CMS renal clearance was related to creatinin clearance (CLcreat) according to CLR=0.85×CLcreat in mL/min. Typical values for CMS non renal clearance were 48 mL/min in healthy volunteers and 27 mL/min in patients. Colistin clearance was almost exclusively non-renal with relatively close typical values in healthy volunteers (CLcoli=49 mL/min) and in patients (CLcoli=35 mL/min).

**Conclusion:** Most inter-patient variability in CMS disposition and therefore colistin plasma concentrations is due to differences in renal function and can be predicted from CLcreat. CMS initial dosing regimen in patients should therefore be adjusted to creatinin clearance.
**P805** Population pharmacokinetics of doripenem in Japanese subjects and Monte Carlo simulation for patients with renal impairment

Y. Matsuo*, T. Ishibashi, R. Kabaoka, T. Wajima (Osaka, JP)

**Objectives:** Doripenem (DRPM), a parenteral carbapenem antibiotic, exhibits broad spectrum of anti-bacterial activity against any aerobic Gram-positive and Gram-negative bacteria and anaerobes. The aim of this study was to evaluate the pharmacokinetics (PK) of DRPM using plasma concentrations in the clinical studies by means of population PK analysis. In addition, dosing regimens for patients with renal impairment were investigated by Monte-Carlo simulation.

**Methods:** A total of 921 plasma concentration data from 92 subjects from 8 phase 1 studies (including studies for subjects with renal impairment and elderly subjects) were used for population PK analysis. Age, body weight and renal function (creatinine clearance, CLcr) were used as covariates on PK of DRPM. Model evaluation was performed using conventional diagnostic plots and a visual predictive check. Final model was used to predict plasma concentration profiles in patients using their covariates to confirm whether the model would be applied to patients. The final model was also employed to simulate the effect of renal function on PK and PK/PD parameters by means of Monte-Carlo simulations.

**Results:** The two compartment model well described the observed DRPM plasma concentrations. CLcr and age were found to be predictors of DRPM clearance and CLcr was the most important influencing factor on PK of DRPM, which was consistent with the previous finding that DRPM is mainly eliminated via kidney. The plasma concentration profiles simulated for patients based on the final model parameters estimated from the phase 1 data were consistent with observed data, suggesting that DRPM PK can be explained by CLcr and age, and that there was no significant PK difference between non-infected subjects and patients. Simulations suggest that 1 g every 12 hours (q12h), 0.5 g every 8 hours (q8h) and 0.25 g q8h for patients with mild, moderate and severe renal impairment, respectively, give similar exposures (AUC) and sufficient the percentage of time above MIC in comparison with 1 g q8h for patients with normal renal function.

**Conclusion:** Our population PK model confirmed renal function is the most important influencing factor on PK of DRPM. Dose adjustment based on CLcr is recommended for patients with renal impairment.

**P806** Lean body weight explains variability in clearance and absorption rate constant of linezolid in cystic fibrosis patients

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**Objectives:** In cystic fibrosis patients (CFP), a relation between body weight and disease state1 has often been described, the latter known to impact the pharmacokinetics (PK) of antibiotic2. Therefore, one might hypothesize that body size could affect the PK of linezolid, a widely used antibacterial treatment option in CFP. The objective of this analysis was to compare different body size descriptors (BSD) as patient specific factors (covariates) in a population PK model to determine the optimal BSD to explain observed variability in PK parameters.

**Methods:** Based on a study of 8 adult CFP exposed to multiple 600 mg twice daily doses, population PK was applied to describe concentration-time profiles using a two-compartment model with concentration-time-dependent clearance inhibition3. The following BSDs [body weight (BW), body surface area (BSA), fat free mass (FFM), lean body weight (LBW), and body mass index (BMI)] were tested on a model without covariates (base model) for their effect on volumes of distribution, clearance (CL), intercompartmental clearance, absorption rate constant (ka), and bioavailability. All modelling and simulation processes were carried out using the nonlinear mixed-effect modelling approach in NONMEM. Selection of the model was guided by goodness-of-fit plots, objective function value (OFV), plausibility and ability to explain variability in parameters.

**Results:** Compared with the base model, incorporation of BSD covariate relations on CL and ka significantly improved the model performance leading to a decrease in the OFV >-10.83 (df=1, p < 0.001). FFM and LBW exhibited a positive relation with CL and ka while significantly and plausibly explaining interpatient variability. But only LBW almost completely explained interpatient variability of ka, stabilized the entire model, and demonstrated a statistically significant influence on the PK of linezolid. For clinical routine, in order to assess only a single BSD in a patient, LBW was incorporated in the final PK model.

**Conclusion:** Body size does have an impact on linezolid PK in CFP, specifically on CL and ka. Adaptation of the linezolid dose to LBW as marker for the disease status might have an impact on safety and tolerability in this special population.

**P807** Population pharmacokinetics of temocillin in intensive care patients and Monte Carlo simulations to evaluate resistance breakpoints

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**Objectives:** Temocillin (TMO) is a narrow spectrum penicillin with good activity against Gram negative micro-organisms including ESBL and AmpC producers. Previous studies indicated that the commonly used dose of 2g every 12 hours could be too low to cover the WildType distributions of Enterobacteriaceae if variation of pharmacokinetics in a patient population was taken into account. Data from a pharmacokinetic study in 11 ICU patients receiving TMO 2g q8h were used to establish a population model and perform Monte Carlo Simulations (MCS) to determine Probabilities of Target Attainment (PTAs) for pharmacodynamic indices (PDI) in order to evaluate and suggest clinical resistance breakpoints.

**Methods:** Blood samples were taken from ICU patients after (t=0.5, 1, 2, 8 h) a 30 m infusion of 2 g TMO (n=11) and afterwards cooled, centrifuged and stored at −70ºC until analysis by HPLC. Protein binding was determined using an ultrafiltration method. Results were used to estimate population pharmacokinetic parameters by NONMEM (version VI, ICON development solutions, USA) and Miclab2.36 (Medimatics, NL) was used to perform MCS (10000 cycles) and obtain PTAs for the unbound fraction including 95% confidence intervals (CI) for the target concentrations. fT >MIC was chosen as the PDI because of the pharmacodynamic properties of TMO.

**Results:** Protein binding was 61%. A two-compartment model best fitted to the data, with estimates (se) of V1 = 440 (2.5) L, CL = 3.69 L/h, (0.46), V2 = 21.7 (4.5) L and Q = 8.45 (1.06) L/h, and omega's for V1 and CI of 0.34 and 0.13 respectively. The breakpoint MIC for a mean fT >MIC of 50% was 32 mg/L. However, MCS – taking the variation in the population into account – and a 95%CI at 50% fT >MIC indicated a clinical breakpoint of 16 mg/L.

**Conclusion:** The population model described the data well. The MCS using population pharmacokinetic estimates indicate a resistance breakpoint for temocillin of >16mg/L provided an administration of 2 g q8h is used.

**P808** Pharmacodynamics of three carbapenems against drug-resistant Gram-negative pathogens isolated from hospitalised patients in Germany using Monte Carlo simulation

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**Objective:** The carbapenems (CARBA) are widely used for empirical therapy of serious infections involving drug-resistant Gram-neg. pathogens like P. aeruginosa, A. baumannii or Enterobacteriaceae expressing an ESBL phenotype or a stably de-repressed AmpC β-lactamase. The objective of this study was to predict the probabilities of attaining...
Population pharmacokinetics of peramivir and dose adjustment for influenza patients with renal impairment

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Background and Objectives: Peramivir is a selective inhibitor of neuraminidases produced by influenza A and B viruses, and is the first injectable agent for treatment of influenza infection which was approved in Japan and Korea in 2010 and is under development elsewhere. The aim of this study was to evaluate the pharmacokinetics (PK) of peramivir based on plasma concentration data in clinical trials by means of population PK analysis. Furthermore, dose adjustment for special populations was investigated by a simulation approach.

Methods: Population PK analysis of peramivir was performed using data from 3199 plasma concentration samples in 332 subjects from 6 clinical studies in Japan and the US, including studies in subjects with renal impairment, elderly subjects and influenza patients. Age, body weight, renal function (creatinine clearance, CLcr) and gender were tested as covariates. PK differences between the Japanese and US subjects and between influenza patients and uninfected subjects were investigated. The final model was evaluated by using conventional diagnostic plots and a visual predictive check. Monte-Carlo simulation was applied to evaluate the effect of influencing factors on the area under the plasma concentration-time curve (AUC) based on the final model.

Results: A three-compartment model described the observed plasma concentration data of peramivir well, and CLcr was found to be the most important influencing factor on clearance (CL). Age and body weight were also found to be the covariates on CL and volume of distribution, respectively. No PK differences were suggested between genders and between Japanese and US subjects. Small PK differences were observed between uninfected subjects and influenza patients. Monte-Carlo simulations indicated that the 1/3-fold and 1/6-fold dose adjustment for patients with moderate (CLcr: 30–50 mL/min) and severe renal impairment (CLcr: 10–30 mL/min), respectively, would give similar AUCs to those in patients with normal renal function. The need for an additional dose after hemodialysis for patients on intermittent hemodialysis was suggested.

Conclusion: A population PK model of peramivir was developed. CLcr was found to be the most important influencing factor on the PK of peramivir. The simulation studies suggested the usefulness of the dose adjustment based on renal function.

[811] Intra-ocular pharmacokinetics of doripenem in rabbits

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Objectives: The aim of this study was to investigate intraocular penetration of doripenem in rabbits.
Serum and urine concentrations of antibiotics in patients
Penetration of moxifloxacin into liver tissue of patients
Pharmacokinetic evaluation of colistin in cerebrospinal
samples, but was measured in HA with a peak at 0.49 ± 0.08 mg/mL. Its clearance (CLu) and volume of distribution (Vu) were both respectively equal to 1.7 ± 1.1 L/h and 0.754 L. Corresponding half-life was 0.31 ± 0.03 h (18.4 min). Doripenem was not detected in any of the UF samples, but was measured in HA with a peak at 0.49 ± 0.08 mg/mL and in CLin,HA/CLout,HA ratio, equivalent to AUCHA/AUCU ratio, equal to 8.3%.

Conclusion: Doripenem distribution is negligible in VF and limited in AH after systemic administration in healthy rabbits, from which it can be concluded that doripenem is likely to be substrate of still undetermined efflux transporters at the blood-aqueous barrier. Clinical trials are now warranted to assess AH penetration of doripenem in patients with endophthalmitis and to compare AH concentrations with bacteria MICs.

Methods: Nineteen New Zealand rabbits received a single 20 mg (close to 10 mg/kg) dose of doripenem intravenously in a marginal ear vein over 60 minutes. Specimens of aqueous humor (AH), vitreous fluid (VF) and blood were obtained at either 30 min. (n = 5), 1 h (n = 5), 2 h (n = 5) or 3 h (n = 4) after starting infusion. Doripenem concentrations in plasma, plasma ultrafiltrates (UF), AH and VF were analyzed by (HPLC). A naïve pool data pharmacokinetic (PK) analysis was conducted in UF and HA (n = 19), with the software WinNonLin (version 5.3 Pharsight Corporation, Mountain View, CA, U.S.A.). In a first step UF concentration-time data were analyzed using a one compartment model with IV infusion. Owing to its small volume, HA will not affect UF data and therefore a forcing function was used in a second step to estimate HA input (CLin,HA). Elimination from the HA compartment was added to estimate output (CLout,HA) clearance responsible for the lower AUC than in UF. HA volume (VHA) in rabbits was set at 0.3 mL.

Results: Doripenem peak concentration in UF was equal to 10.4 ± 2.1 mg/mL. Its clearance (CLu) and volume of distribution (Vu) were both respectively equal to 1.7 ± 1.1 L/h and 0.754 L. Corresponding half-life was 0.31 ± 0.03 h (18.4 min). Doripenem was not detected in any of the VF samples, but was measured in HA with a peak at 0.49 ± 0.08 mg/mL and in CLin,HA/CLout,HA ratio, equivalent to AUCHA/AUCU ratio, equal to 8.3%.

Conclusion: Doripenem distribution is negligible in VF and limited in AH after systemic administration in healthy rabbits, from which it can be concluded that doripenem is likely to be substrate of still undetermined efflux transporters at the blood-aqueous barrier. Clinical trials are now warranted to assess AH penetration of doripenem in patients with endophthalmitis and to compare AH concentrations with bacteria MICs.

Serum and urine concentrations of antibiotics in patients implanted with gentamycin and vancomycin PMMA spacers: data from the first 50 patients (multi-centre study)
E. Bertazzoni Minelli*, A. Renini, T. Della Bora (Verona, IT)

Objectives: The resistance to aminoglycosides is increasing and the combination with vancomycin (V) is useful in orthopaedic infections. Gentamicin (G) and V are known to have a potential nephrotoxic effect, especially when administered in combination. Toxic serum levels are >10 mg/L for G and >60 mg/L for V, respectively. Industrial spacers loaded with gentamicin and vancomycin (1.9% ± 1.9%) were studied in an international multicenter clinical trial (Italy, Spain, Switzerland). The primary endpoint was to assess their safety when implanted in patients with prosthetic joint infection (two-stage revision), also when the devices are utilized along with the systemic antimicrobial therapy. The secondary endpoints were related to the mechanical safety and to the functional effectiveness (joint mobility and re-implantation).

Methods: Fourteen centres specialized in Orthopaedics and Traumatology were involved in the study. G and V concentrations in serum and urine were determined at different time (24, 48, 72 hours for serum and 24, 72, 120 hours for urine) after spacer implantation. Parameters (renal clearance, serum creatinine, and BUN), inflammatory markers (CRP, ESR) and clinical examination (X-ray, etc.) were also considered during and after the study. Serum and urine concentrations of G and V were determined by FPIA method.

Results: Fifty patients (mean age 67.0 ± 5.0 yrs, mean ± SD) were implanted with 34 knee spacers and 16 hip spacers. The serum (range from 11.00 to 49.3 mg/L) because of the parenteral co-administration of V; however these values are in the therapeutic range. 72.5% of patients showed serum levels of V below sensitivity limits (2.0 mg/L). Renal parameters were in normal range during the study.

Conclusions: The implant of industrial spacers loaded with G and V determined very low concentrations of both antibiotics in patients serum, below the risk of renal toxicity. The release of G and V from spacers exerted synergistic local antimicrobial activity, without toxic systemic effects.

Therefore, the use of industrial spacers loaded with G and V in two-stage revision surgery for the treatment of prosthetic joint infections should be considered generally safe.

Penetration of moxifloxacin into liver tissue of patients undergoing liver resection
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Objective: Moxifloxacin (MXF) may be a candidate for antibiotic prophylaxis in the case of hepatobiliary interventions as well as for treatment of bacterial liver abscesses. The aim of the study was to provide data on the pharmacokinetics (PK) of MXF in serum and liver tissue of patients undergoing liver resection due to primary or secondary tumour of the liver.

Methods: After informed consent, patients scheduled for planned liver resection were enrolled into the study. The patients received MXF 400 mg as one hour intravenous infusion at randomized timed intervals prior to liver resection. Blood and healthy liver tissue was sampled in 34 patients (21 m/13 f, age 22–79 years, body weight 56–125 kg, height 155–187 cm) 1.5–26 h after administration of MXF. Plasma was sampled concurrently. In a subgroup of 19 patients, additional serum specimens were obtained after 2, 4, 8, 12, 24, 36 and 48 h to establish the PK. The pharmacokinetic parameters of MXF were calculated applying a two-compartment model.

Results: The mean (SD) pharmacokinetic parameter were as follows: Cmax 6.06 (1.93) mg/L, AUC 50.1 (15.1) mg*h/L, t1/2 11.9 (2.7) h, Vd 133 (36) L, Vd/kg 1.55 (0.21) L/kg, CL 8.73 (2.77) L/h. Compared with historical data (SmPC, AVELOX, Nov 2009), mean Cmax and AUC were 25% higher, Vd normalized to body weight and CL 25% lower. The PK parameters were significantly correlated with weight and height, best Vd R² = 0.80, p < 0.001 and R² = 0.55, p < 0.001, respectively. The mean (SD) tissue concentrations were 9.13 (4.15, n = 13) mg/kg after 1.6–2.4 h, 7.62 (2.54, n = 9) mg/kg after 2.6–4.9 h, 7.48 (2.76, n = 7) mg/kg after 5.6–10.0 h, and 6.24 (3.66, n = 5) mg/kg after 22.9–26.5 h. The mean tissue to serum quotients were 2.9, 3.4, 5.0 and 12.3, respectively. The apparent half-life in tissue was 47 h, almost fourfold compared to serum half-life. This difference indicates non-linear PK, most likely due to saturated excretion of MXF into bile which contaminates the tissue homogenate.

Conclusion: The PK parameters of MXF in patients undergoing liver surgery were moderately altered compared to healthy volunteers. Disposition of MXF into liver tissue apparently follows non-linear kinetics.

Pharmacokinetic evaluation of colistin in cerebrospinal fluid after endoventricular administration of colistin methanesulfonate at different regimens in patients with multidrug-resistant CNS infections
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Objectives: The increasing multidrug resistance to Gram-negative bacteria and the lack of new active antibiotics have forced clinicians to reconsider the use of colistin. Due to poor penetration through the blood brain barrier, colistin methanesulfonate (CMS) is used via endoventricular or intrathecal route. We evaluated the pharmacokinetic of colistin in cerebrospinal fluid (CSF) after endoventricular CMS administration.

Methods: Six critically ill patients (5M, 1F, aged 26–70 years) were investigated. CSF samples from each patient were collected at the steady-state (sampling day range 3–23) before dosing and at time intervals after the end administration. Patients no. 1, 5 and 6 received concomitant CMS intravenously, not significantly influencing our data. Colistin concentrations were measured by HPLC with fluorescence detector.
Results: Patient no. 1 receiving CMS 30000 IU/24h had a Cmax and C trough CSF level of 7.9 and 0.99 mcg/ml, respectively. Patients no. 2 and no. 3 receiving 60000 IU/24h had a mean ± SD Cmax and C trough CSF level of 14.1±6.5 and 2.2±1.1 mcg/ml, respectively. Patients no. 4 and no. 5 receiving 30000 IU/12h had a mean ± SD Cmax and C trough CSF level of 12.8 ±0.4 and 5.8 ±0.4 mcg/ml, respectively. Patient no. 6 receiving 60000 IU/12h had a Cmax and C trough CSF level of 16.8 and 7.6 mcg/ml, respectively. Mean clearance and Vd were 0.025 L/h (range: 0.018–0.033 L/h) and 0.44L (range: 0.20–0.54L), respectively. Interpatient variability (CV%) of clearance and Vd were about 20.7% and 28.6%, respectively. The different amount of CSF spontaneously drained during the study period is in part responsible for such variability. Patients’ positive response to therapy was demonstrated by the decrease in the CSF white blood cell count over the following days and by subsequent sterilization of CSF.

Conclusions: Intravenous administration of colistin in critically ill patients was effective in the eradication of the Gram-negative bacteria from the central nervous system. Since the AUC/MIC ratio seems to be the most relevant surrogate parameter of efficacy (assuming a MIC value ≤1mcg/ml), our data suggest that the 60000 IU/24h (AUC/MIC=209.2), 30000 IU/12h (AUC/MIC=278.4) and 60000 IU/24h (AUC/MIC=316.4) dose regimens achieved the optimal exposure, while the administration of CMS 30000 IU/24h (AUC/MIC=99.6) did not. Further studies are needed to clarify which is the optimal regimen to use when CMS is administered endoventricularily to treat CNS infections.

**P815 Microdialysis study of metronidazole cerebral distribution in patients with acute brain injury**

**D. Frasca, C. Daubert-Fizelier, O. Mimoz, B. Debaene, W. Coutet*, S. Marchand (Poitiers, FR)**

Objectives: Metronidazole is part of the standard therapy of bacterial brain abscess and considered to penetrate well blood-brain barrier (BBB) [1]. However dosing regimens are based on plasma and few cerebral spinal fluid (CSF) pharmacokinetic (PK) studies [2,3]. As infections mainly occur in tissue extracellular fluid (ECF), corresponding unbound ECF antibiotic concentrations are responsible for the antimicrobial effect. This study aims to explore metronidazole distribution in patients with acute brain injury, by comparing their unbound concentrations in brain and plasma.

Methods: After local ethic approval and written informed consent, four brain injured patients, sedated, mechanically ventilated, monitored by cerebral microdialysis (CMA 71, membrane length 10 mm, membrane diameter 0.6 mm, molecular cut-off 100 kDa; CMA, Stockholm, Sweden) and receiving metronidazole for an infection or prophylaxis, were enrolled. PK study succeeded to 500 mg of metronidazole over 30 minutes and brain dialysates and blood samples were collected over 400 minutes. In vivo probes recoveries were evaluated individually by retrodialysis. Metronidazole was assayed by HPLC.

Results: Mean metronidazole brain to plasma AUC ratio was 0.86±0.14 (range from 0.74 to 1.06). All patients had metronidazole concentrations versus time curves in brain delayed (mean time-to-peak = 69±30 min) and peaks were smoother than corresponding curves in plasma with mean Cmax in brain and plasma of 14.5±1.2 and 19.1±2.4 mcg/mL, respectively. Mean half-lives was 379±74 min in plasma; Cmm were 7.2±4.0 mcg/mL in plasma and 5.5±1.3 mcg/mL in brain. Mean probe recovery was 78±1.3%.

Conclusion: Our findings confirm previous studies in CSF, metronidazole penetrates well BBB. Indeed, in acute brain injury patients, unbound metronidazole AUCs in brain and plasma are close. Therefore unbound metronidazole concentration in plasma could be a good surrogate of metronidazole active concentration in brain for PK monitoring in routine.

**P816 Study on the penetration of levofloxacin into the prostatic tissue**

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Background: Levofloxacin (LVFX), a fluoroquinolone marketed by Daiichi Sankyo Co., Ltd., has been widely used in Japan, Europe and the United States. The recommended dosage of LVFX was originally 100 mg two to three times daily in Japan. After a review, based on the PK-PD theory, the regimen was changed to 500 mg once daily in July 2009.

Objective: The efficacy of fluoroquinolones for genitourinary tract infection has been widely recognized. In the treatment of acute bacterial prostatitis (ABP), however, both excellent penetration of a drug into prostatic tissues and strong antimicrobial activity against the causative pathogens are needed. In order to investigate the penetration of LVFX into prostatic tissues, we conducted the present post-marketing clinical study in compliance with GCP and measured the drug concentration in prostatic tissues after administration of 500 mg.

Subjects and Methods: Among the patients with prostatic hyperplasia who presented to participating institutions during the period from December 2009 to September 2010, 10 who fulfilled the following two conditions were enrolled: 1) transurethral prostatectomy (TUR-P) was judged to be necessary and 2) they gave written informed consent. The subjects had a mean age of 73.9±9.09 years (mean±SD), while their body weight was 59.12±8.02 kg and CLcr was 62.01±20.66 ml/min. After preoperative administration of one 500-mg LVFX tablet 3 hours prior to the surgery, each patient underwent TUR-P and 1−2 g of prostatic tissue was obtained. At the same time, a blood sample was collected from each patient to determine the ratio of the tissue drug concentration to that in the plasma, which was separated from the blood sample. LVFX concentrations in the prostatic tissues and plasma were measured by liquid chromatography tandem mass spectrometry and by high-performance liquid chromatography respectively.

Results: The concentration of LVFX in prostatic tissues was 6.44±1.79 mcg/g (mean±SD) and the tissue/plasma concentration ratio was 1.16±0.26 (mean±SD).

Conclusions: It was demonstrated that LVFX, when administered orally at 500 mg, showed good penetration into prostatic tissues. Improper use of fluoroquinolones may promote the development of fluoroquinolones resistance in _Escherichia coli_, which is a current problem in the clinical field. LVFX (500 mg once daily) promises to be effective for ABP both in terms of its good antimicrobial activity and also by inhibiting the emergence of resistant bacteria.
Clinical long-term microdialysis study with voriconazole in healthy volunteers

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Objectives: Interstitial fluid (ISF) concentrations of voriconazole (VRC) were to be investigated representing the target site drug disposition after sequence dosing of the antifungal drug. Microdialysis (MD), as a minimally invasive method to facilitate the direct access to the unbound ISF concentrations, was employed in a long-term setting with extensive sampling over several days. Plasma ultrafiltrate (UF) sampling was also planned in order to determine the distribution to the target site by comparing unbound plasma and tissue concentrations.

Methods: 6 healthy volunteers (age 21–46 yrs) were entered into the study after written informed consent. VRC was administered as iv infusion of 6 mg/kg over 2 h (2 loading doses, dosing interval t=12 h) followed by 4 mg/kg over 1.3 h (2 doses, tau=12 h) and 200 mg VRC tablets (tau=12 h). MD sampling over 84 h was performed in abdominal subcutaneous adipose tissue using concentric catheters (CMA60, cut-off 20 kDa) at a flow rate of 2.0 mL/min. Relative recovery (RR) was determined by retrodialysis. Plasma samples were ultrafiltrated (cut-off 30 kDa). MD and UF VRC concentrations were determined by a validated HPLC assay. The genotype analysis of blood samples of the study individuals with regard to the CYP2C9(*2/*3) and CYP2C19(*2) was carried out.[1]

Results: Continuous MD was successfully applied over 84 h (except in 1 individual: 60 h). RR revealed low intra- (0.7%–5.8% CV) and interindividual variability (1.8%–3.2% CV) across all samples and showed an overall high RR of 85.3%. UF (n = 309 > lower limit of quantification (LLOQ); n = 19 < LLOQ) and ISF concentrations (n = 328 > LLOQ; n = 10 < LLOQ) were calculated: UF ranging from 0.17–3.27 mg/L and ISF from 0.17–3.13 mg/L. Individual concentration-time profiles revealed that variability between individuals significantly increased over time with one individual displaying the highest concentrations (genotype: homozygous mutant for CYP2C19*2). VRC showed favourable distribution to the target site with predominately AUC ratios between ISF and UF of ≥ 1.

Conclusion: The concentration-time profiles of VRC in ISF, determined by the use of MD, and in plasma allowed characterisation of the pharmacokinetic behaviour and future assessment of pharmacokinetic-pharmacodynamic relationships both in circulation and at the target site.

Microdialysis for the study of ertapenem muscle distribution and protein binding in healthy volunteers

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Objective: Evaluate the relevance of microdialysis to assess muscle distribution and plasma protein binding of ertapenem, as a representative drug with extensive binding.

Methods: CMA 60 and CMA 64 1View microdialysis probes were respectively inserted in the quadriceps and arm vein of young healthy volunteers (n = 6). Probes recoveries were determined individually by retrodialysis by drug. Ertapenem (1g) was then administrated intravenously over 30 min and assayed by HPLC at various time points (n = 13) in plasma before (total concentrations) and after ultrafiltration (free concentrations) with Centrifree® devices. Free ertapenem concentrations were also determined in blood and muscle dialysates (n = 14) and corrected for probes recoveries.

Results: Probes recoveries varied between 22.8±8.7% and 77.8±4.9% in blood and 39.2±2.3% and 86.0±3.3% in muscle. Mean±SD unbound muscle to plasma AUC ratio was equal to 3.1±1.2 [1.7–3.9] using free plasma concentrations estimated by ultrafiltration with Cenntifere® devices. Free ertapenem concentrations were also determined in blood and muscle dialysates (n = 14) and corrected for probes recoveries.

Penetration of moxifloxacin into wound tissue and wound exudate obtained during topical negative pressure therapy

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Objectives: Measurements regarding penetration of antibiotics into third space fluids (TSF) are of paramount importance for assessment of drug exposure. Different experimental methods exist, including microdialysis and skin blister fluids, but their use is limited in patients. Topical Negative Pressure (TNP) therapy is a convenient and ethical approach to obtain intermittent fluid under clinical conditions. Moxifloxacin (MXF) is approved for treatment of soft tissue infections, but data on its penetration into the site of infection are limited.

Methods: After given informed consent, 21 patients treated with TNP because of chronic wound healing disturbances or decubitus ulcer were enrolled into the study. The patients received MXF 400 mg intravenously over 1 hour. In 18 patients (median, range: 51, 21–70 years; 75, 60–98 kg; 170, 160–180 cm) plasma and TNP wound exudate (WE) could be determined by blood microdialysis. These discrepancies could be due to limited non specific adsorption of ertapenem on the ultrafiltration membranes.

Results: A total of 12 patients (2m/10f, age 25–61 years; body weight 98–166 kg, height 151–183 cm, BMI 43.0–58.2 kg/m²) completed the study. The oral bioavailability (F) was 78.5 (11.5)%). The mean (SD) PK were on day 1/ day 4: AUCov/AUCss 34.4 (8.9)/45.0 (12.2) mg·h/L, 11/2 9.9 (2.8)/12.3 (2.2) h, Vd/FVd 169 (34)/162 (29) L, CL/F/CL 12.3 (2.8)/ 9.4 (2.0) L/h. Compared with historical data from healthy subjects (Stass & Kubitzka. J Antimicrob Chemother. 1999;43 Suppl B:83–90) AUC was 30% higher, CL 25% lower. Absolute Vd was like in healthy subjects, but halved when normalized to body weight (1.04 L/kg as 2 L/kg in normal weight subjects). Accordingly, Vd was correlated with height (R² p.o/i.v. = 0.84±0.61; p<0.01) more than with weight (R² p.o/i.v. = 0.64±0.50; p<0.01). The quotient (SD) of the tissue to the concomitant plasma concentrations was 2.22 (0.44) in small intestine, but only 0.267 (0.064) in omentum majus and 0.224 (0.044) in subcutaneous fat, indicating that adipose tissue is not a relevant compartment for MXF.

Conclusion: Surprisingly, the PK of MXF were not substantially altered in adult morbidly obese patients compared to subjects with normal weight, and no dose adjustments seem to be required for obese patients.
sampled before and 1–3, 12 and 24 hours after drug administration. The pharmacokinetic parameters were determined in plasma using a one-compartment model. WE data were evaluated descriptively.

Results: The mean (SD) pharmacokinetic parameters were similar to data obtained from healthy subjects as described in the SmPC AVELOX Nov 2009: Cmax 3.27 (0.90) mg/L, AUCCo 39.0 (16.7) mg*hr/L, t1/2 8.1 (3.8) h, Vd/kg body weight 1.64 (0.42) L, CL 12.6 (6.7) L/h. Half-life and Vd/kg were somewhat lower, but this may be due to the short sampling period of only 24 hours. Penetration of MXF into the WE as expressed by AUC was 62%, the quotient (mean (SD)) of fluid to plasma concentrations was 0.76 (0.56, n=14) after 1–3 hours, 0.74 (0.34, n=12) after 8.6–13.8 hours and 0.69 (0.48, n=14) after 24–30h.

Conclusion: The concentrations of MXF in WE were similar to the unbound fraction in plasma (60%) and could be indicative for the concentrations in interstitial fluid in a clinical setting.

MRSA: trend, surveillance, genetic characterisation

Objective: The earliest reports of community associated meticillin resistant Staphylococcus aureus (CA-MRSA) involved indigenous people living in remote Western Australian communities. Although genetically diverse, these early strains primarily carried the type IV or type V SCCmec element. Internationally a variety of “atypical” SCCmec elements have recently been reported. The aim of this study was to determine if novel SCCmec element types have been acquired by S aureus isolated in the Western Australian community.

Methods: Since 1989, 84 pulsed-field gel electrophoresis (PFGE) CA-MRSA strains with 46 multilocus sequence types (MLST) have been identified in Western Australia (WA). These sequence types are from 19 MLST clonal complexes and two singleton lineages. SCCmec typing was performed on these strains by PCR and microarray DNA, and the nomenclature as proposed by the International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements was used.

Results: The 84 PFGE CA-MRSA strains correspond to 67 CA-MRSA MLST/SCCmec clones. Several SCCmec types and subtypes, novel SCCmecs, and composite SCCmecs were identified. Forty six PFGE strains carried SCCmecIVA-d [2B] (31 Iva, 2 Ivb, 9 Ivc, 4 Ivd), 12 strains SCCmec V [5C2] and two strains SCCmec VIII [4A]. Two strains had non typeable SCCmec IV subtypes and four strains had a SCCmec element with a novel ccr gene complex including three with a class B mec gene complex and one with a class A mec complex. Eighteen strains carried SCCmec elements with composite ccr gene complexes including twelve with SCCmec V [5C2&5] (5C2 plus ccrC1 allele 8), three with SCCmecIVA [2B] & 5 (2B plus a type 5 ccr gene complex), one with SCCmecV [5C2] & 2 (5C2 plus a type 2 ccr gene complex) and two with SCCmec V [5C2&5] & 2 (a composite SCCmec V element plus a type 2 ccr gene complex).

Conclusion: Although SCCmec IV and V are the predominant SCCmec element types isolated in the Western Australian community, 30% of strains carry novel or composite SCCmec elements. Although several SCCmec elements have been acquired by multiple S aureus lineages from which many CA-MRSA clones have emerged, only a few of these clones have successfully adapted to the Western Australian community environment.
remained constant over the study period, used in an average 62.99% of episodes. Most MRSA bacteraemia are community strains, and antibiotic susceptibility/strain types will be reported.

**Conclusion:** *S. aureus* bacteraemia still represents a huge burden in both patient morbidity and financially. While a large proportion of *S. aureus* bacteraemia are still hospital acquired, MRSA and possibly MSSA case ascertainment through admission screening, with appropriate isolation and decolonisation, remains critical.

**Genotypic characterisation of *Staphylococcus aureus* isolates causing bacteraemia at Tygerberg Hospital, Western Cape Province, South Africa**

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**Objectives:** There is a paucity of studies on the genotypic characterisation of invasive *S. aureus* strains and the incidence of community-acquired methicillin resistant *S. aureus* (CA-MRSA) infections in South Africa. In this study we characterized *S. aureus* isolates from bacteraemia episodes using molecular methods and prospectively collected demographic and clinical data on these patients.

**Methods:** Consecutive non-duplicate *S. aureus* blood culture isolates were prospectively collected over one year. A multiplex PCR was used for the detection of the spa, mecA and pvl genes. The spa gene was sequenced and Ridom StaphType® used to determine spa-types and spa clonal complexes (spa-CC). All cases were categorised by clinical data as either hospital acquired (HA), health-care associated (HCA) or community acquired (CA) *S. aureus* infections. Data were analysed by using the Statistica® χ² test. A p value less than 0.05 was considered to be statistically significant.

**Results:** 113 *S. aureus* isolates (70% MSSA, 30% MRSA) were collected from 104 patients. 86 bacteraemia episodes were classified as HA (58%), HCA (27%) and CA (15%). According to clinical data, all CA infections were due to MSSA and no CA-MRSA was detected in our study. In the MSSA subgroup, 45% of cases were classified as HA, 33% HCA and 22% CA. Furthermore, all PVL-positive isolates were MSSA (22.7% of all MSSA). MRSA strains clustered mainly in CC701 and CC012, whereas CC002 only consisted of MSSA (p = 0.0016). The predominant source for *S. aureus* bacteraemia was catheter-related sepsis (39%). Skin and soft tissue infections and pneumonia were predominantly associated with MSSA strains.

**Conclusion:** Approximately one third of *S. aureus* bloodstream isolates were MRSA in our setting, a rate comparable to the University Hospital of Würzburg, Germany. None of the isolates were clinically categorized as CA-MRSA; the majority of isolates were derived from cases defined as HA and the major source was catheter-related sepsis. This information is useful for more targeted infection control and prevention practices to reduce *S. aureus* bacteraemia.

**MALDI-TOF mass spectrometry: a useful tool for typing *Staphylococcus aureus* strains in the context of cystic fibrosis**

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**Objectives:** Respiratory infections remain a major threat to cystic fibrosis (CF) patients and lungs represent a specific ecological niche that is chronically colonized by various bacteria especially *Staphylococcus aureus* that are known to be highly adapted to this microenvironment during evolution of the disease. In this study, we used MALDI-TOF MS on whole cells as a typing method to look for the possibility of a specific clustering of *S. aureus* isolates recovered from sputum samples of CF patients as compared to non-CF clinical isolates of *S. aureus*.

**Methods:** A total of 523 *S. aureus* isolates were analyzed including 324 isolates from CF patients collected from 2006 to 2010 at Marseille (CF group), as well as 195 isolates from other patients in Marseilles and four reference strains (non-CF group). All strains were cultivated on COS petri dishes at 37°C overnight before analysis by MALDI-TOF MS (AutoFlex apparatus, Bruker Daltonics®). Each of four replicates of spectra was used to create profiles that were compared and analyzed using Biotyper 2.0 software (Brucker Daltonics®) to generate a dendrogram.

**Results:** All isolates were correctly identified by MALDI-TOF MS with scores >1.9. The dendrogram obtained using Biotyper software clustered the strains in five different clusters with an arbitrary distance level >500. Statistical analysis revealed that cluster 1, 2 and 3 were significantly associated to isolates recovered from the CF group (p < 10−3) (Figure). Cluster 4 was composed of 42 strains both from CF and non-CF group. Conversely, cluster 5 was significantly composed of isolates recovered from the non-CF group (p < 10−6) (Figure). Looking more precisely at the composition of these clusters for the CF group we found that cluster 3 was significantly associated to isolates from adults (age >18 years, p = 0.007) (Figure).

**Conclusion:** Our results suggest that MALDI-TOF MS may be useful for the differentiation of isolates of *S. aureus* isolates from CF patients. This should be compared with isolates from other CF centers to exclude the possibility of a specific epidemiology of strains in our region. The difference of clustering of isolates observed between children and adults CF patients may be due to selective pressures in the CF patient’s lungs during chronic infection, such as host responses and repeated antibiotic treatment that may act as a driver for microevolution.

**Methicillin-resistant staphylococci in the Republic of Belarus: results of the National Surveillance System (2008–2010)**

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**Background:** *Staphylococcus aureus*, especially its oxacillin-resistant variant (MRSA), is the most important cause of multidrug resistant health-care-associated infections and mortality. In Belarus the national antimicrobial resistance (AMR) surveillance program was established in 2003 on the base of Laboratory for Clinical and Experimental Microbiology (LCEM).

**Objectives:** To investigate the prevalence and levels of an antimicrobial resistance of MRSA circulating in hospitals at the Republic of Belarus.

**Materials and Methods:** LCEM collects routine antimicrobial susceptibility results of MRSA strains, isolated in regional laboratories of the ministry of health. The data from the database of Vitrek 2 Compact (BioMerieux) of 943 isolates were also used. Minimal inhibitory concentrations (MIC) for 15 antibiotics were detected automatically. Results were analyzed using OBSERVA software (BioMerieux) and biological statistics methods.

**Results:** The prevalence of MRSA isolates in hospitals varied from 20 to 45% and depends on the size of hospital, antibiotics prescribing and consumption policy. MICs 90% (mg/l) for next antibiotics were detected: benzylpenicillin (>0.25−0.5), oxacillin (>2.0−<4.0), tetracycline (>8.0−<16.0), erythromycin (>4.0−<8.0), ciprofloxacin (>4.0−<8.0), levofloxacin (>2.0−<4.0), moxifloxacin (>1.0−<2.0), gentamicin (8.0,
HA-MRSA epidemiology in an Italian paediatric ICU: an outbreak caused by the Southern Germany clone

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Objectives: Methicillin-resistant Staphylococcus aureus (MRSA) is increasingly isolated in healthcare associated (HA-MRSA) and community-acquired (CA-MRSA) infections. The aim of the present study was to detect the presence of MRSA clones in the Intensive Care Unit (ICU) of a Pediatric Hospital.

Methods: Nasal swabs were collected from 60 patients and 88 healthcare workers in the ICU during a 7 months period of time. All putative isolates were identified as Staphylococcus aureus by selective culture media and positive results of the SlideX Staph Plus. MRSA isolates were identified using Oxa Screen Test Agar and Brillance MRSA medium. SCCmec typing was performed in order to properly characterize CA-MRSA and HA-MRSA, and all strains were analyzed with Multi Locus Sequence Typing (MLST) in order to identify known epidemic clones (Oliveira DC et al. 2002, Enright MC et al., 2000). The antibiotic susceptibility profile of MRSA strains was evaluated by disk diffusion test on Mueller-Hinton agar and E-test.

Results: Twenty-four out of 148 (16.2%) nasal swabs were positive for the presence of MRSA. Fifteen out of 24 (62.5%) isolates were HA-MRSA. Three different clones were delineated. ST228-MRSA-I shared by three patients, ST30-MRSA-III shared by two healthcare workers, and ST45-MRSA-III shared by a healthcare worker and a patient. Our attention was focused particularly on ST228-MRSA-I, the Southern Germany clone.

This clone characterized by high rifampicin resistance has been described in different European countries. MRSA strains isolated in Italian hospital since 1990 were sensible to rifampicin. The Southern Germany clone responsible of this Italian outbreak showed a susceptibility to rifampicin characteristic of Italian isolates.

Conclusion: ST228-MRSA-I has not been previously highlighted as responsible of outbreaks in Italy. This finding suggest that the epidemiology of MRSA infections in critical care units need to be carefully monitored, in order to detect and prevent the spreading of new epidemic strains.

An interaction between clone type and vancomycin MIC influences risk of endocarditis associated with MRSA bloodstream infection

C. Miller, O. Tosas, R. Batra, J. Otter, G. French, J. Edgeworth* (London, UK)

Objectives: Bacterial virulence mechanisms and antimicrobial susceptibilities may impact on risk of complications during treatment of MRSA bloodstream infection. Isolates with a vancomycin minimum inhibitory concentration (MIC) at the upper end of the susceptible range (1.5–2 μg/mL) have been associated with treatment failure, and some single centre studies report an increase in population vancomycin MIC profile towards 1.5–2μg/mL: a phenomenon called MIC creep.

Methods: Spa typing, staphylococcal cassette chromosome mec allo-typing and vancomycin and teicoplanin E-test-susceptibility testing were performed on first-isolates from 821 consecutive MRSA bloodstream infection episodes occurring between 1999 and 2009. This period overlapped with implementation of a successful hospital-wide MRSA control programme leading to a 90% reduction in MRSA bacteremias. Clinical and demographic data including focus of infection were available for 695 clinically significant episodes. Bayesian model averaging (BMA) for multinomial logistic regression was used to determine associations between clone type, MIC and focus of infection.

Results: Typing placed isolates in three groups; clonal complex (CC) 22 (n = 273), CC30 [n = 349] and non-CC22/30 [n = 198]. Over 11 years there was a significant increase in proportion due to CC22 and non CC22/30 and a decrease due to CC30, although the absolute number decreased for all groups. Vancomycin MIC of all isolates was <2μg/mL, however there was a significant trend increase in vancomycin MIC for each clonal group (CC22, OR 1.50 (95% CI 1.28–1.80), p < 0.001; CC30, OR 2.08 (95% CI 1.56–2.96), p < 0.001; non-CC22/CC30, OR 1.21 (95% CI 1.05–1.41), p = 0.024), which was not observed for teicoplanin. BMA indicated that a vancomycin MIC of 1.5–2μg/mL (n = 111), was associated with endocarditis with a posterior probability of 96% depending on clone type. Specifically, endocarditis was at least three times more likely in patients with a bacteremia due to CC22 with an MIC of 1.5–2μg/mL (95% CI 2.99 – 58.0) than other clone types. Clone and/or MIC did not predict bone and joint or other infection focci.

Conclusion: Vancomycin creep has occurred on the background of an effective MRSA control programme. In this setting clones have emerged that have a strong association with endocarditis. The link between clone type, higher MIC and a single distal focus site suggests that treatment parameters alone are unlikely to account for this phenomenon.

Genetic characterisation of early MRSA isolates in England

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Objectives: Since their emergence in the UK in the early 1960s, Methicillin-Resistant Staphylococcus aureus (MRSA) have spread worldwide. Fifty years on, they remain a major cause of hospital acquired infection. To provide a benchmark for MRSA evolution in hospitals in the UK, we have characterised some of the first MSRA isolates collected in England.

Methods: 29 MRSA recovered from hospitalised patients during 1960–61 in South London and the surrounding area, were collected, and used for genetic analyses including pulsed-field gel electrophoresis (PFGE) profiling, Multilocus Sequence Typing (MLST), spa and agr typing, toxin gene profiling and Staphylococcus cassette chromosome mec (SCCmec) typing. Microarray analyses were carried out on a subset of eight isolates using StaphyType kit (Alere Technologies). MICs of a wide range of antibiotics were determined and susceptibility interpreted according to British Society for Antimicrobial Chemotherapy (BSAC)/European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria.

Results: Multiple pulsetypes were identified, but were closely related and belonged to MLST CC8. ST250-SCCmec I (belonging to spa types t008 and t121) was predominant (n = 27) and was recovered from each hospital studied. A second lineage, ST247-SCCmec I (single locus variant of ST250) was associated with spa t051. All isolates were positive for enterotoxin B and were agr type 1. Microarray analyses highlighted divergences in the SCCmec element, antimicrobial resistance traits and virulence-associated markers. All isolates were resistant to β-lactams, as the MIC of oxacillin >16 μg/mL, plus tetracycline (tetK-positive); two isolates were non-susceptible to erythromycin (ermA).

Conclusion: Collectively, these data support the clonal expansion of two closely related early ancestral MRSA strains, ST247 and ST250. Two decades following their emergence, they were dubbed UK EMRSA-5 and -8 in recognition of their epidemic potential in UK hospitals. Understanding the emergence, epidemiology and evolutionary biology
of these resistant clones will provide important insights into the driving force behind the expansion of MRSA clones.

**P830 Temporal trends in the incidence of Staphylococcus aureus bacteraemia. A multi-national population-based study**

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Objectives: The epidemiology of Staphylococcus aureus has been changing in recent years especially with the emergence of methicillin-resistant (MRSA) strains. However, it is unclear whether the overall incidence of S. aureus bacteraemia is increasing. The objective of this study was to determine the population incidence of S. aureus bacteraemia and assess trends in incidence over time.

Methods: Population-based surveillance for all incident S. aureus bacteraemias was conducted nationally in Finland and in Calgary, Canada, Canada, and three regions in Denmark (North Denmark Region, Copenhagen County, and Copenhagen City) during 2000–2008. Incidence rates were age- and gender-standardized to the European Union 27-country 2007 population.

Results: During 76,300,753 person-years of surveillance 17,046 episodes of S. aureus bacteraemia were identified of which 16,364 (96.0%) were methicillin-sensitive (MSSA) and 682 (4.0%) were MRSA. The very young and the elderly were at highest risk. Overall males were at significantly increased risk for development of S. aureus bacteraemia [crude incidence 27.9 vs. 17.0 per 100,000; incidence rate ratio (RR) 1.63; 95% confidence interval (CI) 1.59–1.69]. The overall age- and gender-standardized incidence rate was 27.0 per 100,000 and was 24.9 and 2.0 per 100,000 for MSSA and MRSA, respectively. The overall standardized incidence rates (per 100,000) were 20.3 in Finland, 28.6 in Calgary, 29.9 in Canberra, 31.0 in North Denmark, 33.1 in Copenhagen County, and 32.1 in Copenhagen City. During the 9 years of the study, the overall incidence (per 100,000) gradually decreased (29.8 in 2000 vs. 27.0 in 2008) and this was attributable to a reduction in disease due to MSSA (28.5 in 2000 vs. 24.9 in 2008). However, MRSA increased from 1.3 per 100,000 in 2000 to 2.0 per 100,000 in 2008, and this increase was marked in some regions.

Conclusions: The overall incidence of S. aureus bacteraemia is decreasing. However, ongoing surveillance is needed to assess regional differences and whether this trend may be reversed in the coming years due to the emergence of MRSA.

**P831 The changing molecular epidemiology of methicillin-resistant Staphylococcus aureus bacteraemia at a London teaching hospital, 1999–2009**

J.A. Otter*, J. Edgeworth, C. Miller, G. French (Andover, UK)

Objectives: Against a background of decreasing incidence, we sought to determine the changing molecular epidemiology of methicillin-resistant Staphylococcus aureus (MRSA) bacteraemia at Guy’s & St Thomas’ Trust (GSTT), a large London teaching hospital.

Methods: We investigated isolates of MRSA bacteraemia recovered at GSTT from 1999–2009. The first MRSA isolate from each patient was characterised by spa type, staphylococcal cassette chromosome mec (SCCmec) allotype and resistance to a range of antimicrobial agents. Clones were defined by using Based Upon Repeat Pattern (BURP) clustering with a stringent calculated cost between lineages of 1. Analysis of contingency tables was performed using χ² tests.

Results: 820 episodes of MRSA bacteraemia were reported during the study period. Annual numbers decreased from 137 in 2000 to 13 in 2009. The mean age of affected patients was 62 years (standard deviation 17), 84% were white, 34% were female and 30% of patients died. Epidemic MRSA-15 (E15) and E16 accounted for 76% of cases overall. There appeared to be a clonal replacement of E16 by E15 and other types (chart). E15, other types and E16 accounted for 29%, 13% and 46% of 634 cases during 1999–2004 compared with 46%, 24% and 22% of 186 cases during 2005–2009, respectively (p < 0.001 comparing the proportion of E15, other types and E16 in these two periods). E15 were SCCmec IV (100%), predominantly 032 (89%) and 65% were resistant to <3 classes of non-β-lactams. E16 were SCCmec II (99%), predominantly 018 (91%), 91% were resistant to >2 classes of non-β-lactams and 40% were mupirocin resistant. 016 was classified as a singleton lineage by BURP; it accounted for 9% of isolates and was associated with a previously reported line-associated outbreak, peaking in 2003 (chart). 0167 isolates were SCCmec III (84%) and 86% were resistant to >4 classes of non-β-lactams. The remaining 15% of isolates were a heterogeneous group comprising 42 different spa types. There were significant differences in clonal distribution of all types in terms of the focus of bacteraemia, patient specialty and antimicrobial resistance.

Conclusion: During a significant decline in MRSA bacteraemias, the relatively drug-susceptible E15 appears to have replaced the multidrug-resistant E16 lineage as the commonest type of isolate and the proportion of clonally heterogeneous and singleton isolates has increased. The reasons for these clonal replacements are unknown and warrant further investigation.

**P832 Mechanisms of adaptation of successful methicillin-resistant Staphylococcus aureus clones**

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Objectives: Methicillin-resistant Staphylococcus aureus (MRSA) is a major cause of healthcare-associated (HA) infections. Most HA-MRSA infections are due to few epidemic successful MRSA clones. Since 2006, we collected and characterized the MRSA strains from infected patients, admitted at the hSR, for infection control purposes and to identify and characterize the major clones. We compared the genetic background and the physiological characteristics of MRSA of “epidemic” and “sporadic” clones to investigate the adaptation mechanisms and the genetic-phenotypic factors involved in the epidemic behaviour of MRSA.

Methods: MRSA strains were characterized by SCCmec-typing, PFGE, spa-typing and detection of Panton-Valentine leukocidin (PVL) genes. Based on PFGE patterns, MLST was performed on selected strains. Biofilm production of isolates belonging to major clones and competitive growth experiments were performed.

Results: We identified 3 major epidemic HA-MRSA clones and we report here, for the first time in Italy, the replacement of the ST228-SCCmec Southern German clone, still the most prevalent clone in Italy, by the gentamicin- susceptible (GS) ST22-SCCmecIV EMRSA-15, that became the leading clone in hSR. We noticed a strong correlation between the ability to produce biofilm and the capacity of spreading and persistence of clones: during the study period, the ST22 predominant type modified its biofilm production from weak to strong producer (p < 0.01). In hSR, there were no particular restrictions on antibiotic use that could explain the replacement of a
The risk factors of mortality for nosocomial *Staphylococcus aureus*

### Objective:

Bloodstream infections (BSI) due to *Staphylococcus aureus* have become increasingly common in hospitals worldwide. *S. aureus* continues to be an important cause of nosocomial bacteraemia. The aim of this study was to evaluate the risk factors for mortality of nosocomial BSI due to *S. aureus*.

### Method:

We analyzed risk factors for mortality of methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) bacteraemia in a prospective case control study in an 1196-bed tertiary referral medical center. One hundred and seventy six patients were identified with clinically significant and microbiologically confirmed nosocomial bacteraemia due to *S. aureus* between July 2006 and January 2009. Logistic regression analyses were used to determine risk factors of mortality.

### Results:

A total of 176 episodes of *S. aureus* BSI were identified: 102 MRSA BSI and 74 MSSA BSI. The crude mortality rate of *S. aureus* bacteraemia was 50.6%. The difference between the mortality rates of MRSA (62.7%) and MSSA bacteremia (31.0%) was 31.7%. Upon multivariate logistic regression analysis, the mortality with MRSA bacteremia was revealed to be 3.02 times higher (p=0.008, RR=3.02, 95%CI=1.34−6.8). Other significant variables were found as potential risk factors for mortality on multivariate logistic regression analysis as follow; age equal or greater than 60 (p=0.015, RR=2.13, 95%CI=1.1−3.39), hospitalization in intensive care unit (p=0.000, RR=5.6, 95%CI=2.9−10.9), total parenteral nutrition (p=0.017, RR: 3.28, 95%CI: 1.24–8.76) and methicillin resistance (p=0.008, RR=3.02, 95%CI: 1.34–6.8).

### Conclusion:

Methicillin resistance lead to higher mortality rates in *S. aureus* BSI. As a consequence of this study we aimed decreasing total parenteral nutrition usage. In addition, we planned to look over infection control precautions and to apply strictly. For decreasing methicillin resistance, we decided to evaluate rational antibiotic usage.

### Reference:

Bastug A.*, Yilmaz G.R., Kayaslan B., Bodur H. (Ankara, TR)
Persistent methicillin-resistant Staphylococcus aureus bacteremia: risk factors analysis and clinical outcomes

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Background: The high mortality attributable to persistent MRSA bacteremia despite glycopeptide treatment has heightened the need for early detection and intervention with alternative agents. The purpose of this study was to determine the clinical characteristics and risk factors of persistent MRSA bacteremia.

Methods: All first episodes of significant MRSA bacteremia were collected at a 710-bed academic medical center during the period November 2009 through August 2010. Blood culture was done at 3rd days and 7th days after initiation of glycopeptide treatment. Clinical characteristics were compared between persistent MRSA bacteremia (>7 days) and non-persistent MRSA bacteremia (<3 days). Time to blood culture positivity (TTP) is defined as time between onset of incubation and growth detection using an automated blood culture system.

Results: Of 84 patients with MRSA bacteremia during the study period, 33 patients (39.3%) had persistent MRSA bacteremia. Persistent MRSA bacteremia group had a significantly higher 30-day mortality than non-persistent MRSA bacteremia group (OR, 17.60; 95% CI, 3.58–86.45; p < 0.001). Multivariante analysis indicated that catheter retention in catheter-related infection (CRI) (OR, 5.48; 95% CI, 1.38–21.86; p = 0.016) and metastatic complication at presentation (OR, 24.36; 95% CI, 4.63–128.21; p = 0.001) were independent predictors for persistent MRSA bacteremia. Patients who had early TTP <12 hours were at a increased risk of persistent MRSA bacteremia (32.2% versus 11.4%, p = 0.039).

Conclusions: High mortality of persistent MRSA bacteremia is noted in patients with glycopeptides. Early removal of catheter and evaluation of metastatic infection should be taken into consideration for reducing the risk of persistent MRSA bacteremia. Further large studies are needed to evaluate the efficacy of early intervention with appropriate alternate agents in MRSA bacteremia of earlier TTP.

Distribution of the arginine catabolic mobile element in staphylococci

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Objectives: The SCCmec-associated arginine catabolic mobile element (ACME) has been recently found in the MRSA strain ST8-MRSA-IVa, “USA300” (Diep, 2006). This element was thought to contribute to the pandemic spread of this strain, e.g., by enhancing survival on human skin. ACME was also described in ST1-MRSA-IVa, ST3-MRSA-II and ST39-MRSA-IVa, ST22-MRSA-IV, ST59-MRSA-IVa, ST97-MRSA-V and ST239-MRSA-III isolates (Goering, 2007; Diep 2008; Ellington, 2008; Ellis, 2009; Shore, ISSSI, 2010; Ghaznavi-Rad, 2010). Aim of the study was to detect ACME in a large collection of staphylococcal isolates.

Methods: Approximately 6,400 S. aureus isolates (including ca. 3,700 MRSA) from diverse geographic locations were genotyped using DNA microarrays (Alere Technologies, Germany) which assigned isolates to MLST clonal complexes and detected virulence-associated genes including the ACME genes arca/B/C/D. This assay was also applied to 185 methicillin-resistant coagulase-negative staphylococci (MR-CoNS) recovered from blood cultures from one Irish and two German hospitals.

Results: ACME was identified in 3.5% of S. aureus isolates tested. The most common and widespread ACME-positive strain was “USA300”, although one-third (57/182 isolates) lacked ACME. Other ACME-positive MRSA strains included two clones, which appear to be restricted to Hong Kong (ST1048-MRSA-IV, ST1774-MRSA-IV), and the above-mentioned ST22-MRSA-IV from Ireland. ACME was found to occur sporadically in several CC45-MRSA strains as well as in CC5-MRSA-II, -IV, -V and ST239-MRSA-III isolates. The only methicillin-susceptible S. aureus harbouring ACME were epidemiologically linked, hospital-acquired CC8-MSSA from South-Eastern Saxony. ACME was also detected in methicillin-resistant S. epidermidis and S. capitis as well as in methicillin-susceptible S. warneri. Among blood culture isolates of MR-CoNS, 28.6% (53/185) were ACME-positive with no significant difference between German and Irish isolates. The highest prevalence of ACME was noted for S. epidermidis (47.3%).

Conclusion: ACME was identified in a small proportion of the S. aureus population but it was more common among CoNS. While it still needs to be clarified whether the presence of ACME really confers a selective advantage to S. aureus, it can be speculated that intra-species transfer of ACME is a rare event and that it may confer some disadvantage or fitness cost which limits its spread within S. aureus.

Is our hospital environment clean enough for the containment of methicillin-resistant Staphylococcus aureus?

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Objective: Transmission of multi-drug resistant pathogens has been associated with hospital hygiene. One of the common hospital acquired infection is methicillin-resistant Staphylococcus aureus (MRSA). This study aimed at examining the level of environmental MRSA contamination in a public hospital in Hong Kong.

Methods: 32 patients with MRSA were randomly selected over one month and each of these cases was matched with one control patient presenting with no MRSA who resided in the same ward. 15 sites in close proximity of the patient bed were selected where environmental samples were obtained with contact slides and swabs. All the MRSA isolates were characterized by molecular tests.

Results: There was significantly higher chance of identifying MRSA at the near-to-patient sites of infected patients than control patients (53% vs 28%, p-value <0.05). Each MRSA patient had an average of 2.1 sites present with MRSA, compared with only 0.5 sites for each control. Hand-touched sites, such as locker handle bar (23%) and bed tilt adjustment handles (19%), most frequently harbored the microbe. Fomites that were often omitted from routine cleaning, for instance, cubical partition ledge and curtain, had substantial contamination of MRSA. Molecular studies showed that most of the isolates belonged to sequence type (ST) 45 (with spa t0811, Staphylococcus cassette chromosome mec (SCCmec) V, agr IV). It constituted 40% (36 out of 90 isolates) of total near-to-patient MRSA. The second largest group comprising 31% (28 isolates) was ST 8/239 (spa t037, SCCmec III/IIIA, agr I).

Conclusions: It is found that MRSA ST45 is the most prevalent in the hospital environment in Hong Kong. The higher rate of MRSA isolated around the infected patients suggests a possible route of MRSA transmission from the patients to their proximal environment. Enhanced and audited cleaning should be advocated to reduce the risk of nosocomial infection.

First report of methicillin-resistant Staphylococcus aureus Cordobes/Chilean clone involved in nosocomial infections in Brazil

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The objective of this study was to describe the molecular epidemiology of methicillin-resistant Staphylococcus aureus (MRSA) involved in nosocomial infections in the city of Porto Alegre, Southern Brazil, and its metropolitan area. We studied patients with MRSA infections identified at a hospital complex from April to December, 2008. The SCCmec complex type was determined by Polymerase Chain Reaction (PCR). Representative isolates were typed by chromosomal DNA macrorestriction followed by pulsed field gel electrophoresis (PFGE). A total of thirty MRSA isolates were obtained from inpatients. Eighteen (60%) of MRSA isolates showed SCCmec type III and PFGE analysis of representative isolates revealed a pulstype which corresponds to the Brazilian Epidemic Clone (BEC). Eleven (36.7%) isolates showed
SCCmec type-I, all belonging to the same clonal group which was related to the Cordobes/Chilean clone by PFGE analysis. Finally, we observed one isolate exhibiting SCCmec type IV that, according to PFGE analysis was not similar to any of the international clones tested. Isolates presenting SCCmec type I were typically multidrug resistant, except for trimethoprim-sulfamethoxazole (TMP-SMX), vancomycin and rifampin, whereas isolates presenting SCCmec type III had a variable pattern of resistance. The rise of the now called Cordobes/Chilean clone was documented in Cordoba, Argentina, more than a decade ago and this clone quickly became predominant over BEC in Argentina in 2001 (53% versus 23% in hospital-acquired infections). In Brazil, besides BEC, the presence of different clones was described, however the occurrence of Cordobes/Chilean clone was not reported so far. All isolates of this study exhibited susceptibility to TMP-SMX and this may be a useful phenotypic marker in the clinical microbiology laboratory as a preliminary criterion to differentiate it from BEC in a suspected outbreak. Our study is the first report of Cordobes/Chilean clone involved in nosocomial infections in Brazil. Considering that this clone successfully replaced BEC in hospitals in Argentina and in other countries, it is important to emphasize the need of surveillance studies based on molecular epidemiology tools allowing us to trace the spread of Cordobes/Chilean clone.

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**P840** Molecular characterisation of methicillin-resistant *Staphylococcus aureus*: ST239 isolates collected over a 12-year period in Hong Kong

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Objectives: To determine the usefulness and discriminatory ability of an extended panel of published VNTR primers when investigating the molecular characterisation of a longitudinal collection of Methicillin Resistant *Staphylococcus aureus* (MRSA); ST239 isolates from Hong Kong.

Methods: Seventy-six non duplicate patient MRSA isolates collected between 1998–2000 from blood culture and non blood culture samples were confirmed as sequence type 239 (ST239) using multiplex PCR. Three multi locus variable number tandem (VNTR) typing schemes (A, B, C) were used to epidemiologically characterise these isolates. The published methods utilised twenty-eight VNTR regions on the *S. aureus* genome, of which twenty-three loci were amplified. The five omitted loci were duplicated targets between all three schemes. Amplicons were analysed using the QIAxcel capillary gel electrophoresis platform to determine size and repeat number of specific products at each locus.

Results: Variability was seen in 20/23 loci. Scheme A, utilised 7 VNTRs with 3 variable loci and resolved 76 isolates into 14 unique molecular fingerprints. Scheme B and C with 5 and 11 VNTRs respectively were variable at all loci and resolved the isolates into 20 and 75 specific profiles respectively. When comparing all 23 VNTRs all isolates were resolved uniquely through a single repeat change at any loci. Overall similarity of all 76 isolates using 23 loci differentiation was ≥24%, with 69 isolates clustering within 85% similarity as determined by BioNumerics.

Conclusion: This study has demonstrated that by increasing the number of VNTRs from a possible seven to 23 loci to characterise *S. aureus* isolates for molecular fingerprinting, further enhances its discrimination as an epidemiological tool. However, opting for a 23 loci typing method potentially may have cost implications, thus further work on identifying the optimal loci within this extended panel for epidemiological differentiation or sub-typing is warranted.

**P841** Clinical features of *Staphylococcus aureus* resistant to linezolid at the Hospital Universitario Infantino Cristina, Badajoz, Spain

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Objective: To report the isolation of methicillin-resistant *Staphylococcus aureus* resistant to linezolid (L-MRSA) in six patients at the Hospital Universitario Infantino Cristina in Badajoz.

Methods: Descriptive analysis of clinical features of infections caused by L-MRSA. At laboratory, susceptibility testing was performed using the WalkAway System (Siemens Healthcare Diagnostics). Resistance to Linezolid (Lz) was confirmed with the method of E-test (AB Biodisk, Solna, Sweden); interpretation of MICs was according to CLSI guidelines.

Results: Between May and October 2009, five patients admitted to the intensive care unit (ICU) and one to the Neurosurgery unit in our center had severe infections caused by L-MRSA: two septic shocks (secondary to nosocomial and to community-acquired pneumonia, respectively), a post-quiurgical meningitis, a cranium encephalic trauma, a cerebral hematoma and an encephalophaty secondary to cardiac arrest. All the patient were men, with ages in a range 47–75 years (mean=56); all them had central catheter in place, 4 of them (57%) had prior surgery; the lenght of hospitalization were 10–44 days (mean=23), all of them died at ICU between 1–23 days (mean=9). The patients treated with linezolid received it for 5–14 days (mean=7). L-MRSA was isolated in blood cultures in three cases, in tracheal aspirate in one case, from CSF in one case and from an eschar in another case. 2 patients were confecced with *A. baumannii* and one with extended-spectrum β-lactamase producing *Escherichia coli*. All the patients except that from the Neurosurgery unit had been treated with Lz prior to the isolation of L-MRSA. All the isolates had resistance to Lz with MIC values between 16 and >258 μg/ml confirmed by E-test. L-MRSA isolates were found resistant to oxacillin, ciprofloxacino, clindamycin, erythromycin, chloramphenicol, and susceptible to teicoplanin, trimethoprim/sulfamethoxazole and vancomycin. Plasmidic cff gene was detected by PCR and confirmed by sequencing, but plasmids harboring this gene are under study. The two patients with L-MRSA septic shock and the one with cranium encephalic trauma died. No new cases were observed after reinforcing infection control practices.

Conclusions: Lz-resistant MRSA isolates affected critically ill patients with and without previous Lz treatment. High MICs of linezolid suggest additional resistance mechanisms and are currently under further investigation.

**P842** Community-acquired methicillin-resistant *Staphylococcus aureus* in Spain: present situation and evolution over the last 7 years in a general hospital

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Objectives: Since the first description in 2003 of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) isolates producing the Panton-Valentine leukocidin (PVL+) in Spain, their incidence seems to have increased. We describe the evolution of PVL+ CA-MRSA over a period of 7 years in our institution, the molecular characteristics of the isolates and analyze the clinical features of patients infected with these isolates.

Methods: From September 2004 to November 2010, we collected 48 PVL-positive MRSA isolates corresponding to patients attending to the emergency department. The isolates were genotyped by pulsed-field gel electrophoresis, SCCmec typing, agr polymorphism, and multilocus sequence typing. Susceptibility to 29 antimicrobials was determined by the broth microdilution method. In addition, the susceptibility to linezolid, tigecycline and daptomycin was determined by the Etest method. Methicillin-resistance was confirmed by the detection of the
meC gene by PCR and pvf genes were detected by PCR amplification of the lukS-PV and lukF-PVF genes.

**Results:** Over the period of study the number of isolates recovered (year/number) was: 2004/4, 2005/2, 2006/4, 2007/10, 2008/8, 2009/11, 2010/10. The isolates belonged to the genotypes: ST8-SCCmec IVc (n = 38); ST8-SCCmec IVa (USA300; n = 3); ST30-SCCmec IVc (n = 3); ST5-SCCmec IVa (n = 1); ST80-SCCmec IVc (n = 1); and 2 singletons. The corresponding agr types were I, I, III, II, I, and I, respectively. In addition to methicillin-resistance, 10 isolates were resistant to tetracycline and doxycycline; 4 to erythromycin and clindamycin; 3 to erythromycin and ciprofloxacin; and 2 to fusidic acid. All isolates were fully susceptible to the new antimicrobials linezolid, tigecycline and daptomycin. The isolates were from children (n = 24) and adults (n = 24), and were associated with skin and soft tissue infections (pyogenic abscesses; n = 44), otitis (n = 2), and secondary bacteremia (n = 2). Twenty-eight patients were from South America and one from Africa. All patients recovered after surgical drainage and/or antimicrobial treatment.

**Conclusions:** CA-MRSA infections are emerging in Spain although their incidence is still low. Half of the patients were not Spanish-native. CA-MRSA isolates belong to different lineages, although the majority of the isolates belong to genotype ST8-SCCmec IVc. At present, the presence of the USA300 clone is anecdotal in our area.

**P845**

**Hospital-onset of community-associated methicillin-resistant Staphylococcus aureus USA300 strain in Vancouver, Canada**

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**Objectives:** USA300 is the predominant “community-associated” methicillin-resistant Staphylococcus aureus (CA-MRSA) strain in the USA, and has spread to Canada, Spain, Germany and other countries worldwide. The epidemiology of CA-MRSA has recently changed and USA300 appears to be emerging as a cause of serious, hospital-onset infections in large American cities. The aim of this study was to determine if transmission of USA300 MRSA was occurring within two Canadian hospitals.

**Methods:** All new MRSA cases detected by a hospital-based laboratory over a 12-month period in 2008/2009 were included in the study. Microbiological specimens submitted for testing included both surveillance and clinical specimens. Chart review was performed and cases were defined as hospital-onset MRSA (HO-MRSA) if positive specimens were collected more than 72 hours after admission. Bacterial isolates from these cases underwent PCR testing for the Panton-Valentine leukocidin (PVL) genes. All HO-MRSA isolates that were PVL positive underwent pulsed field gel electrophoresis (PFGE) fingerprinting.

**Results:** In total, 841 new MRSA cases were detected during the study period. Of these, 126 were HO-MRSA isolates and underwent testing for PVL. Overall, 39 of 126 (31%) HO-MRSA isolates were found to be PVL positive, and were confirmed as the USA300 strain by PFGE.

**Conclusion:** Our findings indicate that transmission of HO-MRSA USA300 amongst hospitalized patients is common in two Canadian hospitals. This study represents the first known documented report of extensive transmission of HO-MRSA USA300 in Canada. Further, our findings indicate that “community-associated” MRSA may not be an appropriate classification when molecular-based definitions of MRSA are available.

**P844**

**Epidemiology and antibiotic resistance of MRSA isolated in Hospital Bologhine Ibn Ziri, Algiers**

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**Objectives:** To evaluate the epidemiology and the antibiotic resistance of the MRSA strains isolated in our university first hospital.

**Method:** We analyzed the strains isolated from different samples from outpatients and inpatient from January first 2007 to September 30th 2010. The culture and identification were performed according to the standards techniques. The susceptibility test was performed according to the disk diffusion CLSI technique against 15 antimicrobial agents; Oxacillin (Oxa), kanamycin (Ka), Fusidic acid (FA), Tetracycline (Te), Cotrimoxazole (SXT), Gentamicin (Gen), Erythromycin (Eb), Ofloxacin (OFl), Clindamycin (DA), Amikacin (Amk), Fosfomycin (Fos), Levofloxacin (Levo), Pristinamycin (Prt), Rifampicin (Rip), Chloramphenicol (Cm) and Tobramycin (Tob), Teicoplanin (Tei) and Vancomycin (Va). MRSA was defined by Cefoxitin resistance (Cefoxitin disk diameter <19mm).

**Results:** 76 strains were isolated from 71 patients. The sexe ratio was 1.73. 63.38% were female and 36.6% were male. MRSA was isolated more frequently from children under the age of 10 years and the age groups of 31–40 years. 37.5% of MRSA was isolated from outpatient samples. In the hospital, MRSA was frequently isolated from patients’ samples from the surgery department 15.5%, followed by the hematology and the intensive care unit 12.25%. Skin and soft tissue samples were the most frequent 55.26%, followed by bacteremia 15.78%. The susceptibility test showed a high resistance to Ka (74%), FA (61%), and Te (51.9%). MRSA was also resistant to SXT (29.9%), Ge (24.7%), E (18.2%), Of (18.2%), DA (14.9%), Amk (10.4%), Fos (5.2%), Levo (5.2%), Prt (2.6%), Rif (1.3%), Cm and TOB (1.3%). 36 different biotypes were identified. The most frequent was K,Te,FA 21% (37.5% in the outpatient and 16.66% in the inpatients). It was followed by the antibiotic K,FA (5.26%) which was isolated essentially from outpatient samples. The other antibiotic were <3.94%. One Strain of MRSA was susceptible to all antibiotics.

**Conclusion:** This study showed that MRSA is most frequently isolated from soft and skin infections. The biotype K,Te,FA which is usually isolated from community infections is the most frequent biotype isolated in our hospital. It seems that it had spread in our hospital. However it is difficult to determine the origin of its acquisition, in the community or in the hospital. Further studies on the carriage of MRSA at the admission are needed.

**P843**

**Clonal lineages of tetracycline-resistant MRSA strains isolated in a Spanish hospital during 2009. Detection of MRSA ST398**

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**Objectives:** To study the clonal lineages, resistance mechanisms and virulence traits of tetracycline resistant (TetR) MRSA strains isolated in a Spanish Hospital during 2009.

**Methods:** 34 TetR MRSA were detected from unrelated patients in Miguel Servet Hospital during 2009, representing 9.5% of MRSA recovered in that period, and 27 of them were included in this study. The antibiotic susceptibility profile to 23 antibiotics was determined by Microscan® system. Presence of ermA, ermB, ermC, emrT, emrF, mrsaA/mrarB, mphC, cfr, Isa(8), TetE, TetL, TetM, TetO, aac(6′)aph(2″), mupA, dfrA, dfrD, dfrF, and dfrG genes was studied by PCR. Sequences of grlA and gyrA genes were analysed in some of the strains. The determination of SCCmec and agr-typing was implemented on all MRSA isolates. Spa-typing was performed by PCR and sequencing. Presence of virulence factor genes lukS/lukF, lukS, st, etb, etd and cna was investigated by PCR.

**Results:** The spa-types identified among the 27 TetR MRSA isolates were (no strains): st01 (12), st1197 (1), st394 (2), st002 (1), st008 (1), st067 (3), st127 (2), st1381 (1), st220 (2), and 2 new spa-types (st613, st577). Fifteen strains (55.6%) were included in spa types related to the livestock-associated sequence type ST398 (st01, st1197 and st394). These strains and one strain with a new spa-type (st577) were typed as SCCmecIV and agr. The other strain with a new spa-type (st613) presented a non-typable SCCmec and agr. The remaining 10 MRSA strains were typed as SCCmecIV and agrI, agrII or agrIII. The following genes were detected (no strains): ermB (7), ermC (13), emrT (2), mrsaA/mrarB (4), mphC (5), mphF (5), tetE (16), tetM (27), aac(6′)aph(2″) (5), dfrA (2) and mupA (3). Mutation in quinolone targets were studied in 10
Clinical outcomes and risk factors for mortality in patients with invasive infection caused by ST72-MRSA-SCCmec type IV strains in South Korea

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Objectives: Even though CA-MRSA in South Korea were known to be the predominance of SCCmec type IV with sequence type (ST) 72 and PVL-negative, the clinical outcomes and risk factors for mortality in patients with CA-MRSA infection have been rarely explored.

Methods: Retrospective cohort study was designed to identify ST72-MRSA-SCCmec type IV from 3417 MRSA isolates stored in Samsung Medical Center from 2007 to 2009. SCCmec typing and multilocus sequence typing (MLST) was performed in selected MRSA infection cases which had been susceptible to fluoroquinolones (FQ), gentamicin (GM), rifampin (RFP) and sulfamethoxazole/trimethoprim (SMX/TMP). Finally, patients who had infection caused by ST72-MRSA-SCCmec type IV were enrolled in this study.

Results: A total of 124 patients were identified to have ST72-MRSA-SCCmec type IV infection from 452 MRSA isolates susceptible to FQ, GM, RFP and SMX/TMP. The most common primary focus of infection was skin and soft tissue infection (SSTI) (51/124, 41%) which when it included surgical site infection (27/124, 21.8%), and followed by pneumonia (34/124, 27.4%) and bone and joint infection (13/124, 10.5%). Eighty-four patients (67.3%) had community-onset (CO) infection with 61 (49.2%) healthcare-associated, whereas 40 (32.3%) had hospital-onset (HO) infection. In comparison of patients between community- and hospital-onset infection, complicated SSTI had been related to community (16/84 vs. 1/40; P = 0.002) and pneumonia to hospital (16/84 vs. 18/40; P = 0.005). The univariate analysis showed that factors associated with mortality were the presence of hematologic malignancy and pneumonia. By multivariate analysis, understanding hematologic malignancy and pneumonia still showed a significant association with mortality (OR, 11.93; 95%CI, 1.41–101.07; P=0.023 and OR, 37.74; 95%CI 6.64–214.65; P<0.001).

Conclusions: Community-genotype strains which were defined as ST72-MRSA -SCCmec type IV, have been commonly observed in healthcare and hospital-settings. Pneumonia was more related to community-onset infection compared to community, whereas SSTI to community compared to hospital. Underlying hematologic malignancy and pneumonia were significantly associated with mortality in patients with ST72-MRSA infection.

Comparison of mortality-associated bacteremia due to methicillin-resistant and methicillin-susceptible Staphylococcus aureus

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Objectives: 1. Study of the prevalence and evolution of methicillin-resistant Staphylococcus aureus (MRSA) in our hospital. 2. Comparison of the mortality risk associated with bacteremia due to MRSA and methicillin-susceptible S. aureus (MSSA).

Methods: Retrospective study of MRSA and MSSA strains isolated from clinical samples of patients attended in the Hospital Universitario Miguel Servet from 2005 to 2009. Isolates were identified and tested for antibiotic susceptibility by microdilution system (MicroScan WalkAway® Siemens). Data from outcome of patients with S. aureus bacteremia were collected for the study.

Results: The total isolates of SAMS were 1242 (65.78%) in 2005, 1342 (69.14%) in 2006, 1441 (67.49%) in 2007, 1520 (64.82%) in 2008 and 1466 (68.05%) in 2009. The isolates of MRSA were 646 (34.22%), 599 (30.86%), 694 (32.51%), 825 (35.18%) and 688 (31.94%). The total isolates of SAMS in blood samples were 99 (7.90%) in 2005, 143 (10.60%) in 2006, 153 (10.61%) in 2007, 136 (8.94%) in 2008 and 122 (8.32%) in 2009. The isolates of MRSA were 43 (6.65%), 39 (6.51%), 69 (9.94%), 40 (4.84%) and 38 (5.52%) respectively. The percentages of patients affected by MRSA and MSSA bacteremia are shown in the table below (table 1). The outcome was studied in the patients with S. aureus bacteremia. We found that mortality was 66.6% in patients with MRSA bacteremia, in 2005 (n=6), 40% in 2006 (n=4), 46.6% in 2007 (n=7), 37.5% in 2008 (n=6) and 50% in 2009 (n=14). In comparison, mortality in patients with MSSA bacteremia data were 31.2% in 2005 (n=15), 37.5% in 2006 (n=27), 28.3% in 2007 (n=19), 37.7% in 2008 (n=24) and 41.8% in 2009 (n = 36).

Conclusions: • The prevalence of MRSA doesn’t change, although the number of patients with MRSA bacteremia increases slightly in the period study. • Patients with MRSA infection had higher mortality risk, compared with patients with MSSA. • Increased effort in the infection control measures and in reliable laboratory screening for resistance is necessary, as MRSA infections are still a cause of concern in our hospitals.

Genetic lineages, virulence and antimicrobial resistance mechanisms detected in methicillin-resistant Staphylococcus aureus of blood origin in a Spanish hospital

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Objectives: To characterize the molecular epidemiology, resistance and virulence in all methicillin-resistant Staphylococcus aureus (MRSA) recovered from blood cultures of unrelated patients at a Spanish hospital (period November 2008 to 2010).

Methods: Thirty-one MRSA isolates were included in this study. Identification was carried out by Microscan and by PCR amplification of nuc gene. The susceptibility testing to vancomycin (VAN), teicoplanin (TEC), streptomycin, gentamicin (GEN), netilmicin (NET), tobramycin (TOB), kanamycin (KAN), trimethoprim-sulfamethoxazole (SXT), ciprofloxacin (CIP), tetracycline (TET), chloramphenicol (CHL), linizolid (LZD), mupirocin (MUP), quinupristin-dalfopristin (Q-D), erythromycin (ERY), and clindamycin (CLI) was tested by disc diffusion method. The presence of resistance genes (e.g. mup, mecA, ant(4′)-Ia, aac(6′)-Ia-aph(2′)-Ia) and virulence factors (e.g. tst-1, pvl, eta and etb) were determined by PCR. The clonal relationship was determined by PFGE/SmaI and the clonal relationships by spa, SCCmec, and agr typing.

Results: The percentages of resistance detected in the MRSA isolates were as follows: CIP (100%), GEN (16%), TOB (81%), KAN (90%), ERY (35%), CLI (16%) and MUP (16%). Three strains presented...
Molecular analysis of Staphylococcus aureus strains isolated in a newborns unit in 2010

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Objective: To characterize Staphylococcus aureus strains circulating in a newborns unit during the summer of 2010, in order to provide information for infection containment.

Methods: Identification of strains was done by using conventional methods, automated VITEK2 system and PCR for mec gene detection. We included mec gene detection in a triplex in house PCR, together with mecA and lukS/F genes detection. For spa typing we used the SeqNet protocol and the Ridom StaphType software (Ridom GmbH). Pulsed Field Gel Electrophoresis was performed according to the HARMONY protocol (Stephen Murchan et al.) and SCCmec typing according to the PCR multiplex updated method (C. Milheirico et al. – AAC, 2007). Disc diffusion was used for antimicrobial susceptibility testing, according to the EUCAST method. For cefoxitin and vancomycin MIC detection we used the E-test.

Results: Bacterial cultures received by our national reference laboratory from a newborns unit were isolated during two summer months, in 2010, from umbilical wounds (nr. = 13), nasal exudate (nr. = 7), and two other body sites. We identified 22 S. aureus strains, from which 2 MSSA and 20 MRSA. All MRSA belonged to the t127 spa type. The 20 MRSA strains were equally distributed between 2 PFGE patterns, with a Dice index of similarity of 87.5%. All MRSA strains were SCCmec type VI or a type VI variant and PVL negative. The MSSA strains were of spa type t616 and t1556, respectively, showing a low similarity index with the MRSA strains. All MRSA strains, except one, showed the same antimicrobial resistance pattern, with resistance to FOX, E, DA (MLSb), CN and TE. One strain was additionally resistant to Ciprofloxacin.

Conclusion: Molecular analysis of MRSA strains isolated from a newborns unit revealed a cluster of strongly related strains, supporting the hypothesis that a success clone is evolving in this care unit. Besides local measures, further studies are needed for deeper understanding the origin of the evolving strain.

Characterisation of oxacillin-susceptible mecA-positive Staphylococcus aureus: could this be a new type of MRSA?

S. Al Johani*, A. Al Awaji (Riyadh, SA)

Objective: Methicillin-resistant Staphylococcus aureus (MRSA) has been defined as S. aureus having the mecA gene or showing a minimum inhibitory concentration (MIC) of oxacillin higher than 4 mg/L. However, some clinical isolates are mecA-positive and oxacillin-susceptible. Therefore, we surveyed the occurrence of S. aureus having the mecA gene and an MIC of oxacillin of less than 2 mg/L (oxacillin-susceptible MRSA; OS-MRSA).

Method: Between August and November 2010; a total of 2835 nasal swabs submitted for MRSA screening. Tests carried out by Xpert MRSA (Cepheid®); all positive samples by Xpert system that exhibit low Ct endpoint (<31) confirmed by routine culture method using Mannitol salt Agar (MSA) and all confirmed staphylococcus aureus samples examined by its susceptibility to cefoxitin disc (30ug; Oxoïd®) as per CLSI guidelines. All strains that susceptible to cefoxitin tested by a third method, Pencillin binding protein 2a (PB2P2a) which directly detects the PB2P2 protein encoded by the mec A gene.

Results: Of the 2835 nasal swabs screened by Xpert MRSA; there were 195 samples reported as positive for MRSA. Of the 195 there were 156 (80%) true positive confirmed by culture and cefoxitin resistance, 19 (9.74%) were false positive and there were 20 (10.26%) samples that has both Xpert MRSA positive, PB2P2a positive and Cefoxitin susceptible with a zone diameter of 25−31 mm. Oxacillin MIC for all 20 isolates were below 2 mg/L. These OS-MRSAs were least resistant to oxacillin among the MRSA strains tested and were within the susceptible range to seven other beta-lactam antibiotics tested. Thus, OS-MRSA may become a high-resistant MRSA upon the treatment of patients with beta-lactam antibiotics.

Conclusion: We notice a prevalence of 10% of all positive MRSA, either new type of resistance. These results questioning the presence of new mecA positive, Cefoxitin susceptible type of Staphylococcus aureus that may be classified as a new type of MRSA. In this study, the occurrence of OS-MRSA at a certain frequency was noted; precautions are called for in the classification of oxacillin-resistant S. aureus and in the treatment of OS-MRSA infection. Further studies needed to genotype these isolates.
The aim of this study was to analyse the genodiversity and presence of virulence factors in *Staphylococcus aureus* isolated from patients with nosocomial blood stream infections irrespective of Methicillin-resistance.

Strains were isolated from patients at two university hospitals participating in the Invasive *Staphylococcus aureus* Infections Cohort (INSTINCT). One-hundred and forty-one strains were clinically Prevailing profiles of virulence factors can be crucial for studying of related genetic background, a considerable proportion belongs to CC different spa-types. Even if about half of the isolates had a MRSA-

The most prevalent superantigens were the egc-cluster toxins (n=79) including 20 different CC. Strain of each CC were assigned to varying spa-types, except of two CC59 isolates (both t216). MRSA included

The predicted CC ranked in the order CC5 (n=21), CC30 and CC45 including 20 different CC. Strains of each CC were assigned to varying spa-types, except of two CC59 isolates (both t216). MRSA included

**Methods:** We received 205 questionnaires, representing 16.8% of the 1217 Italian hospitals, and 39% of the hospitals ≥200 beds, from 19/20 Italian regions, accounting for 42% of the national admissions. 74 hospitals (36%) had written guidelines, and further 17 hospitals referred to CDC or WHO guidelines on multi-resistant microorganisms, for a total of 91 centres (44%). Local guidelines were based mostly on CDC guidelines (78 hospitals, 86%), WHO (48, 53%), and/or SHEA (30, 33%). A hand hygiene program was ongoing in 95 hospitals (46%), 33 (16%) had indications on contact precautions, 87 (43%) analysed and fed back surveillance data, 66 (32%) screened for MRSA high risk wards and/or patients, 42 hospitals (20%) performed MRSA decolonization, and 41 (20%) had an ongoing antimicrobial stewardship program. One hospital (0.5%) was compliant with all indications, while in 59 facilities (29%) no intervention has yet been organized. Thirteen hospitals (6%) adhered to 5 indications, 15 (7%) to 4, 39 (19%) to 3, and 78 (39%) to 1 or 2.

**Conclusion:** Although some level of MRSA control within hospitals has been implemented in over 40% of the participating hospitals, a well organized system, adhering to >4 interventions recommended by the Italian MRSA bundle is available in 1 out of 7 hospitals in the country. A higher level of organization is needed, and the forthcoming national Italian MRSA recommendations are welcome.
context in which infections occur and patient characteristics are necessary to assess possible consequences for antibiotic management.

**Table 1:** MRSA rates 2009 from German Antimicrobial Resistance Surveillance (ARS) from hospital and outpatient care stratified by ward type, sample type, age and sex.

| Hospital care | Outpatient care |
|---------------|-----------------|
|               | Number of S. aureus isolates | Number of S. aureus isolates |
|               | % | n | % | n |
| **Total**     | 21.6 | 22,251 | 12.5 | 14,825 |
| **Ward type** |               |               |               |               |
| General ward  | 20.7 | 19,916 | n.a. | n.a. |
| Intensive Care Unit | 24.8 | 2,335 | n.a. | n.a. |
| **Medical department** |               |               |               |               |
| General ward  |               |               |               |               |
| Surgical      | 18.2 | 6,624 | n.a. | n.a. |
| Medical       | 24.4 | 5,546 | n.a. | n.a. |
| Other         | 19.2 | 4,044 | n.a. | n.a. |
| **Intensive Care Unit** |               |               |               |               |
| Surgical      | 31.8 | 358 | n.a. | n.a. |
| Medical       | 35.4 | 872 | n.a. | n.a. |
| Other         | 23.7 | 1,105 | n.a. | n.a. |
| **Sample type** |               |               |               |               |
| Urine         | 33.0 | 2,412 | 22.4 | 1,289 |
| Other swaps   | 26.9 | 7,528 | 10.4 | 8,218 |
| Respiratory samples | 26.7 | 5,528 | 12.9 | 4,585 |
| Wound swaps   | 23.8 | 7,623 | 16.1 | 4,880 |
| Blood         | 22.1 | 1,610 | 21.4 | 70 |
| Punctures     | 17.8 | 7,715 | 9.6 | 157 |
| Other         | 25.3 | 3,304 | 24.7 | 179 |
| **Age group** |               |               |               |               |
| <125          | 4.9 | 2,044 | 3.0 | 2,064 |
| 16-59         | 13.6 | 6,262 | 6.5 | 6,182 |
| >60           | 27.7 | 13,945 | 21.2 | 6,539 |
| **Gender**    |               |               |               |               |
| Male          | 18.5 | 7,120 | 12.1 | 6,045 |
| Female        | 16.5 | 5,257 | 10.2 | 5,985 |
| Unknown       | 25.7 | 9,844 | 16.6 | 3,385 |

1. Copy–strain-rate: 1 isolate per species per patient/quarter
2. not applicable
3. Copy–strain-rate: 1 isolate per species per patient/quarter

Travel medicine: tropical and parasitic diseases

**P855** Liver dysfunction and its outcome in patients with Dengue infection

S. Kumarasena, A. De Silva, R. Premaratna*, J. de Silva (Ragama, LK)

Objective: To describe the spectrum of liver dysfunction, identify possible predictors of acute liver failure, and outcome in patients with dengue infections.

Methods: All serologically confirmed dengue patients admitted from January 2009 to March 2010 were included in the study. Relevant clinical and other details were obtained by analyzing their patient records. Patients with DFH and DSS were managed in accordance with WHO guidelines. Management of acute liver failure included supportive management and intravenous N-acetyl-cysteine (NAC).

Results: Of 349 patients [54.6% female, mean age 36 years] with serologically confirmed dengue, 187 (53.6%) had dengue fever, 113 (32.4%) Dengue haemorrhagic fever (DHF) and 49 (14%) Dengue shock syndrome (DSS). Serum transaminases (ALT), total serum bilirubin, alkaline phosphatase (ALP), γ-glutamyl transpeptidase, and International Normalized Ratio (INR) were observed in 81.9%, 75.9%, 7.7%, 5.4%, 9.7%, and 17.7% of patients respectively. 299 (85.6%) patients had elevated hepatic transaminases, and 278 (93.9%) of them had an AST:ALT ≥1. Most (54.8%) had elevated transaminases ≤3 times the upper limit of normal (ULN), 31.4% >3 to <25 ULN, and 41 (13.7%) had elevations above 1000 IU/L. Of these 41 patients, 16 developed acute liver failure (ALF). Early predictors ALF among patients with transaminase levels above 1000 IU/L were presence of nausea and vomiting, elevated bilirubin, elevated INR and elevated ALP. There were three deaths; two had ALF and one had dengue shock syndrome and multi-organ failure without ALF.

Conclusions: Liver function is commonly impaired in dengue fever, and severe liver dysfunction does not seem to be uncommon. The presence of nausea and vomiting, elevated bilirubin, elevated INR and elevated ALP in patients with very high hepatic transaminases should alert the physician to the possibility of impending acute liver failure.

**P856** The effects of benznidazol treatment in the autonomical and immunological response in chronic Chagas disease

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Evidences of cardiac autonomic dyautonomia and the main immunological response in Chagas’ disease (ChD) are still controversial, and factors that determine the different clinical outcomes, leading to a mild or to severe forms of the disease are not fully understood. Benznidazole treatment (Bz) induces parasite destruction and antigenic spreading, resulting in immune and autonomic changes that may have beneficial or detrimental effects on prognosis of Chagas’ disease. In order to elucidate this issue, we performed a longitudinal study to evaluate the parasitological, immunological and cardiac autonomic profile before and during Bz-treatment. Three groups were selected, 17 patients with indeterminate ChD (ICHD), 12 patients with a determinate ChD (DCDD) (7 cardiac, 1 digestive and 4 cardio-digestive) and 29 healthy control subjects. All chagasic patients received specific treatment and were follow-up for 60 days. Cardiac autonomic condition was assessed by a short-term heart rate variability analysis (HRV) during basal conditions, cold face test (parasympathetic stimulus) and passive orthostatism – Tilt Test (parasympathetic stimulus) analyzing temporal, geometrical, spectral and non-linear indices by Kubios HRV 2.0 software. The production of plasmatic cytokines was evaluated by ELISA. HRV parameters were significantly decreased in basal conditions in both chagasic groups when compared to the control group. During the cold face test, the IChD group presented a lower variation and the DCDD a higher variation of the sympathetic parameters. Tilt tests did not showed any alteration. During Bz-treatment was observed an unbalanced sympato-vagal modulation, and a subsequent relative sympathetic and parasympathetic modulation at the end of the treatment. During Bz-treatment, was observed an increased expression of pro-inflammatory cytokines in both chagasic groups, but at the end of the treatment the IChD group expressed a predominant proinflammatory profile modulated by a subsequent production of antiinflammatory cytokines. In summary, both chagasic groups present abnormal vagal modulation with similar but not equal cytokine profiles before specific treatment, and during Bz-administration each group present a different modulation of the cytokine/autonomic profiles, probably, by an inflammatory response triggered by parasite antigens or by a drug side effect.

**P857** Severity of confirmed adult Dengue in Singapore: comparison of World Health Organization 1997 and 2009 criteria

V. Gan*, T.L. Thein, W.W. Lin, D. Lye, Y.S. Leo (Singapore, SG)

Objective: In 2009, World Health Organisation (WHO) published new guidelines on the classification of dengue, using non-severe dengue with and without warning signs, and severe dengue. We evaluated its correlation with WHO 1997 criteria of dengue fever (DF), dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS).

Methods: This is a retrospective study of 1278 adults with polymerase chain reaction-confirmed dengue at our centre managed with a
standardised clinical pathway in 2004 (predominantly dengue serotype 1) and 2007 (predominantly dengue serotype 2).

**Results:** The two WHO criteria identified significantly different subpopulations (Bhapkak test for marginal homogeneity p < 0.001). The new criteria classified more cases as severe (McNemar test of overall bias p < 0.001), with 2.7% (0.7–7.8%) DSS vs. 16% (11.5–27.4%) severe dengue. Isolated bleeding or organ failure without evidence of plasma leakage accounted for the increase in severe dengue. A majority of cases classified as severe by one set of criteria were classified as mild by the other, indicating significant disagreement between the two classifications: 60% (30–85%) of DHF were classified as non-severe by WHO 2009. Conversely 56.3% (56–68%) of severe dengue by WHO 2009 were classified as DF. Difference in definition of plasma leakage and shock led to 45% of DSS being classified as non-severe dengue.

**Conclusion:** The two WHO criteria are significantly different. The 2009 criteria rely on clinical judgment more than 1997. Further work is needed to standardise the case definitions in WHO 2009, as subjective judgement will undermine its value in surveillance and comparison. The WHO 1997 criteria are less useful in assessing clinical severity but may be better in surveillance. More extensive and rigorous evaluation of the new criteria should be undertaken before abandonment of WHO 1997 criteria.

**P858** The utility of warning signs in predicting Dengue severity in confirmed adult Dengue: number needed to diagnose

V Gan*, T.L. Thein, W.W. Lin, D. Lye, Y.S. Leo (Singapore, SG)

**Objectives:** World Health Organisation (WHO) proposed new guidelines for dengue management in 2009, including using warning signs to identify patients at risk of severe dengue. These criteria, derived from expert consensus, differ substantially from previous guideline (WHO 1997) and require validation.

**Methods:** All adult dengue patients with positive dengue polymerase chain reaction managed with a standardised clinical pathway at our centre in 2004 and 2007 were retrospectively studied. Dengue serotype 1 predominated in 2004, and dengue serotype 2 in 2007.

**Results:** Using the six of seven warning signs identified by WHO 2009 that we were able to analyse, having any one warning sign had sensitivity of 88–100% and specificity of 25–67% in predicting subsequent dengue haemorrhagic fever (DHF) (WHO 1997). The sensitivity was 74–97% and specificity 63–68% for progression to severe dengue (WHO 2009). The three gastrointestinal warning signs (abdominal pain, persistent vomiting and hepatomegaly) were each not significantly associated with either DHF or severe dengue (p > 0.05). The number needed to diagnose (NND) is the number of patients that need to be tested to give one correct result. Mucosal bleeding was the best predictor for DHF, with NND=1.1–2.6 (p < 0.001). Concurrent rise in haematocrit or a drop in platelet count was next best with NND=6.74–8.80 (p < 0.01). In predicting severe dengue (WHO 2009), haematocrit rise with platelet drop had a lower NND=2.5–2.9 (p < 0.001) with mucosal bleeding next best with NND=3.0–6.1 (p < 0.001). Cases with clinical fluid accumulation were too few to provide meaningful quantitative estimates.

**Conclusion:** Both mucosal bleeding and a rapid concurrent haematocrit rise with a drop in platelet count are good predictors of dengue severity using WHO 1997 classification of DHF and WHO 2009 category of severe dengue. Using the presence of any one warning sign to identify at-risk patients, the NND ranges from 1.4–7.3. While warning signs are associated with dengue severity, there is scope for increasing the specificity of criteria to reduce the burden on healthcare systems, as the presence of warning signs is an admission criterion in the new WHO 2009 guideline.

**P859** Echinococcus multilocularis in the Netherlands: what about humans?

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**Introduction:** In 2008 the first autochthonous case of alveolar echinococcosis was diagnosed using Em specific PCR and serology. The patient lived in an area in the South of the Netherlands where the prevalence of infection in foxes was determined to be more than 12% and where the wormload is increasing. The incidence of Echinococcus multilocularis (Em) in foxes in neighbouring countries is increasing. Em is not a reportable disease in the Netherlands and it is not known if the diagnosis in patients is missed.

**Objective:** To determine the seroprevalence of Echinococcus multilocularis antibodies in areas at risk in the Netherlands.

**Methods:** 1581 human serum samples of 6 municipalities in areas at risk and 5 control municipalities were tested. Antibodies against Echinococcus spp. were detected using a commercial available Em2plus ELISA (Bordier) and an in house E. granulosus IgG ELISA. All positive samples were tested in an in house Immunoblot E. granulosus IgG1 to confirm the reactivity.

**Results:** 169 out of 1581 sera tested positive in the ELISA, 6 were positive in both ELISAs. The reactivity of the ELISA positive samples (Em or Em or both) could not be confirmed by westernblot.

An unexplained high reactivity was seen in children of 1–4 years and 5–9 years: 4.8–23.5% were positive, depending on the cut off level. This reactivity was strongest in the E. multilocularis serology. 36 of 48 Em seropositive children are autochthonous Dutch.

**Conclusion:** We have found no evidence for specific antibodies in this selection of the dutch population. The seroprevalence is still low (<1:1581). Serology alone, without imaging or clinical examination is not a good tool to determine if E. multilocularis is a threat for a population. We advise not to study the population but to investigate people at risk that live in regions with infected foxes.

**P860** Burden of anaemia associated to helminthiases: partial results from an ongoing Venezuelan survey

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**Objective:** Helminthiases are known aetiological factors in tropical anaemia; however the extent to which their presence might interact to further enhance the risk of anaemia is poorly understood. The aim of this study was to determine the prevalence of helminthiases-associated anaemia in asymptomatic individuals from 11 states in the context of a national survey of growth and development (2007–2009).

**Methods:** In the context of a Venezuelan Study on Human Growth and Development (SENACREDH), a cross-sectional, probabilitistic study, of 4,779 asymptomatic children and adults (~60-ylold) was done. Sampling was random, adjusted for age, sex and location. Results represented population weighted estimates (5,295,762 pop.), 2,935,070 male, 2,360,692 female. Study area was 71 municipalities in 11 states of North-Central Coastal, South and Andean Venezuelan regions. Intestinal parasites were diagnosed in stool samples after being preserved in MIF media. Anaemia was classified according WHO criteria after measure haemoglobin in blood samples.
Results: Hookworms (Necator americanus/Ancylostoma duodenale) and whipworm (Trichuris trichiura) were significantly associated with anemia. Hookworm prevalence was 1.35% (71/540) (95%CI 1.34–1.36%). Hookworms was 0.57% (10/1,853) (95% CI 0.56–0.58%). Anemia prevalence was 12.92% (684/5,054) (95% CI 12.88–12.95%), being significantly higher in those with hookworm infection (26.9%, 95% CI 26.8–26.93%) (p < 0.001) (OR=2.5, 95% CI 2.4–2.6). Similarly was found for whipworm (16.4%, 95% CI 16.36–16.43%) (p < 0.001) (OR=1.3, 95% CI 1.2–1.4).

Conclusions: Anemia is one of the most widespread and common health conditions afflicting individuals living in the tropics. The consequences of anemia are particularly severe for children and pregnant women. For these reasons multiple level preventive interventions at national scope should consider intestinal parasite surveys as this study shown.

P861 The comparison of microscopy, antigen detection and real-time polymerase chain reaction tests for the diagnosis of Pneumocystis jirovecii pneumonia: evaluation of clinical parameters

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Pneumocystis jirovecii pneumonia (PCP) causes serious infections, especially in patients with immunosuppressive diseases. We aimed to evaluate the results of bronchoalveolar lavage (BAL) samples of PCP suspected patients with three different methods. The BAL samples sent to the our Parasitology Laboratory were investigated with microscopically examination using Gram Weigert and Giemsa stains, direct fluorescent antigen detection test (DFA, Pneumo Cel Indirect) and Real Time Polymerase Chain Reaction (PCR) tests. We designed specific primers and probes according to partial sequence of PCP mitochondrial large subunit ribosomal RNA gene and DNA samples were analysed by Real Time PCR & melting curve analyses methods, respectively. Demographic, clinical and laboratory data were recorded. The BAL samples were studied in 42 patients (24 male, age: 31.29±26.43). There were totally 17 positives either one of the tests. Sixteen, 3 and samples were detected positive by PCR, microscopically and DFA, respectively. Tests were positive in one case by all three methods, whereas only DFA test was positive in one case and only PCR test was positive in 13 cases. Clinical data is shown in the table. HRCT analyses were showed fine noduler pattern in 1, pulmonary infiltration in 12 and ground-glass appearance in 6 cases. In comparison of positive and negative groups, HRCT results were found to have a supportive affect on the clinical evaluation of PCP patients. Co-trimoxazole was prescribed in 11 PCP cases diagnosed by laboratory test and 6 of them died. Besides this, PCR positive 6 cases were not treated with no clinical signs compatible with PCP and they diagnosed as foreign body aspiration (n=3), fungal pneumonia (n=1), alveolar carcinoma (n=1) and H1N1 pneumonia (n=1).

Table 1: Clinical and laboratory data

| PCP positive patients | PCP negative patients | Total |
|-----------------------|-----------------------|-------|
| Fever                 | 12                    | 15    | 27    |
| Respiratory distress  | 14                    | 16    | 30    |
| Hemoptysis            | 1                     | 1     | 2     |
| Lobar pneumonia       | 6                     | 11    | 17    |
| Leucopenia            | 1                     | 4     | 5     |
| CRP >0.5 mg/dl        | 13                    | 18    | 31    |

Risk factors:

- Malaria
- Immunosuppressant drugs use
- Diabetes Mellitus
- Chronic renal failure
- Renal transplantation
- Lung transplantation
- Other

Preparedness for malaria prevention in relief camps for flood affected – a cross-sectional survey from Pakistan

B. Ahmed*, K. Nanji (Karachi, PK)

Objectives: The monsoon floods in Pakistan have caused massive destructions and affected about 3.2 million people, including 1.4 million children and 133,000 pregnant women (UNICEF 2010). And approximately 1.3 million people were internally displaced. Flood waters had spread beyond the rivers and irrigation channels into poorly-drained, low-lying areas, creating stagnant pools that form a breeding ground for multidisciplinary approach is required for early PCP diagnosis. The amount and intensity of samples affects the success of laboratory tests. It's important for definitive diagnosis of PCP to see parasites directly as well as detection of specific antigen and DNA. It's shown that DNA amplified by PCR increases the sensitivity of diagnosis in our study. However, high PCP carrier rate in the society should take in consideration and clinical findings must be evaluated carefully for treatments in cases have PCR-positive samples. In our study we concluded that at least one diagnostic method besides PCR should be applied in PCP pneumonia suspected cases and disease symptoms should be evaluated carefully for treatment.
Cryptosporidiosis in Iranian immunocompromised patients:

Objective: Cryptosporidium spp. is a major cause of diarrhea in developing countries mainly affecting people with compromised immune system especially HIV-infected individuals with low CD4+ T-cell counts. The infection is self-limiting in immunocompetent hosts, but can be severe and persistent in the immunocompromised. There are limited studies about cryptosporidiosis and Cryptosporidium genotypes in Iranian immunocompromised patients. Also no information on risk factors for disease exists. We undertook this study to identify prevalence, genotypes and risk factors for cryptosporidiosis in immunocompromised patients.

Methods: 183 patients was sampled three times and processed with modified Ziehl-Neelsen staining methods and 18S ribosomal RNA gene amplification and sequencing.

Results: The overall infection prevalence was 6%. C. parvum was identified in isolates from five HIV-infected patients, one patient with Bone Marrow Transplantation and one with C. hominis was identified in isolates from two HIV-infected patients and two ALL patients. In the univariate analysis factors statistically significant were diarrhea (OR=21.7, CI=2.83–78.4, P < 0.003), CD4+ cells less than 100 cells/mm³ (OR=41.3, CI=13.45–114.82;83–78.4, P < 0.0001), other microbial infections (OR=7.13217, CI=1.97–25.732, P = 0.006), Weight loss (OR=73.78217, CI=15.5–35083;78.4, P < 0.0001), Abdominal pain (OR=10.29, CI=2.81–37.74, P = 0.001), Dehydration (OR=72.1, CI=17.6–341.5, P < 0.0001), Vomiting (OR=4.87, CI=1.4–16.94, P = 0.015), Nausea (OR=9.4, CI=2.38–37.24, P < 0.001), Highly Active Antiretroviral Therapy (HAART) (OR=0.089, CI=0.01–0.8, P = 0.015) and Diarrhea of household members (OR=7.37, CI=2.94–26.66, P = 0.001). After multivariate analysis and a back ward deletion process, only CD4+ cells less than 100 cells/mm³ maintained a significant association with infection.

Conclusion: We surmise that the presence of this infection should be suspected in patients with diarrhea, weight loss, and dehydration and especially diarrheal individual with CD4+ T-lymphocytes counts less than 100 cell/mm³.

Tropical infections in Coventry, UK

Objectives: We present 3 fascinating tropical cases masquerading as other diagnoses. The diagnostic conundrum lead to delays in diagnosis. We also treat 2 of the patients with unconventional therapy.

Methods and Results: Case 1: A 56 year old man returned from his holiday in Majorca with multiple ‘insect bites’ over his body. He failed to improve with steroids, however a skin biopsy several months later showed granulomas. Although special stains for mycobacteria were negative, a Leishmania donovani PCR was eventually positive. As he was a pilot instructor, conventional therapy with systemic Sodium Sibogluconate was contraindicated. We thus administered miltefosine and the lesions are now resolving.

Case 2: A 43 year old man from Nigeria presented to the acute medical team with fever and painful legs. He had an elevated white cell count and C-reactive protein. IV antibiotics were commenced for ‘cellulitis’. A more extensive cutaneous assessment revealed hyperpigmented skin lesions on both legs, thickened auricular, ulnar and peroneal nerves, and nodular lesions on the face. A slit-skin smear of the earlobe showed numerous acid-fast bacilli and confirmed our suspicions of multibacillary leprosy. It became apparent that he had been previously treated and in fact the skin lesions on his legs were consistent with Erythema nudaum leprosum He is now being treated with dapsone and clofazimine.

Case 3: An 85 year old Englishman presented with a 3 week history of fever and pancytopenia. He had travelled to Portugal for an 8 day holiday, 4 months prior to his presentation. A peripheral film and a bone marrow showed possible myelodysplasia and haemophagocytosis. He therefore was commenced on systemic steroids with partial resolution of his symptoms. 6 weeks after the initial presentation, a delayed leishmania serology eventually came back positive. A retrospective analysis of the bone marrow showed definite leishmania-donovani bodies and the PCR was positive for Leishmania donovani. In view of his age and impaired renal function, he was given a single dose of Ambisome 5mg/kg and a test of cure bone marrow biopsy will be performed at 6 months.

Conclusions: These cases show that:

1. A thorough history is paramount in considering tropical infections in the differential
2. Migration and increasing world travel means rare conditions may present in Northern Europe
3. A high index of suspicion is required

The outcome of the unusual treatment in Case 1 and 3 will be presented.

Neurocysticercosis in Italy

Objectives and Methods: To report two cases of imported neurocysticercosis (NCC) recently hospitalised in our department and to stress the importance of NCC outside endemic areas.

Results: The two patients were male African immigrants, strict Muslims, living in Italy for several years. Both patients were admitted with fever and pleural and pericardial effusions and reported previous episodes of seizure. One patient was HIV2 positive and on HAART, but had treatment failure and severe immunosuppression (CD4 24/mcl). Laboratory analysis revealed increased IgE titters and mild eosinophilia in both cases. A tubercularetiology of the pleuro-pericardial effusion was ruled out (direct acid-fast bacilli examination, polymerase-chain reaction and culture of fluid resulted negatives). MRI of the brain revealed the presence of hypointense cystic lesions in the frontal lobes. In the HIV-negative patient, NCC was diagnosed by histological examination of a
biotic sample. In the HIV-positive patient, brain biopsy was avoided because of a very low platelets count (5000 plt/mmc). The diagnosis of NCC was made instead on the basis of MRI results, failure of anti-toxoplasma, anti-TB and antifungal therapy, and regression of the cystic lesion upon empiric therapy with albendazole. In both patients, ova and parasites stools examination, colonoscopy and specific serological tests for cestode infection were negative.

**Conclusion:** NCC should be taken into account as a possible diagnosis in all patients born and raised in endemic areas showing seizures and compatible lesions in brain imaging. The lack of dietary intake of pork meat should not be considered a valid epidemiological criterion to exclude taenia solium infection, inter-human transmission having been proven in African countries. A negative serology does not exclude forcestodeinfectionwerenegative.The response to empiric treatment should be taken into consideration when a direct diagnosis is not obtainable, beenproveninAfricancountries.Anegativeserologydoesnotexclude compatible lesions in brain imaging. The lack of dietary intake of parasites stools examination, colonoscopy and specific serological tests for cestode infection reported in the literature as response to the reactivation of the infection, requires further study.

**Objectives:**

**Loa Loa**

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**Methods and Results:** First case: a 27-years-old woman, who had migrated to Italy from Cameroon since 2002, presented complaining with the sensation of something crawling over her left eye. On examination a worm-like body was seen moving in the inferior fornix. Physical examination demonstrated as an healthy pregnant woman at 16 weeks of gestational age. Blood, urine and stool investigations were unremarkable except for a mild eosinophilia (9%). Fresh peripheral blood examination revealed the presence of highly motile microfilariae. Examination of a Giemsa-stained peripheral blood smear revealed the presence of a moderate number of sheathed microfilariae. During the pregnancy woman was not treated. She gave birth an healthy baby girl. The microscopic examination of both umbilical cord's blood and neonate's peripheral blood demonstrated no circulating microfilariae. Second case: a 25-years-old female from Cameroon. She has been living in Italy since 2003. She presented complaining of a foreign body sensation in her left eye. On ophthalmological examination a motile worm was visualized subconjunctivally. Repeated samples of blood for fresh peripheral blood examinations, Giemsa-stained peripheral blood smears and Knott concentration technique were demonstrated negative for the presence of microfilariae.

**Conclusion:** One of the main characteristics of human infection with *Loa Loa* is that a certain proportion of subjects remain microfilaricerci. The diagnosis of second case was made in an amicrofilaricerci woman who had a history of either living in an endemic area and eye worm migration. In microfilaricerci patients such as first case, the diagnosis of loaisis may be made by microscopic examination of Giemsa or hamatoxylin stains of peripheral blood. The microfilariae may stain poorly and not to be identified correctly, since Giemsa stain does not stain the microfilarial sheath adequately. The appearance of “halos” around the microfilariae does not always indicate the presence of a sheath because this effect might be due to shrinkage. The Knott concentration technique and filtration of blood are also useful techniques to make this diagnosis.

**Molecular investigation of Cryptosporidium spp. using the TRAP-C2 gene**

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**Objectives:** At present 18 *Cryptosporidium* species are identified as valid but *Cryptosporidium parvum* and *C. hominis* are the common species in human. The aim of this study was to establish the genotypes of *Cryptosporidium* spp. among children with diarrhea using the TRAP-C2 gene.

**Methods:** Fecal samples were collected from 1263 children less than 12 years with diarrhea. After determine the presence of *Cryptosporidium* oocysts by Ziehl-Neelsen acid fast staining, genomic DNA was extracted of positive samples and nested PCR/RAFLP was performed to amplify the TRAP-C2 gene.

**Results:** Out of 1263 samples, *Cryptosporidium* oocysts were found in 31 (2.5%) sample. RAFLP analysis showed *C. parvum* in 25 (80.6%) isolates, *C. hominis* in 5 (16.1%) and mix infection pattern of both *C. parvum* and *C. hominis* in 1 (3.2%).

**Conclusion:** In conclusion the use of TRAP-C2 primers could be sensitive enough to conduct a routine detection study. The nested PCR method using the TRAP-C2 gene sequence can be an alternative diagnostic method to identify human infected with *Cryptosporidium* and its genetic diversity.
**Epidemiology of intestinal parasitosis in eleven states of Venezuela: partial results of an ongoing national survey**

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Objective: Last national survey of prevalence of intestinal parasites in Venezuela was held 2 decades ago (1989–92). The aim of this study was to determine the prevalence of intestinal parasite species isolated from faeces samples in asymptomatic individuals from 11 states in the context of a national survey of growth and development (2007–2009).

Methods: In the context of a Venezuelan Study on Human Growth and Development (SENACREDH), a cross-sectional, probabilistic study, of 6,437 asymptomatic children and adults (<60 y-old) was done. Sampling was random, adjusted for age, sex and location. Results represented population weighted estimates (7,120,744 pop.). Study area was 71 municipalities in 11 states of North Central Coastal, South and Andean Venezuelan regions. Intestinal parasites were diagnosed in stool samples after being preserved in MIF media.

Results: We found prevalences, for pathogen parasite infections, ranging 0.257–7.426% (see Table). However opportunistic parasite Blastocystis hominis was found in 45.632%. Highest protozoan infection prevalence was found for Giardia intestinalis, 7.246% (being higher in those <6 y-old, 12.9%). Highest helmintic infection prevalence was found for Ascaris lumbricoides, 3.974% (being higher in those <6 y-old, 6.7%).

Conclusions: Intestinal parasitoses continues to be one of the most common infections worldwide spread, especially in tropical countries. In 1992 national prevalence was 20% whilst in this study, for 11 states (of 24) it was less than 10% for pathogenic infections. During last years a significant reduction would be linked to improvement in housing, tap-water access and in general, to the country development.

**Splenial abscess due to Salmonella enteritidis**

P.871

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Objectives: Splenic abscess is a very rare complication of nontyphoid salmonellosis in persons with comorbidities.

Case: A 63-year-old woman from east part of Turkey was admitted with the complaint of back pain, vomiting and nausea since 20 days. She had not any other complaint. Physical examination was normal. She had diabetes mellitus and hypertension. In Laboratory examination White blood cell (WBC) count was 14.670/mm³ (Neutrophils 88.9%) and C-reactive protein (CRP) level was 58 mg/L (normal value <5 mg/L). Erythrocyte sedimentation rate was 76 mm/h. An abdominal ultrasound revealed a hypoechoic cystic structure of 61 × 62 mm in the upper part of the spleen with calcifications, confirmed on abdominal CT scan compatible with abscess. The patient underwent an exploratory laparotomy and splenectomy was performed. Salmonella enteritidis was yielded from the culture of the abscess obtained during the operation. After the isolation of Salmonella enteritidis, the patient questioned in detail, it was learned that the patient had diarrhoea and fever existed 2 days and recovered without antibiotic treatment one month ago. Ciprofloxacin was administered 500 mg bid po for 10 days postoperatively. The patient recovered well after surgery. There were no recurrent symptoms during the follow up.

**Conclusions:** Salmonella gastroenteritis is usually a self limiting disease. Although bacteraemia develops in less than 5% of all patients with Salmonella enteritidis, patients with comorbidities are at increased risk of invasive infection. The treatment of splenic abscess includes antibiotic treatment and surgery. Splenectomy is still the most accepted standard surgical treatment of a splenic abscess.

**A case of Alkhurma haemorrhagic fever virus infection in an Italian traveller returning from Egypt**

P.872

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Objectives: The Alkhurma haemorrhagic fever virus (AHFV) is a recently described member of the tick-borne haemorrhagic fever group of the genus Flavivirus. To date, AHFV has been detected only in Saudi Arabia, it is an emerging pathogen causing symptoms such as fever, headache, thrombocytopenia; severe cases may present with haemorrhagic fever manifestations and encephalitis which can result in death (case fatality rate as high as 25%).

Methods: A traveller, who spent one week in a touristic village in southern Egypt, while visiting a camel and dromedary market, was bitten by an unidentified arthropod. Two days after, the patient developed high fever, shaking chills, anorexia, malaise, nausea and vomiting, and blurred vision. Over the following 5 days his symptoms worsened and he was admitted to the department of infectious diseases in the Ospedali Riuniti di Bergamo.

Results: Laboratory test results showed leucopenia, thrombocytopenia and increased liver enzymes. The patient was started on paracetamol, fever and general malaise progressively decreased over the following 5 days. He was discharged 11 days after hospitalization in good general condition despite persistence of asthenia. Acute and convalescent sera sent to the virology laboratory of the ‘L. Spallanzani’ in Rome to be tested for Dengue and West Nile virus infection. IgG and IgM for both viruses were detected by immunofluorescence, with a titre of ≥1:640 and ≥1:20 respectively, in both serum samples. There was no evidence of rising antibody titers in the convalescent serum sample, arising the suspicion of a cross-reactivity due to a previous Flavivirus infection or to the Yellow Fever vaccinination. A genus-specific RT-PCR targeted to the NS5 gene of Flaviviruses was positive in the acute serum and the sequence analysis of the amplicon showed high similarity with AHFV sequences posted in GenBank This unexpected result called for further investigations to confirm the diagnosis of an AHFV infection. To this aim, an AHFV-specific nested RT-PCR targeted to a wider region of a different gene (E) was performed.

Conclusions: The finding that the distribution of the causing agent is wider than previously thought should prompt further investigations to better assess the real danger for local populations and international travellers. Veterinary and entomological investigations are also necessary to further understand the geographic distribution of AHFV.

**Two cases of visceral leishmaniasis treated with a single-dose of liposomal amphotericin B**

P.873

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Objectives: Liposomal Amphotericin B (LAmB) is the first line therapy for Visceral Leishmaniasis (VL). The standard treatment in immunocompetent patients are: 3 mg/kg/day at days 1–5 and 10 in Mediterranean area; 2 mg/kg/day for 5 days in India. Recent trials indicate that high dose and shorter duration regimes of LAmB therapy show comparable results to conventional treatment and are not associated to higher rates of renal toxicity. We report two VL cases in immunocompetent patients treated with single-dose of LAmB.

Case 1: A 35-year-old Angolan man presented with remitting fever (39–40°C) and acute renal failure. The histological examination of the renal biopsy revealed a collapsing variant of focal segmental glomerulosclerosis. The patient was treated with a single-dose of LAmB (3 mg/kg) with a good clinical response. A renal biopsy performed 4 months after treatment showed a complete resolution of the histological abnormalities.

Case 2: A 25-year-old Portuguese patient presented with a single-dose of LAmB (3 mg/kg) followed by a second dose 5 days later due to the appearance of a fever and due to the suspicion of a cross-reactivity due to a previous Flavivirus infection. The patient was discharged with complete resolution of the clinical symptoms and with negative parasitological tests.
Melioidosis diagnosed by mass spectrometry in a tourist returning from Martinique Island

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Case report: A 35-year-old healthy man visited Martinique from 2010, November 13th to 23rd. To practise surfing, he crossed recently flooded areas, while having multiple scratched mosquito bites on his legs. He presented to our hospital on the 25th with fever, chills, headache, abdominal cramps, mild diarrhea and lower back pain evolving for 48 hours. Suspecting typhoid fever, he received ceftriaxone, which was changed after 24h to high dose imipenem-cilastatine. Indeed, blood cultures were positive. Two hours after subcutverting, Mass spectrometry (MS) was performed on a very thin layer of material growing on the blood agar, allowing the identification of *Burkholderia pseudomallei* and leading to the presumption of melioidosis. MS was performed later the same day on a well-grown colony and allowed the identification of *B. thailandensis* (score >2), MS cannot distinguish *B. thailandensis* from *B. mallei* or pseudomallei. While breathing normally at admission, the patient developed severe abdominal abscess-forming pneumonia, and died on the 27th from septic shock with multi-organ failure and ARDS (acute respiratory distress syndrome), despite the addition of granulocyte colony-stimulating factor (G-CSF), low-dose steroids and intensive care support including extracorporeal membrane ventilation. Sequencing of the 16S-rDNA confirmed the presence of *B. pseudomallei* in all blood cultures. Epidemiological data obtained from the patient was transmitted on November 26th to the local clinicians and international medical information networks, along with the diagnosis of melioidosis. Following our immediate reporting to local hospitals, two additional cases were suspected with immediate adaptation of antibiotic therapy.

Discussion: Melioidosis is endemic in South-East Asia and Northern Australia, but cases are increasingly recognised in other parts of the world. *Burkholderia pseudomallei* is a non-fermenting saprophyte living in soil and surface water, usually transmitted percutaneously with an incubation period of 1−21 days. Clinical presentation can range from fulminant sepsis to chronic disease. The most common manifestations are pneumonia, sepsis along with abscess of the prostatic gland and others organs. Prognosis is poor in cases with severe sepsis (30−50% mortality). Known predisposing medical conditions, are diabetes, cirrhosis, alcoholism or renal failure. Mass spectrometry provides rapid identification, with the potential to improve prognosis of fulminant melioidosis.

Cutaneous and visceral tropisms of *Leishmania tropica* strains isolated in Turkey in murine model

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Objectives: Visceral leishmaniasis (VL) caused by *Leishmania* (L) infantum, and cutaneous leishmaniasis (CL) by *L. tropica* and *L. infantum* have been recorded in Turkey. The possibility of genetic exchanges between *Leishmania* species that may lead to the formation of hybrid strains has recently been suggested. The aim of this study was to examine tissue tropisms and clinical manifestations of *Leishmania* isolates obtained from 5 CL and 1 VL patients from different endemic areas of Turkey on a murine model. All were identified as *L. tropica* by isoenzyme analysis.

Methods: After the preparation of suspensions (1×10^8 promastigotes/ml), 15 and 10 microl of each suspension were injected into the left footpads of Balb/C mice subcutaneously (SC Group, 6 groups, 36 mice) and into another mice group (IV Group, 6 groups, 36 mice) intravenously. Footpads of SC Group were followed for 6 months and checked for the presence of amastigotes in every 15 days. IV Group was sacrificed after 30 days, livers and spleens were removed. All samples were stained with Giemsa and inoculated to NNN medium.

| Variable | Normal Range | CASE 1 | CASE 2 |
|----------|--------------|--------|--------|
| Temperature (°C) | < 37.5°C | 39 36 36 36 36 36 | T0 T0 T0 T0 |
| WBC (×10^3/mm) | 4 - 9.5 | 1.96 6.00 4.25 4.50 1.28 3.48 4.92 4.91 |
| Hb (g/dl) | 14 - 18 | 6.29 11 12.2 12.5 7.1 12.2 14.3 13.5 |
| PLT (×10^3/mm) | 150 - 400 | 240 300 267 228 81 200 227 190 |
| ESR (mm/h) | 1 - 15 | 136 62 21 11 66 75 - 12 |
| CRP (mg/l) | 0 - 5 | 138.5 3.5 4.2 <3 9.06 0.7 0.7 0.64 |
| Creatinine (mg/dl) | 0.4 - 1.3 | 3.70 1.51 1.45 1.34 0.9 0.9 1.0 0.9 |
| Urea (mg/dl) | 8 - 45 | 45 42 41 20 10 12 11 9 |
| Total Protein (mg/dl) | 6 - 8.5 | 6.7 7.4 6.8 6.97 10.84 10.14 9.75 8.88 |
| Proteinuria (g/24h) | 0 - 0.2 | 3.83 2.5 3.05 2.96 1.92 0.13 0.14 |
| Weight (Kg) | 67 70 70 74 72 73 78 83 |
| Spleen size (cm) | 9 - 12 | 14 11.5 - 11.2 22 16 - 12 |
| Stereology IFAT | 1/300 1/640 | 1/90 1/320 1/160 |
| Microscopic examination of bone marrow aspirate: presence of amastigotes | Neg. | Pos. |
| Bone marrow aspirate: Leishmania culture | Neg. |
| Bone marrow aspirate: Leishmania PCR | Neg. |

*p:* positive, *neg:* negative

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Results: After 30 days, lesions with erythema and swelling were seen on footpads of all mice in SC Group; microscopy and cultures were positive after 45 days. No lesion occurred on mice of VL isolate in SC Group. All mice of VL isolate in IV Group showed visceralization with symptoms, and 4 of 5 CL isolates showed visceralization in IV Group. Real-time PCR on ITS-1 gene region was conducted, followed by melting curve analyses for genotype identification. All 6 isolates showed peaks specific to *L. tropica*, similar to isoenzyme analysis, while lesion aspiration smear samples obtained from the same patients showed two peaks specific to both species, *L. tropica* and *L. infantum*, or one peak specific to *L. infantum*.

Conclusions: In this study, murine models were established using local Leishmania strains. Four isolates that presented dermo/viscерotropism in murine model were evaluated as hybrid Leishmania strains. This is the first study that reports clinical manifestations and different tropisms of Leishmania isolates in murine model using local strains. We further plan; (a) advanced molecular analyses on different gene regions and proteomics analyses, (b) detailed assessments of clinical manifestations in animal models, (c) advanced examinations of clinical conditions of patients.

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Not just Lake Malawi. Katayama Fever at the Hospital of Tropical Diseases, London, UK, over the last 12 years

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Objectives: Our objective was to describe epidemiological and clinical features of Katayama Fever in the era of improved serological testing for Schistosomiasis.

Methods: We performed a retrospective review of cases of Katayama Fever that have been seen over the last 12 years at the Hospital for Tropical Diseases, London, UK. We identified patients from a specifically designed database of all cases of Schistosomiasis as well as searching through the hospital inpatient and outpatient databases and our own personal records.

Results: We have complete data on 57 patients from 1998 to 2008. We will have further data on at least a further 8 patients prior to May 2011. 73% (42) are male and the median age at presentation was 27 years (range 19-60 years). Only 38% had been to Malawi though all reported freshwater exposure. With the exception of one all had been to Africa. Swimming on holiday was the main reason for exposure in 56% of patients. The others were either expatriates or on business trips. Time from exposure to symptoms was established in 32 patients and the median was 6 weeks. The diagnosis was made on the clinical presentation. Eosinophilia was present in 79% of patients though interestingly 12 patients presented with a normal count which subsequently increased. Schistosomal serology was positive in 91% of patients. This was tested in all patients within 12 weeks of their exposure. The majority were tested at the time they had symptoms of Katayama fever. Only 10% of patients had detectable ova in their urine or stool. All were treated appropriately with praziquantel and had a good clinical outcome.

Conclusions: Exposure to Schistosomiasis occurs throughout Africa, not just Lake Malawi. Katayama Fever should be considered in patients presenting with any of the typical features. A normal eosinophil account does not preclude the diagnosis. The serology is positive in the majority of patients at the time of their symptoms. Maps, tabular presentation of the clinical features with the additional patient data incorporated and a graphic of the Schistosomiasis life cycle will form part of the poster presentation.

![P877] Laboratory diagnosis of malaria and leishmaniasis: conventional or molecular methods?
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Objectives: The application of molecular methods for the laboratory diagnosis of malaria and leishmaniasis was evaluated in our laboratory as compared to conventional methods.

Methods: Malaria: Blood samples (1,467) from 928 patients with clinical suspicion of imported malaria were subjected to thin-film microscopy (acridine orange and Giemsa stain) and to different 18S-rDNA PCRs alternatively used during the period 2000–2010, including nested- and real-time PCR assays.

Leishmaniasis: Different samples (27 bone marrow, 13 cutaneous and 1 splenic biopsies) from 36 patients with clinical suspicion of leishmaniasis were subjected to conventional methods (microscopy and culture) and a 18S-rDNA real-time PCR assay, during 2006–2010.

Results: Malaria: By microscopy 208 cases of malaria were diagnosed [173 Plasmodium falciparum (Pf) (83.2%), 11 P. oocye (Po) (5.3%), 12 P. vivax (Pv) (5.8%), 9 P. species (4.3%) and 3 mixed infection (4.4%)], whilst 215 were diagnosed by PCRs [174 Pf (80.9%), 21 Po (9.8%), 8 Pv (3.7%), 3 P. malariae (1.4%) and 9 mixed infections (4.2%)].

Leishmaniasis: A total of 2 cases of leishmaniasis (1 cutaneous and 1 visceral) were diagnosed by both conventional methods and PCR. Regarding the cutaneous case, 2 biopsies were collected including 1 negative by conventional methods.

Conclusion: Malaria: Despite microscopy remains the reference diagnostic method (rapid and inexpensive), in some cases molecular assays are the only ones allowing a correct diagnosis of malaria, particularly to detect infections by species other than Pf and mixed infections. PCR proved to be more sensitive and specific than microscopy and changed malaria epidemiology in our area detecting 7 single and 6 mixed infections missed by microscopy, revealing 5 single and 2 mixed infections incorrectly diagnosed by microscopy and giving speciation in 9 cases in which microscopy had limited the result to genus identification. Then, the association of microscopic and molecular assays demonstrated to be essential for an accurate diagnosis of malaria and to administer a targeted therapy.

Leishmaniasis: The application of the real-time PCR did not lead in our study to a dramatic improvement in the accuracy of the diagnosis of leishmaniasis, even if the number of the analysed samples was limited. However, real-time PCR is very rapid and specific and it could be successfully associated to the conventional methods in particular in order to improve the diagnosis of visceral leishmaniasis.

![P878] Serological screening for Chagas disease in Latin American pregnant women in Barcelona South Metropolitan Area

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Introduction: Chagas disease was almost unknown in our country until recent migratory flow coming from Latin America. The risk of vertical transmission by pregnant women represents a new challenge in public health because the first cases of congenital Trypanosoma cruzi infection in Spain have already been described.

Since 2010 serological diagnosis of Chagas disease in pregnant women coming from Latin America has been included in the serological screening in Catalonia (Spain).

Objective: The aim of this study was to evaluate the results after the implementation in 2010 of the serological screening for Chagas disease in pregnant women coming from Latin America in the Barcelona South Metropolitan area.

Methods: The Laboratori Clinic L’Hospitalet of the Catalan Institute of Health in the Barcelona South Metropolitan area attends around 700.000 inhabitants with a 5.6% of Latin American population. From May to December 2010, pregnant women from Latin America, who received prenatal care in the public health system, were tested for the presence of
T. cruzi antibodies. The screening assay performed was a recombinant enzyme immunoassay (r-EIA, Bioelisa Chagas Biokit®) using antigens with four immunodominant epitopes of T. cruzi. Positive sera were confirmed by a second assay, a native enzyme immunoassay (n-EIA, Ortho® T. cruzi Elisa Test System) using a lysate of antigens prepared from T. cruzi. According to the manufacturers sensitivity and specificity of both methods were respectively >95% and 95–98%.

Results: From a total of 804 pregnant women tested, 17 (2.1%) were positive by r-EIA and all were confirmed by the n-EIA. The level of antibodies by de r-EIA technique was three times greater than the cut-off value in all the positive sera. Most of the Latin American pregnant women studied were from Ecuador, Colombia and Bolivia. However, all the infected women were Bolivian. None of the 17 women with positive tests were known previously to suffer from Chagas disease.

Conclusions: The presence of pregnant women with Chagas disease justifies the performance of this test during pregnancy in women coming from endemic areas. There is a good agreement between recombinant and native EIA techniques used. It is important to identify infected pregnant women in order to achieve a prompt diagnosis and treatment of the children with congenital Chagas disease.

**May immunochromatographic assay allow accurate detection of antibodies against Echinococcus granulosus?**

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**Objectives:** Echinococcus granulosus (E. granulosus) is the causal agent of cystic echinococcosis (CE). Serologic assays, together with imaging techniques, are most frequently used for diagnosis. The indirect hemagglutination test (IHA) and enzyme-linked immunosorbent assay (ELISA) seem most satisfactory. In this study, a new immunochromatographic assay (ICA) for total E. granulosus antibodies, VIRapid® HYDATIDOSIS, has been evaluated.

**Methods:** Two main groups of samples were analyzed to check the test performance: 137 sero-negative serum samples from Turkish hospitals and 85 sero-positive serum samples from different areas. A new ICA, VIRapid® HYDATIDOSIS, (Vircell, Spain) designed for the qualitative detection of antibodies against E. granulosus both in serum and plasma samples. It is a single strip assay two lines, test and control. The test performance was visually read out after incubation for 20 min at room temperature. An IgG ELISA (DRG, Germany) and an IHA (Fumouze, France) tests were used as references methods.

**Results:** 81 out of 85. positive sera displayed a distinct red test line in the ICA. 137 out of 136 negative sera showed no reactivity on the test line in the ICA. Sensitivity, specificity, positive predictive value and negative predictive value were calculated for the new VIRapid® HYDATIDOSIS assay as 95.29%, 99.27%, 98.78% and 97.14% respectively.

**Conclusion:** We obtained good predictive values in detection of anti-CE antibodies in human serum samples. The test offered a fast and easy to use performance with high sensitivity and specificity. There is no need for elaborate instrumentation, special storage condition and the results are easy to interpret.

**The pinworm: a Trojan horse for Dientamoeba fragilis?**

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**Objective:** Despite over 90 years have passed since its first description, the mysterious journey of Dientamoeba fragilis trophozotes through human gastric juice before reaching their target site, the colonic lumen, and their survival outside the human body are yet to be defined. Helminths may play a role in its transmission and the pinworm, Enterobius vermicularis, has been blamed for its transmission to humans. We previously reported that 9.6% of pinworm-infected patients were coinfected with D. fragilis, whereas 2.4% of the non-pinworm infected patients had a D. fragilis infection. Similarly, 25.4% of D. fragilis-infected patients were coinfected with the pinworm as compared to 10.1% in non-D. fragilis infected patients in our study group.

Results: As previously described by Verweij. Sequence analysis was conducted with the amplicons in “BigDyTerminator (Applied Biosystems)” and the results were analysed with ABI 3900. Obtained DNA sequences were assembled and compared to reference sequences using “CodonCode Aligner” (Codon Code Corporation) and MEGA 4.0.6.

**Conclusion:** These initial results suggest a much higher percentage of co-infection with D. fragilis in pinworm positive patients than previously found in our population. Therefore, pinworm positive patients with symptoms suggestive of D. fragilis co-infection should be thoroughly examined for D. fragilis.
wolves and that the zoonotic Assemblage A was common in this wild animal. These findings suggest that wolves may play an important role in zoonotic transmission cycles of the parasite in north of Portugal.

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[**P882 Detection of Acinetobacter sp. in human lice from Ethiopia**

M. Kempf*, E. Angelakis, A. Abdissa, G. Diatta, J.F. Trape, D. Raoult (Marseille, FR; Jimma, ET)

**Objective:** The human body louse (Pediculus humanus humanus) and the head louse (P. h. capitis) are closely related obligate parasites that feed exclusively on human blood. Acinetobacter baumannii readily infect, multiply and proliferate in body lice, producing a generalized infection. The objective of this study was to assess the rate of infection to Acinetobacter sp. of body and head lice collected in Ethiopia.

**Methods:** We collected head and body lice from seven locations with different altitudes (from 1450 to 2400 meters) in Ethiopia. Total genomic DNA of each louse was extracted and used as a template in a real-time PCR assay targeting the rpoB gene of *Acinetobacter sp.* 2. In order to identify the genotype of body and head lice, the mitochondrial gene, cytB (cytochrome b) was amplified and sequenced. For data comparison, EpInfo version 6.0 software was used (Centers for Disease Control and Prevention, Atlanta, GA, USA). A p value <0.05 was considered significant.

**Results:** A total of 115 head and 113 body lice were collected from 134 patients (109 females and 25 males) and tested. All body lice were grouped in phylotype A and all the head lice, which were all black, belonged to phylotype C. *Acinetobacter sp.* was found in 62 head (53%) and 69 body (66%) lice. No difference was found in the presence of *Acinetobacter sp.* between head and body lice (p=0.58) or between males and females patients (p=0.34).

**Conclusion:** Our study is the first showing presence of *Acinetobacter sp.* in head and body lice in Ethiopia. The percentage of lice infection in Ethiopia was much higher than in other countries such as in France or the Netherlands where respectively 18% and 32% of body lice were infected with *A. baumannii* [3]. Head and body lice can be differentiated into 3 deeply divergent mitochondrial clades, each having unique geographic distribution. The first contains both head and body lice and is worldwide in distribution; the second occurs only in head lice and has been found in the New World, Europe, and Australia; and the third has been found only in black head lice from Nepal and Ethiopia [4]. In our study, we have shown that all head lice presented the phylotype C and all body lice presented the phylotype A.

[**P883 Performance of a multiplex PCR to identify diarrhoeagenic Escherichia coli (DEC) in patients with diarrhoea**

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**Objectives:** Accurate diagnosis of diarrhoeagenic *Escherichia coli* (DEC) in patients with diarrhoea is hindered by standard detection methods. This is particularly outstanding in subjects with traveller’s diarrhoea in whom DEC is the main enteropathogen bacteria involved. Our objective was to analyze the prevalence of DEC by a new multiplex PCR (E. coli DEC PCR, Statens Serum Institut) in our Hospital specialized in Tropical Medicine.

**Methods:** From June 2009 to October 2010, stool samples from patients suffering acute or persistent diarrhoea were investigated. Samples were analyzed for enteropathogen bacteria by standard procedures. In addition, a commercial multiplex PCR was performed to detect genes associated with DEC: verocytotoxins 1 (vtx1) and 2 (vtx2), intimin (eae), heat-stable enterotoxin (esta) and heat-labile enterotoxin (elta) and invasive plasmid antigen (ipaH). Epidemiological data, including recent travels and country of destination was also recorded.

**Results:** A total of 105 samples corresponding to 100 patients were included. Most patients were travellers: 38 came from Africa, 15 from Central America, 15 from Asia and 12 from South America. The remaining 20 subjects did not report any travel in the last weeks. The mean age of the study population was 38±14 years and 5% of patients were children (<15 years old). The gender distribution was equal. The main enteropathogen bacteria identified were DEC (7 patients) followed by *Salmonella* spp (5 patients), *Campylobacter* spp (3 patients, 2 *Campylobacter jejuni* y 1 *Campylobacter coli*), *Shigella sonnei* (2 patients) and *Plesiomonas shigelloides* (1 patient). *eae* gen was the only found in subjects with DEC strains. All of them were adult travellers from Asia (4 patients), Africa (2 patients) and South America (1 patient). Three of them presented coinfection with other enteropathogens (*Campylobacter jejuni*, rotavirus and *Giardia lamblia* in each case). Of note, ipaH was detected in two patients in whom *Shigella sonnei* was cultured. These subjects did not report any travel in recent weeks and one of them was coinfected with HIV-1.

**Conclusion:** DEC was the main enteropathogen bacteria responsible of traveller’s diarrhoea with a remarkable prevalence (7%). The multiplex PCR is an useful and simple tool for detecting these strains. Our data supports that investigation of DEC should be recommended as a routine diagnostic test for patients in this clinical setting.

[**P884 Disseminated Penicillium marneffei infection in a non-HIV-infected woman**

R. Plongla*, K. Assawath, P. Leethong, A. Chaindamporn, C. Saunkratay (Bangkok, TH)

**Objectives:** *Penicillium marneffei* is a dimorphic fungal infection endemic in Southeast Asia. It is one of the commonest opportunistic infection in AIDS patients in Thailand, especially in the northern part. Infection in non-HIV-infected patients is uncommon.

**Results:** A total of 105 samples corresponding to 100 patients were included. Most patients were travellers: 38 came from Africa, 15 from Central America, 15 from Asia and 12 from South America. The remaining 20 subjects did not report any travel in the last weeks. The mean age of the study population was 38±14 years and 5% of patients were children (<15 years old). The gender distribution was equal. The main enteropathogen bacteria identified were DEC (7 patients) followed by *Salmonella* spp (5 patients), *Campylobacter* spp (3 patients, 2 *Campylobacter jejuni* y 1 *Campylobacter coli*), *Shigella sonnei* (2 patients) and *Plesiomonas shigelloides* (1 patient). *eae* gen was the only found in subjects with DEC strains. All of them were adult travellers from Asia (4 patients), Africa (2 patients) and South America (1 patient). Three of them presented coinfection with other enteropathogens (*Campylobacter jejuni*, rotavirus and *Giardia lamblia* in each case). Of note, ipaH was detected in two patients in whom *Shigella sonnei* was cultured. These subjects did not report any travel in recent weeks and one of them was coinfected with HIV-1.

**Conclusion:** DEC was the main enteropathogen bacteria responsible of traveller’s diarrhoea with a remarkable prevalence (7%). The multiplex PCR is an useful and simple tool for detecting these strains. Our data supports that investigation of DEC should be recommended as a routine diagnostic test for patients in this clinical setting.

**Figure:** A: multiple discrete erythematous painless subcutaneous nodules distributed on her face. B and C: arthritis and redness at the fifth PIP joint of left hand and the ulnar side of right wrist without deformities. Bone radiographs showed juxta-articular osteopenia, multiple osteolytic lesions at bilateral fibula shafts, erosions and osteolytic lesions at styloid process of right ulnar bone (long arrow) and left fifth PIP joint (short arrow) with soft tissue swelling without periosteal reaction. A and B: Wright stain from pus showed multiple 2–4 μm yeast-like organisms with transverse septum. C: Silver stain. D: cultures of pus from right wrist grew the fungal colonies which formation of a soluable red pigment of *Penicillium marneffei*. 
Methods and Results: We described a 48-year-old HIV-negative woman who presented with fever, multiple painless subcutaneous nodules, multiple osteolytic lesions, and alteration of consciousness due to severe hypercalcemia. *Penicillium marneffei* was isolated from the pus of her subcutaneous nodules (see Figures). She recovered following eight-week treatment with amphotericin B.

Conclusion: *Penicillium marneffei* infection in non-HIV-infected patients presented differently from HIV-infected patients. Clinical manifestations can be vary from mild skin papules to severe disseminated infection. However, common findings included fever, weight loss, skin manifestation, and ostearticular involvement. Although, *Penicilliosis in non-HIV-infected patients is uncommon*. By reporting this case, we hope to raise the awareness of this fungus in the clinical practice.

**P885**

Occupational outbreak of tropical rat mite (*Ornithonyssus bacoti*) dermatitis in laboratory personnel, in southern Italy (Apulia region)

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Objectives: The tropical rat mite (TRM), *Ornithonyssus bacoti* is a non-burrowing, temporary blood-sucker mite infesting a variety of domesticated and wild mammals worldwide, including rodents. It can also bite humans and cause a non-specific dermatitis. We report an occupational outbreak of TRM dermatitis in laboratory personnel and discuss the failed parasitological diagnosis.

Methods: Five researchers (4 women, 1 man) working in the animal facility of a local University complained of pruritus and sporadic, tiny papulap of two weeks' duration involving mainly arms and hands. They had also experienced the sensation of “bites and stings” at work when handling the laboratory mice. Due to intense itching, one of the researchers inspected her body and collected arthropods from her underclothes similar to those previously observed on the rodents' cages. Inspection of the vivarium revealed several parasites which were then sent to the Istituto Zooprofilattico of Foggia for the identification.

Results: The mites were identified as *O.b.* The animal room and the rodents' cages were disinfested using pyrethroids. A professional exterminator was recommended. There was no evidence of mites or dermatitis during the follow-up.

Conclusions: Medical and parasitological textbooks rarely describe *O.b.* outbreaks and only sparse information was found in manuals on laboratory rodents maintenance and their infestations. Due to the difficulty of achieving correct identification and to the lack of information on this mite in several European countries, personnel working with laboratory rodents may be unaware of the existence of TRM infestation and therefore fail to implement any preventive/control measures. The mite often gains access to the animal research building on feral rats and then lives in crevices adjacent to laboratory cages. Laboratory rodents and man then become a food source. TRM should therefore be listed as a zoonotic biological agent in all European regulations regarding occupational safety, while TRM dermatitis should be listed as an occupational risk for individuals working with laboratory rodents. This study also highlights the need to train personnel working in animal facilities about the zoonotic ectoparasites of laboratory rodents with particular emphasis on TRM. As pet rodents have also bite humans and cause a non-specific dermatitis. We report an occupational outbreak of TRM dermatitis in laboratory personnel, in southern Italy (Apulia region) and Coimbra regions, and Madeira Island, all in Portugal, and Cape Verde, and were morphologically identified as *Planorbarius metidjensis*. Genomic DNA was extracted according to Stothard (1996). Two mitochondrial genes, COI and 16S, and the nuclear ITS region were amplified by PCR and sequenced in both directions. The sequences obtained were edited with BioEdit version 7.0.0, aligned with homologous sequences, obtained from GenBank and phylogenetic trees were produced using MEGA 3.1.

Results: On a BLAST search the most similar sequences were of *Helisoma sp*. All sequences were very similar to each other, but it was possible to identify separate robust clusters on the rooted phylogenetic trees. Specifically, one cluster included samples from Cape Verde, which were very closely related, another samples from Madeira, which were more diverse, and two separate clusters from mainland Portugal.

Conclusions: Snails morphologically identified as *P. metidjensis* were genetically characterized using one nuclear and two mitochondrial regions, which suggested a higher similarity to the genus *Helisoma*. The population from Cape Verde was very homogeneous, suggesting a recent bottleneck or founder effect. Conversely, that from Madeira was polymorphic, suggesting multiple introductions from the same region or an ancient introduction. Although these seemed related to the populations from mainland Portugal, it was much more polymorphic. Mainland Portugal samples did not show geographical patterns, but were divided into two populations, which may have distinct biological characteristics and, thus, implications for current and future transmission of helminth parasites.

Funding: UPMM

**P886**

Molecular analysis applied to mitochondrial DNA from the family Planorbidae (Pulmonata: Basommatophora) snails, potential intermediate hosts of trematode hosts of trematode parasites of great importance in human health and/or veterinary medicine. For example, the snail hosts of *Schistosoma* spp. belong to the genera *Biomphalaria*, *Bulinus* and *Planorbarius* (Gracio, 1983, 1981). The schistosomes cause considerable morbidity and mortality in humans, and schistosomiasis is considered a neglected disease. Our objective was to compare populations of family Planorbidae snails, from different geographical areas of Portugal (Mainland and Madeira Island) and Cape Verde, a former Portuguese colony, through molecular analysis of mitochondrial DNA.

Material and Methods: The snails were collected in the Alentejo and Coimbra regions, and Madeira Island, all in Portugal, and Cape Verde, and were morphologically identified as *Planorbarius metidjensis*. Genomic DNA was extracted according to Stothard (1996). Two mitochondrial genes, COI and 16S, and the nuclear ITS region were amplified by PCR and sequenced in both directions. The sequences obtained were edited with BioEdit version 7.0.0, aligned with homologous sequences, obtained from GenBank and phylogenetic trees were produced using MEGA 3.1.

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Conclusions: Snails morphologically identified as *P. metidjensis* were genetically characterized using one nuclear and two mitochondrial regions, which suggested a higher similarity to the genus *Helisoma*. The population from Cape Verde was very homogeneous, suggesting a recent bottleneck or founder effect. Conversely, that from Madeira was polymorphic, suggesting multiple introductions from the same region or an ancient introduction. Although these seemed related to the populations from mainland Portugal, it was much more polymorphic. Mainland Portugal samples did not show geographical patterns, but were divided into two populations, which may have distinct biological characteristics and, thus, implications for current and future transmission of helminth parasites.

Funding: UPMM

**P887**

Ocular parasitosis: diagnosis and treatment

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Objectives: Our aim was to establish the parasitic etiology for ocular pathological developments, to monitor the evolution as well as to set out the most efficient therapy.

Methods: We collaborated with different clinics and laboratories and we investigated 76 patients with ocular affections, between 2001 and 2009. To establish the diagnosis we used ophthalmological and serological methods as well as ocularchiography and angiofluorography for several cases. The treatment included the association of praziquantel and sulfadiazine, or praziquantel and azithromycin for toxoplasmosis as well as other schedules that will be presented, detailed and discussed.

Results: Common clinical features were blurred vision, scotoma, ocular pain, photophobia and epiphora. Most ocular parasitosis were produced by *Toxoplasma gondii* (89.47%). We identified one case of cysticercosis, orbital hydatid cyst, deroflariasis (periorbicular granuloma), and the rest were determined by *Toxocara sp*. The most frequent cases of toxoplasmosis were chorioretinitis.

For most of our patients we used 2 or 3 courses of treatment as presented in methods. We would like to underline the efficacy of azithromycin. Systemic corticosteroids were used when lesions involved the macula, optic nerve or papillomacular bundle.

Conclusions: The collaboration between clinicians of different specialty is of outstanding importance. In larva migrans ocularis syndrome the antibody levels are decreased in comparison with larva migrans visceralis syndrome. ELISA results are not a sufficient argument in order to start the treatment. It is extremely important to have a precocious diagnosis and treatment to obtain good results in ocular toxoplasmosis.
Visceral leishmaniasis in a patient on haemodialysis

Introduction: Leishmaniasis is an endemic disease in many underdeveloped countries and also in some Mediterranean countries. Leishmaniasis is recognized as a serious opportunistic infection in some immunosuppressed patients. We report a rare case of Visceral Leishmaniasis (VL) in a hemodialyzed patient successfully treated with amphotericin B.

Case report: A 78-year-old male with end-stage renal failure due to diabetes mellitus on haemodialysis for the past nine years, was admitted in our department with one-month history of anorexia, fatigue, malaise and weight loss. Physical examination revealed only splenomegaly. Laboratory tests revealed pancytopenia (hemoglobin 8.4 g/dl, total leucocytes 3.9 × 10^3 c/dl, platelets 90 × 10^3 c/dl) and hyper-globulinaemia. The prothrombin, activated partial thromboplastin time and fibrinogen were normal. The liver transaminases and total bilirubin were normal. Mantoux and sputum test for acid-fast bacilli were negative. HIV test was also negative. Thoracic radiographs were normal and an abdominal ultrasound study showed splenomegaly (16 cm in its maximum diameter). Platelets dropped further up to 50 × 10^3 c/dl on the next days and a bone marrow aspirate was performed and demonstrated amastigotes of *Leishmania donovani*. Peripheral blood smear showed no parasites. Liposomal Amphotericin B was initiated at doses of 150 mg/day intravenously for 5 continues day with no side effects. A short course of treatment repeated five days later. Bone marrow aspiration and bone marrow culture 3 months after the end of the treatment were negative. One year later there has been no relapse.

Conclusion: Visceral Leishmaniasis has been reported as a complicating infection in some immunosuppressed patients. However, to the best of our knowledge, only three cases of VL in a haemodialysis patient have been reported. It has been suggested that impaired cell-mediated immunity and inhibition of macrophage function which are important in the elimination of intercellular microorganisms may be predisposing factors for this kind of infection. Pentavalent antimonials remain the drug of choice for VL. However, the trend in southern Europe is shifting towards using liposomal amphotericin B as the preferred treatment, even though the response rate is still around 90% for antimonials. VL should be considered in the differential diagnosis of patients who present with fever, hematological abnormalities or hyper-globulinaemia, especially in endemic areas.

Development of new anti-Leishmania drugs: activity of heterocyclic derivatives, pyrroles and porphyrin synthetic compounds on *L. infantum* and *L. tropica* promastigotes

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Protozoan parasites of the genus *Leishmania* cause visceral, cutaneous and mucosal diseases in humans, which are collectively referred to as leishmaniasis. These diseases affect more than 12 million people worldwide with approximately 350 million people at risk. Considering the toxicity, side effects, rate of relapse, cost, length of the treatment and the resistance that parasites develop against drugs, more attention should be given to the development of new compounds for leishmaniasis.

In the present work we focused on the activity of heterocyclic derivatives, thieno pyrroles and porphyrin synthetic compounds. Some of these compounds seem to have pharmaceutical potential: heterocyclic derivatives have cytotoxicity for cancer cell lines by apoptosis mechanisms associated with caspases signalling and DNA topoisomerases inhibition and porphyrins showed good properties as a sensitizer in photodynamic therapy. Inducers of apoptosis, anticancer drugs and inhibitors of DNA topoisomerases seem to be an advantageous approach to the development of leishmanicidal drugs.

The antileishmanial assays was performed on promastigotes cultures of *L. infantum* and *L. tropica* incubated in growth medium, RPMI 1640 medium enriched with 10% fetal bovine serum, with different concentrations of compounds. The promastigotes proliferation was assessed by counting the total cells in haemocytometer and the viability by the tetrazolium-dye colorimetric method (MTT). The experiments were performed in triplicate and in at least six independent assays. Results were expressed as concentrations that inhibit parasite growth or viability by 50% (IC50).

For the all tested compounds (19) only two heterocyclic derivatives and one porphyrin revealed activity against *Leishmania* promastigotes with IC50 values ranging from 22 μg/ml to 130 μg/ml. There were distinct and considerable differences in molecular structure of compounds that showed activity against *Leishmania*. The 4-dimethylaminophenyl substituent present on of the heterocyclic derivate and the Cl atom that bind to methyl group on the other heterocyclic compound seems to be responsible for the biological activity. In relation to the porphyrins, the presence of carboxyl substitues was crucial to the anti-protozoa activity. The results suggest that the active synthetic compounds may serve as models for the design of new active molecules for leishmaniasis therapy, including cutaneous and visceral forms.

This work was supported FCT POCI (FEDER)

Increased level of interleukin-10 and tumour necrosis factor-a associated with severe scrub typhus

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Objectives: Scrub typhus is a chigger-borne illness caused by *Orientia tsutsugamushi* that causes a systemic infection with various severity degrees. We aimed to identify the risk factors associated with severe scrub typhus and compare the cytokine levels between severe and non-severe scrub typhus groups.

Methods: We conducted a prospective study from September to December 2009. Scrub typhus was confirmed using indirect immunofluorescent assay. We developed scrub typhus severity index (STSI) to evaluate the severity of illness and defined patients with STSI ≥3 as having severe scrub typhus. The concentrations of cytokines were measured using enzyme-linked immunosorbent assays.

Results: A total of 63 patients were confirmed with scrub typhus and 13 patients were denoted as severe scrub typhus. Thrombocytopenia, hypalbuminemia and hyponatremia were more frequently associated with severe than with non-severe scrub typhus. Mean age and CRP increased more in severe scrub typhus group. However, multivariate analysis demonstrated no factors were significantly associated with severe scrub typhus. The concentrations of interleukin (IL)-6, IL-10, IL-12p40, interferon (INF) − g, and tumor necrosis factor (TNF) − a were increased in patients with scrub typhus compared with those of normal control. IL-10 and TNF-a increased significantly more in severe than in non-severe scrub typhus group (p=0.006).

Conclusion: Whereas there were no clinical features indicative of severe scrub typhus, measuring the concentrations of IL-10 and TNF-a may predict disease severity in adults with scrub typhus.

Clinical and epidemiological peculiarities of severe leptospirosis in Georgia

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Objectives: Leptospirosis is a zoonotic diseases of a worldwide distribution. Frequent circulating serotypes of *Leptospira interrogans* as an etiologic agent in Georgia are: *L. icterohaemorrhagiae*, *L. canicola*, *L. grippotyphosa*, *L. ballum*. During recent years new serotypes have been identified: *L. mankarso*, *L. wolffii*, *L. autumnalis*. Incidence during last 5 years is 0.63. Overall lethality is 7–14%. This research aimed to identify clinical and epidemiological aspects of severe leptospirosis.
Severe Mediterranean spotted fever complicated by bilirubin nephropathy

Methods: The research retrospectively studied the cases of severe leptospirosis admitted at the emergency department of the Infectious disease, aids and clinical immunology scientific practical centre of Georgia, Tbilisi during 2005–2010. Serological confirmation included: ELISA, MAT.

Results: Totally 13 patients (11 males, 2 females) were registered, among them 4 cases (31%) were lethal (3 males, 1 female). Age of the patients were from 26 to 71 years. Source of infection were: contact with a natural water reservoir-54%, ground contaminated with rodent excretions-8%, cattle-raising activity-8%, unknown source – 15.5%.

Clinical finding: fever – 100%, jaundice – 100%, hepatomegaly – 100%, myalgia – 85%, increased creatinin – 85%, oligo-anuria – 77%, pneumonia – 69%, chills – 61.5%, cutaneous and mucosal haemorrhages – 61.5%, splenomegaly – 54%, infectious toxic shock – 46%, oedema – 38.5%, proteinuria – 38.5%, convruitivitites – 31%, macrohaematuria – 23%, haemoptoe – 16%, haematomatisis – 8%, cutaneous rash – 8%. Mortality was induced: severe infectious toxic shock, acute renal failure, hepato-cellular failure, acute lung injury, thrombo-haemoragic syndrome. Such important concomitancies as HCV chronic carrier, oncological pathology, body mass deficit with lung TB and atopic dermatitis in non-lethal cases were noticed to induce prolonged course of illness and afterwards – reconvalencecence.

Conclusion: Leptospirosis remains as an important protozoon for Georgia. Severe leptospirosis is characterized with high mortality in Georgia. Most frequent signs are: fever, jaundice, hepatomegomy, myalgia, oligo-anuria, elevated creatinin, pneumonia, chills, cutaneous and mucosal haemorrhages. During recent years new serovars have been registerd in Georgia, which have not been circulating before.

Severe Mediterranean spotted fever complicated by bilirubin nephropathy

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Mediterranean spotted fever (MSF) is a tick-borne acute febrile disease caused by Rickettsia conorii. Recently, complicated cases have been more frequently reported. We describe the case of severe MSF complicated by bilirubin nephropathy and liver function test anomalies.

53-year-old male patient presented at hospital with a 7-day history of chills, shivering; fever (40°C), severe fronto-occipital headaches, fatigue, generalized maculopapular rash, and muscle weakness. He confirmed having a dog in his garden and ticks on it, but denies any tick bite on himself. His noticed maculopapular rash first on the forearms and extended to the whole body involving palms and soles. An inooculation scar (“tache noire”) was noticed on the stomach. He had currently medicated with cefuroxime and ampicillin, but had no relief. On physical examination, he had painful abdomen on the epigastric region with hepatomegaly and splenomegaly, sonographically confirmed. Based on a presumptive diagnosis of rickettsiosis, he was put on doxicycline 100mg bid PO. On day 3, the patient presented mental confusion, agitation, hallucination, disorientation, nausea and vomiting. The presence of meningitis signs led to a lumbar puncture, revealed nopleocytosis and normal protein count. Cranial MRI finding was not correlated with meningoenecphalsis. Due to urinary retention, the patient was undergone to catheterization, revealing a yellowish urine and had no urine for the following 6 hours. Urine specimen showed bilirubin containing hyaline slenders. Laboratory analyses showed the leukocytosis with predominant neutrophils, liver transammase level increase; aspartate aminotransferase (AST): 87 IU/L; alanine aminotransferase, 89 IU/L; lactate dehydrogenase (LDH), 438 IU/L; serum creatinine (Cre), 4.12 mg/dL; C-reactive protein (CRP), 304 (<5). Patient was given intravenous NAHCO3 solution and diuresis started. Liver enzymes, Cre and CRP levels returned to normal in three days. Biopsy of the papular lesion revealed a dense inflammatory infiltrate, composed by histiocytes, lymphocytes and neutrophils, with diffuse and perivascular distribution, present in a “tache noire”. Weil-Felix test was negative on day 0 but positive on day 7. Seven days after initiation of the antibiotic therapy, clinical conditions, including the headache, nausea and rash started to ameliorate. The antibiotic treatment continued for 10 days, and eventually the patient was discharged without any complaint.

Two cases of Toscana virus meningitis in Algarve, Portugal

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Since the discovery of Toscana virus (TOSV) in 1971 in Turkey, sandy-borne TOSV has become recognized as a leading cause of acute meningitis in central Italy during the summer months from May to October, far exceeding enteroviruses. France, Spain, Greece, and Cyprus have also reported cases of TOSV infection. In Portugal there are 2 cases reported in travellers to Algarve and 2 more others cases from authors personal experience in the last 5 years. These 2 new concomitant cases reported here alert clinicians to the possible emerging of this agent in the region of Algarve. TOSV should be included in the differential list of viral pathogens among patients who seek treatment with symptoms consistent with meningitis or encephalitis living or having recently travelled to Mediterranean areas, including Portugal.

Visceral leishmaniasis: a final diagnosis of an intermittent febrile syndrome with 3 years of evolution.

Case report

M. Ribero*, J. Louro, L. Riem (Barcelos, PT)

Background and Objectives: Visceral Leishmaniasis (VL) caused by the intracellular protozoan Leishmania infantum is an endemic infection in Portugal with 15 to 20 cases diagnosed a year in immunocompetent patients. Dogs are considered the main reservoir for human visceral infection and parasites are transmitted by the bite of phlebotomine sand flies, being Phlebotomus perniciosus and P. ariasi the proven vectors in Portugal. Onset of symptoms may be insidious or subacute weight loss, enlargement of the spleen and liver, and decreases in the production of blood cells that can lead to anemia, bleeding and infections with other microorganisms. Without treatment, this form of the disease is nearly always fatal. The objectives of this study are to report a VL case in a patient that initiated his condition with an intermittent febrile syndrome and liver involvement and to review the aspects related to this disease.

Case report: A 38 years old male patient. His past medical history includes psoriatic arthritis, hypertension and 2 previous admissions (in 2006, May and 2007, October) for fever of unknown origin: he had increased liver enzymes and negative microbiological/immmunologic studies. In 2006 his abdominal ultrasound (US) showed hepatic steatosis and in 2007 had evidenced hepatomegal. Liver biopsy performed at that time was compatible with chronic liver disease. In both times fever broke only with steroids and the cause was not found. In 2009, October our patient presented with fever and weight loss. Work up
revealed pancytopenia, raised ESR and liver enzymes. He had hepatic steatosis and splenomegaly in abdominal US. After three bone marrow biopsies performed was possible to visualize amastigotes in the last one, ensuring the diagnosis of leishmaniasis. He had responded well to the administration of liposomal amphotericin, without recurrence of fever up to date.

Discussion: The chronicity of complaints and the existence of prolonged fever periods for which it was not possible to determine the etiology and which were not repeated after treatment with amphotericin lead us to think about the possibility if it is a chronic leishmaniasis.

Conclusion: Symptomatic disease is subacute or chronic and diverse in presentation and outcome. Definitive diagnosis requires the demonstration of parasite by smear or culture in tissue.

**P895** Evaluation of therapeutic potential of methanolic extract of stem bark of *Acacia nilotica* (Linn) for the treatment of African trypanosomiasis

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Objectives: The objective of this work was to evaluate the therapeutic potential of methanolic extract of stem bark of *Acacia nilotica* (Linn) in the treatment of human African trypanosomiasis (sleeping sickness).

Methods: Powdered stem bark of *A. nilotica* was extracted in 70% v/v (Methanol/Water) and partially purified using column and thin layer chromatography. Both crude and partially purified extracts were then evaluated for their therapeutic effects in experimental *Trypanosoma brucei* brucei infection in mice. Blood and cerebrospinal fluid infectivity tests were done by drawing blood from the cured mice, sub inoculating each into healthy mice, and then monitoring inoculated mice for establishment of infection. Acute toxicity studies was done by administering 2000 mg/kg bw to a group of four healthy mice which were then observed for 48 hours for mortality.

Results: The crude extract at a dose of 400 mg/kg bw per day completely cured experimental *Trypanosoma brucei* brucei infection in mice within eight days, while a dose of 50 mg/kg bw per day of the partially purified extract completely cured experimental *Trypanosoma brucei* brucei infection in mice within two days. Sub inoculation of blood and cerebrospinal fluid drawn from the cured mice and inoculated into healthy mice failed to produce infection within 28 days of post inoculation. Administration of 2000 mg/kg-lbw of the partially purified extract led to the death of half the number of the experimental animals used in the acute toxicity studies.

Conclusion: It is concluded that methanolic extract of *A. nilotica* stem bark is sufficiently trypanocidal to allow further studies to pave way for possible development of phytomedicine or synthetic safer drug through modification of isolated bioactive constituent for use in the treatment of sleeping sickness.

**MRSA in animals**

**P896** Occurrence and characteristics of methicillin-resistant *Staphylococcus aureus* in pig farming in the Czech Republic

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Objectives: The aim was to carry out the first nationwide survey on the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) strains on Czech pig farms, in accordance with the Commission Decision 2008/55/EC. Presented are results of the study, including characteristics of MRSA isolates.

Methods: On 283 selected Czech pig farms, samples of dust on metal partitions separating pens were collected using special swabs. Bacteriological analysis was performed using methods published in the Commission Decision 2008/55/EC to obtain MRSA isolates. The PCR method was used to study their ability to produce enterotoxins, exfoliatins, PVL and TSST. Macrocotidermination analysis using pulsed-field gel electrophoresis was carried out using the Smal restriction enzyme. MRSA isolates were characterized by SCCmec typing, spa typing and MLST. The minimum inhibitory concentration values for MRSA isolates to selected antibiotics were determined by the standard broth microdilution method.

Results: MRSA was detected on 5 Czech farms localized in 5 different districts. The prevalence of MRSA in herds of breeding pigs in the Czech Republic was 1.8%. None of the MRSA strains carried a gene for the production of the studied toxins. Using macrorestriction analysis, all the isolates were classified as nontypeable. Molecular typing revealed that all isolates belonged to ST398, showing 3 different, but closely related, spa types (t034, t2346, and t4659). Two isolates sharing spa type t3346 differed by SCCmec types (types IV and V); the remaining 2 isolates possessed SCCmec type V. The tested isolates were resistant to at least 4 types of antibiotics and all of them were resistant to tetracycline.

Conclusion: The prevalence of MRSA on Czech pig farms (1.8%) does not pose a significant general epidemiological risk for the human population. However, the colonization of pigs with MRSA ST398 has been identified as an occupational health risk for farmers, veterinarians and their families. Monitoring of the occurrence of MRSA should be recommended in other commodities, in particular milk and dairy products.

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**P897** Methicillin-resistant *Staphylococcus aureus* isolated from swine and farm workers in Spain

C. Potel-Alcarellos, L. Constella-Carames, A. Moreno-Flores, C. Lopez-Coton, E. Comesaña-Da-Vila, L. Eiroa-De-La-Puente, S. Perez-Castro, M. Alvarez-Fernandez* (Vigo, Pontevedra, ES)

Objectives: To determine the prevalence and molecular characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) colonization among swine and related workers.

Methods: The nares of 197 swine and 8 workers from four production systems comprising 2,900 live pigs were sampled. The swabs were cultured on CNA and MRSA selective agar (bioMerieux). *S. aureus* strains were identified by the tube coagulase test. MRSA was confirmed by cefoxitin and oxacillin disk diffusion test. The MRSA strains were characterized by pulsed field electrophoresis (PFGE) using EagI restriction enzyme, spa typing, and multilocus sequence typing (MLST). PCR was used to determine the SCCmec type and the presence of the mec and the pvl genes.

Results: The swine ages were 3 (n= 28, MRSA 21.4%), 8 (n= 45, MRSA 26.6%), 12 (n= 10, MRSA 30%), 16 (n= 75, none MRSA), and 24 (n= 25, MRSA 8%) weeks. Additionally 14 adult sows were studied being the MRSA prevalence 14.3%. A total of 25 (12.7%) MRSA isolates were recovered from swine and 6 (7.5%) MRSA were recovered from workers. All the strains were ST398. The only SCCmec identified was type V. The most common spa-type among pigs was t011 (84%), all of them belonged to the same farm and were SCCmec V positive. PFGE classified the t011 strains in four types. The workers were colonized by the same PFGE types than the related animals except in one case. All the strains were pvl negative.

Conclusions: The results showed that the colonization of swine by MRSA is common, being the nasal colonization among workers very frequent. PFGE results showed diversity within the spa t011 strains. Swine could be an important reservoir for MRSA representing a challenge for human health care systems.
**P898** Livestock-associated MRSA responsible for human colonization and infection in a northern region of Italy
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**Objectives:** Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a pathogen of increasing importance in health-care settings (HA-MRSA) and in community (CA-MRSA). Livestock-Associated MRSA (LA-MRSA) belonging to ST398 lineage, common among pigs and other animals, emerged in Central and Northern Europe, becoming a new risk factor for MRSA among farm workers. Strains belonging to ST398 can be responsible for human infections, mainly in areas with high livestock-farming. The aim of this study was to investigate the occurrence of LA-MRSA human colonization and infections in a Northern region of Italy with high density of pig and cattle farming.

**Methods:** In the period March-April 2010, at Manerbio Hospital (Lombardia Region) in the North of Italy, 879 nasal swabs were sampled from patients submitted to a pre-admission screening. In the period March-August 2010, at the same Hospital, MRSA isolated from outpatients infections were collected. By PCR assays, the species *S. aureus*, methicillin-resistance and the presence of Panton-Valentine (PVL) toxin genes were confirmed. Molecular characterization included SCCmec typing, spa typing and, on selected strains, multi-locus sequence typing (MLST).

**Results:** Out of 879 nasal swabs examined, 9 (1%) yielded MRSA. No strains were positive for PVL toxin genes. Six isolates harboured SCCmec type IV and 3 strains SCCmec type V. Five strains were assigned to t009 (3 isolates), t108 (1 isolate) and t222 (1 isolate), all belonging to ST398 and therefore categorized as LA-MRSA. Three strains were 008-ST8 and 1 strain was t688-ST5, likely of hospital origin. A total of 10 MRSA were detected from outpatient infections of which 9 were skin and soft tissue infections (SSTI). Of these, 4 strains were PVL positive CA-MRSA which harboured SCCmec type IV (3 strains), type V (1 strain) and belonged to different clones (008-ST5, 008-ST8, 021-ST8 and T445-SST72). Other 5 strains were PVL negative, SCCmec type IV, 008 (2 strains), t127 (2 strains) and t515 (1 strain). One MRSA from ear infection was PVL negative, SCCmec type IV, t099 and ST398, hence categorized as LA-MRSA.  

**Conclusion:** In areas with high density of pig and cattle farming LA-MRSA is able to colonize the population and also to produce infections along with typical and more common CA-MRSA. Since animal contact is an established risk factor for LA-MRSA, close surveillance combined to appropriate control measures should be employed to avoid LA-MRSA spread.

**P909** Occurrence of methicillin-resistant *Staphylococcus aureus* in dairy products of Apulia region Italy
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**Objectives:** *Staphylococcus aureus* is one of the most important foodborne pathogens and its pathogenicity is related to the production of staphylococcal enterotoxins (SEs). The pathogenicity of some strains is greatly increased by antimicrobial resistance. The use of antibiotics in veterinary practices, could determine the selection of antibiotic-resistant clones of *S. aureus*, including methicillin-resistant *S. aureus* (MRSA). In this report are noted the results of the characterization of *S. aureus* isolates from milk and cheeses produced in Italy.

**Methods:** 110 strains of *S. aureus* were isolated from dairy products during 2008–2009 in Apulia (Italy). These strains were characterized in order to determine the presence of staphylococcal enterotoxin(s) genes (sea, seb, sec, sed, see, seg, seh, sei, sej, sem, sen, seu) by PCR, the antibiotic-resistance profile using the disc agar diffusion method (Kirby-Bauer), and the detection of mecA gene by PCR. Different PCR were used for Staphylococcal cassette chromosome mec (SCCmec) typing.

**Results:** Overall out of 110 analysed strains, 42 (38.2%) *S. aureus* isolates were found to be positive for SE genes. Most of the isolated strains carried, alone or in association, the sed gene (15.5%), followed by efg cluster: seg, sei, sen, see (13.6%), sej (10.9%), sea (9.1%), seb (6.4%), sec (5.5%), seb (0.9%). Strains carrying see gene were not found. A strong resistance to ampicillin/penicillin G (40%), streptomycin (28.2%), erythromycin (17.3%), novobiocin (16.4%), tetracycline (14.5%), kanamycin (11.8%) and bacitracin (10%) was found in the analyzed *S. aureus* strains. Two *S. aureus* strains resulted resistant to methicillin (1.8%) and mecA positive. These strains carried the SCCmec type V.

**Conclusion:** The presence of enterotoxigenic strains of *S. aureus* in food produced in Italy represents a potential risk for consumers. A remarkable level of resistance to several antibiotics was found in the *S. aureus* strains analyzed in this survey. The total percentage of multi-resistant strains (resistance to three or more antibiotics) was found to be 31.8% and this confirms that dairy products still remain a major source of antibiotic resistance in *S. aureus*. In our study two *S. aureus* presented methicillin resistance (1.8%). Both strains were positive for the presence of mecA and harboured the SCCmec type V. The aim of this work was to contribute to the knowledge on the prevalence of MRSA in dairy products. (Work supported by IZSPB 06/08.)

**P900** High prevalence of methicillin-resistant *Staphylococcus aureus* in pigs and slaughterhouse workers
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**Objectives:** Methicillin-resistant *Staphylococcus aureus* (MRSA) strains belonging to clonal lineage sequence type (ST) 398 are being reported at an increasing frequency in Europe and other countries. This new MRSA type has been isolated from colonized and infected animals and humans. This study aimed to determine the prevalence of nasal MRSA carriage in pig and slaughterhouse workers.

**Methods:** A total of 200 pigs and 25 workers of the slaughterhouse of Tenerife (Canary Islands, Spain) was prospectively included in the study. Animals were transported to the slaughter and placed in separate stalls according to different farm origin. All of them were slaughtered within 12 hours.

Nasal samples were collected in the slaughter by using Amies-Rayon swabs (Deltalab™). Swabs were enriched in Brain-Heart Infusion with 7% NaCl at 37°C for 24 hours and subsequently streaked onto MRSA chromID plates (BioMerieux). They were incubated at 37 °C for 24–48 hours.

Suspected MRSA colonies were first screened using Slide™ Staph Plus agglutination and subsequently confirmed with PB2 agglutination (MRSA™ Screen). Molecular typing of microorganisms was performed by pulsed field gel electrophoresis (PFGE) using the restriction enzyme Apal (Promega™). Allele and sequence type (ST) designations were made by using the MLST website.

**Results:** We found 91% (18/200) in pigs to carry MRSA in their nares. The overall prevalence of nasal MRSA carriage in employees of pig slaughterhouse was 8% (2/25) All the MRSA isolates belonged to the swine strain ST398.

**Conclusion:** The prevalence of MRSA in nasal samples from pigs in our area is even higher than those found in other studies. All the MRSA isolates belonged to MRSA strain ST398. The high prevalence found in our study suggests implementation of screening measures, surveillance and control in livestock facilities. Further studies are needed to evaluate the pig livestock as MRSA reservoir.

**P901** Diversity of rep-families in *Staphylococcus aureus* of food, animal and human origin
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**Objective:** To reveal the plasmid content of a collection of *S. aureus* strains of human and animal origin by expanding the classification system for plasmids described for enterococci and other Gram-positive
bacteria (Jensen et al., 2010). This classification system was based on PCR amplification of conserved areas of rep genes encoding replication initiating proteins.

**Methods:** A total of 112 rep sequences from Staphylococcus aureus retrieved from GeneBank were aligned using Bionumeric program. Nucleotide- and protein-based phylogenetic trees were generated. A cut-off value of 75% identity was chosen to determine the rep-families. A total of 15 rep-families and 10 unique sequences were defined. A collection of 92 S. aureus with different antibiotic resistance phenotype and genotypes and of different clonal lineages were included in this study. Forty-three methicillin-resistant S. aureus (MRSA) were isolated from Spain, human, animal and food origin. The remaining 49 strains (27 MRSA and 22 methicillin-susceptible S. aureus [MSSA]) were from animal origin obtained in Denmark. Primers were designed for new defined rep-families and unique sequences. Multiplex PCRs from the previous study together with newly designed PCRs were performed in the 92 strains. Some of the amplicons obtained were sequenced to confirm amplification of the correct product.

**Results:** Positive amplicons were obtained from rep-families (no strains): 2 (1), 5 (6), 6 (1), 7 (49), 7b (5), 9 (2), 10 (35), 10b (2), 13 (4), 15 (2), 16 (2), 20 (5), 21 (31), 22 (28) and from unique sequences pE194 (1) and pKKS825 (5). Eight strains did not yield amplicons for any of the rep-families or unique sequences studied. The rep2, 6, 9 families (specific of other Gram-positive genera) were detected in MSSA CC398 strains from Denmark. Some rep-families and unique sequences were only detected in strains from Spain (rep5, 7b, 10b, 15, 16, 20 and pKKS825) or Denmark (rep2, 6, 9 and pE194). Only 3 rep-families were identified in MSSA strains (rep7, 10 and 21).

**Conclusion:** A classification system for plasmids from Gram-positive bacteria has been expanded for S. aureus. Predominant rep-families were identified. rep-families of other genera were found in S. aureus strains demonstrating plasmid transfer between species. Differences in rep family content were detected according to the origin. Association between resistant phenotype and rep-families was often observed.

**Molecular tools for Staphylococcus aureus: from epidemiology to diagnosis**

**Presence of Panton-Valentine leukocidin, toxin genes and Agr typing of methicillin-resistant Staphylococcus aureus strains isolated from bloodstream infections at Hacettepe University Adult Hospital, Ankara, Turkey**

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**Objectives:** To investigate the presence of Panton Valentine leukocidin (PVL), and toxin (enterotoxin A-J, staphylococcal toxic shock toxin-a) genes, and accessory gene regulator (agr) types of mecA-positive methicillin-resistant Staphylococcus aureus (MRSA) strains isolated from hospital-acquired (HA) bloodstream infections.

**Methods:** A total of 112 non-duplicate MRSA strains isolated from HA bloodstream infections between January 2004-January 2010 at Hacettepe University Adult Hospital were included in the study. Hospital acquisition was defined on the basis of CDC definition criteria. The isolation and identification of the isolates were made by automated system (Phoenix, Becton Dickinson, USA) at the central laboratory of the hospital and then they were stored at −80ºC until the beginning of the study. They were tested for methicillin resistance by oxacillin (30µg, Oxoid, UK) disk diffusion test according to the CLSI criteria and for the presence of mecA, PVL, enterotoxin, TSST genes and agr types by PCR method.

**Results:** All MRSA isolates were mecA-positive. None of them were positive for PVL or TSST genes. Eleven (10.1%) isolates was not carrying any of the enterotoxin genes. Five isolates (4.5%) was carrying sea gene alone. The remaining 95 isolates was positive for combination of enterotoxin genes as follows: sea+sec+see+sei (48 isolates, 50.5%), sea-see (35 isolates, 36.8%), and sea+sec+see+sei (6 isolates, 6.3%), other combinations (6 isolates, 6.3%). Agr typing revealed that 36 (32.4%) were type I, and four (3.6%) were type II. The remaining 66 isolates (59.5%) was carrying two or three agr groups: 32 (28.8%) were types I and III; 34 (30.6%) were types I, II, and III. In 5 isolates, agr groups could not be identified.

**Conclusion:** Enterotoxin genes (especially sea) and complex agr types were prevalent among HA-blood-stream MRSA isolates in our hospital. Besides, none of them were positive for PVL or TSST genes.

**Identification of methicillin-resistant staphylococci by PNA FISH directly from positive blood cultures**

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**Background:** In Denmark, the prevalence of methicillin resistant Staphylococcus aureus (MRSA) is very low (1.6% in 2009) whereas the prevalence of methicillin resistant coagulase negative Staphylococcus (MR-CNS) is high. Rapid identification (S. aureus vs. CNS) and determination of resistance to methicillin (MR vs. MS) of positive blood cultures containing staphylococci is important for optimal patient therapy.

S. aureus/CNS PNA FISH is a rapid method for routine identification of S. aureus and CNS using RNA as target. In this study, we evaluated a novel assay – mecA PNA FISH – targeting mecA messenger RNA (mRNA) in staphylococci performed in parallel with S. aureus/CNS PNA FISH for rapid identification of MRSA, MSSA, MR-CNS, and MS-CNS within 2hrs.

**Method:** Initially, 172 reference strains and clinical isolates spiked into negative blood culture bottles were used to assess the analytical sensitivity and specificity of mecA PNA FISH. Subsequently, the clinical performance of mecA PNA FISH (prototype) in parallel with S. aureus/CNS PNA FISH (AdvantDx) was assessed using 66 positive blood cultures (Rigshospitalet, Copenhagen) containing Gram-positive cocci in clusters (GPCC). Results were compared to routine identification obtained following subculture and determination of resistance to methicillin using cefoxitin disk diffusion and mecA EVIGENE.

**Results:** 66 GPCC positive cultures comprised 49 CNS (33 S. epidermidis, 11 S. haemolyticus, 1 S. hominis, 1 S. warneri, 3 mixed cultures (2 CNS + E. faecium and one CNS + CNS)), 16 S. aureus and 1 M. luteus. As expected, 100% (16/16) of S. aureus were MSSA and 90% (44/49) of CNS were MR-CNS. mecA PNA FISH showed 86% (38/44) sensitivity and 100% (22/22) specificity compared to disk diffusion and mecA EVIGENE.

**Conclusions:** mecA PNA FISH is a promising tool for identification of MR-CNS and potentially MRSA directly from positive blood cultures (2hrs). Less than 100% sensitivity for MR-CNS may be explained by low or delayed expression of mecA. Low expression and potential link to low level resistance is being studied and may be of therapeutic importance.
Discrimination of staphylococci using an rpoB-consensus PCR coupled with a microarray hybridisation assay

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Objectives: The objective of the study was to develop a DNA microarray based assay for the molecular discrimination of staphylococci based on rpoB sequences.

Methods: BLAST analysis revealed rpoB sequences of all available staphylococci, additional isolates were sequenced. PCR primer and microarray probes were designed using Clonland Oligo design software. Resulting 3‘aminomethylated oligonucleotides were synthesised and used for the fabrication of ArrayStrip microarrays. A total set of 296 clinical staphylococcal isolates and reference strains were characterised using this assay. An automated algorithm for pattern recognition led to the identification of the species. Identifications were confirmed by rpoB sequencing, MALDI TOF and/or biochemical profiles (VITEK).

Results: All relevant rpoB sequences were annotated in a local database and aligned. After alignment, the most conserved sequence windows were calculated for primer design. The sequences between the primers were used to produce all possible oligo sequences from these data within a certain range of calculated melting temperatures. A set of probe sequences was selected in order to achieve the strongest possible discriminating power. In practical microarray experiments, the stringency was adjusted to be as close as possible to theoretical experiments derived from rpoB sequenced isolates. The assay was then applied to CNS or S. aureus isolates which were identified by sequencing rpoB (n = 34, concordance 100%), by MALDI TOF (n = 12, concordance 91.7%), by VITEK-2 (n = 148, concordance 83.3%) or for which identifications have been provided by strain collections (DSMZ, n = 29, concordance 89.7% and NARSa, n = 7, concordance 100%).

Conclusion: Based on intensive bioinformatic calculations and optimisation of reaction conditions and stringencies, array hybridisation targeting rpoB and subsequent pattern recognition rather than sequencing allows identification of staphylococcal species using the high throughput format ArrayStrip/ArrayMate and adapted software. Despite an overall good concordance, the methods for pheno- or genotypic species identification can differ in their results. This can be attributed to the presence of mis-identified or atypical isolates in the available databases or to principal discrepancies between geno- and phenotypic species definitions. Further investigation, especially with regard to rare species, is warranted.
Molecular tools for Staphylococcus aureus: from epidemiology to diagnosis

Conclusions: The correlation between our molecular and phenotypic data supports the possibility of screening for delta-hemolysis, in the presence of vancomycin, to quickly and accurately detect potential hVISA or VISA isolates of diverse agr-genotypes. Studies are ongoing using various sheep blood media.

P908 meca expression in clinical MRSA strains upon cefoxitin induction
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Objective: A clinically highly relevant diagnostic tool based on PNA FISH on mRNA was developed for identification of methicillin-resistant Staphylococcus aureus (MRSA). This study examines the expression of meca in clinical MRSA strains upon cefoxitin induction by both PNA FISH and reverse transcriptase quantitative polymerase chain reaction (RT-qPCR).

Methods: Clinical isolates of MRSA and MSSA were grown with shake at 35 °C for 40 min with or without 3 μg/mL cefoxitin. PNA FISH slides were prepared, total RNA extracted from 1 mL culture and cDNA synthesized. cDNA was used in qPCRs targeting the meca and 16S rRNA genes respectively. Using qPCR data the baselines for cefoxitin induced and uninduced cells and the mean fold changes (MFC) in meca expression were calculated (Shang et al., 2010). In order to evaluate the effect of induction on meca expression as measured with RT-qPCR uninduced and induced baselines were compared by paired Student’s t-tests.

Results: Table 1 shows the cefoxitin minimum inhibitory concentrations, baselines, P-values for t-tests, MFC and PNA FISH results obtained for each strain. 7 strains showed significantly increased meca expression upon cefoxitin induction (P < 0.05).

Conclusion: Even though inducability of the strains varied PNA FISH detected all strains with MIC >12 μg/mL. No correlation between the SCCmec type and the meca expression was observed. There was a tendency for strains with a low cefoxitin MIC to have a low meca expression. The results of PNA FISH and RT-qPCR corresponded well.

Table 1: Cefoxitin minimum inhibitory concentration (MIC) calculated baselines for cefoxitin induced and uninduced cells, P-values for paired comparisons of induced and uninduced baselines, calculated mean fold change, and results of PNA-FISH microscopy and RT-qPCR analysis for clinical MRSA and MSSA strains.

| Strain | SCCmec type | Cefoxitin MIC (μg/mL) | Induced baseline | P-value | MFC | PNA FISH |
|-------|------------|-----------------------|------------------|---------|-----|----------|
| 155 (MRSA) | - | 3 | 0 | 1.00 | 0 | Negative |
| 156 (SCSA) | - | 4 | 0 | 0.00 | 0 | Negative |
| 9 | I | 256 | 69 ± 19 | 0.21 | 1.4 ± 0.5 | Positive |
| 1489 | I | 256 | 67 ± 49 | 0.25 | 1.0 | Positive |
| 430 | I | 256 | 153 ± 64 | 0.04 | 30 ± 22 | Positive |
| 39 | II | 128 | 138 ± 28 | 0.01 | 24 ± 13 | Positive |
| 11 | II | 256 | 34 ± 10 | 0.03 | 11 | Positive |
| 2 | III | 256 | 33 ± 32 | 0.35 | 7 ± 6 | Positive |
| 13 | BIA | 256 | 49 ± 11 | 0.06 | 6 ± 3 | Positive |
| 827 | IV | 24 | 51 ± 21 | 0.05 | 5 ± 1 | Positive |
| 895 | IV | 48 | 9 ± 5 | 0.11 | 1 ± 0 | Positive |
| 590 | IV | 48 | 9 ± 5 | 0.00 | 5 ± 4 | Positive |
| 557 | IV | 64 | 74 ± 95 | 0.06 | 52 ± 2 | Positive |
| 330 | IV | 256 | 48 ± 17 | 0.02 | 3 ± 1 | Positive |
| 256 | IV | 256 | 80 ± 11 | 0.01 | 9 ± 2 | Positive |
| 396 | V | 12 | 9 ± 3 | 0.11 | 7 ± 3 | Weak positive |
| 460 | V | 12 | 13 ± 2 | 0.004 | 16 ± 2 | Weak positive |
| 251 | V | 06 | 36 ± 16 | 0.00 | 12 ± 2 | Positive |

P910 Comparison of two molecular methods, Xpert MRSA assay and MRSA Advanced Test, for methicillin-resistant Staphylococcus aureus nasal screening
P Stano*, M. Acioi, R. De Rosa, M. Modolo, A. Camporese (Pordenone, IT)

Objective: Colonization with methicillin resistant Staphylococcus aureus (MRSA) is a risk factor for subsequent infections, leading to increased morbidity and mortality, as well as healthcare costs, especially in patients admitted to intensive care units (ICU). Infection control guidelines recommend (Calfee DP et al. Infect Control Hosp Epidemiol, 2008) nasal screening for MRSA to prevent and limit the spread of infections. Recently, the introduction of various molecular tests for rapid identification of MRSA carriers have improved infection control procedures by providing results in hours rather than days, as the time required for culture-based methods. In this study we evaluate the accuracy of a rapid molecular test, MRSA Advanced Test (Roche), to detect MRSA colonization among ICU patients, compared with the Xpert MRSA Assay (Cepheid), FDA (Food and Drug Administration) approved test and used routinely in our laboratory.

Methods: From December through April 2010, a total of 128 patients were screened for MRSA carriage at admittance to ICU, by using a double-shaft swab to sample both nostrils. All nasal swabs were analyzed by GeneXpert MRSA (Cepheid) and, at the same time, with MRSA Advanced Test (Roche). Both methods are able to amplify by real-time PCR the target sequence for MRSA at the SCCmec-orfX junction.

Results: Among the 128 patients examined for MRSA, 12 had a positive screening result by both molecular methods. Patients who had negative results were 104 by MRSA Advanced Test and 106 by Xpert MRSA admissions. Service was offered from 8am – midnight and 7days a week. The rates of MRSA infections have since reduced by 78% (40/2007–08 to 9/2008–09); 80% (8/2009–10); 1 bacteremia[April 2010 till date]. Since August 2010, this screening of emergency admissions by PCR was extended to include MSSA and MRSA.

PCR is an integral part of any busy Microbiology department. During times of serious financial turbulence in NHS and subsequent budget cuts, we have attempted to find an innovative solution. We present a case for optimisation of laboratory services and money savings by incorporation of MRSA/MSSA PCR services from bacteriology to virology section of the department.

Methods: Economic modelling based on quantum of work, shift patterns and expected turn-around-times. This included the Virology department staffing structure of 5 dedicated technical members of staff [Band 4s used where appropriate]. Extending working day to 8am till midnight. Timing and fine tuning batching of specimens for MRSA/MSSA PCR to optimise the benefits of 8am – midnight service, reducing costs and reduce TAT. Planned spreading of work from 8am till midnight. Serological tests conducted between MRSA PCR runs; Data analysis [period before and after shift of MRSA PCR service to virology section].

Results: Between Aug-Nov 2010 [First 3 months of introducing dual MRSA/MSSA PCR screening] reduction in MSSA positives from 27.3% to 20.5%.

Since Jan 2010: MRSA positives reduced from 5% to 1.8%; reduction of intermittent results and 60% reduction in failed runs; optimal batching permits 20% more tests from each kit.

Savings of up to £260K from bringing back in-house costly tests and additional rapid tests which had been previously sent away and helped reduce turn-around times.

Conclusions: Optimisation of services by economic modelling, service and demand management can help maintain high quality of service and reduce costs in these financially challenging times. Cost benefits from reduction in MRSA/MSSA infections through saved bed-days & bringing in-house expensive tests offsets the staffing costs while reducing TAT. Further cost saving/service optimisation options are open to explore including splitting out-of-hours on call service between virology [dual bacteriology/virology trained] between 8pm-midnight and bacteriology from midnight-8am bringing a potential saving of £30K.

P911 MRSA PCR screening and optimisation of laboratory services: an innovative way for saving precious pennies in times of financial turbulence in NHS
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Background: Blackpool Victoria Hospital introduced a PCR based MRSA screening service in March 2008 for all emergency hospital
Assay. Initially 2 patients had invalid results in the MRSA Advanced Test and confirmed negative after repeat testing.

**Conclusion:** In conclusion we demonstrate here that MRSA Advanced Test, compared to Xpert MRSA Assay, has similar sensitivity and specificity. Both tests show high efficiency and efficacy compared with culture methods. In fact they require few hours to complete the analysis allowing a shorter turnaround time (TAT) and, by improving patient management, a better clinical and therapeutic outcome. However the Xpert MRSA Assay remains a real molecular point of care considering its remarkable low complexity, the short time required to perform the analysis and the best time-to-result (less than 70 minutes). This is a start point for infection control strategies to prevent MRSA spread and for developing more focused therapeutic measures in all colonized patients.

**References:**

1. KFSH-Dammam, a tertiary care hospital, has guidelines for hospital wide screening of new hospital admission for Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) to limit the spread of antimicrobial resistance within certain high risk populations. The aim of this study was to evaluate the diagnostic performance of BD GeneOhm vanR assay, a rapid real time PCR test that detects the presence of vanA and/or vanB genes. We evaluated also the performance of BD GeneOhm MRSA which detect the staphylococcal cassette chromosome mec (SCCmec cassette) carrying the mecA gene and *Staphylococcus aureus* specific sequence located within the orfX gene.

**Methods:** Three hundred duplicate rectal swabs collected consecutively between January 2009 & June 2009 were analyzed for the presence of VRE by culture and BD GeneOhm VanR Assay PCR. 2267 duplicate swabs were collected (728 nasal and 1539 groin swabs) between January 2008 & June 2009 and analyzed for the presence of MRSA by conventional cultured method and BD GeneOhm MRSA.

**Results:** Compared to culture, the BD GeneOhm vanR assay showed a sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 100%, 91.1%, 23.5% and 100% respectively. The BD GeneOhm MRSA assay revealed sensitivity, specificity, PPV and NPV of 97%, 99.4%, 89.5% & 99.9% respectively for nasal swabs. For groin swabs, it was 100%, 98.4%, 60.9% and 100% respectively.

**Conclusion:** The BD GeneOhm vanR assay is a good screening test for rapid exclusion of VRE carriers in hospitals. In our population with predominantly vanB colonized patients and poor positive predictive value, results of the assay should be confirmed by another method for the presence of VRE. The BD GeneOhm MRSA assay represents a reliable screening test when applied to nasal and groin specimens. The true strength of the BD GeneOhm assay for MRSA and VRE is its exceptionally high NPV making the test an ideal tool for rapid exclusion of MRSA and VRE carriers in hospitals. As a consequence, this would dramatically shorten the patient isolation time.

**Development of a rapid and simple isothermal molecular assay for the qualitative detection of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* directly from blood culture on a non-instrumented platform**

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**Background and Objectives:** *Staphylococcus aureus* (SA) is responsible for approximately 25% of all bloodstream infections, amongst those 26% to 47% are caused by Methicillin-resistant strains (MRSA). The resulting bacteraemia has a mortality rate of 25%-35%, thus the timely identification of SA and MRSA is necessary in order to provide effective antibiotic therapy. Current traditional methods for identification of SA and MRSA include culture and agglutination testing followed by oxacillin susceptibility testing, which takes between 16 to 48 hours in order to obtain results. PCR based methods have been developed that can be performed in less than two hours, however, these assays require expensive instrumentation and must be performed in a highly-complex molecular lab, rather than in a microbiology laboratory, a resource that many small to medium hospitals do not have access to.

**Methods and Results:** Helicase dependent amplification (HDA) is an isothermal nucleic acid amplification platform that utilizes a helicase detection device (BEST™ cassette) that allows for the rapid detection of labeled amplicons generated by primers and probes specific for the Ess A gene (SA) and Mec A cassette (MRSA) simultaneously in only three pipetting steps. An aliquot from a positive blood culture tube is diluted and heated to lyse the cells and transferred to a tube containing the lyophilized HDA reagents. The sample is then incubated at 64°C for one hour and transferred to the BEST cassette wherein the result is read via a lateral flow strip. The entire assay is performed “on-demand” in less than 1.5hrs without the need for batching of samples. Initial analytical sensitivity testing determined that the assay can detect down to 30 copies of both SA and MRSA, and does not cross-react with any non-SA organisms that have been evaluated. Preliminary evaluation of the assay with previously characterized clinical samples found that 18/18 MRSA samples and 18/18 SA positive/Mec A negative samples were correctly identified.

**Conclusion:** The HDA-based SA/MRSA assay is a simple and sensitive molecular assay that is capable of differentiating between SA and MRSA infections and can be performed in a variety of laboratory settings without the need for costly equipment.

**Evaluation of the impact of a rapid MRSA point-of-care test**

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**Objectives:** MRSA screening of hospital admissions is now a requirement of hospitals in England; however, the optimal methodology for screening has not been defined. Sandwell and West Birmingham Hospitals NHS Trust have chosen to screen emergency admissions by a rapid point of care PCR method and this study attempts to pull together data on the impact of this approach on a number of outcomes.

**Methods:** Patients included in this study were those admitted to an assessment unit and screened by MRSA POCT. Target patients eligible for testing were either admitted for >24 hours or transferred to another ward. During the period of study a total of 4707 screens were undertaken; 312 were positive. 250/312 MRSA positive patients were included in an audit. Patients environments were screened for MRSA using broth culture and compared to the environment of patients found to be MRSA positive by routine culture.

**Results:** The screening programme has been accompanied by a steady and sustained fall in MRSA bacteraemia, although many other initiatives may also have contributed to this. An interesting finding has been a steady decline in positivity rates from MRSA screening. Overall, 53% of patients sampled had a screen result available within 12 hours of admission and 91% within 24 hours. Overall, 76% of MRSA positive patients received their first dose of mupirocin within 24 hours of their screen result and 65% received their first application of chlorhexidine within 24 hours. Most patients responded well to their experience of the screening programme. A small study of environmental sampling suggests that there might be less environmental contamination with MRSA in the environment of patients identified as carriers by the POCT screening compared with a group who had not been identified as carriers.

**Conclusions:** Our study has shown that the introduction of rapid POCT MRSA screening was accompanied by a sustained fall in MRSA bloodstream infections and an overall fall in positivity rates for screening. This may be due to the rapid awareness of MRSA positivity status and early institution of decolonisation treatment. Preliminary results
also suggest a possible reduction in environmental contamination around patients who have had this early intervention.

**P914** Genotypic and phenotypic characteristics of methicillin-resistant *Staphylococcus aureus* blood isolates with higher MIC (3 mg/L) of vancomycin in Taiwan

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**Background:** *Staphylococcus aureus*, especially methicillin-resistant *S. aureus* (MRSA), is a major pathogen that causes bacteraemia and sepsis with significant morbidity and mortality. Infections by MRSA with higher minimal inhibitory concentration (MIC) of vancomycin (VA) were reported with treatment failure. Objectives: The goals of this study were to identify and delineate the genotypes and phenotypes of MRSA isolates with higher VA MIC from sterile tissue.

**Methods:** MRSA isolates from sterile sites were obtained from 2006 to 2009 collection of Tigecycline In-vitro Surveillance in Taiwan (TIST) and tested of VA MIC with agar dilution. Isolates with higher VA MIC (≥3 mg/L) were selected for genotyping and phenotyping experiments including pulse-field gel electrophoresis (PFGE), staphylococcal cassette chromosome mecA (SCCmec), multilocus sequence typing (MLST), genes encoding protein A (spa) and accessory gene regulator (agr), direct repeat unit (dru), E-test to daptomycin, disk diffusion to varied antibiotics, D-test, gene encoding Panton-Valentine leucocidin (pvl), and superantigenic toxins.

**Results:** A total of 596 MRSA isolates from sterile site were retrieved with mean VA MIC of 1.46 (range: 1–3 mg/L), in which most were isolated from blood (551, 92.4%). Ten isolates with VA MIC 3 mg/L were selected, and all were multidrug resistance and negative pvl. Six isolates were SCCmecIII-ST239-spa t037-agrI-dru7 (1 isolate) and dru14 (5 isolates), 2 isolates were SCCmecII-ST5-spa t3520-agrII-dru4, and one isolate was SCCmecII-ST89-spa t3520-agrIII-dru7 and the other isolates was SCCmecIV-ST59-spa t437-agrII-drul, respectively. Only one isolate had positive D-test and three isolates were susceptible to daptomycin (MIC ≤1 mg/L). Five isolates possessed sea-seIk-seIq, in which 4 belonged to SCCmecIII-ST239-spa t037-agrII-drul. Five pulsotypes were also identified in ten isolates.

**Conclusions:** Neither vancomycin-intermediate *S. aureus* (VISA) isolate nor vancomycin creep was identified in the 4-year survey, but elevated VA MIC in MRSA isolates was noted in Taiwan. MRSA isolates with VA MIC 3 mg/L were few with limited genotypes and corresponding phenotypes.

![Figure 1. Distribution of minimal inhibitory concentration (MIC) of MRSA isolates from sterile tissue (mean ± standard deviation). *: Mann-Whitney-U test for statistic comparison of mean MIC.](image-url)

**P915** Detection of small colony variants among methicillin-resistant *Staphylococcus aureus* blood isolates

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**Objectives:** *Staphylococcus aureus* small colony variants (SCVs) are associated with chronic and persistent infections. Methicillin-resistant *S. aureus* SCVs cause more severe infections and mortality rates are higher in comparison with infections caused by MRSA. Our objective was to document the prevalence and phenotypical characteristics of SCVs among MRSA blood isolates.

**Methods:** MRSA strains isolated from blood during 1999–2009 are evaluated. Among 299 MRSA isolates, SCV suspected colonies were inoculated onto Columbia blood agar and Schaedler agar. Columbia blood agar was incubated in normal atmosphere and Schaedler agar in 5–10% CO₂, both at 35°C. If the small, non-pigmented, non-hemolytic colonies on Columbia blood agar were seen as normal sized, hemolytic and pigmented colonies on Schaedler agar, they were considered as MRSA SCVs.

**Results:** Among 299 isolates evaluated, six MRSA SCVs are detected. When subcultures are made, four of them reversed to phenotypically normal *S. aureus*, but two isolates were stable as SCV phenotype. As a result, the prevalence of SCVs among MRSA blood isolates was found as % 0.67 (2/299).

**Conclusion:** This is the first report of detection of SCVs among MRSA blood isolates from Turkey. As the clinical significance of MRSA infections is well documented, evaluation of MRSA SCVs in clinical samples, especially from intensive care patients and those who have chronic and persistent infections is important to consider.

**P916** Susceptibility patterns of *S. aureus* as predictors of Panton-Valentine leukocidin positivity

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**Objectives:** Resistance to some antibiotics (e.g. fusidic acid) is frequently used as an indicator for Panton-Valentine Leucocidin (PVL) in *Staphylococcus aureus*. However its diagnostic value has not been thoroughly evaluated. This study examines the usefulness of antimicrobial susceptibility patterns in predicting PVL.

**Methods:** All *S. aureus* strains that were tested for PVL between Sept. 2006 and Nov. 2010 at our institution were retrospectively analysed. Susceptibility patterns of PVL positive and -negative strains were compared to each other. Both methicillin-resistant (MRSA) and methicillin-susceptible *S. aureus* (MSSA) strains were evaluated separately. Identification and susceptibility testing of *S. aureus* was performed by Vitek-2. All isolates were furthermore tested for the presence of SA442 (*S. aureus* specific gene), mecA and hlf gene (encoding production of PVL) by PCR.

**Results:** Of 133 strains, mostly from abscesses and blood cultures with clinical evidence of a possible role of PVL, the hlf gene was detected in 47 (35.3%) strains (17 MRSA, 30 MSSA); 86 isolates were negative for PVL (16 MRSA, 70 MSSA). PVL positive MRSA strains were significantly more often ciprofloxacin and clindamycin susceptible (p < 0.05) and occurred more often in younger patients (median age 33 years for PVL+, 62 for PVL-), p < 0.001), mostly men (82.4%). Sensitivity, specificity and positive predictive value of susceptibility patterns (PPV) were 47.1%, 93.8% and 88.9% for ciprofloxacin and 52.9%, 81.3% and 75.0% for clindamycin. In MSSA, PVL was also more common in younger patients (median age 28.5 years, p < 0.001), regardless of gender.

**Conclusion:** PVL is an important virulence factor of *S. aureus* and was frequently detected (35.3%) in strains which were selected for testing based on clinical evidence. Most of the positive strains were from young patients. In our setting, fusidic acid was not a good predictor of PVL, in contrast to reports from other studies.

Susceptibility patterns such as ciprofloxacin and clindamycin susceptibility in MRSA may serve as an additional aid to initiate PVL testing when there is an appropriate clinical context (PPV 88.9% and 75%). However,
the sensitivity and negative predictive value of the described markers is low and diagnostic algorithms based on antimicrobial susceptibility patterns alone are therefore of limited utility for the detection of PVL.

Methods: Purified native or overexpressed staphylococcal enterotoxins A and B, toxic shock syndrome toxin, staphylococcal haemolysins α and β, staphylokinase and Panton Valentine leukocidin (PVL, F-component) were used to generate different specific sets of monoclonal antibodies via phage display. These antibodies were purified after over-expression in E. coli, characterised initially by ELISA and spotted in different dilutions in microtiter strip-mounted protein microarrays.

Results: For each toxin, all possible combinations of capture and detection antibodies were tested with microarrays using different protocols and antigen concentrations in order to find the most specific and sensitive antibody combination as well as the fastest possible protocol. These arrays together with a specifically designed software algorithm allow one to measure concentrations of single or multiple staphylococcal toxins in culture supernatants after calibration using recombinant and native toxins. In a first series of experiments, the expression of PVL by different S. aureus strains under uniform culture conditions was targeted. PVL from human isolates yielded another reaction pattern than leukocidin (lukM/lukF-P83) from bovine strains.

Conclusions: The simultaneous measurement of staphylococcal toxins in high throughput format allows studying toxin gene regulation at different experimental conditions, strain or clonal complex-specific variations and a possible interference between different toxin genes. These issues as well as, e.g., a potential clinical significance of hyper-producing strains warrant further study.

### P918

**Extending rapid PCR screening to all Staphylococcus aureus (MSSA and MRSA): an innovative project at Blackpool Victoria Teaching Hospital, UK**

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**Background:** Rapid and accurate identification of *Staphylococcus aureus* (SA) in hospital admissions is essential for timely decisions on optimal treatment, isolation/bio-burden reduction, and reducing the potential for cross transmission and self-acquisition of healthcare-associated infections (HCAI). Significant reductions in MRSA bacteraemias have been consistently reported from our hospital in previous 2-years. Here we present the case for extending the PCR based screening programme to MSSA and MRSA; evaluation of third year of MRSA screening programme and the innovative trust investment tracking programme.

**Methods:** Retrospective analysis of infections with SA [MSSA/MRSA]: trends in pre & post 48h MRSA infections; evaluation of clinical/collateral benefits and hospital investment benefit tracking programme.

**Results:** 40 MRSA bacteraemias were recorded in 2007–08 against a target of 26. Reductions of 78% [9bacteraemias]2008–09; 80% [8bacteraemias]2009–10; 1 bacteraemia [Apr–Nov 2010]. In 2009–10, a 28% reduction [120/414] in MRSA wound infections. The rates of MSSA infections including bacteraemia have remained static over the previous years [2008–10] while consistent reductions in MRSA infections. Following from the phenomenal success with reductions in MRSA infections in previous years, a case was made for extending the trust screening programme to SA [MSSA and MRSA] since August 2010. The trust has an innovative investment tracking programme to evaluate the economic benefits. 4-months is a short period for evaluation, however, a consistent decline in MSSA bacteraemias [7, 6, 5 and 2] are recorded during Aug–Nov 2010. Details to be presented.

**Conclusions:** Rapidly available SA [MSSA/MRSA] results are routinely used to complement clinical decision making and optimise treatment in our hospital. All emergency admissions to hospital are screened using the PCR method and all electives using the chromogenic culture method. The cost effectiveness of any HCAI programme is proportional to its success. The key to success in this model is the teamwork between CEO, infection control teams, laboratory services and clinical teams (medical and nursing staff). The body of evidence which supports the efficacy of rapid screening is growing. A sound business case and return on investment for the use of rapid diagnostic methods in our hospital can be made. These technologies also enhance clinical quality, improve patient safety and reduce the overall cost of SA infections.
Emergent C. difficile PCR ribotype 027. After the recognition of this, the majority of the affected countries have developed surveillance studies to monitor the spread of this strain and strict infection control measures have been implemented. In Hungary, the first recognized infection due to C. difficile PCR ribotype 027 was detected in 2007, after this case, isolate belonging to this PCR ribotype could not be found among those strains, which were sent to the Anaerobe Reference Laboratory for further analysis.

Methods: Up to this time, 203 C. difficile strains isolated from diarrhoeal faeces of both inpatients and outpatients in 2010 were analysed. The presence of toxins A and B was detected in local laboratories, while PCRs were used to detect the presence of tcdA, tcdB and binary toxin genes in the reference laboratory. If the binary toxin gene PCR gave positive result, PCR ribotyping was performed.

Results and Conclusion: Until this time, among the examined 203 strains, 102 isolates proved to be positive for binary toxin genes. 100 of them were PCR ribotype 027. 79 strains carried the genes coding toxins A and B, these strains did not harbour binary toxin genes. In the case of 22 isolates, tcdA, tcdB and binary toxin genes could not detected. The majority of strains belonging to PCR ribotype 027 were isolated from inpatients (93 strains) and from patients over 60 years of age (81 strains). On the basis of recent findings, increased prevalence of hypervirulent PCR ribotype 027 could be observed in hospitals of North and Northwest Hungary, but because of the lack of culture in several laboratories and the low sensitivity of some commercial toxin assays, the outbreak situation will go from bad to worse.

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Enterococci

Colonisation with vancomycin-resistant Enterococcus faecium in a neonatal intensive care unit in Greece

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Objectives: The aim of this study was to determine the incidence of fecal colonization and the molecular characterization of vancomycin – resistant Enterococcus faecium (VRE) in a 30-bed, university-affiliated, level II-IV Neonatal Intensive Care Unit (NICU) at a large pediatric hospital in Athens.

Methods: Routine surveillance rectal cultures of 312 neonates, hospitalized in our NICU during one year period (from 1 December 2009 to 31 November 2010) were performed by using the campylo sel agar®. Fecal samples were obtained upon their admission and then weekly, during their stay. The isolation and identification were done according to the CLSI criteria. The MICs to vancomycin and teicoplanin were determined by Etest®. The detection of van genes was assessed by PCR, while the molecular identification was based on Multi Locus Sequence Typing.

Results: During the study period, a total of 390 neonates were admitted in our NICU from various maternity hospitals. A total number of 12 Enterococcus faecium (VRE) isolates were recovered from respective patients (3.08%). Four resistant patterns were observed. The individual MICs for vancomycin were >256 mg/L, whereas for teicoplanin ranged from 32–64 mg/L. All isolates carried the vanA gene. Molecular identification showed that the isolates belonged to two main clones; four belonged to ST17, seven to ST192 and one to ST64. The strains of ST17 were isolated from April to May 2010, while the strains of ST192 were isolated the first week of September 2010. The strain of ST64 was isolated upon admission of a neonate referred from a maternity hospital. All but one strains belonging to two main clones, were NICU – acquired. Environmental samples were negative for VRE. Strict infection control measures, such as hand hygiene and patient cohorting, were established during the two micro-outbreaks in order to eradicate the dissemination of such strains.

Conclusion: Active surveillance cultures, together with implementation of other infection control measures were instrumental in controlling VRE transmission in our NICU.

High-level aminoglycoside resistance in vancomycin-sensitive and -resistant enterococci

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Objectives: Despite the interpretation “resistant” for aminoglycosides (AMGs) streptomycin (S) and gentamicin (G) can be used in combination therapy for the treatment of infections with Enterococci, like Endocarditis. However these strains can develop high level resistance (HL), which renders the respective treatment useless. We wanted to analyse the data of the EPICENTER network database to see the development of resistance to vancomycin (VAN) and to aminoglycoside high level resistance in vancomycin resistant and sensitive Enterococci.

Methods: All three Laboratories participate in the network using the automated BD PHOENIX-systems measuring MICs. The BD EPICENTER Data-Management-System is used for the evaluation of the data in the laboratory and for the transfer of the data for join analysis. Here the data are interpreted using, appropriate breakpoints. Copy strains are excluded. Quality control is mandatory. We analysed the data of two german and one italien laboratory. For this purpose we used EUCAST break points. High level resistance was determined with gentamicin (G) ≥500 and streptomycin (S) ≥1000 mg/L as selective criteria.

Results: We analysed 13930 E. faecalis and 4320 E. faecium isolates. Vancomycin resistance was decreasing over the years from 2005 to 2010 in E. faecalis from 4.3% to 1.2%. 50% of the E. faecalis VRE strains have high level AMG Resistance. 28% of the strains are highly resistant to gentamycin and streptomycin (table). The data for vancomycin sensitive strains are similar: 59 and 27% for high level AMG and combined high level gentamicin and streptomycin resistance respectively.

Vancomycin resistance in E. faecium decreased over the period from 15 to 9%. High level resistance in these strains is more common than in E. faecalis. In VAN sensitive strains 77% of the strains had a high level AMG resistance. In VAN resistant strains however the incidence of HL AMG resistance was even 88%.

Conclusion: Vancomycin resistance in E. faecalis and E. faecium is decreasing. A specific problem is the high level aminoglycoside resistance in both VAN resistant and sensitive Enterococci. As high level aminoglycoside resistance is similar in VAN sensitive and VAN resistant strains both characters probably occur independently from each other.

Focus and outcome for community-acquired Enterococcus faecalis bacteremia

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Objectives: To determine the epidemiology and outcome of community-acquired bacteremia with Enterococcus faecalis.

Methods: We performed a retrospective population-based study of all patients diagnosed with community-acquired bacteremia due to E. faecalis at two departments of clinical microbiology in the Copenhagen area (approximately 1,250,000 inhabitants or 25% of the Danish population, serving nine public hospitals (3800 somatic beds) during the period 2006–2009. If a patient had more than one episode, only the first episode was included. Also, Patients with cases of poly-microbial bacteremia were excluded. Clinical data (age and sex of the...
patient, focus of infection, medical specialty) for patients with positive blood cultures were collected prospectively and registered in a bacteremia research database. Vital status and 30-day mortality were obtained from the Danish Civil Registration System. Results: During the 4 years study period, 169 patients were identified. Twenty-nine percent were females. The median age of the patients was 79 years (range 22−98 years). The foci of infection were as follows: Urinary tract was the focus in 43%, infectious endocarditis in 26%, intra-abdominal infection in 5%, and 26% unknown focus. The total 30-day mortality was 21%, significantly highest for patients with unknown focus 35% (OR: 2.4 (1.1−5.3), p=0.036), followed by intra-abdominal infections with 22%, focus in the urinary tract with 18%, and 11% for patients with infective endocarditis. Two percent of the patients required intensive care. Most of the patients, 77% were admitted to a medical ward. Ten patients had relapse of the bacteremia. All the E. faecalis isolated were susceptible to ampicillin. Two isolates were resistant to vancomycin, and 26% of patients with infectious endocarditis had a gentamicin high-level resistant isolate of E. faecalis.

Conclusion: Infective endocarditis was surprisingly often, in 26%, the focus for the community-acquired E. faecalis bacteremia. From this finding, we recommended that echocardiography to all patients with community-acquired E. faecalis bacteremia should be performed. Most of the patients were women, 71%, the median age was 79 years, and the most frequent focus seen was the urinary tract. Our results show that it is very important for the prognosis to identify the focus of infection, as patients with, unknown focus have a significant worse prognosis.

P923 Increased prevalence of high-level gentamicin resistance in invasive Enterococcus faecium strains in Norway is associated with hospital-adapted genetic lineages carrying virulence determinants and aac(6′)-Ie-aph(2″)-l-a containing transferable megaplasmids

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Objectives: During 2003−2008 a 10-fold increase in the prevalence of high level gentamicin resistance (HLGR), to above 50%, in blood culture isolates of E. faecium in Norway was reported by the Norwegian Surveillance System for Antimicrobial Resistance (NORM). Here, we present a molecular epidemiological study of representative invasive E. faecium strains collected through the NORM system during 2008, aiming molecular typing and mechanisms involved in the increased prevalence of HLGR.

Methods: A total of 100 E. faecium strains were genotyped by PFGE and multilocus sequence typing (MLST). Antimicrobial susceptibility against clinically relevant antibiotics was determined by agar diffusion and agar dilution (vancomycin) tests. The presence of HLGR encoding genes, putative virulence determinants, plasmid replicon (rep) types and plasmid stabilizing systems were examined by PCR and partial DNA sequencing. Plasmid linkage of rep- and resistance genes was analysed by Southern hybridization of S1 treated total DNA. PFGE was performed by SmaI and S1 nuclease PFGE and genes. VanB-transfer studies were performed by filter mating, and transconjugants were analysed by SmaI and S1 nuclease PFGE and Southern hybridization.

Results: PFGE revealed the presence of a polyclonal collection of E. faecium, although MLST-results showed that >90% of the strains belonged to hospital adapted genetic lineages of E. faecium. A high prevalence of ampicillin resistance (84%), HLGR (56%) and presence of several virulence associated genes was detected. Neither vancomycin nor linezolid resistance was observed. HLGR was strongly associated with the presence of the aac(6′)-Ie-aph(2″)-l-a gene. This resistance gene was linked to non-rep-typable transferable megaplasmids (>200 kb) often carrying the ace-txe plasmid stabilization system.

Conclusion: There is a significant increased prevalence of hospital adapted ampicillin resistant, HLGR E. faecium among invasive strains in Norway. High level gentamicin resistance is present in 56% of the strains and linked to transferable megaplasmids carrying the aac(6′)-Ie-aph(2″)-l-a gene encoding the bifunctional aminoglycoside modifying enzyme.

P924 The widespread VRE outbreak in Swedish hospitals 2007–2009 was associated with clonal E. faecium CC17 genogroup strains harbouring several virulence traits and transferable vanB pRUM-like repA plasmids

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Objectives: To perform a molecular characterization of vancomycin resistant (VRE) and susceptible (VSE) Enterococcus faecium isolates from the dominant PFGE clone causing a widespread outbreak in Swedish hospitals in 2007−09.

Materials and Methods: Outbreak isolates were analysed by PFGE. Eighteen VRE isolates from 3 Swedish counties as well as two VSE clinical isolates isolated before the outbreak belonging to the same PFGE clone were included in the study. All isolates were analysed by E-test, MLST, and PCRs specific for vanB2, Tn5382 and virulence genes. VanB-transfer studies were performed by filter mating, and transconjugants were analysed by SmaI and S1 nuclease PFGE and Southern hybridization.

Results: All VRE-isolates showed vancomycin MICs ranging from 8 to >256 µg/L and susceptibility to teicoplanin, consistent with the vanB2 genotype. 18 isolates including the two VSE belonged to ST192 and two isolates were single (ST78) and double (ST17) locus variants. Filter mating with donors containing plasmids of 70 kb resulted in transfer of larger sized (110−150 kb) pRUM-like repA plasmids harbouring vanB2 integrated in the conjugative transposon Tn5382. The VSE isolates contained a plasmid with only pRUM-like repA. All isolates contained 8−11 virulence genes and were ampicillin and high-level ciprofloxacin resistant, which all are traits associated with the CC17 genogroup.

Conclusion: The dominant vanB clone of the widespread VRE outbreak in Swedish hospitals in 2007−09 belonged to the successful hospital associated CC17 genogroup which contained several resistance and virulence traits as well as transferable pRUM-like repA plasmids with acquired vanB2 as part of the conjugative transposon Tn5382.

P925 Emergence of vancomycin-resistant Enterococcus gallinarum strains carrying acquired glycopeptide resistance determinants in Stockholm, Sweden

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Objectives: The emergence and spread of glycopeptide resistance in enterococci has become a significant clinical concern. VanA and VanB are the most common types of the acquired glycopeptide resistance. VanC-type glycopeptide resistance is intrinsic in Enterococcus gallinarum, Enterococcus casseliflavus and Enterococcus faecium. The acquisition of the vanA- or vanB-mediated glycopeptide resistance in enterococcal species other than Enterococcus faecalis and Enterococcus faecium has only been sporadically reported. Here we report the detection of three vancomycin-resistant E. gallinarum isolates carrying both the vanC1 gene and acquired glycopeptide resistance genes (vanA or vanB) in Stockholm, Sweden.

Methods: The isolates were detected in our routine screening for vancomycin-resistant enterococci (VRE) by a broth-PCR assay. The strains were then isolated on the ChromID VRE plates, and further identified by biochemical test, mobility test, Vitek 2 system and PCR for the determination of species and resistance genes. Antimicrobial susceptibilities were determined by disk-diffusion method or Etest. To verify the vanA gene in one of isolates, DNA sequencing of the PCR amplicons was performed. Pulsed-field gel electrophoresis (PFGE) was applied to investigate the clonal relationship of the isolates.

Results: Three vancomycin-resistant E. gallinarum were detected. Violet colonies were observed on the ChromID VRE plates. In addition to the vanC1 gene, the vanA determinants were detected in two of them and
vanB in one isolate. Both vanA isolates were resistant to high levels of gentamicin (MIC >256 mg/L). One of the vanA-harboring isolates (EG08001) presented a phenotype otherwise typically seen in VanB-type strains, with MICs of vancomycin and teicoplanin at 32 mg/L and 4 mg/L, respectively. The vanA identity of strain EG08001 was confirmed by sequencing. The two vanA-carrying VRE isolates displayed not identical but similar PFGE patterns.

**Conclusion:** This is the first report of the detection of vancomycin-resistant Enterococcus faecium in drinking water in our area. All VRE strains belonging with the vanA or vanB genotype may represent an additional threat for the control of enterococcal infections, which warrants further attention for these microorganisms.

**P926** Prevalence and rep-PCR typing of vancomycin-resistant enterococci VanA-type recovered from sewage in Austria

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**Objectives:** The importance of vancomycin-resistant enterococci (VRE) as a nosocomial pathogen has increased globally in the last decade in correlation with their ability to acquire high-level resistance against glycopeptides. Enterococci are also able to reach the environment persisting in sewage. The aim of the study was to determine the presence of VRE VanA in sewage and to analyze their genetic relationships to clinical isolates.

**Methods:** In 2009 a total of 115 sewage sludge samples from five different treatment plants located in the region of Styria were screened for the presence of VRE. All specimens were cultured on VRE Screen agar, after enrichment in Enterococcus Broth. Routine methods for identification and susceptibility testing were employed. Eight clinical isolates were recovered from faeces (n = 4) and wounds (n = 4) of inpatients at the Institute of Hygiene/MD Graz. The detection of the vanA-gene was done by PCR. To generate molecular fingerprints of the VanA-strains a semi-automated rep-PCR (DiversiLab) was performed. Isolates with >97.5% similarity and maximum one band difference were characterized as indistinguishable.

**Results:** 70% of the sewage sludge samples were VRE positive whereas the majority of the 228 isolated strains were E. gallinarum and E. casseliflavus with intrinsic low level resistance to vancomycin. 9% of the recovered strains were E. faecium and 7% were E. faecalis. A total of 11 (5%) E. faecium strains isolated from sewage sludge of three treatment plants (A,B,C), harboured the vanA-gene.

Genotyping: Sewage and clinical isolates were discordant. For the 11 sewage isolates the rep-PCR distinguished two clusters including each three strains from plant A, and one cluster including one strain from plant A and two from plant B. Two strains were unique. The fingerprints of the clinical isolates showed minor concordance. They were distributed in seven different patterns and only two strains had the same genotype.

**Conclusion:** In the present study no clonal relationships between sewage and clinical VRE VanA isolates could be observed. Nevertheless we could prove that VRE is able to persist in sewage. Environmental spread via sewage sludge, disposed in agriculture, can not be excluded. Therefore further surveillance of VRE is considered to be important.

**P927** The vancomycin-resistant enterococci in drinking water

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**Objectives:** Enterococci are members of the normal intestinal micro flora in humans and animals. It has been suggested that the environmental enterococcal strains could serve as a reservoir of the antimicrobial resistance genes, which can be transferred to other potentially pathogenic bacteria. The aim of study was to investigate the prevalence of vancomycin resistant enterococci (VRE) in drinking water in our area.

**Methods:** 2000 samples of drinking water were analyzed in East Macedonia-Thrace region of northern Greece from October 2009 to November 2010. Water samples (100mL) were processed by a membrane filtration procedure and filters were placed in Slanetz-Bartley agar plates for growth of Enterococcus spp. Membranes with typical colonies were transferred into a plate of bile -aeoculin-azide agar and incubated in 440C for 2h (ISO 7899). For each positive sample, colonies were stored at −80ºC in a medium containing 15% glycerol. The strains isolated were identified by the automated system Vitek2 and antimicrobial susceptibility testing was carried out by Vitek2 and E-test (Biomeurieux). Culture of enterococcal strains, grown in blood agar, were diluted in saline to 0.5 McFarland and spread on Mueller-Hinton agar (MHA). Furthermore, VRE strains were diluted in broth to 2 McFarland and spread on Brain Heart Infusion agar (CLSI M100-S20 Vol. 30 No 1).

**Results:** Enterococci (n = 121) were found in 6% of the samples. On the basis of their biochemical profiles, 11 strains were identified as E. faecium, 49 as E. faecalis, 10 as E. durans, 10 as E. hirae, 20 as E. gallinarum and 20 as E. casseliflavus. Resistance to vancomycin according to results of Vitek2 was detected in 22 (18.2%) of the isolates examined, 7 strains belonging to E. casseliflavus and 15 to E. gallinarum. Only 4 strains on MH agar with 0.5 McFarland were found resistant with MIC <8 mg/dL. In contrary with 0.5 McFarland methods, all strains were found resistant with MIC from 8 to 16 mg/dL. Resistance to teicoplanin was not detected in any of the isolates examined.

**Conclusion:** Consistent with these findings, VRE were detected in samples from drinking water in our area. All VRE strains belonging to E. casseliflavus and E. gallinarum with vanC phenotype according to results of Vitek2.

**P928** In vitro susceptibility of 104 vancomycin-resistant Enterococcus faecium isolates from clinical specimen against daptomycin, linezolid and tigecycline

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**Background:** Vancomycin resistant Enterococcus faecium (VREF) has become an important nosocomial pathogen world wide. Because of rising infection rates and limited treatment options we compared the local in vitro activity of Daptomycin (D), Linezolid (L) and Tigecycline (T) against VREF.

**Methods:** 104 VREF were collected between 2004 and 2009 at the University Hospital Aachen, Germany. The strains were isolated from clinical specimens only (43 urine, 33 wounds, 15 blood-cultures, 12 catheter-tips, 1 respiratory secretion). Copy strains were excluded. For identification the Phoenix expert system (BD, Germany) was applied and in vitro activity was detected by E-test-method. Because E. faecium breakpoints for D and T do not exist, susceptibility values given by CLSI and FDA for E. faecalis were adopted. Breakpoints for L for E. faecium were applied according to CLSI.

In vitro susceptibility of 104 Vancomycin resistant Enterococcus faecium isolates for Daptomycin, Tigecyclin and Linezolid

Results: The MIC values are shown in Figure 1. All strains were susceptible to D, 103 out of 104 strains (99%) were susceptible to L and 99 out of 104 strains (95.2%) to T.
MIC90 values for D were 3 μg/mL, for L 1.5 μg/mL and for T 0.19 μg/mL, respectively. Strains with limited sensitivity to L and T had MIC values only one step above the breakpoints.

Considering pharmacokinetic data we calculated the rates of Cmax to MIC90 for the tested antibiotics. Values for Cmax were taken from the literature (D dose 6 mg/kg, L 600 mg iv and T 100 mg: 95 μg/mL, 15 μg/mL and 1.5 μg/mL). The correlating Cmax: MIC90 values for D, L and T were: 32, 15 and 8, respectively.

Conclusion: D, L and T had excellent in vitro activity against VREF strains in our hospital. For D the correlation of Cmax:MIC90 was 2x and 4 times higher in comparison to L and T, respectively.

Considering the emergence of multiply resistant pathogens the local epidemiological data are of importance.

Author Disclosure Information: This study was sponsored in part by Novartis, Germany. S.L. is a member of the Advisory Board of Novartis.

**P929** Susceptibility of 234 clinical isolates of vancomycin-resistant *Enterococcus* spp. to teicoplanin, daptomycin, tigecycline, doripenem and linezolid

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Background: Vancomycin-resistant enterococci are dreaded pathogens of invasive infections in immunocompromised patients. The frequency of the isolation of Vancomycin-resistant enterococci from clinical specimens has been dramatically increasing during the last 5 years at the University of Vienna.

Objective: The aim of this study was to investigate in vitro activity of teicoplanin (TEI), daptomycin (DAP), tigecycline (TIG), doripenem (DOR) and linezolid (LIN) against vancomycin-resistant *Enterococcus* spp. (VRE) isolated at the University Hospital of Vienna during 2006–2010.

Methods: Enterococcal strains, isolated from clinical specimens were collected and saved at –80 degrees, and then tested against TEI, DAP, TIG, DOR and LIN using the microdilution method. The minimal and maximal minimal inhibitory concentration (MIC) (range) was measured, along with MIC90 and MIC50.

Results: A total of 234 enterococcal isolates were tested against TEI, DAP, TIG, DOR and LIN, the MIC were determined for each isolates. For TEI, the MIC50 was 64 μg/mL (range 0.125–256) and the MIC90 was 125 μg/mL. For DAP, the MIC50 was 4 μg/mL (range 0.06–256) and the MIC90 was 8 μg/mL. For TIG, the MIC50 was 0.5 μg/mL (range 0.03–32) and the MIC90 was 2 μg/mL. For DOR, the MIC50 was 256 μg/mL (range 1–256) and the MIC90 was 256 μg/mL. For LIN, the MIC50 was 2 μg/mL (range 0.25–256) and the MIC90 was 4 μg/mL.

Conclusion: Overall, nearly all isolates were resistant to TEI and exhibited very high MIC against DAP and DOR. Fifty percent of isolates exhibited MICs >0.5 μg/mL against TIG. LIN is still most active against VRE as 95.3% of the isolates exhibited a MIC ≤4 μg/mL.

**P930** Antimicrobial susceptibility of enterococci and streptococci from United States hospitals: a 9-year summary of the Daptomycin Surveillance Program (2002–2010)

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Objectives: To evaluate the activities of daptomycin (DAP) and comparator agents tested against enterococci and streptococci (β-haemolytic [BH] and viridans group [VGS]) collected from USA hospitals in 2002–2010. DAP was first approved by the USA-FDA in 2003 and by the European Medicines Agency (EMEA) in 2005; and has increasingly been used to treat bacteremia and acute bacterial skin and skin structure infections (ABSSSI) worldwide.

Methods: Unique patient strains of clinical significance were consecutively collected in 31 USA medical centers and susceptibility (S) tested in a central reference laboratory against DAP and various comparators by CLSI broth microdilution methods. Mueller-Hinton broth was supplemented to 50 mg/L of calcium when testing DAP.

Results: 14,044 strains were evaluated, including 5,977 *E. faecalis* (EF; 95.2% vancomycin [VAN]-S), 3,735 *E. faecium* (EFM; 79.6% VAN-S), 3,246 BHS and 1,086 VGS. Isolates were mostly from bacteremia (62%), ABSSSI (15%) and urinary tract infections (13%). DAP-S rates were 99.97, 99.60, 100.0 and 99.63% for EF, EFM, BHS and VGS, respectively. VAN resistance (R; MIC, >4 mg/L) increased progressively from 64.9% in 2002 to 79.1% in 2010 among EFM, while VAN-R EF increased from 2.8% in 2002 to 6.4% in 2008, but decreased to 2.9 and 3.3% in 2009 and 2010, respectively. Only 17 DAP-non-S enterococci (0.18%) were observed. 2 EF (0.03%) and 17 EFM (0.40%). Importantly, VAN-S and VAN-non-S enterococci exhibited very similar DAP MIC distributions (Table). DAP was very active against VAN-non-S EF (MIC50/90, 0.5/1 mg/L) and EFM (MIC50/90, 2/4 mg/L). Linezolid was also very active against EF (MIC50/90, 1.2/2 mg/L; 99.85% S) and EFM (MIC50/90, 1.2/2 mg/L; 95.5% S). BHS was very S to DAP (MIC50/90, ≤0.120/2.5 mg/L; 100.0% S) and most other antimicrobials tested. DAP was also highly active against VGS (MIC50/90, 0.25/0.5 mg/L; 99.63% S).

Conclusion: DAP demonstrated sustained activity against an extensive sampling of clinical isolates of enterococci (including >3,000 VAN-R strains) and streptococci from numerous USA medical centres over the last 9 years (99.85% S overall). DAP activity was not adversely influenced by R to other antimicrobial classes, including VAN among enterococci.

**P931** Evaluation of enterococcal broth for detection of vancomycin-resistant *Enterococcus* at Royal North Shore Hospital Sydney, Australia

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Objective: In Australia vancomycin-resistant enterococci are mainly *Enterococcus faecium* of the vanB phenotype. Vancomycin MICs are often low, and the sensitivity of detection is greatly improved using enrichment techniques. Screening may be of value for particular at-risk patients. Knowledge of VRE colonisation may also prevent subsequent clinical VRE infection as well as avoid transmission to other patients. Recent studies using VRE broth enrichment have used a 24-hour incubation period. However would a 48-hour incubation protocol be more effective in a routine clinical laboratory setting?

Methods: In this study a method using enterococcal broth culture containing 6 mg/L vancomycin with up to 48-hour incubation was evaluated retrospectively for 5156 specimens submitted for VRE screening during 2009 at Royal North Shore Hospital in Sydney, Australia.

Results: Patient specimens included faeces (n=64), rectal swabs (n=1974) and swabs from other body sites (n=289). Swabs collected from the hospital environment were also screened (n=2847). Overall 285 patient specimens and 44 environmental specimens were positive for VRE. These included two *E. faecalis* vanB, three *E. faecium* vanA and 289 *E. faecium* vanB as confirmed by PCR. PCR was not performed on 35 repeat positive isolates from patient specimens. Since sensitivity testing of vancomycin is influenced by the test medium, MICs were determined, by a gradient on six different media. Of patient specimens that tested positive for VRE, 125 (43.9%) had broth with black appearance following overnight incubation (up to 24 hours). An additional 160 (56.1%) had black appearance after a further
In vitro activity of linezolid and comparators against Enterococci phenotypes: Europe, 2007–2010

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Background: Enterococcus faecium and E. faecalis are significant pathogens both in community and hospital patients causing infections of the urinary tract, skin/skin structure and blood stream. The increasing prevalence of vancomycin-resistant Enterococcus sp. worldwide dictates the continued monitoring of these phenotypes in all countries. The Tigecycline European Surveillance Trial (T.E.S.T.) evaluated the activity of linezolid, tigecycline and comparators to over 4100 Enterococcus isolates in 25 European countries 2007–2010.

Methods: In Europe 489 cumulative sites in 25 countries collected 4197 significant Enterococcus species during 2007–2010. MICs were performed at each site using prepared broth microdilution panels and interpreted according to EUCAST guidelines. Results: The % susceptible and MIC90 (mg/L) of tigecycline, linezolid and comparators to Enterococcus sp. including vancomycin-susceptible and -resistant phenotypes are shown in the following table.

Conclusions: In Europe 13.0% and 1.3% of E. faecium and E. faecalis, respectively, were vancomycin resistant during 2007–2011. Linezolid demonstrated potent in vitro activity against both vancomycin susceptible and resistant isolates with percent susceptible ranging from 98.8–100% and MIC90 of 0.25 mg/L regardless of phenotype. Tigecycline and linezolid continue to demonstrate in vitro activity again all phenotypes of Enterococcus sp.

First detection of the trimethoprim resistance gene dfrK in Enterococci isolates

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Objectives: The objectives of this study were to investigate a collection of vancomycin-resistant and -susceptible enterococci of different species and origins for the presence of trimethoprim resistance genes with particular reference to the gene dfrK and its genetic environment.

Methods: In total, 166 Enterococcus isolates of different species (88 E. faecium, 15 E. faecalis, 14 E. durans/E. hirae, and 49 E. gallinarum/E. casseliflavus), recovered from food (50), clinical (50) and healthy human (34), animal (31) and sewage (1) samples, were studied (vanA/vanB2: 93, vanC1/2: 59; susceptible: 24). Susceptibility to trimethoprim and trimethoprim-sulfamethoxazole was assayed by disk diffusion and agar dilution methods. The gene dfrK and the linkage of the tetracycline resistance gene tet(L) with dfrK were detected by PCR and sequencing. One E. faecium isolate was chosen for further analysis to determine the dfrK environment by inverse PCR, cloning and sequencing. Screening for other dfr genes known to occur in enterococci (dfrA and dfrG) was done by PCR.

Results: The gene dfrK was detected in 89/166 isolates (54%), including 41 E. faecium, 12 E. faecalis, 11 E. hirae/E. durans and 18 E. gallinarum/E. casseliflavus isolates. The linked tet(L)-dfrK genes were detected in 17 isolates (19%) (7 E. faecium/vanA, 1 E. faeciam/vanB2, 8 E. gallinarum/vanC1 and 1 E. hirae/vanA). Strain E. faecium C1723 was selected for further analysis. This strain showed a MIC of trimethoprim of 8 mg/L and harboured the dfrK gene, but was negative for the tet(L)-dfrK linkage. S1 nuclease digestes and PFGE-ICE1-hybridization revealed the chromosomal location of dfrK. The genetic environment of dfrK corresponded to a Tn559-like element integrated into the rpdC gene. This dfrK-containing Tn559-like element was also detected in 14 other dfrK-positive enterococci. All possible combinations of dfr genes were detected among the enterococci tested: dfrA, dfrG, dfrF, dfrK-dfrG, dfrK-dfrF, dfrF-dfrG, dfrF-dfrG-dfrK.

Conclusion: This is the first detection of the dfrK gene in Enterococcus. These data suggest that enterococci may act as recipients of trimethoprim resistance genes known to occur in other Gram-positive bacteria. The presence of dfrK in enterococci of different origins and its association with transposon Tn559 – recently identified in the chromosomal DNA of Staphylococcus aureus ST3398 – underlines the resistance gene transfer between enterococci and staphylococci.

Emergence of tigecycline and linezolid resistance in a vancomycin-resistant isolate of Enterococcus faecium

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Objectives: E. faecium is an opportunistic, nosocomial pathogen which can cause infections of the urinary tract, wounds and bloodstream. Over 30% of UK isolates of E. faecium are resistant to glycopeptides; mostly with the acquired VanA phenotype. Resistance to linezolid occurs but is less common, whereas tigecycline resistance has not previously been described in E. faecium. We investigated the emergence of linezolid and tigecycline resistance in a clinical strain of vancomycin-resistant E. faecium.

Methods: An isolate of E. faecium (designated EF082) was recovered from the bloodstream of a post-second liver transplant patient who presented septic with hepatic artery thrombosis and hepatic abscesses. Linezolid therapy was commenced two days later and continued for two weeks. A second isolate was recovered from the same site one week after the first and was designated EF083. Three months later, after a second episode of sepsis, the patient received a course of tigecycline. After nine days of tigecycline therapy a third isolate (designated EF294) was recovered from a hepatic abscess aspirate. MICs were determined by agar dilution and Etest on IsoSensitest agar and interpreted according to BSAC guidelines. PFGE was used to determine isolate relatedness. PCR was used to screen for tetX and vanA genes. A G2576T mutation in 23S rRNA genes was sought by PCR-RFLP analysis. Tigecycline resistance in Staphylococcus aureus has been associated with up-regulation of the MATE pump, MepA and expression of a mepA-like gene was examined by real-time RT-PCR.

Results: MICs are detailed in Table 1. All isolates were: (i) indistinguishable by PFGE; (ii) PCR-positive for vanA, but negative for the tigecycline-modifying determinant, tetX. Isolate EF294 had resistance to tigecycline and linezolid, also to chloramphenicol and tetracyclines. The G2576T mutation was identified in 23S rRNA genes of EF294, explaining its linezolid resistance. RT-PCR identified a mean eight-fold increase in mepA-like transcript in EF294 compared with EF082 and EF083.

Conclusions: We report the emergence of resistance to linezolid and tigecycline, in a vancomycin-resistant clinical isolate of E. faecium, from a patient treated with these drugs. The linezolid resistance was mediated by a defined mutation in 23S rRNA genes while tigecycline resistance was associated with up-regulation of a MATE-type efflux pump. The
mechanism(s) responsible for the reduced susceptibility to telavancin remain undefined.

| Isolate | UNZ | TSIC | VAN | TEI | TLU | CHL |
|---------|-----|------|-----|-----|-----|-----|
| EF294   | 8.9 | 8    | >32 | >32 | 2   | 32  |
| EF083   | 2   | 0.125| >32 | >32 | 0.125| 4   |
| EF082   | 2   | 0.125| >32 | >32 | 0.125| 4   |

Table 1. Antibiotic susceptibilities of E. faecium isolates used

Methods: Three Enterococcus faecium strains VRE ATCC 51559, VRE 12311, and VRE SF 12047 were evaluated in a 7 day 1-compartment in vitro PK/PD model at a starting inoculum of 10^8 CFU/mL. Simulated regimens were DAP 6 mg/kg/day (Cmax=7.9 μg/mL, T1/2=8 h), and DAP 10 mg/kg/day (Cmax=15.2 μg/mL, T1/2=8 h). Samples were plated daily on Mueller Hinton agar (MHA) supplemented with 50 μg/L CaCl2+ and containing DAP 16 μg/mL to assess for the emergence of DAP resistance. For each strain, the recovered mutant with the highest DAP MIC value was then evaluated for changes in relative surface charge (cytochrome c binding assay), cell wall thickness (transmission electron microscopy), DAP induced potassium release and cytoplasmic membrane depolarization (utilizing the membrane potential sensitive fluorescent dye DiSC3).

Results: DAP MIC values were 4 μg/mL for all 3 strains. A dose dependent response and regrowth was observed for DAP 6 and DAP 10 against all 3 strains. Mutants of VRE ATCC 51559 (MIC = 128 & 64 μg/mL) and VRE 12311 (MIC = 256 and 32 μg/mL) were recovered from DAP 6 and DAP 10, respectively. For VRE SF 12047, a mutant (MIC = 32 μg/mL) was recovered from the DAP 6 model. All mutants displayed an increase in relative surface charge compared to their respective parent strains. The DAP resistant mutants displayed a significant (p < 0.0001) increase in cell wall thickness from 30.3 nm vs. 43.5 nm; 22.2 nm vs. 32.4 nm; 24.9 nm vs. 39.5 nm for VRE SF 12047, VRE 12311, and VRE ATCC 51559, respectively. DAP induced potassium release and membrane depolarization decreased by 10−36% for the DAP resistant mutants compared to their parent strains.

Conclusion: VRE with elevated DAP MIC values displayed increased surface charge, increased cell wall thickness, and decreased depolarization by DAP which is consistent with previous findings for Staphylococcus aureus. Based on MIC values for enterococci, higher dosages for invasive infections may be required to improve effective kill and prevent the emergence of resistance.

Coagulase-negative staphylococci

These bacteria are usually only opportunist pathogens for immune-compromised patients, they may serve as a reservoir of antibiotic resistance genes for more important organisms that co-exist with them (e.g., S. aureus). Data regarding the resistance genes in CNS strains colonizing healthy people are lacking. Here, we present a systematic analysis of antibiotic resistance genes of CNS collected from healthy volunteers (HVs).

Methods: 5 HVs from UK who had not received antibiotics during the last year were screened for the presence of CNS strains. Nasal samples were collected using swabs with Amies-Chocarol transport medium. Skin samples were obtained using a 2-mm slice of gelatine which was pressed against the skin and transferred to 2 mL PYG buffer. Samples were serially diluted in PBS buffer and plated on blood agar (BA) plus amoxicillin [8 mg/L] and BA plus minocycline [0.5 mg/L]. Species identification was achieved using MALDI-TOF. Genomic extraction was obtained using a bacterial DNA kit. A microarray platform was used to analyze up to 110 antibiotic resistance genes already described among Gram-positives. Genome sequencing was carried out using the 454 technology.

Results: All HVs were colonized in the nose with at least one CNS strain harbouring multidrug-resistant (MDR) genotype (i.e., at least 3 genes conferring resistance to different classes of antibiotics). In particular, 4 out of 5 subjects were colonized with isolates carrying the mecA gene. These isolates also contained genes conferring resistance to penicillins [blaZ], tetracyclines [tet(K)] and trimethoprim [dfr(A)]; association with genes conferring resistance to macrolides [erm(C)] and aminoglycosides [ant(4)-Ia] was also observed in isolates from 2 HVs. Colonization of the skin by MDR CNS strains was seen in only 2 HVs, one of them a mecA-positive S. hominis. The whole genome sequencing of this isolate was obtained.

Conclusion: The present analysis is part of a larger study (ANTIRESD-DEV, 7th RTD Framework of the European Commission) that will involve more HVs. These preliminary data indicate that healthy people in the UK can harbour in the nose and skin CNS isolates with an extended assortment of antibiotic resistance genes. The clinical impact of such a reservoir of gonorrhea and their ability to be shared with other Gram-positives remains to be evaluated.

Table 1. Antibiotic susceptibilities of E. faecium isolates used

| Isolate | UNZ | TSIC | VAN | TEI | TLU | CHL |
|---------|-----|------|-----|-----|-----|-----|
| EF294   | 8.9 | 8    | >32 | >32 | 2   | 32  |
| EF083   | 2   | 0.125| >32 | >32 | 0.125| 4   |
| EF082   | 2   | 0.125| >32 | >32 | 0.125| 4   |

P935 Characterising vancomycin-resistant Enterococcus strains with varying daptomycin resistance developed in an in vitro PK/PD model

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Objectives: Evaluate two DAP regimens (6 and 10 mg/kg/day) in a pharmacokinetic/pharmacodynamic (PK/PD) model and evaluate recovered mutants for changes in phenotypic characteristics including membrane surface charge, membrane depolarization, and cell wall thickness.

Methods: Three Enterococcus faecium strains VRE ATCC 51559, VRE 12311, and VRE SF 12047 were evaluated in a 7 day 1-compartment in vitro PK/PD model at a starting inoculum of 10^8 CFU/mL. Simulated regimens were DAP 6 mg/kg/day (Cmax=7.9 μg/mL, T1/2=8 h), and DAP 10 mg/kg/day (Cmax=15.2 μg/mL, T1/2=8 h). Samples were plated daily on Mueller Hinton agar (MHA) supplemented with 50 μg/L CaCl2+ and containing DAP 16 μg/mL to assess for the emergence of DAP resistance. For each strain, the recovered mutant with the highest DAP MIC value was then evaluated for changes in relative surface charge (cytochrome c binding assay), cell wall thickness (transmission electron microscopy), DAP induced potassium release and cytoplasmic membrane depolarization (utilizing the membrane potential sensitive fluorescent dye DiSC3).

Results: DAP MIC values were 4 μg/mL for all 3 strains. A dose dependent response and regrowth was observed for DAP 6 and DAP 10 against all 3 strains. Mutants of VRE ATCC 51559 (MIC = 128 & 64 μg/mL) and VRE 12311 (MIC = 256 and 32 μg/mL) were recovered from DAP 6 and DAP 10, respectively. For VRE SF 12047, a mutant (MIC = 32 μg/mL) was recovered from the DAP 6 model. All mutants displayed an increase in relative surface charge compared to their respective parent strains. The DAP resistant mutants displayed a significant (p < 0.0001) increase in cell wall thickness from 30.3 nm vs. 43.5 nm; 22.2 nm vs. 32.4 nm; 24.9 nm vs. 39.5 nm for VRE SF 12047, VRE 12311, and VRE ATCC 51559, respectively. DAP induced potassium release and membrane depolarization decreased by 10−36% for the DAP resistant mutants compared to their parent strains.

Conclusion: VRE with elevated DAP MIC values displayed increased surface charge, increased cell wall thickness, and decreased depolarization by DAP which is consistent with previous findings for Staphylococcus aureus. Based on MIC values for enterococci, higher dosages for invasive infections may be required to improve effective kill and prevent the emergence of resistance.

P936 Coagulase-negative staphylococci carrying mecA and other antibiotic resistance genes among healthy volunteers in the UK

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Objectives: Coagulase-negative staphylococci (CNS) represent part of the normal bacterial flora that colonizes the nose and skin. Although healthy dogs in Spain: novel genotypic and phenotypic characteristics of this emerging multi-resistant pathogen

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Objectives: To identify the methicillin-resistant coagulase-positive staphylococci (MRCS), nasal carriage rate of dogs in Spain, to determine their antimicrobial resistance and virulence traits, and to characterize the recovered isolates by different molecular techniques.

Methods: 196 nasal samples from healthy dogs were obtained in La Rioja (Spain). Following enrichment in nutrient broth with 6.5% NaCl, samples were cultured on ORSAB plates (OXOID). Isolates were identified by species-specific PCRs, PCR-RFLP (MboI) and sequencing of sodA and hsp60 genes. All isolates were characterized by spa-, SCCmec- and MLST/agr-typing, SmaI-PFGE, antimicrobial susceptibility by disk-diffusion test to 16 antimicrobials, determination of 33 antimicrobial resistance genes and toxin gene profiling (38 toxin genes).

Results: Methicillin-resistant Staphylococcus pseudintermedius (MRSP) were recovered from 9 of 196 samples (4.6%), being the only MR isolates obtained. Seven isolates were typed as ST71(MLST)-Hl(agr-A(PFGE)-t02(eta)-II-III(SCCmec) and showed 4 closely-related SmaI-PFGE profiles. One was typed as ST92-II-B-t06-V and the last one was ST262-I-C and spa and SCCmec non typeable. All ST71-MRSP isolates presented an unusual inducible cefoxitin resistance phenotype when placed next to oxacillin disk. MRSP were resistant to [resistance gene/number isolates]: β-lactams [mecA-blαZ/9], tetracycline [tet(K)/7], tet(M)/2, macrolides-lincosamides-streptogramins B [erm(B)/9], aminoglycosides [aac(6′)-Ie-apH(2)-Ia-apH(3′)-II-ant(6′)-Ia/9], streptothricin...
Dissimilar distribution of cassette chromosome recombinase allotypes among methicillin-susceptible coagulase-negative staphylococci

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Objectives: The essential components of the mobile genetic element Staphylococcal Cassette Chromosome mec (SCCmec) are the mec complex and the cassette chromosome recombinases (ccr). The mec complex contains the determinant of methicillin resistance, mecA (with or without the regulators mecA and mecR1), and the genes of the ccr complex are responsible for SCCmec mobility. Eight SCCmec types have been described so far in S. aureus that result from the combination of different ccr allotypes and mec complex classes. However, it is not clear how SCCmec has been assembled and evolved.

In order to better understand the role of different CoNS species in the evolutionary history of SCCmec, we compared the ccr allotype content of some methicillin-resistant coagulase-negative staphylococci (MR-CoNS) and methicillin-susceptible coagulase-negative staphylococci (MS-CoNS).

Methods: A collection of 427 S. epidermidis (233 MRSE and 194 MSSSE), 46 S. haemolyticus (36 MRSH and 10 MSSH) and 45 S. hominis (34 MRSSH and 11 MSSH) and were analyzed. The ccrAB and ccrC presence was determined by Southern hybridization and the ccrAB2 allele was determined by amplification of the mec and ccr complexes by PCR.

Results: SCCmec type IV (2B) was the most frequent among MSSSE (93/233, 40%), SCCmec 1A was the most common among MRSH (13/34, 38%) and SCCmec V (5C) (10/36, 28%) was the most widespread among MRSH. Among the susceptible strains of the three species analyzed 36% of MSSSE (69/194), 73% (8/11) of MSSH and 60% (6/10) of MSSE carried ccr genes. The ccrBA2 allele was almost exclusively found among MSSE and ccrBA4 and ccrAB1 were exclusively found among MSSH. The ccrC allele was most frequently identified among MSSH (50%, 5/10), but was also present among MSSSE (36%, 25/69) and MSSH (27%, 3/11). The nucleotide sequence of ccrB from MSSE and ccrB1 and ccrB4 from MSSH had a high homology (>95%) with that of ccrB from SCCmec types IV, I, and VI/VIII from S. aureus, respectively.

Conclusion: Our results suggest that methicillin susceptible S. hominis might have been the donors of ccrAB1 and ccrAB4 for the assembly of SCCmec types I, VI, and VIII in S. aureus, and that methicillin susceptible S. epidermidis might have been the donors of ccrAB2 for SCCmec IV.

Prevalence of coagulase-positive staphylococci and methicillin-resistant coagulase-negative staphylococci in healthy humans and their companion animals: 6 cases of interspecies transmission

E. Gómez-Sanz*, C. Lozano, C. Aspiriz, C. Torres, M. Zaragaza (Logroño, Zaragoza, ES)

Objectives: To detect and identify the CPS and/or MRCCNS nasal carriage rate of healthy humans and their pets and to determine the potential staphylococcal interspecies transmission.

Materials: 32 unrelated pet-owning households were screened for CPS and MRCCNS nasal carriage in La Rioja (Spain) from June-2009/July-2010. Forty-four owners and 46 pets (39 dogs, 7 cats) were sampled. Following enrichment in nutrient broth with 6.5% NaCl, samples were cultured on ORSAB and Mannitol-Salt-agar plates. Two colonies per selective plate were analysed. Isolates were identified by species-specific PCRs, PCR-RFLP, and sequencing of sodA gene. All MRCCNS isolates were tested for mecA gene by PCR. All S. aureus were characterized by spa- and agr-typing. MLST and Smal-PFGE was conducted in all isolates suspected of interspecies transmission.

Results: 21 of the 32 households (66%) were positive for CPS and/or MRCCNS. A maximum of 3 distinct strains per individual were obtained. Twenty owners of the 44 (46%) studied carried CPS and/or MRCCNS with 24 isolates obtained: 17 S. aureus; 2 S. pseudintermedius, and 5 MRCCNS. Twenty-two of the 46 studied pets (48%) were positive for CPS and/or MRCCNS with 27 isolates obtained: 6 S. aureus; 12 S. pseudintermedius, and 9 MRCCNS. In 11 of 21 positive-households (52%), both owners and their pets were colonized by CPS and/or MRCCNS. All 14 MRCCNS carried the mecA gene and only one methicillin-resistant (MR) CPS (S. pseudintermedius) from a dog was obtained. In 9 positive-households (43%) there were mecA-carrying strains. The agr-types of S. aureus were: agrI (10 isolates), agrII (4), agrIII (?), and agrIV (2). Sixteen different spa-types were obtained. No isolates when more than one): t073 (5), t021 (2), t002 (2), t209 (2), t151, t440, t159, t015, t091, t1504, t148, t037, t3711, t3906, and a new spa-type (2). In six cases identical strains by PFGE were isolated from both owners and their pets: 1. S. aureus (spa)t073-(agrI)-(MLST)ST45- (Clonal Complex)CC45; 1 S. aureus (209-II-ST109-C9); 1 S. aureus (202-I-III-ST1654(new)-singleton; 1 S. pseudintermedius; 1 MR S. lenta, and 1 MR S. haemolyticus.

Conclusions: A high rate of households investigated (66%) carried CPS and/or MRCCNS. CPS and/or MRCCNS are common colonizers of healthy humans and their pets, although MR-CPS occurrence is low. In 6 pet-owning households (19%) both owner and pet carried the same strain what raises concerns on the potential interspecies transmission of these microorganisms.
performed by VITEK2 system and internal transcribed spacer PCR; species identification was ascertained by RNA16S for specific cases. All isolates were characterized by pulsed-field gel electrophoresis, SCCmec typing for the mec complex class and ccr allele by PCR and sequencing strategies.

Results: Seventeen different Staphylococcus species were identified among the 528 CoNS isolates collected. S. epidermidis, S. haemolyticus, S. hominis, and S. saprophyticus, together, accounted for 70% of the isolates. Methicillin resistance (MR) was observed in 11 out of 17 species identified, with an overall prevalence of c.a. 40%, ranging from 4 to 74%. The same individual could be colonized with as many as 5 different CoNS species and 8 different strains. Moreover, MR and susceptible isolates of the same species could be isolated simultaneously from the same host. SCCmec types 1A, 2A, 3A, 4B, 5A, 5C, and 7A were identified and several isolates were non-typeable. At least 25 different ccr alleles were found among 68 isolates. Interestingly, the great majority of isolates colonizing the same individual did not share the same SCCmec type.

Conclusion: CoNS isolates colonizing a single host were found to be highly diverse in terms of genetic background and type of SCCmec carried. The results suggest the absence of SCCmec transfer between highly diverse in terms of genetic background and type of SCCmec isolates colonizing the same individual. Differences in terms of genetic background and type of SCCmec isolates colonizing the same individual did not share the same SCCmec type.

AMR in Gram-positives

Objective: To evaluate the in vitro activity and spectrum of daptomycin (DAP) and comparators tested against clinical isolates from European (EU) hospitals. DAP is a cyclic lipopeptide approved by EU Medicines Agency (EMA) for the treatment of complicated skin and skin structure infections (cSSSI) and S. aureus (SA) bacteremia and endocarditis.

Methods: 36,759 consecutive strains were collected in 34 medical centres located in 13 EU countries, Turkey and Israel, including S. aureus (SA; 18,352, 27.2% MRSAs); coagulase-negative staphylococci (CoNS; 6,874, 76.6% oxacillin [OXA]-Resistant [R]), Enterococcus spp. (ENT; 7,241, 9.4% vancomycin [VAN] + R), β-haemolytic (BHS; 3,009), and viridans group streptococci (VGS; 1,176), and S. bovis/gallolyticus (SB; 1,07). The organisms were isolated mainly from patients with bacteremia (56%) or cSSSI (23%). The strains were tested for susceptibility (S) against DAP and comparators by CLSI broth microdilution methods in cation-adjusted Mueller-Hinton broth with 50 mg/L of calcium for DAP tests.

Results: DAP was very active against SA and CoNS (MIC50/90, 0.25−0.5 mg/L for both organisms) and its activity was not adversely influenced by OXA-R. MRSAs varied from 1.3% in Sweden to as high as 61.3% in Portugal and 55.9% in Greece. MRSAs exhibited high R rates to levofloxacin (88.7) and clindamycin (40.2%), and...
high S rates to DAP (MIC90, 0.25/0.5 mg/L; 100.0% S), linezolid (LZD; MIC90, 1/2 mg/L; 99.9% S), tigecycline (TIG; MIC90, 0.12/0.25 mg/L; >99.9% S) and VAN (MIC90, 1/1 mg/L; 100.0% S). All E. faecalis were S to DAP. VAN-R E. faecium (VREFM) was observed in 13 of 15 countries evaluated and was highest in Ireland (62.4%) and UK (44.2%). DAP (MIC90, 2/2 mg/L; 100.0% S), LZD (MIC90, 1/2 mg/L; 99.7% S) and TIG (MIC90, 0.06/0.12 mg/L; 99.5% S) were the most active agents tested against VREFM. VAN-S and -R ENT were equally S to DAP. DAP was also active against BHS (MIC90, 0.06/0.25 mg/L; 100.0% S), VGS (MIC90, 0.25/0.5 mg/L; 99.8% S) and SB (MIC90, 0.06/0.12 mg/L; 100.0% S).

Conclusions: DAP was highly active against a large collection (36,759) of Gram-positive (GP) organisms isolated in European hospitals and its activity remained stable across the 7-year period evaluated (2003–2009) using reference methods. Decrease in DAP potency has not been observed since EMEA approval and widespread clinical use, and emerging R to other compounds did not adversely influence the DAP potency against GP species.

P944 Fusidic acid activity and coverage of Gram-positive pathogens associated with acute bacterial skin and skin structure infections in the USA (2008–2010)
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Objectives: To determine the susceptibility (S) rates and activity of fusidic acid (FA; CEM-102) tested against Gram-positive pathogens that cause ABSSSI, isolated in the USA during 2008–2010 (16,033 strains) without significant change in the S rate (99.73% at c1 mg/L). MRSA and methicillin-S SA had the same S to DAP, LZD, ERY, and TIG, respectively, against BHS. The lowest S rates among BHS isolates were observed against CLI (90.2%) and ERY (82.5%).

Results: Ceftaroline was very active against all S. aureus (24.6% MRSA; highest MIC, 2 mg/L). Oxacillin-S S. aureus (MSSA; MIC90, 0.25 mg/L) had lower MICs than MRSA (MIC90, 1/2 mg/L). Against MSSA, ceftaroline was 2-, 4-, 8-, 16- and 16-fold more potent than daptomycin (DAP), vancomycin (VAN), linezolid (LZD), ceftazidime (CRO) and cefepime (CPM), respectively. MRSA isolates demonstrated 100.0% S to LZD, VAN, DAP and tigecycline, but high resistance to levofloxacin (LEV; 89.4%), erythromycin (ERY, 65.6%) and clindamycin (CLI; 32.1%). Against BHS, all isolates were inhibited at <0.03 mg/L of ceftaroline, with the greatest activity (MIC90; mg/L) observed against group A (<0.008 mg/L), followed by other BHS (0.015 mg/L) and group B (0.03 mg/L). Ceftaroline was 8-, 16-, 32- and 32-fold more potent than DAP, VAN, LZD and LEV, respectively, against BHS.

Conclusion: Ceftaroline demonstrated strong broad-spectrum activity against the most common ABSSSI pathogens (S. aureus, inclusive MRSA and BHS) isolated from patients in EU medical centres in 2009. These data warrant continued clinical evaluation of ceftaroline as a therapeutic agent for CSTI.
Comparative in vitro potency of torezolid (TR-700) and linezolid against key Gram-positive pathogens of European origin (2009–2010)

C. Pillar, D.F. Sahn, K. Bartizal* (Chantilly, San Diego, US)

Objectives: Terezolid (TR-700) is an investigational oxazolidinone currently under development for the treatment of acute bacterial skin and skin structure infections (ABSSSI). Target ABSSSI pathogens of terezolid include S. aureus, β-hemolytic streptococci, and enterococci. As part of its development, it is important to establish the activity profile of terezolid relative to that of linezolid, the only currently marketed oxazolidinone. This study was conducted to analyze such activity as it pertains to European isolates of target skin pathogens.

Methods: Non-duplicate, non-consecutive clinical isolates of S. aureus (N=444), E. faecium (N=33), S. pyogenes (GAS; N=30), S. agalactiae (GBS; N=33), and Group C/F/G streptococci (C/F/G; N=10) were collected from 18 sites distributed across nine European countries from 2009–2010. Isolates underwent susceptibility testing against terezolid, linezolid, and other relevant comparators in accordance with CLSI M7-A9 guidelines at a central laboratory (Eurofins Medinet, Chantilly, VA).

Results: The MIC50 and MIC90 values (mg/L) of terezolid and linezolid against the evaluated pathogens are presented in the table below. Terezolid had an MIC50 and MIC90 of 0.5 and 2.0 mg/L, respectively, compared to linezolid at 2.0 and 4.0 mg/L, respectively. This trend should be monitored.

| Pathogen | Terezolid (mg/L) | Linezolid (mg/L) |
|----------|------------------|-----------------|
| Staphylococcus aureus | 0.25 | 2.0 |
| MRSA | 0.25 | 2.0 |
| GAS | 0.12 | 0.5 |
| GBS | 0.25 | 0.5 |
| E. faecium | 0.06 | 0.5 |

Conclusions: These data demonstrate that the in vitro potency of terezolid (based on MICs) against key Gram-positive pathogens is several-fold greater than that of linezolid. It will be of interest to determine the level of terezolid activity against linezold non-susceptible isolates, thus continued surveillance monitoring of oxazolidinone susceptibility is essential.

P947

In vitro activity of telavancin and comparators against Gram-positive bacteria isolated from bloodstream infections

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Objective: The antimicrobial activity of telavancin against 315 clinical Gram-positive cocci obtained primarily from patients with bloodstream infections hospitalized at the University of Cologne was evaluated and compared with other anti-Gram-positive reference drugs.

Methods: Antimicrobial susceptibility testing was investigated by E-test on 315 non-duplicate, Gram-positive bacterial isolates for telavancin, vancomycin, teicoplanin, daptomycin, linezolid, and oxacillin. 89% of isolates were obtained from bloodstream specimens between 2000 and 2010. Isolates included methicillin-susceptible Staphylococcus aureus (MSSA) (n=40); MRSA (57, including heteroVISA); vancomycin-susceptible (VAN-S) Enterococcus faecalis (20); VAN-S E. faecium (20); vancomycin-resistant (VAN-R) E. faecium with vanA phenotype (40); Van-R E. faecium with vanB phenotype (20); oxacillin-susceptible coagulase-negative staphylococci (CoNS; 47); oxacillin-resistant CoNS (41); penicillin-susceptible Streptococcus pneumoniae (20); and penicillin-non-susceptible S. pneumoniae (10).

Results: The MIC50 and MIC90 values (µg/ml) of the 315 Gram-positive isolates are summarized in the table.

| Pathogen | TR-700 (µg/ml) | Linezolid (µg/ml) |
|----------|----------------|-----------------|
| S. aureus | 0.25 | 2.0 |
| MSSA | 0.25 | 2.0 |
| MRSA | 0.25 | 2.0 |
| GAS | 0.12 | 0.5 |
| GBS | 0.25 | 0.5 |
| C/F/G | 0.06 | 0.5 |

Conclusions: These data demonstrate that the in vitro potency of telavancin (based on MICs) against key Gram-positive pathogens is several-fold greater than that of linezolid. It will be of interest to determine the level of terezolid activity against linezold non-susceptible isolates, thus continued surveillance monitoring of oxazolidinone susceptibility is essential.

P948

Prostate-derived Propionibacterium acnes isolates are sensitive to potentially useful antibiotics

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Objective: Bacterial infections of the prostate are increasingly recognized as a potential risk factor for development of proliferative diseases as benign prostate hyperplasia and cancer. We and others have identified the Gram-positive facultative anaerobic bacterium Propionibacterium acnes as a frequent inhabitant of prostate tissue. Currently, we are investigating several aspects of this bacterial infection, as: prevalence in prostatectomy tissue, genetic variance of isolates from prostate contra other loci, and the inflammatory and proliferative effects on prostate epithelium. Antibiotic treatment aimed to eradicate the bacterium might be a therapeutic choice in an empiric strategy for
Determination of the postantibiotic and postantibiotic sub-MIC effect of vancomycin and daptomycin on *Staphylococcus aureus* and *Enterococcus faecalis* by isothermal microcalorimetry

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**Objectives:** The postantibiotic effect (PAE) and the postantibiotic sub-MIC effect (PA-SME) are important pharmacodynamic parameters helping to optimize dosing of antibiotics. The current gold standard for the determination of PAE and PA-SME is the viable counts method, which is laborious and time consuming. We developed and evaluated a new method for determination of PAE and PA-SME based on the measurement of bacterial growth-related heat production. Microcalorimetry allows an accurate determination of the PAE and PA-SME. In comparison with the conventional viable count method, microcalorimetry is less labor-and time-consuming. Further experiments will aim at determining the PAE and PA-SME of various drug/pathogen combinations.

**Methods:** Postantibiotic-derived Propionibacterium *acnes* strains and a panel of control strains collected from skin and deep infections were assessed, using Etest®, for susceptibility to ciprofloxacin, tetracycline, azithromycin, clindamycin, sulfamethoxazole and trimethoprim, penicillin G, metronidazole, fusidic acid, moxifloxacin, linezolid, vancomycin, imipenem, piperacillin/tazobactam, gentamicin, rifampicin, daptomycin, tigecycline, erythromycin, and inducible clindamycin resistance.

**Results:** In the reference panel, 5 out of 25 isolates had acquired macroline resistance with cross-resistance against azithromycin, clindamycin, and erythromycin. One of these was also resistant against tetracycline. None of the 24 prostate derived *P. acnes* strains exhibited acquired resistance to the tested antibiotics. All strains were resistant against metronidazole, and poorly susceptible to ciprofloxacin, fusidic acid, moxifloxacin, gentamicin and daptomycin.

**Conclusions:** *P. acnes* strains recovered from prostate exhibited less acquired antibiotics resistance when compared with strains from other localities.

**Table 1.** PAE and PA-SME of vancomycin and daptomycin (in hours) against methicillin-susceptible (MSSA) or methicillin-resistant (MRSA) *S. aureus* and *E. faecalis*.

| Strain          | Vancomycin | Daptomycin |
|-----------------|------------|------------|
| *S. aureus* ATCC 29213 (MSSA) | 1.5 ± 0.3 | 3.2 ± 0.3 |
| *S. aureus* ATCC 35037 (MRSA) | 1.5 ± 0.3 | 3.2 ± 0.3 |
| *S. aureus* ATCC 29213 (MSSA) | 1.5 ± 0.3 | 3.2 ± 0.3 |
| *S. aureus* ATCC 35037 (MRSA) | 1.5 ± 0.3 | 3.2 ± 0.3 |
| *E. faecalis* ATCC 49403 | 1.5 ± 0.3 | nd |

**Results:** Table 1 summarizes the PAE and PA-SME of vancomycin and daptomycin determined by microcalorimetry. In comparison with the conventional viable count method, microcalorimetry showed excellent repeatability, with vancomycin PAE of ca. 1.5h for all strains. The daptomycin PAE was significantly higher, of ca. 4.5h (for *E. faecalis*). The PA-SME increased with higher sub-MIC concentrations.

**Conclusion:** Microcalorimetry allows an accurate determination of the PAE and the PA-SME. In comparison with the conventional viable count method, microcalorimetry is less labor-and time-consuming. Further experiments will aim at determining the PAE and PA-SME of various drug/pathogen combinations.

**P951** In vitro development of high-level resistant Viridans group streptococci upon exposure to daptomycin

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**Objectives:** Viridans group streptococci (VGS) are a leading cause of infective endocarditis and bacteremia. Limited data exists regarding the activity of newer antimicrobials against these organisms. We evaluated daptomycin (DAP) 8 mg/kg and 6 mg/kg doses, linezolid (LZD) and vancomycin (VAN) against four clinical strains of viridans group streptococci in a pharmacodynamic simulated endocardial vegetation model (SEVM) and in kill curves (KCs).

**Methods:** Four clinical isolates, *Streptococcus mitis* 1643, *Streptococcus oralis* 1647 and 1648 and *Streptococcus gordonii* 1649, and 1 ATCC strain, *Streptococcus oralis* 35037 were utilised. MICs were determined by broth microdilution per CLSI guidelines. For SEVM, vegetations, approximately 10^10 CFU/g were added and samples were taken over 72 hours for colony counts. KCs assessed activity at 1/2, 1, 2, 4 and 8 times MIC over 24 hours. Antibiotic peak/trough concentrations utilised: DAP (8 mg/kg) 133/17 and DAP (6 mg/kg) 80/10 mg/L; LZD 18/4.5 mg/L; VAN 40/15 mg/L. Simulated half-life for DAP/LZD/VAN was 8-6.6 hours, respectively. Organism regrowth at 72 hours was assessed for development of resistance by re-evaluating MICs and by plating samples on 4 and 8 times DAP-containing plates.

**Results:** MICs (mg/L) for DAP were 1–2 for the clinical strains and 0.25 for 35037. MICs for LZD/VAN were 1/0.5 for all clinical isolates and 1/1 for 35037. Despite susceptible MICs all DAP and LZD regimens in SEVM achieved approximately a 1–2 log kill for all clinical isolates except DAP (8 mg/kg) against 1649 (2.7 log kill). Two regimens/strains increased in CFU/g at 72 hours (DAP 6 mg/kg versus 1643 and DAP 8 mg/kg versus 1648). VAN achieved approximately 2–3 log kill for all isolates. Initial reductions in CFU were noted at 8 hours but within 24–32 hours significant regrowth was noted in all SEVM and KCs using clinical strains except 1649 in DAP (8 mg/kg) in SEVM. 35037 had significant regrowth by 48 hours in DAP 8 (mg/kg) with post-exposure MIC >256 mg/L but not in DAP (6 mg/kg). Post-exposure MICs for all clinical isolates except 1649 (6 mg/kg) SEVM increased to >256 mg/L.

**Conclusions:** Susceptible isolates achieved minimal in vitro kill for all agents and were similar between the SEVM and KCs. There appears to be high level resistance in VGS to DAP induced upon drug exposure. Further characterisation of this phenomenon is warranted to determine mechanism and clinical relevance.
fever developed and he was treated with linezolid for an additional 8 days. Subsequent blood cultures grew MSSA resistant to linezolid (≤4 mcg/mL by MicroScan, 8 mcg/mL by broth microdilution, but only 2 mcg/mL by Phoenix). Treatment was changed to nafcillin and the bacteremia eventually cleared.

The isolate had a phenotype consistent with cfr, displaying resistance (≤64 mcg/mL) to chloramphenicol, clindamycin, erythromycin, and tiamulin. PCR for cfr was positive. There was no evidence of G2576U mutation.

**Conclusion:** To our knowledge this is the first reported case of a linezolid-resistant, cfr positive, clinical isolate of MSSA. All previous cases have been in MRSA, presumably reflecting the selective use of linezolid in this setting. As in most prior cases of linezolid resistance in both staphylococci and enterococci, associated either with ribosomal mutations or cfr, the case was associated with protracted therapy. It is noteworthy that the BD Phoenix system missed the linezolid resistance. As cfr is located on a mobile genetic element, future cases are assured. Consequently, continued vigilance is warranted, especially in the context of prolonged linezolid use, even in cases of infection due to MSSA.

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**P953** Study of frequency of metallo β-lactamase among *Pseudomonas aeruginosa* strains isolated in clinical samples in Ilam hospitals, Iran

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**Objectives:** Carbapenems are the most potent β-lactam agents with a broad-spectrum activity against Gram-negative bacteria specially *P. aeruginosa*. This study focuses on the frequency of metallo β-lactamase including VIM and IMP among *P. aeruginosa* strains isolated in clinical samples and positive strains which produced VIM or IMP and or both of these genes has been affected against tannin.

**Methods:** Two hundred and forty isolates of *P. aeruginosa* were obtained during one-year period from January 2008 to December 2009 in Ilam Hospitals. Identification of organisms was done by the standard laboratory techniques. Detection of MBL was performed by phenotypic and genotypic methods. *P. aeruginosa* producing MBL were tested against tannin extract.

**Results:** Of two hundred and forty isolates of *P. aeruginosa*, 26.6% of them were MBL positive by phenotypic method. Of sixty-four MBL positive isolates, 3.1% (n=2) and 28.1% (n=18) were positive for IMP and VIM genes respectively, moreover, 1.5% (n=1) of them had both IMP and VIM genes. *P. aeruginosa* producing VIM and IMP was inhibited in 10μg/mL and 15μg/mL concentration of tannin respectively.

**Conclusion:** This study showed that frequency of MBL among clinical strains of *P. aeruginosa* was high and most of MBL positive strains had VIM genes.

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**P954** Microbiological characteristics of clinically significant Gram-positive bacilli from soft-tissue infections

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**Objectives:** This study was conducted to microbiologically characterize aerobic Gram-positive bacilli obtained from surgically-acquired samples in patients with soft-tissue infections.

**Methods:** Study isolates consisted of aerobic, Gram-positive bacilli isolated from operative clinical samples in patients with soft-tissue infections. Only isolates that grew in pure culture were included in the study. Clinical data was collected from electronic medical records. Conventional phenotypic identification was initially performed using a commercial identification kit (API Coryne, bioMérieux). Genotypic identification was performed by comparing ≥400bp sequence of the 16s ribosomal gene against datasets available in the BLAST database (Pubmed). Antimicrobial susceptibility testing was performed for penicillin, erythromycin, amoxicillin-clavulanate and moxifloxacin by Etest. Categorical susceptibility was determined by applying CLSI breakpoints for *Corynebacterium* spp. or *Staphylococcus aureus*.

**Results:** Forty-two clinical isolates were available for inclusion in the study over a two-year period. The most common isolates identified by 16sRNA sequencing were aerobically-growing *Actinomyces* spp. (n=28, 67%), and *Corynebacterium* spp. (n=8, 19%). 36% of isolates were not identifiable by API Coryne, and a further 19% were mis-identified. Only 32% of *Actinomyces* spp. were successfully identified to genus level by the API test kit, compared with 75% of *Corynebacterium* spp. The most common infections involving *Corynebacterium* spp. were pilonidal abscesses, ischio-rectal and other cutaneous soft-tissue infections. Over two-thirds of the infections with *Actinomyces* spp. were located around the waist, ischio-rectal and perineal regions. The range of infections involving *Corynebacterium* spp. was more varied. *Actinomyces* spp. were uniformly susceptible to penicillin and amoxicillin-clavulanate, but some isolates were not susceptible to erythromycin (36%) and moxifloxacin (43%). Antibiotic susceptibilities were more varied for the other genera isolated.

**Conclusion:** Aerobic *Actinomyces* species may be an under-reported cause of bacterial soft-tissue infections, as they represented two-thirds of all Gram-positive bacilli isolated from mono-microbial soft-tissue infections and are poorly identified by phenotypic identification kits. Most study isolates remain susceptible to penicillin and amoxicillin-clavulanate, while the activities of erythromycin and moxifloxacin are more variable.
**Streptococcus bovis**

**Results:** The results of the antibiotic susceptibility tests are presented in table 1. In total, multiresistance (i.e. resistance to $\geq 3$ antibiotic classes) was 24% in group I, 15% in group 2 and 100% in group 3. Whereas no specific spa type dominated in group 1, 1084 was commonly found among the isolates in group 2 in all three counties. Combined resistance to both cldimycin and tobramycin (group 3) was rare, except for in Östergötland County, where the majority of these isolates belonged to spa type 0084. This calls for further investigation.

**Conclusion:** The ECT-R clone (0082) continued to be strongly associated with hepatobiliary disease (33.7% and 55.6% of all isolates in biotypes I and II, respectively). In contrast, no specific spa type dominated in group 1, t084 was commonly found among the isolates in group 2 in all three counties. Combined resistance to both cldimycin and tobramycin (group 3) was rare, except for in Östergötland County, where the majority of these isolates belonged to spa type 0084. This calls for further investigation.

**Methods:** A 5-year case-control study was carried out at King Chulalongkorn Memorial Hospital, Bangkok, Thailand. We retrospectively reviewed all available medical records of adults older than 15 years who had **S. bovis** infection from January 2005 to December 2009.

**Results:** One-hundred and fifty-two patients had **S. bovis** infection during the study period. Of this, 12 (7.9%) and 140 (92.1%) infections were caused by **S. bovis** biotypes I and II, respectively. There was no difference in the mean age between the 2 groups (65.8±17.95 and 61±17.00 years in biotypes I and II, respectively). **S. bovis** biotype I infection was significantly associated with colonic cancer, compared to biotype II infection (16.7% and 2.2% of biotypes I and II, respectively) (RR = 2.5, 95% CI 0.66–9.51, p = 0.17). The clinical spectrum of infections caused by the 2 biotypes was not significantly different: primary bacteremia (66.7% and 51.1% of biotypes I and II, respectively), endocarditis (8.3% and 8.9%). Except spontaneous bacterial peritonitis and biliary tract infection were only found in **S. bovis** type II infection (15.6% and 8.9%, respectively). According to the MIC values, all isolates were susceptible to penicillin, vancomycin, cefotaxime, and meropenem. The mortality was comparable (25% and 20% in biotypes I and II).

**Conclusions:** Our study showed that **S. bovis** infection is frequently observed in the elderly. Each biotype has the unique features. **S. bovis** biotype I infection is associated with colonic cancer, whereas **S. bovis** biotype II infection tends to be associated with hepatobiliary disease. Even though all isolates were susceptible to all available antibiotics, the mortality was relatively high.

**Table:** The association between **S. bovis** biotypes I and II in colonic cancer, hepatobiliary tract disease, and infective endocarditis

|                | **S. bovis** biotype I | **S. bovis** biotype II | Relative risk | 95% CI     | P-value |
|----------------|------------------------|-------------------------|---------------|------------|---------|
| Colonic cancer | 16.7%                  | 2.2%                    | 8.8           | 0.72–106.85 | 0.046*  |
| Hepatobiliary  | 33.7%                  | 56.6%                   | 2.5           | 0.86–9.61  | 0.170   |
| Infective       | 0.3%                   | 8.9%                    | 1.0           | 0.11–10.60 | 0.942   |
| endocarditis    |                        |                         |               |            |         |

95%CI: 95% Confidence Interval

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**Objectives:**

- Group B streptococci (**Streptococcus agalactiae**) is the causative agent of neonatal meningitis and sepsis and colonise frequently the genital tract of women. The aim of the present study was the determination and comparison of the genotypes of a nationwide collection of group B streptococci isolated from invasive neonatal infections and cervical smears of pregnant women.

- **Methods:** A total of 214 group B streptococcal isolates were collected from Greek hospitals during the period 2007–2010; 45 were isolated from invasive neonatal infections and the rest from cervical smears of pregnant women. Isolation and identification of isolates was performed at each hospital. Isolates accompanied with demographic and clinical data were collected at the Department of Microbiology of the University of Larissa, Greece. Serotyping was performed by agglutination test with specific antisera. The genetic relatedness of the isolates was assessed by Multilocus sequence typing (MLST). Alleles and sequence types (STs) were compared and determined with those deposited at the international database (www.mlst.net). Clonal complexes were identified by using the e-burst software (www.ebust.mlst.net).

- **Results:** Group B streptococci isolated from invasive disease of neonatal infections were of serotype III and belonged to distinct clonal complexes of ST17 (62%) and ST19 (18%). Group B streptococci colonised pregnant women belonged to STs 1 (31.5%), 23 (28%), 19 (14%), 12 (10.5%) 8 (9.5%) and 17 (6.5%). There were no differences in the incidence of STs among different regions of the country.

- **Conclusions:** Whereas ST17 is the most frequent ST isolated from neonatal invasive infections, its prevalence among colonised pregnant women is low. Therefore, there is no obvious correlation between the colonisation of pregnant women and neonatal group B neonatal infections.

**References:**

1. J. Verhaegen*, W. De Buckel, B. Delaere, J. Flamaing, W. Peertmans, P Van Damme, K. Van Herck, Y. Van Laethem, F. Surmont on behalf of the Adult IPD Study Group

**Objectives:** An ongoing Invasive Pneumococcal Disease (IPD) surveillance network in Belgium started in 2009 aiming to:

- document the distribution and antibiotic susceptibility of **Streptococcus pneumoniae** serotypes responsible for IPD in adults $>50$ y of age
- document the mortality and morbidity of IPD in the same population
- estimate the potential coverage provided by the 13-valent conjugated pneumococcal vaccine.

- **Methods:** Prospective, active surveillance of IPD in hospitalized adults older than 50 years of age. Isolation of **S. pneumoniae** from culture of a normally sterile site by hospital microbiological laboratories. Fifty hospitals (44% of acute hospitals) participated in the surveillance network. The clinical presentation, complications and death caused by an IPD was evaluated and documented during hospital stay, at discharge and at 1 month thereafter.

- **Results:** A total of 551 patients older than 50 (mean age 71.7 range 50–88) with IPD were identified in 1 year. Of these, 442 patients were evaluable. 340 (76.9%) had pneumonia with bacteremia, 39 (8.8%) had empyema, 23 (5.2%) had meningitis, 21 (4.8%) had primary bacteremia. The mean duration of hospitalization was 21 days. ICU admission was
required for 31% of patients, with a mean duration of 12.6 days. 14% of patients had sequelae that persisted more than 1 month in 50% of patients. A total of 78 (18%) died during or within 1 month (6) after hospitalization.

Serotypes 3 and 19A accounted for 22% of all IPD. The most frequent types were 19A and 3 in pneumonia and 7F (15%) in empyema. Serotype 19A showed penicillin non-susceptibility in 12% of isolates. The highest mortality was found with serotypes 1, 3 and 5.

61% of the serotypes identified are included in the 13-valent conjugated pneumococcal vaccine.

Conclusion: This study shows that serotypes 3 and 19A are the most frequently isolated in invasive pneumococcal infections in Belgium. IPD has a high morbidity and mortality with a case fatality rate of 1 in 5. The serotypes present in the 13-valent pneumococcal vaccine account for 61% of IPD's.

**P960**

Penicillin- and erythromycin-non-susceptible Streptococcus pneumoniae serotypes in a Greek paediatric population, 2005–2009: replacement of 19F by 19A and 6A

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**Objectives:** Despite adoption of the heptavalent (7V) anti-pneumococcal vaccine in 2004 for the <2yo paediatric population in Greece, the ‘Taiwan’ 19F-14/ST236 clone, largely penicillin-non-susceptible and erythromycin-resistant, had persisted well into 2006 (Mavroidi et al., Clin Microbiol Infect 2007). Surveillance of *Streptococcus pneumoniae* in children was continued, to assess whether this trend would eventually change with time.

**Methods:** Over 2005–2009, 951 infection and carriage isolates from children accessing two children's hospitals in the Greater Athens area (~3,500,000 total population) were serotyped by the Quellung reaction using commercially available antisera (Statens Serum Institute, Copenhagen, Denmark). Their susceptibility to antimicrobial drugs, following 2009 CLSI breakpoints, was assessed by disk test (penicillin, amoxicillin, cefotaxime, erythromycin, clindamycin, tetracycline, cotrimoxazole, chloramphenicol, levofloxacin, rifampicin) and, for selected isolates and drugs, by the strip gradient method (Etest, bioMerieux). MLSB phenotypes were characterized by the disk approximation method (erythromycin-clindamycin). This study was conducted within CAREPNEUMO (FP-Health-2007-B/22311).

**Results:** Of the 31 serotypes observed, six were represented by >5% of the isolates: 19F (20.3%), 19A (8.3%), 6B (7.1%), 23F (6.7%), 14 (6.6%) and 6A (5.3). Comparing 2005 to 2009 across all serotypes, the erythromycin resistance rate had remained relatively stable (from 42% to 47.6%), whilst that of non-susceptibility to penicillin showed an upward trend (45% to 66.7%), entirely attributable to resistance rather than intermediate susceptibility. During this period, the 7V vaccine serotypes decreased, whilst non-7V serotypes increased: most notably, 19A from 2.7% to 24.8%, and 6A from 3.1% to 11.4%. The rate of resistance to erythromycin was lower amongst 19A and 6A (17% and 55.3%, respectively) than in the initially dominant 19F (85%), as was the rate of non-susceptibility to penicillin: 66.1% in 19A and 55.2% in 6A, compared to 88.8% in 19F.

**Conclusion:** Whilst the initially persisting 19F serotype declined from 2007, non-7V vaccine serotypes 6A and 19A increased. This trend was accompanied overall by relatively stable resistance rates to erythromycin but increased penicillin non-susceptibility rates, due to all serotypes’ contributions. Surveillance will be continued to monitor the effects of introducing newer vaccines against more serotypes.

**P961**

Characteristics of invasive Streptococcus pneumoniae samples

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**Objectives:** To study evolution of the sensibility of *Streptococcus pneumoniae* in the last 10 years, and analyze the presentation, clinical course and serotypes.

**Methods:** Retrospective observational study of *S. pneumoniae* isolated from invasive samples (blood and CSF) in the HCU Virgen de la Victoria of Málaga, from January 2001 to November 2010. The blood culture system used was the BD BACTEC 9600 (Becton-Dickinson). The sensibility study was carried out by E-test method and the interpretation of the cut-off points were used CLSI standards 2008. Serotyping was performed on all isolates from 2004 (Instituto Salud Carlos III).
**Results:** We can see the characteristics of the patients in Table 1. The sensitivity to penicillin was 99% for non-meningal sites, representing a MIC50 of 0.016 to 0.5 (range: <0.016–3) and 99.7% for meningeal location. As for cefotaxime, 95.5% of the cases were sensitive to locations not meningeal, MIC50 of 0.016 to 0.25 (range: <0.016–2) and 93.5% for meningeal infections. Sensitivity to other antibiotics, erythromycin, tetracycline, clindamycin, moxifloxacin, levofloxacin and ceftriaxone was 76.8%, 77.7%, 82.8%, 99.3% and 64.5% respectively.

The crude mortality was 25%, associated with sepsis and/or septic shock (57.6%), 24.3% with meningitis and 16.1% with CAP. Mortality in the first 48 hours was 48.2% and due to sepsis and/or septic shock in 67% of cases and 25% CAP, however, if it occurred after 48 hours in a 74.2% of the cases was due to CAP. As the most common serotypes associated with mortality within 48 hours to 50% were not included in the heptavalent vaccine (1, 14, 19A, 4, 7F, 3).

**Conclusions:**
1. *S. pneumoniae* bacteremia are more common in men with a mean age of 60 years, being the most common clinical presentation of CAP.
2. The high mortality (25%), especially in the first 48 hours (50%) was due to serotypes not included in the heptavalent vaccine.
3. *β*-lactams is still first-line treatment at present for both meningeval and non-meningeval infections.

**Table 1:**

| Total | 528 |
|-------|-----|
| Sex   | Male |
| Mean age | 3 (1-65) |
| Mean stay | 15 days |

**Source of isolates**

- Blood: 91.7%
- CSF: 5.2%

**Serotypes**

- 1 (12.5%)
- 19F (11.5%)
- 8 (11.1%)
- 2 (8.4%)
- 16 (4.9%)
- 4 (4.3%)

**Cultural mortality**

2.8%
in Cantabria (Spain), (3 hospitals, a total of 1500 beds). All patients ≥2 years old in whom *S. pneumoniae* was isolated from any normally sterile site were identified by prospective surveillance in the microbiology laboratories of the three National Health System hospitals in Cantabria. Isolates of *S. pneumoniae* were serotyped by the Microbiology National Center of Spain. For each case, one control was matched according to age (±5 years), gender, site of hospitalization and admission date (±2 months) and the condition that constituted the indication for vaccination. Matched OR, prevention fraction in exposed (etiologic fraction) and their 95% confidence interval were calculated.

**Results:** From January, 2003 to December 2008, 144 cases and 144 controls were included in the study. The annual incidence was 8.7 cases per 100,000 population, and the four serotypes more frequently isolated were 3, 14, 4 and 19A, all of them are included in polysaccharide vaccine. In 13.2% of patients were isolated serotypes that were not included in this vaccine. The pneumococcal polysaccharide vaccine effectiveness in this case-control study was 11.7% (95% CI = −48.6% to 47.8%).

**Conclusion:** Pneumococcal polysaccharide vaccine effectiveness was only 12%. Vaccine effectiveness increased in the months of December, January and February (coincidence with influenza season) being 40%. In addition in patients in whom influenza vaccine was administered simultaneously the effectiveness of 23-pneumococcal polysaccharide vaccine was 31%. This study was partially financed by the Fondo de Investigaciones Sanitarias (FIS) of Spain, Project number PI021754.

**P965** Prognostic factors and mortality of invasive pneumococcal disease in Cantabria (Spain)

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**Objectives:** *S. pneumoniae* infections are among the leading causes of illness and death worldwide. The objective of this study was to identify the main factors associated with mortality in patients with IPD in Cantabria with special attention to the impact of the vaccination with pneumococcal polysaccharide vaccine in mortality. Cantabria is a region in the north of Spain, with a current immunization schedule since the year 2000 that includes the pneumococcal polysaccharide vaccine.

**Methods:** A prospective multicenter community-based study was done from January 2003 to December 2008 in the 3 National Health System hospitals in Cantabria (Spain). All patients in whom *S. pneumoniae* was isolated from any normally sterile site were included. They were identified by prospective surveillance in the microbiology laboratories. Data about risk factors, microbiology, clinical course, and mortality were prospectively collected. Comparison of the means (Student’s t) for continuous variables and Fisher’s exact test for categorical variables were applied. Risk Ratios and their 95% Confidence Intervals were calculated.

**Results:** During the study period, 335 cases of IPD were identified (60.0% male). Underlying disease was presented in 65.4% of patients, been diabetes (21.3%) and COPD (15.5%) the most frequent. Pneumococcal Bacteremia occurred in 89.6% of cases and meningitis in 8.1%. Attributable mortality of IPD occurred in 35 cases (10.4%). Mortality related to bacteremia was 10.3%, whereas mortality in patients with meningitis was 20.0% (p = 0.17). The pneumococcal polysaccharide vaccine was administered in 22.1% of the cases, and there was not significant differences in mortality rate in this group compared with the group of non-vaccinated patients (RR=1.2; 95% CI=0.6−2.5; p = 0.66).

Patients older than 65 years had higher mortality rate than younger patients (RR= 3.0, 95% CI = 1.4–6.1; p = 0.002). The development of any complication during hospitalization was the strongest factor associated with mortality (p < 0.001).

**Conclusions:** The overall case-fatality rate of IPD was 10.4%, lower than the reported in other countries. The highest mortality occurred among the elderly and patients with any complication during hospitalization. The use of polysaccharide vaccine was not associated with a lower mortality.
diagnosis of CAP caused by *St. pneumoniae*, sitafloxacin (50 mg × 2, or 100 mg × 1) was orally administered for 7 days.

**Results:** Sixty-three patients were eligible for this study. In 56 patients, *St. pneumoniae* was cultured from the sputum and only urinary antigen was positive in 7 patients. The urinary antigen test was positive in 46 patients among 62 patients (46/62, 74.2%), however, urinary antigen test was not performed in 1 patient. Clinical efficacy of sitafloxacin could be evaluated in 62 patients among 63 patients (clinical follow-up data could not be available in 1 patient). Clinical improvement of CAP was obtained in 60 patients among 62 evaluable patients (60/62, 96.8%). Bacteriological effect of sitafloxacin could be evaluated in 56 patients, and eradication of *St. pneumoniae* was observed in 54 patients among 56 evaluable patients (54/56, 96.4%). In 2 patients, eradication of *St. pneumoniae* was not achieved instead of clinical improvement of CAP.

**Conclusion:** These findings demonstrated the clinical as well as bacteriological effectiveness of sitafloxacin in the treatment of CAP caused by *St. pneumoniae*.

**FOCUS 1 and 2:** *Streptococcus pneumoniae* subset analyses from two phase III trials of ceftaroline fosamil vs ceftiraxone in the treatment of community-acquired pneumonia

**Objective:** Ceftaroline fosamil (produg of the active component ceftaroline [CPT]) is a novel, parenteral, broad-spectrum cephalosporin that has previously demonstrated efficacy and safety in the treatment of community-acquired pneumonia (CAP) in the FOCUS trials (File et al., 2010). Herein we present a subset analysis of those patients who had *Streptococcus pneumoniae* identified as a baseline pathogen.

**Methods:** FOCUS 1 and FOCUS 2 were global, double-blind trials in subjects who were hospitalised for moderate to severe CAP (Pneumonia Outcomes Research Team [PORT] risk class III or IV) requiring intravenous (IV) therapy. Subjects were randomised to receive IV CPT fosamil 600 mg q12h or ceftiraxone (CRO) 1 g q24h for 5 to 7 days. Treatment groups were comparable in terms of dosing days and PORT scores; 51% of CPT and 47% of CRO subjects had CAP of PORT Risk Class IV. Clinical cure and microbiological response were evaluated 8 to 15 days post-therapy at the test-of-cure visit in the CE and MITTE populations. This subset analysis further evaluated results by *S. pneumoniae* baseline MIC, serotype, and bacteraemia. Susceptibility testing was performed by broth microdilution and disk diffusion tests.

| Response Rates by Pathogen | Ceftaroline fosamil | Ceftiraxone |
|-----------------------------|---------------------|-------------|
| Clinical cure (mITT)        | n/N (%)             | n/N (%)     |
| All *S. pneumoniae* (base-line isolates) | 59/69 (88.5) | 48/70 (68.6) |
| MDRSP                      | 4/4 (100)           | 2/9 (22.2)  |
| Positive by urinary antigen only | 25/28 (89.3) | 23/31 (74.2) |
| Positive by culture*        | 34/41 (82.9)        | 25/39 (64.1) |
| Favourable microbiological response (mITT) | All *S. pneumoniae* (base-line isolates) | 60/69 (87.0) | 51/70 (72.9) |
| MDRSP                      | 4/4 (100)           | 4/9 (44.4)  |
| Positive by urinary antigen only | 25/28 (89.3) | 23/31 (74.2) |
| Positive by culture*        | 35/41 (85.4)        | 28/39 (71.8) |

*Includes* *S. pneumoniae* isolates that were identified by both culture and urinary antigen. CE = clinically evaluable; MDRSP = multidrug-resistant *S. pneumoniae*; mITT = microbiological modified intent to treat efficacy.

**Results:** *S. pneumoniae* was identified among 69 of 182 baseline pathogens from the CPT group (24 tested; MIC ≤ 0.06 mcg/mL; range, ≤0.004–0.25 mcg/mL) and 70 of 188 pathogens from the CRO group (27 tested; MIC ≥ 0.06 mcg/mL; range, ≤0.015–2 mcg/mL). *S. pneumoniae* was identified by positive urine antigen alone for 40.6% (28/69) of the isolates in the CPT group and 44.3% (31/70) of the isolates in the CRO group. Rates of clinical cure and favourable microbiological response in subjects with baseline *S. pneumoniae* were 85.5% and 87.0%, respectively, in the CPT group compared with 68.6% and 72.9% in the CRO group (Table). Clinical response was consistent across a range of *S. pneumoniae* MICs and diverse serotypes, including serotypes 10, 15A, and 19A. Of the 29 cases of bacteraemia caused by *S. pneumoniae*, rates of clinical cure were 82.4% (14/17) in the CPT group and 66.7% (8/12) in the CRO group.

**Conclusion:** CPT fosamil was associated with higher clinical cure rates than those for ceftiraxone among subjects with CAP caused by *S. pneumoniae*, including subjects infected with MDRSP. CPT was efficacious across the MIC distribution and diverse serotypes of *S. pneumoniae*, including non-vaccine serotypes, and for patients with *S. pneumoniae* bacteraemia in the FOCUS trials.

**P968** Nasopharyngeal carriage of pneumococcal and other respiratory bacteria in community-dwelling asymptomatic elderly

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**Objectives:** *Streptococcus pneumoniae* is a common cause of pneumonia, sepsis, and meningitis among the elderly. The aim of this study was to evaluate the prevalence of nasopharyngeal carriage of *S. pneumoniae* and its serotype distribution in asymptomatic non-institutionalized elderly in the Netherlands prior to the introduction of the 7-valent conjugate vaccine for infants in the national immunization programme in June 2006. Additionally, carriage rates of other common respiratory bacteria and possible risk factors for pneumococcal carriage were evaluated.

**Methods:** A cross-sectional study was conducted in a random sample of 330 community-dwelling subjects aged 65 years or older (median age: 72.7; IQR: 68.7–79.0). Information on possible risk factors were obtained by questionnaire. Both transnasal and transoral nasopharyngeal samples were collected. Nasopharyngeal carriage of *S. pneumoniae* was identified by conventional culture and PCR (lytA and ply). Pneumococci identified by culture were serotyped by Quellung reaction. Pneumococci detected by PCR were molecularly serotyped.

**Results:** Nasopharyngeal pneumococcal carriage rate, using both conventional culture and PCR, was 22.8% (95% CI: 18.3–27.4). Pneumococci were detected more frequently in transorally obtained samples (18.8%; 95% CI: 14.6–23.1) than in transnasally obtained samples (11.1%; 95% CI: 7.7–14.5). Pneumococcal carriage rates detected with conventional cultures (25 positive isolates; 7.6%; 95% CI: 4.7–10.4) were lower than with PCR analyses (71 positive PCR-results; 21.9%; 95% CI: 17.4–26.4). Strains covered by the 13-valent pneumococcal conjugate vaccine were frequently carried (40.6% of all serotyped isolates by Quellung reaction; 76.7% by PCR). Of all potential risk factors, only regular contact with children under the age of five appeared to increase nasopharyngeal pneumococcal carriage (OR 1.62; 95% CI: 0.95–2.78; p = 0.077). Carriage rates for *S. aureus* were 17.0% (95% CI: 12.9–21.0), *H. influenzae* 10.0% (95% CI: 6.8–13.2), and *M. catarrhalis* 8.5% (95% CI: 5.5–11.5). Carriers of *S. pneumoniae* were more likely to be co-colonized with *S. aureus* (OR 3.73; 95% CI: 1.59–8.87; p = 0.001) and *H. influenzae* (OR 6.59; 95% CI: 2.63–16.47; p < 0.001).

**Conclusion:** Approximately one in five elderly carries *S. pneumoniae* and those persons have increased risks of colonization with other respiratory bacteria. Regular contact with children under five years of age appears to increase pneumococcal carriage.
A pilot study of serotype distribution and antimicrobial resistance of nasopharyngeal isolates of S. pneumoniae from healthy toddlers in Evros, Greece
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Objective: To study the serotype distribution and antibiotic susceptibilities of nasopharyngeal (NP) isolates of Streptococcus pneumoniae (SP) in children attending day care centres (DCC) in the area of Alexandroupolis, Thrace, Greece.

Methods: From February 4 through March 17, 2010, we obtained NP samplings from 184 healthy children aged 2 to 78 months (median age 65 months) attending 13 DCC. These children constituted 41% of the total number of children enrolled in these DCC. Antibiotic susceptibility testing was performed by E-test. Results were interpreted according to the 2010 CLSI criteria, except for ciprofloxacin, where the Eucast testing was performed by E-test. Results were interpreted according to the 2010 CLSI criteria, except for ciprofloxacin, where the Eucast 2010 breakpoints were used. Multi-resistance was defined as resistance to ≥3 classes of antibiotics. Pneumococcal isolates were serotyped by the Quellung reaction using the 12 pooled antisera Pneumotest panel and specific factor sera.

Results: Approximately 98% of the children had received ≥1 dose of PCV7. Overall, 48 (26%) of the children sampled carried SP in their nasopharynx. The percentage of SP carriers ranged between 0 and 69.2% among the DCC sampled. Among the 48 SP isolates, 16 belonged to PCV7 serotypes, i.e., 6B (4), 14 (2), 19F (3) and 23F (7), 16 belonged to the 6 additional serotypes of PCV13, i.e., 3 (6), 7F (3), and 19A (7), while the remaining 16 serotypes were non-PCV related, i.e., 9N (1), 10A (2), 11A (4), 15B (6), 17F (2), and 35A (1). 20.8% of the isolates had decreased susceptibility to penicillin, but no isolate had high level resistance. All isolates were susceptible to amoxicillin, second and third generation cephalosporins, carbapenems, chloramphenicol, vancomycin, linezolid and all quinolones tested except ciprofloxacin. Macrolide resistance was seen in 31.3% of the isolates, with the M phenotype being the most common (93.4% of macrolide-resistant isolates). Some resistance to TMP/SMX was seen in 60.4% being high level in 39.6% of the isolates. Eight SP isolates (16.7%) were multi-drug resistant (MDR) and all these isolates except one (serotype 15B) were PCV-related.

Conclusion: PCV13 will cover against two thirds of pediatric NP isolates in our area, where macrolide resistance (M phenotype) is prominent. Continued serotyping and antimicrobial susceptibility surveillance of NP carriage isolates of SP is essential for designing rational immunization strategies and for providing guidance for appropriate treatment options for pneumococcal infections.

Evaluation of different techniques to detect S. pneumoniae in hospitalised adults with community-acquired pneumonia across Europe
K. Loens*, A. Vanderstraeten, K. Bergo, H. Goossens, M. Ieven (Antwerp, BE)

Background: S. pneumoniae is the most common cause of community-acquired pneumonia (CAP). Early and accurate diagnosis of pneumococcal pneumonia remains difficult due to the limitations of conventional diagnostic methods. Several PCR have been employed with varying degrees of success, using primers specific to repetitive regions and genes. In recent years, real-time quantitative PCR (Q-PCR) has improved diagnosis.

Objectives: To compare the sensitivity and specificity of conventional sputum culture, blood culture, S. pneumoniae urinary antigen test (uAg), and qualitative and quantitative real-time PCR (Q-PCR) for the detection of S. pneumoniae applied to a single throat swab (TS) from hospitalised patients with CAP.

Methods: In a prospective study (November 2002-May 2003), 216 adult hospitalised patients with CAP collected all over Europe, were enrolled. CAP was confirmed by chest X-Ray. The following samples were collected: sputum and blood for conventional culture, urine for S. pneumoniae uAg-testing (Binax Now), and a TS for qualitative PCR and Q-PCR. Samples were stored locally and batch-wise transported to the central laboratory in Antwerp for analysis in 2003. Q-PCR was applied in 2010. A sample was considered positive if positive by sputum culture, blood culture and/or uAg-test.

Results: 20/216, 6/216, 39/216, 72/216, 44/216 patients were found to be positive by sputum culture, blood culture, uAg test, qualitative PCR and Q-PCR respectively. For 131 patients no S. pneumoniae positive result was obtained. The bacterial load in the TS varied between 0.01 and 30500 DNA copies/ml. Samples with a Ct-value above 33.5 in qualitative PCR were in general negative when analysed by Q-PCR. No clear correlation was found between the results of Q-PCR and a positive sputum and/or blood culture. Storage at −70°C had little influence on the DNA quality, generally resulting in a 0.5 Ct drop in the qualitative PCR. Sensitivities and specificities are shown in the table.

Conclusion: In conclusion, S. pneumoniae may be rapidly diagnosed by analyzing respiratory specimens by (Q) PCR and this may be particularly valuable in patients in whom antibiotic therapy was initiated before sampling. However, the main difficulty is the quality of the sample, the TS in this study. Quality assessment of the sample is needed. Q PCRs warrants further evaluation in clinical settings and the suitability of the test should be evaluated on different types of respiratory specimens.

The impact in Ireland of the 7-valent pneumococcal conjugate vaccine on invasive pneumococcal disease within two years of its introduction
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Aims: The Invasive Pneumococcal Disease (IPD) Typing Project was established in April 2007. The primary aim was to determine the serotype distribution of IPD isolates in Ireland before the introduction of the 7-valent pneumococcal conjugate vaccine (PCV7) and to subsequently monitor its impact on the epidemiology of IPD.

Methods: Streptococcus pneumoniae isolates from blood and CSF were serotyped using multiple PCR and serology. Before PCV7 refers to the time period from April 2007-September 2008, while post-PCV7 refers to the time period from April 2009-September 2010. Penicillin susceptibility was assessed using the Etest method.

Results: Between April 2007 and September 2010, 1101 invasive S. pneumoniae isolates were serotyped. Forty four different serotypes were identified. The most common serotypes before PCV7 were 14, 4, 9V and 7F. Post-PCV7 serotype 14 was replaced by 7F as the commonest cause of infection, 14 ranked second, followed by 19A and 8. A reduction of 56% and 22% in the IPD burden (all serotypes included) occurred in children <2 years of age and in all age groups, respectively. After PCV7 introduction nine serotypes were associated with reduced susceptibility to penicillin (MIC >0.12 mg/L), three of which were non-PCV7 serotypes. The most common penicillin non-susceptible serotype was 14 followed by 9V.

Conclusions: A dramatic reduction in IPD burden has occurred in Ireland since 2007. However, the high prevalence of serotype 7F and 19A, contained in PCV13, supports the decision to introduce this vaccine to the immunisation programme in December 2010.
Trends in serotypes causing invasive pneumococcal disease in Canadian adults, 2000–2009

W. Rudnick*, A. McGeer, K. Green, S. Pong-Porter, A. Plevneshi, M. Romilowych, D.E. Low on behalf of the Canadian Bacterial Surveillance Network

Objectives: The Canadian Bacterial Surveillance Network (CBSN) is a network of microbiology laboratories that submit bacterial isolates to a central lab for serotyping and susceptibility testing. CBSN has monitored (ST) trends in Streptococcus pneumoniae (SPN) since 1993. Routine pediatric PCV7 was introduced in Canadian provinces between 2002 and 2005.

Methods: Labs submit one SPN isolate per invasive pneumococcal disease (IPD) case for serotyping and susceptibility testing (CLSI standards; non-mening R breakpoints: penicillin (pen) \( \geq 3 \mu g/mL \), ceftriaxone (ctr) \( \geq 4 \mu g/mL \)).

Results: From 2000–9, 6783 SPN isolates from adult (\( \geq 15y \)) IPD cases were submitted from 80 microbiology labs in all 10 provinces (blood:6246, CSF:173, pleural fluid:167, other:197). During this time, the percent of IPD due to ST in PCV7 decreased significantly (56 to 18%), while the percent STs not in PCV7, but in PCV13 increased (18–43%), as did the percent due to non-conjugate vaccine (NPCV) STs (26–40%). The majority of the increase in PCV13 STs was due to 19A (1 to 15%). In 2009, 19A, 7F and 3 were the most common STs in adult IPD, representing 15%, 13% and 10% of isolates. The most common NPCV STs were 22F (16%), 23A (8%) and 9N (8%).

In 2007–09, 19A & 7F were the most common Trends in serotypes (STs) from blood (11%,9%); 19A & 3 the most common in CSF (12%,12%); 22F & 3 were most common from pleural fluid (9%,9%); and 6A & 3 from other sterile sites (12%,10%). Isolates from pleural fluid were least likely to be covered by PCV13 (33 v 60% for blood).

From 2000–09, resistance to at least one antibiotic class increased from 1 to 27%, and multidrug R (MDR to \( \geq 3 \) classes) increased from 1 to 15%. In 2009, 19A, 7F and 3 were the most common STs in adult IPD and RSPN (69%,68%,53%,52%).

Conclusion: IPD due to PCV7 STs in adults decreased in association with the introduction of pediatric PCV7 programs. The most common STs in IPD in '09 are included in PCV13. ST distribution differs from blood, CSF, pleural fluid and other sterile sites. Resistance continues to increase. STs 15A, 33A, 9V, and 19A are the most likely to be antibiotic resistant.

Streptococci: from diagnosis to pathogenesis

P974 Serotype-specific trends in population-based surveillance for invasive and respiratory pneumococcal disease in adults, Toronto, 2000–2009

K. Green*, W. Rudnick, D.E. Low, S. Pong-Porter, A. Plevneshi, M. Romilowych, A. McGeer on behalf of the Toronto Invasive Bacterial Diseases Network

Objectives: TIBDN performs population-based surveillance for invasive pneumococcal disease (IPD) in Toronto/Peel (pop 4M) since 1995. From 2002, respiratory tract isolates have been collected. PCV7 was licensed for children in Canada in 2001 and publicly-funded routine vaccination began 1/2005.

Methods: Serotyping and broth microdilution susceptibility testing to CLSI standards is performed on one isolate per IPD/respiratory case. Clinical data are collected from patient/physician interview and chart review.

Results: From 2000–9, 7080 adult (\( \geq 15y \)) cases of respiratory disease (RPD) and IPD were identified. Older adults (\( \geq 65y \)) accounted for 46% of IPD (1585/3343) and 44% (1621/3647) of RPD. The rate of IPD in adults \( \geq 65y \) decreased from 34 to 21/100000/y between 2002 & 2005, then increased to 25/100000/y in 2009. The rate of IPD stayed constant for adults <65y (5.3/100000/y) in 2002 & 2009. From 2002–9, the rate of IPD due to PCV13/nonPCV7 serotypes (STs) has increased from 1.0 to 2.3/100000/y in adults \( \geq 65y \) and from 5.3 to 9.6/100000/y for those \( \geq 65y \). Non-PCV (NPCV) ST disease has increased from 1.2 to 2.0/100000/y in adults <65y and from 9.7 to 12/100000/y in those \( \geq 65y \). From 2002–9, the proportion of disease due to PCV7 STs decreased from 57 to 17% for IPD and from 44 to 15% for RPD. The proportion of isolates from STs not in PCV7 but in PCV13 increased from 17 to 44% in IPD and 18 to 27% in RPD. The ST distribution of IPD s differs from RPD: 19F, 11A, & 23A/2 are more likely isolated from RPD; 22F, 19A, & 7F are more likely isolated from IPD. ST distribution also differs for nursing-home acquired (NH) and nosocomial (noso) cases. From 2005–9, STs 19A/F, 6A, & 14 were more common in NH IPD and STs 11A, 24 & 1/7F were more common in NH RPD compared to community disease in other adults \( \geq 65y \). STs 35B and 6A/8 are more common in noso IPD and 19F is more common in noso RPD. Isolates from noso/NH cases were also more likely antibiotic R (35% NH/noso IPD R to at least one class vs 23% for community cases; 37 vs 30% RPD isolates).

Conclusions: Since introduction of routine pediatric PCV7, the incidence of adult IPD due to PCV7 STs has decreased while that of PCV3 and NPCV isolates has increased. In 2009, STs in PCV7 comprised 17% of IPD and 15% of RPD isolates; STs in PCV13 comprised 61% of IPD and 41% of RPD, ST distribution differed from IPD and RPD, as well as for nursing-home acquired and nosocomial cases.

Streptococci: from diagnosis to pathogenesis

P975 Can we distinguish Streptococcus pneumoniae from other streptococci in the milk-group using the lytA gene? A comparison of bacterial strains by real-time PCR and phylogenetic analysis

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Objectives: The purpose of this study is to validate the use of the lytA-gene in distinguishing S. pneumoniae from other streptococci. S. pneumoniae, one of the most virulent human pathogens, is a part of the milk-group streptococci which otherwise consist of predominantly avirulent commensals. The lytA-gene is found in a small group of the streptococci belonging to the milk-group. The introduction of rapid molecular diagnostic tests has raised the question whether the commonly
used lytA-gene primers and probes are exclusive to *S. pneumoniae*. We have done a comprehensive work in the validation of the lytA-gene as a PCR-target in the streptococci using a practical and theoretical approach.

**Methods:** The real-time-PCR used for the practical work was developed for detection of *S. pneumoniae* using the lytA-gene (Sheppard et al. 2004). Three panels of bacteria were tested. (1) 51 strains not belonging to the *S. pneumoniae/mitis-group*. (2) 41 strains of *S. pneumoniae*. (3) 22 strains of the *mitis-group* (incl. five *S. pneumoniae* strains), which were kindly provided by M. Kilian and blinded to us while working with them. The theoretical work consisted of a lytA-gene sequence analysis of orthologous gene clusters within the *mitis-group*. 52 sequences were left for phylogenetic analysis after a primary reduction of the search result by comparison of sequence similarities and gene function.

**Results:** The PCR found 45/46 *S. pneumoniae* strains positive (test sensitivity, 97.9%). No positive test results were found in the non *S. pneumoniae* strains (0/48) incl. the 17 *mitis-group* strains (test specificity, 100%). The PPV was 100% and the NPV was 98%. The theoretical approach showed that the lytA-gene is present in many of the *mitis-group* strains. The gene is in general different in *S. pneumoniae*, but a continuum of gene variants seems to be present in the *mitis-group* (Figure 1). 1/44 *S. pneumoniae* strains was found within a *mitis* cluster and did not match the primers and probes (theoretical sensitivity, 97.7%). 0/3 non-*S. pneumoniae* streptococci were found in the *S. pneumoniae* clusters and none of them matched the primers and probes (theoretical specificity, 100%). The theoretical PPV was 100% and the NPV was 89%.

**Conclusion:** It is possible to design sensitive and specific primers and probes using the lytA-gene to distinguish *S. pneumoniae* from other *mitis-group* streptococci. Differences within the gene are in general larger between species than within a species in the *mitis-group*.

**Methods:** The real-time-PCR was incorporated to the procedure and 139 samples have been processed from November 19 to December 17 (period 2).

**Results:** The results were as follows:

- **Period 1:** Sensitivity: 84.6%, specificity 98.4%, PPV = 91.7%, NPV = 96.8% with an undetermined results of 29.9%.
- **Period 2:** Sensitivity 69.5%, specificity 97.2%, PPV = 84.2%, NPV = 93.8% with an undetermined results of 5%.

**Conclusion:** We showed a significant improvement in the number of indeterminate results using a practical step to remove the inhibition of PCR. The percentage of unresolved results is about 5%, a value that is acceptable to a PCR technique in intrapartum screening.

**Streptococcus pneumoniae: a Multiplex-PCR challenged by rough variants**

**Objective:** The three species *S. pneumoniae*, *Streptococcus mitis* and *Streptococcus oralis* are phylogenetically closely related, especially *S. pneumoniae* and *S. mitis*. Rough pneumococcal strains pose a special challenge. A Multiplex-PCR test was attempted made, separating pneumococcal strains from the other closely related species, based on possible differences in presence of the ply gene and two distinct base pairs on the 16S rRNA gene (16S) and applied on rough pneumococcal strains using gdh sequence analysis as definite separation between species.

**Materials:** Multiplex-PCR and gdh sequence comparisons: The Multiplex-PCR tested for presence of the ply-gene and two previous extensively BLAST screened differences at positions 220 and 641 (both *E. coli* numbering) in the 16S rRNA gene; respectively base pair “C” and “G” for *S. pneumoniae*. Partial gdh sequence analysis was used for definite species identification.

**Results:** Eight collection strains (representing the species *S. pneumoniae*, *S. mitis*, *S. oralis* and *S. pseudopneumoniae*) and all strains (*n* = 47) received in 2008 as rough pneumococcal strains on the basis of optochin sensitivity and bile solubility.
**P980** The streptokinase gene as a candidate target for differentiation between Streptococcus dysgalactiae subsp. equisimilis causing infections in humans and horses

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**Objectives:** In humans, Streptococcus dysgalactiae subsp. equisimilis (SDSE) causes a variety of infections ranging from mild to severe diseases. In horses, SDSE is considered opportunistic, although recent studies hypothesize a role for SDSE in causing mild respiratory disease. A comparison between the characteristics of human and equine SDSE is possible only if these can be accurately differentiated. The aim of this work is to evaluate if the streptokinase gene (skg) of SDSE is a valid target to develop PCR tests to distinguish between SDSE isolated from humans and horses.

**Methods:** The sequences of the skg of SDSE isolated from humans and horses deposited in GenBank have been aligned and compared. Primers specific for the skg of human SDSE have been designed and validated by BLAST. SDSE isolated from humans (n = 20), SDSE isolated from horses (n = 30), S. pyogenes (n = 8), S. canis (n = 10) and other Streptococcus spp. strains (n = 20) have been screened by PCR to detect the species S. dysgalactiae, the skg of human SDSE (hskg), and the skg of equine SDSE (eskg).

**Results:** All the 50 SDSE of human and animal origin resulted positive for S. dysgalactiae by PCR. The 20 SDSE of human origin were also positive for hskg but negative for eskg, while the 30 SDSE of equine origin were positive for eskg but negative for hskg. All other streptococci were negative for all three PCR products.

**Conclusion:** On the basis of these results, the skg gene is a valid target to distinguish SDSE of human and equine origin. In the past, an SDSE skg sequence similar to those of equine SDSE was found in the lung of a human patient. The availability of a PCR protocol to specifically detect human SDSE and to distinguish between human and animal strains has not only a diagnostic value, but can also support further epidemiological studies and can allow the evaluation of the zoonotic potential of animal SDSE. The comparison between human and equine SDSE could increase the knowledge about the pathogenic mechanisms of SDSE in their host species and could help to explain the variability in disease severity observed in different hosts.

**P981** Distribution of pathogenicity island XII of group B streptococci

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**Objectives:** Group B Streptococci (GBS) are the major cause of newborn infections. It is well known that there are several pathogenicity islands in the genomes of GBS which usually carry various the genes of various virulence determinants. Pathogenicity island XII carries genes of several virulence factors including scpB, lmb and sspB1. The latter gene was previously shown to be common for the GBS invasive strains isolated in Russia. The aim of the study was to further evaluate the distribution of the island XII among GBS and to investigate the organization of the putative virulence factor genes on this large genetic element.

**Methods:** The collection of 74 clinical GBS strains isolated in St. Petersburg, Russia was investigated by PCR and hybridization. All the strains were genetically typed employing multiplex PCR.

**Results:** 74 recently isolated in St. Petersburg Russia clinical GBS strains have been studied for the presence of genes considered as possible virulence factors. The following genes located on the virulence island XII (PAI XII) have been tested: toxin, Zn-finger, helicase, sspB1, transposon Tn5252, gene of type IV secretory system. The genes of interest have been found only in 16 of 74 strains. Interestingly some of the strains carried different number of the genes under study in their genomes. Multiplex analysis for serotype affiliation allowed determining only 56 strains in the collection. 16 strains belonged to the type Ia, 8 – II, 11 – III, 16 – IV, 5 – V. Types of 18 strains could not be identified. PAI XII was found in different types of GBS.

**Conclusion:** Pathogenicity island XII was found in 22% of clinical strains of GBS under study. The genetic organization of the determinants located on the island was found to be different. There was no correlation between presence of pathogenicity island XII and type of GBS.

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**P982** Non-haemolytic GBS on Granada medium: there might be a solution for this problem!

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**Objectives:** Today, Group B streptococcus (GBS) still is an important cause of neonatal sepsis. Screening for maternal GBS colonisation is generally advised at 35–37 weeks gestation. One of the methods recommended by the CDC (RR-10) for laboratory detection of GBS is the chromogenic Granada medium, which utilizes the ability of GBS to produce its unique orange carotenoid pigment. The reading of this plate is easy and clear cut. However, approximately 5% of all GBS do not produce this orange pigment, a characteristic closely linked to absence of hemolysis. Therefore, we evaluated the addition of an oxacillin disk in an attempt to pick up these challenging non-hemolytic GBS strains.

**Methods:** In a prospective study, 483 vulvo-rectal swabs from pregnant women were tested for GBS by use of Granada medium® (bioMérieux) and Lim Broth® (Beckton-Dickinson) enrichment. At arrival, the samples were directly plated on Granada medium with addition of an oxacillin disk (1 μg), and were inoculated in Lim Broth. After overnight incubation, the Granada medium was evaluated. In case of absence of orange colonies, the Lim Broth enrichment was plated onto a second Granada medium with again addition of an oxacillin disk, and another overnight incubation was performed. After this second overnight incubation, the direct plated Granada media were evaluated again and the media, plated after enrichment, were evaluated once. All white colonies which were oxacillin-susceptible, were tested for catalase. If catalase testing was negative, subsequent agglutination test with group B antisera (Diamondal Strep Kit®) was performed.

**Results:** After 48h of incubation, a total of 91 samples showed the typical orange coloured colonies. Of the 392 white strains, 48 showed an oxacillin-susceptibility zone. By means of catalase testing and
Faster diagnosis by antigen detection for group B Streptococcus carriage in pregnant women

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Objectives: In the new Revised Guidelines of 2010, CDC underlines that more rapid techniques for identifying GBS directly from enrichment broth have been developed. In a previous study we already verified the possibility of avoiding plate subculture, by the detection of GBS antigen within 24 hours directly from the enrichment broth after overnight incubation. The aim of this study was to evaluate the chance of further reducing the time of diagnosis within only 8−13 hours after incubation in the enrichment broth.

Methods: Between 1 April and 30 October 2010, 265 pregnant women were monitored at delivery, by placing into a Todd-Hewitt broth with nalidixic acid and colistin (LIM, Copan) a rectal and a vaginal swab. The samples were incubated at 35ºC immediately by the midwives in the delivery room. Twice a day (8 am and 4 pm) and after at least 8 hours incubation, the broth was subcultured onto ChromId Agar strept B (Biomerieux) and the detection of GBS antigen was performed directly from the enrichment broth, according to the kit procedure (Bio MK). The samples were incubated again until 18−24 hours and the subculture and the antigen research were repeated after the second incubation.

Results: We examined 265 pregnant women, 54 positive and 211 negative for GBS culture. Antigen after a mean of 13 (8−23) hours confirmed 50 of the culture positive results. The 4 cases with the false-negative antigen showed less than 100 CFU/ml in the subcultures. Among 211 negative cultures, 2 cases were positive by antigen. Taking as reference the culture, antigen showed 92.6% sensitivity, 99% specificity, NPV 98% and PPV 96%.

Conclusions: Clinicians ask for a rapid test for the detection of GBS that can be used at the time of delivery. Currently, there are rapid molecular methods, some even faster and simple but expensive. The detection of GBS antigen allowed to identify 50/54 positive cases and showed a PPV of 96%. Only in 4/54 cases the antigen was false-negative after shortened incubation with the growth of only less than 100 CFU/ml. The reading of antigen was always easy and the colorimetric reaction of sample-line resulted weak with a risk of equivocal interpretation in 4 of 211 negative cases. GBS antigen detection was rapid, reliable, easy-to-perform and able to identify 92.6% of colonised pregnant women already within a mean of 13 hours after sample collection.

AMR in fastidious micro-organisms

Antimicrobial resistance in Helicobacter pylori positive patients after therapy failure and in Helicobacter pylori positive untreated patients

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Objectives: Patients with peptic ulcers who are also infected by Helicobacter pylori receive antimicrobial therapy, based on different antibiotic combination including clarithromycin in addition to antisecretory drugs. Eradication is however difficult because of primary and secondary resistance. The aim of the study is to evaluate in vitro susceptibility of H. pylori strains isolated in a group of symptomatic patients with failure of previous eradication treatments and the primary resistance in a group of untreated symptomatic patients.

Methods: Group A: 107 adult patients (36 males and 71 females, mean age 53.5 years and range 25−77) with peptic ulcers and other gastric disorders after failure of previous treatment regimens. Group B: 80 untreated patients. During endoscopy, four biopsies were taken from antrum for histology, rapid urease test and culture and from two for fundus for histology and culture. The specimens for culture were homogenized and streaked on fresh blood agar plates, incubated in a microaerophilic atmosphere at 37ºC for 4 days up to 10−14. Fresh culture of 3 days were tested by E-test method for susceptibility to clarithromycin, amoxicillin,
Results: *H. pylori* susceptibility test was performed from 59/107 (55%) patients of group A and from 33/80 (41%) patients of group B (14 males and 19 females, mean age 29.7 years and range 4–66). In the other subjects the culture showed no growth or contamination. Clarithromycin resistance was found in 80%, metronidazole in 71% and amoxicillin in 2% of group A patients. Clarithromycin, metronidazole resistance was respectively 19% and 15% in group B, where levofloxacin resistance was observed in only one patient. All strains were susceptible to tetracycline. Resistance to at least one drug was observed in 95% of subjects of group A and in 36% of group B, resistance to both clarithromycin and metronidazole was found in 53% and 0% respectively.

Conclusion: The percentage of clarithromycin resistant strains from patients with previous treatments failure is very high (80%), while the percentage of metronidazole resistant strains was 7–19%. Resistance to tetracycline was never observed in both group. As clarithromycin resistance affects the efficacy of first-line therapy and tetracycline should be used in combined alternative treatment, this antibiotic should be considered in susceptibility testing.

### Table 1. Percentages of resistance of gonococcal isolates (2005-2010)

|          | 2005 (n=17) | 2006 (n=18) | 2007 (n=22) | 2008 (n=23) | 2009 (n=20) | 2010 (n=34) | p     |
|----------|-------------|-------------|-------------|-------------|-------------|-------------|-------|
| PEN (+R) | 29          | 6           | 9           | 25          | 10          | 15          | 0.883 |
| Beta-lactamase positive | 0 | 100 | 100 | 33 | 0 | 0 | 0.211 |
| CTX      | 0           | 0           | 0           | 0           | 0           | 0           |       |
| CIP (+R) | 43          | 56          | 23          | 55.5        | 45          | 44          | 0.904 |
| RIF      | 29          | 33          | 32          | 20          | 15          | 38          | 0.296 |
| PEN+CIP  | 14          | 0           | 0           | 22          | 0           | 0           | 0.366 |
| PEN+RIF  | 14          | 0           | 5           | 9           | 5           | 6           | 0.904 |
| CIP+RIF  | 14          | 33          | 18          | 18          | 0           | 6           | 0.007 |
| PEN+CIP+RIF | 14 | 0 | 0 | 0 | 0 | 0 |       |

Results: The 124 isolates corresponded to 120 patients, 116 males and 4 females. Overall, the percentages of resistance to penicillin (PEN), ciprofloxacin (CIP) and rifampin (RIF) were 15%, 48% and 39.5%, respectively. No significant increases in antimicrobial resistance were detected over the study period (Table 1). Resistance to ciprofloxacin ranged between 23% in 2007 to 56.5% in 2008. Among the penicillin-resistant isolates, 9.7% were β-lactamase producers. All strains were fully susceptible to cefotaxime (CTX) (MIC$_{90}$ ≤0.06 mg/mL). One isolate showed combined resistance to penicillin, ciprofloxacin and rifampicin.

Conclusion: The high resistance rate of NG to ciprofloxacin in our area precludes the use of fluoroquinolones for empiric therapy. Cefotaxime remains a highly effective antimicrobial agent for the treatment of gonorrhoea. Surveillance of antimicrobial resistance of NG isolates is essential in order to prevent the spread of resistant isolates and to adjust empirical treatments.
**P098** Cefixime resistance in *Neisseria gonorrhoeae* isolates from 2006 to 2010: correlation of MIC values with target gene alterations, the Italian experience

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**Objectives:** Reduced susceptibility to cephalosporins, now recommended as the mainstay of treatment, along with some treatment failures with cefixime have been reported for *Neisseria gonorrhoeae*. Aim of this study was to test the cefixime and ceftriaxone susceptibility of strains isolated from 2006 to 2010 in Italy. Analyses of genetic mechanisms for resistance and the genetic relationship among the isolates have been also carried out looking for correlation with MICs.

**Methods:** Minimum inhibitory concentrations (MIC) for Ceftriaxone and Cefixime were determined by the E-test method (AB biodisk, Solna Sweden) and agar dilution on 265 *N. gonorrhoeae* strains isolated from 2006–2010. The EUCAST 2010 (MIC >0.12 mg/L) breakpoint, (MIC >0.25 mg/L) breakpoints were taken into account. PenA, mtrR, porB1B and ponn01 sequences together with NGRAM sequences have been also determined.

**Results:** The majority of the gonococci were susceptible to both antibiotics. According to the EUCAST breakpoint 12% resulted resistant to cefixime compared to only 1% according to the CLSI reference breakpoint. However, the number of gonococci with cefixime MICs of 0.125–0.25 mg/L has increased in Italy during 2009–2010. These MICs lie on the edge of the breakpoints. The complete nucleotide sequence of penA from 45 isolates (either susceptible or with decreased susceptibility/resistance) were determined. Five amino acid sequence patterns in PBP2 were identified. These sequences were compared and isolates with MIC >0.125 mg/L all shared the sequence pattern XXXXII. The majority of strains with MIC >0.125 mg/L belonged to ST1407 already described in cefixime resistant strains worldwide. The other target genes did not show any specific alterations compared to susceptible strains. All the examined strains were fully susceptible to ceftriaxone.

**Conclusions:** An upward rate of cefixime resistant or with decreased susceptibility has been found among Italian gonococci isolated from 2006–2010. Our findings show that the PBP2 is involved in resistance or reduced susceptibility equally. The remaining target genes do not influence the genetic mechanism of resistance or decreased susceptibility to cefixime. These data underline once more the usefulness of individual testing and local surveillance of *N. gonorrhoeae* in order to update treatment recommendations and therefore help in disease control.

**P089** Macrolide-resistant *Mycoplasma pneumoniae* in Japan

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**Objectives:** Recent epidemiological studies in Japan have demonstrated that the incidence of macrolide-resistant *M. pneumoniae* is increasing gradually in pediatric patients. The purpose of this study was to clarify the frequency and clinical characteristics of macrolide-resistant *M. pneumoniae* in adults.

**Methods:** A total of 30 children and 40 adults with *M. pneumoniae* infection confirmed by serology and PCR who visited to Kawasaki Medical School hospital from June 2005 to April 2010 were enrolled in this study. Primers for domain V of 23S rRNA gene such as A2063G and A2064G. All of them were treated with macrolides initially. Fever in nine children with *M. pneumoniae* without point mutations at 2063, 2064, or 2617 disappeared within 48 hours of treatment; however, fever persisted for more than 48 hours after treatment in 21 patients with *M. pneumoniae*. Minocycline was effective in these patients with macrolide-resistant *M. pneumoniae*. In contrast to pediatric patients, only three macrolide-resistant *M. pneumoniae* were detected in adult patients.

**Conclusion:** Macrolide-resistance rate of *M. pneumoniae* may be increasing to as high as 70% in children, but macrolide-resistance rate was low in adult patients. However, monitoring of *M. pneumoniae* strains seems to be necessary in order to recognize early changes in the antibiotics resistance pattern of this important agent of human respiratory tract infections. Tetracycline was effective to these macrolide-resistant *M. pneumoniae* infections. Response of fever by antibiotics correlated very well to response of reduction in number of *M. pneumoniae* at nasopharynx.

**Emerging infectious diseases**

**P990** Clinical epidemiology and manifestations of *Campylobacter concisus*

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**Objectives:** *Campylobacter jejuni* is a major cause of bacterial diarrhoea throughout the western world. After the acute gastroenteritis some patients have sequelae like irritable bowel syndrome, reactive arthritis, inflammatory bowel disease and Guillain Barré syndrome. Likewise, *Campylobacter concisus* has been proposed to cause diarrhoea especially among children and immunocompromised patients, but the epidemiology and burden of disease in humans are not clarified. In this community-based study we describe the epidemiology and clinical manifestations caused by *C. concisus*. We describe the differences and similarities of the clinical presentations caused by *C. jejuni* and *C. concisus* respectively.

**Methods:** *Campylobacter concisus* was isolated from diarrhoeal stool samples with use of the filter method. Patients with *C. jejuni* and *C. concisus* were included in the study. The study period was two years and started January 2009. Clinical data were reviewed by use of the patient’s medical records as well as a questionnaire survey with a follow up for six months.

**Results:** In the first 22 months 10.388 faecal samples were cultivated with use of the filter method as well as the routine methods for cultivating pathogenic enteric bacteria. The most prevalent pathogenic enteric bacteria was *C. jejuni* with 456 patients followed by *C. concisus* with 378 patients (36/100,000/year). *Clostridium difficile* and *Salmonella* spp. were isolated from 349 and 206 patients, respectively. There was an almost constant monthly prevalence of *C. concisus* during the study period. The age-specific incidence showed that *C. concisus* was frequent among small children and elderly patients. *C. jejuni* was most common among young adults. Two third of the samples with *C. concisus* were from general practice, the remaining were from hospitals. Patients with *C. concisus* presented a more prolonged duration of diarrhoea compared to *C. jejuni*.

**Conclusion:** *C. concisus* was frequently isolated from faeces from patients suspected for bacterial gastroenteritis and suggest a pathogenic potential of this *Campylobacter* spp. The infection occurs at any age, but in contrary to *C. jejuni*, it is more frequently among infants and the elderly. Many patients with *C. concisus* reported long-lasting diarrhoea, especially among the elderly patients. Laboratory, as well as clinical studies, are required to illuminate the molecular pathogenesis by *C. concisus* and to describe if the patients may benefit of antibiotic treatment.
A study on Chikungunya virus infection in north India
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Objectives: Since re-emergence of chikungunya virus (CKV) infection in Indian subcontinent in 2005, it has become a major public health threat.

Methods: Clinically suspected cases (as per NICD, New Delhi, India) visiting outpatient or inpatient Departments of Pediatrics, Medicine and Rheumatology and referred to Microbiology Department for diagnosis of CKV were prospectively enrolled after obtaining written consent. Detailed clinical history and examination findings were recorded in a pre designed questionnaire from 248 such patients during September 2009 to May 2010. Results of hematological and other investigations were extracted from medical records. IgM ELISA for CKV was done using IgM antibody capture ELISA (NIV, Pune, India). Reverse Transcriptase PCR for CKV was performed on 53 randomly selected samples. Tests for Malaria antigen, IgM against Dengue, Japanese encephalitis (JE) virus and typhoid were done as per referring clinician’s request.

Results: 12.1% (30/248; 21 true positives; 9 borderline cases) patients were positive for CKV IgM and 17/53 were RT-PCR positive (4/45 IgM negative; 1/2 IgM positive; 2/6 borderline IgM positive). Overall CKV infection was present in 44/248 suspected patients. Mean duration of fever was 6.68 ± 1.83 days. Frequent clinical features were fever (100%); arthralgia (95%); headache (85%); rash (57%); pleuritic chest (36.5%); arthritis (25%); lymphadenopathy (16%) and hemorrhagic manifestation (15.9%). Involvement of elbow, wrist and hip joint, pain and redness in eyes and photophobia had significant association (p < 0.05) with CKV positivity. Clinical presentation was different in adults and pediatric age groups. Neurological involvement with encephalitis and seizures was present in 7 cases and was more common in young children. 16/44 cases had co-infection by dengue virus, 4 with JE virus and 3 had concurrent malaria. Mortality rate amongst CKV infected was 4.5% (2/248, both children).

Conclusions: In absence of pathognomonic features, clinical overlap with other infections prevalent in this part of the world; clinical diagnosis becomes difficult. Unusual features like encephalitis were present in significant proportion. Considerable number of patients had co-infection with other pathogens hence even if patient is diagnosed with other prevalent infections, CKV should be tested for. Both, serology and molecular diagnosis should be used in conjunction for better case detection.

The burden of invasive disease with non-b Haemophilus influenzae serotypes in Alaska and Northern Canada, 2000–2009
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Background: Prior to introduction of the Haemophilus influenzae type b (Hib) conjugate vaccines, rates of Hib disease among indigenous people living in Alaska (AK) and Northern Canada (N Can) were among the highest reported in the world. Routine vaccination has reduced these rates; however, serotype replacement with non-type b strains is of particular concern in the North American Arctic.

Methods: We identified cases of invasive Hi disease in AK and N Can from 2000–2009 through the International Circumpolar Surveillance (ICS) network. Medical charts were reviewed on laboratory-confirmed cases using standardized forms to verify clinical presentation. AK and N Can estimated populations as of 2008 were 679,720 and 145,493 respectively; indigenous people comprised 20% of the population in AK and 60% in N Can.

Results: During the study period, a total of 258 cases of invasive Hi disease were reported from AK (152) and N Can (106). Among the 159 (62%) invasive Hi cases with serotype information available; 73 (46%) were serotype a, 47 (30%) were serotype b, 20 (13%) were serotype f. Among Hia isolates, 63 (86%) occurred in indigenous people; median age was 1.0 year (range 2 mo-74 years); 60% were male. Four Hia cases (one adult/5 children) were fatal. Common clinical presentations included: meningitis (32%), pneumonia (27%), and septic arthritis (12%). There were no cases of epiglottitis. Overall annual Hia incidence was 0.3, and 3.7 cases/100,000 population in AK and N Can, respectively. Annual incidence rates among indigenous children <2 years old in AK and N Can were 17 and 104 cases/100,000 persons, respectively.

Conclusions: Serotype a is now the most common Hi serotype seen in the North American Arctic, with the highest rates among indigenous children. Further research is needed to investigate regional differences in rates, and to determine sequelae, risk factors, and the utility of chemoprophylaxis.

Tertiary care hospital candidemia: epidemiology and emerging species
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Objectives: The present study was conducted in order to know the epidemiology of candidemia and the characterization of some emerging species of candida in a 5 year period in our hospital.

Methods: All candida blood culture isolates between 1 January 2005 and 31 December 2009 were included, in a 800-bed community teaching hospital. Subsequent episodes one month before the first were not included. Blood cultures were processed using the Bactec 9240 system (Becton Dickinson, Franklin Lakes, USA). The yeasts were inoculated in Sabouraud-Chloramphenicol agar and in Sabouraud-Chloramphenicol-Acetidina agar (Bio-Rad, Hercules, CA, USA) and also in a differential isolation medium CHROMagar Candida (Becton Dickinson, Franklin Lakes, USA). Further identification was performed using the API ID 32C system (bioMérieux, Marcy l’Etoile, France).

C. albicans was differentiated from C. dubliniensis by PCR and restrict digestion. Candida parapsilosis isolates were confirmed as C. parapsilosis, C. metapsilosis or C. orthopsilosis by RAPD-PCR. C. glabrata was differentiated from its phylogenetically related species C. nivariensis and C. bracarensis by a multiplex PCR.

Results: A total of 203 isolates of Candida were identified, that accounted for 4.2% of all positive blood cultures, with a distribution of 33 in 2005 (3.6%); 41 in 2006 (4.4%); 57 in 2007 (5.4%); 40 in 2008 (4.3%) and 32 in 2009 (3.3%). Males, 118 (58.1%), were more prevalent than females, 85 (41.9%). The majority of the episodes were due to C. albicans 36.9% and C. parapsilosis 34% followed by C. tropicalis 13.3%, C. glabrata 8.3%, C. krusei 2.5%, C. guilliermondii 2.9%, C. lypolitica 1%, C. lusitaniae 0.5% and C. sake 0.5%. The prevalence of parasilosis group was as follows: 94.2% (65) for C. parapsilosis, 1.4% (1) for C. orthopsilosis and 4.3% (3) for C. metapsilosis. We did not find any C. dubliniensis, C. nivariensis nor C. bracarensis among our isolates.

Conclusions: C. albicans was predominant in our hospital, followed very closely by C. parapsilosis. This is in consonance with other countries in Europe but not with data from USA where the more prevalent non-candida species is C. glabrata. Though the number of isolates is limited, the prevalence of the new species in parasilosis group is very low and they are in agreement with recent works in Spain and Portugal. The same as C. nivariensis or C. bracarensis, that represented only the 0.2% of C. glabrata isolates distributed globally.
Clinical and molecular epidemiology of emerging *S. aureus* ST398 strains associated with bloodstream infections in France

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Since 2000, a survey of bloodstream infections (BSI) has been under way in France (JCM.2009.49:2863–71,2007.45:851–7 & 2004.42:5650–7). Since 2006, an increase with incidence involved methicillin-sensitive *S. aureus* strains, associated since 2009 with emergence of ST398 strains (CID.2011.52:152–3). In 2010, incidence of BSI associated with ST398 highly increased into short stay units (0.008/1000 PD in 2009, 0.015 in 2010) (Figure 1).

Our objective was to characterize the clinical and molecular epidemiology of ST398 and non ST398 strains involved with 613 BSI cases diagnosed since 2007.

**Methods:** Antibiotic susceptibility testing & PFGE were performed for all strains. ST398 strains were further characterized (arg type, spa type & MLST). Demographic and clinical data were collected for all patients: age and sex, portal of entry (skin, surgical site, lungs, urine, intravascular device, or digestion), community-associated/hospital-acquired BSI, death within 7 days of BSI diagnosis, duration of hospital stay.

**Results:** Clinical & molecular epidemiology of ST398 and non-ST398 strains differed strongly. Microbiological characteristics. Compared with non-ST398 strains, ST398 strains were more often susceptible to meticillin (10/12, 83%, versus 446/601, 74%) and only resistant to erythromycin (7/12, 58%, versus 40/601, 7%; p < 0.001). ST398 strains were characterized by different spa-types mainly t571, a susceptibility to tetracycline and a lack of PVL production, indicating that they differed from European pig-borne strains (spa-types 011 or 034, TetR) and shared similarity with Chinese-type (spa-type 571, TetS). Clinical data. ST398 BSI cases were mostly hospital-acquired (11/12, 92%), but observed in patients hospitalized in unrelated hospitals, thus excluding an outbreak. Compared with non ST398, ST398 BSI cases were significantly diagnosed following surgery (5/12, 42% versus 56/601, 9%; p = 0.004) or associated with a digestive portal of entry (3/12, 25% versus 15/601, 3%; p = 0.004). Examination of patient history revealed exposure to animals in only one ST398 BSI case (a fatal idiopathic community-acquired BSI in a 84-year old man living in a farm growing one pig), thus questioning us how the present ST398 strains were transmitted.

**Conclusions:** We bring data over the emerging and rapid spreading clone of ST398 strains in human infectious diseases.

Low prevalence of *Coxiella burnetti* endocarditis in patients with a history of valve surgery or cardiac valve prosthesis in a Q-fever endemic area in the Netherlands

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**Objectives:** Q fever is a zoonosis, caused by *Coxiella burnetti*. Following primary infection, which is often asymptomatic, 1–5% of patients develop chronic Q fever, of which endocarditis is the most common manifestation. Q fever endocarditis requires long-term antibiotic treatment and has poor prognosis if untreated. The estimated risk of developing Q fever endocarditis after primary infection for patients with pre-existent valvulopathy was 39% in a retrospective study, with the highest risk for patients with prosthetic valves. In the Netherlands there has been a large outbreak of acute Q fever with over 4000 notified cases since 2007, which allows for a more precise risk estimation of chronic Q fever in high-risk groups. We studied the prevalence of chronic Q fever in an endemic area in patients with a history of cardiac valve surgery, including valve prosthesis.

**Methods:** We selected all patients with a history of cardiac valve surgery from our cardiology outpatient clinic and invited them by letter for microbiological screening. IgG antibodies to phase I and II antigens of *C. burnetti* were tested by immunofluorescence assay. If phase I IgG antibodies were positive, polymerase chain reaction (PCR) on blood for *C. burnetti* DNA was also performed. Chronic Q fever was considered probable if phase I IgG antibody titre was ≥1024 and definite in case of positive *C. burnetti* PCR in blood.

**Results:** A total of 663 patients were identified with a history of valve surgery and unknown Q fever serostatus. As of December 2010, 200 patients had been invited for screening. In total, 172 patients (86.0%) responded and were available for serological screening. Of these, 31/172 patients (18.0%) had phase I and/or phase II IgG antibodies against *C. burnetti*, indicating a previous *C. burnetti* infection. In this group, 2/31 patients (6.5%) had phase I antibodies titres ≥1024, indicating probable chronic Q fever. *C. burnetti* PCR was negative for both patients.

**Conclusion:** Despite a seroprevalence of IgG antibodies against *C. burnetti* of 18.0% in patients in an endemic area with a high risk of developing chronic Q fever, only 6.5% of these patients had probable chronic Q fever. Compared to the previously reported risk of 39% in case of valvulopathy, we found a considerable lower percentage of patients who progressed to chronic Q fever after *C. burnetti* infection. However, as chronic Q fever can develop years after primary infection, further follow-up of seropositive patients is warranted.

The initial presentation of soft tissue anthrax

D. Hamilton*, G. Jones (Dumfries, UK)

**Objectives:** To describe the early clinical presentation of anthrax in a cluster of cases related to recreational injected drug use.

**Methods:** Case notes and available laboratory data were accessed to determine common features of five confirmed anthrax cases.

**Results:** Five cases of anthrax were identified in Dumfries and Galloway Royal Infirmary during early 2010. The following features were common to at least four patients. Mild soft tissue swelling close to a point of injection. The swelling was painful, soft and diffuse but not associated with erythema, eschar or discharge. On debridement these lesions had a distinctive gelatinous appearance with fat necrosis. Fascia was affected but bone and skin were not. The patients were either apprised or had a borderline fever never above 38 degrees centigrade. The neutrophil count and other blood cell indices were within normal ranges; Creatinine, Urea, Sodium, Potassium and Liver Function Tests were normal. The CRP was raised (median 60 mg/L, range 48–70). Diagnosis was by PCR (5 cases), culture (three cases) and serology (all five cases). Each received four antimicrobials from: benzylpenicillin, ciprofloxacin, clindamycin, metronidazole and fluocinocilin, as per the Scottish Guidelines. Following early debridement serous fluid loss from the
Cryptosporidiosis in Kuwaiti children: association of clinical characteristics with Cryptosporidium species and subtypes

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Objectives: To determine the association of clinical characteristics with Cryptosporidium species and subtypes.

Methods: Fecal specimens from 2548 children with diarrhea were screened by microscopy for Cryptosporidium spp. and the positive specimens were genotyped and subtyped by PCR-restriction fragment length polymorphism.

Results: Eighty seven of 2548 (3.4%) children had cryptosporidial diarrhea by microscopy and the majority (41.4%) of the infected children were between 4–8 year-old age group. Molecular characterization showed that C. parvum was the most commonly identified species (72.5%) and consisted of 4 subtypes, IId, IId, and the commonest (78.7%) followed by IIC and IID. Twenty-two (26.5%) of the children had C. hominis and showed three subtypes, IId was the most common (54.5%) followed by Ia (36.4%) and Ic. Associated clinical manifestations varied among different Cryptosporidium spp. Diarrhea associated with subtype IId, the most commonly identified C. hominis subtype, was more severe than that associated with other sub-types.

Conclusion: Our study confirms a very different Cryptosporidium genotype and subtype distribution, with a predominance of C. parvum IId and IId among the Kuwaiti children with diarrhea suggesting that the anthroponotic transmission is likely to be an important mode in the epidemiology of cryptosporidiosis in Kuwait. In addition, subtype IId of C. hominis was associated with more diverse and severe clinical manifestations in infected children suggesting that parasite genetics may play an important role in the clinical manifestations of human cryptosporidiosis.

Deep-brain stimulation hardware related infection: an emerging infectious disease

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Background: Deep brain stimulation (DBS) is increasingly used for the treatment of severe movement disorders. As a consequence, DBS hardware-related infections have emerged as a challenging complication of this medical progress. Better knowledge of DBS hardware-related infections characteristics is required to optimize patients’ management.

Methods: DBS hardware-related infections diagnosed at Rennes University Hospital were identified through computerized database system. Data were retrospectively extracted from medical charts. We performed a systematic review of the literature through MEDLINE databases using the search terms brain stimulator AND infection, hardware-related, device-related, or abscess.

Results: Eleven patients were diagnosed with DBS hardware-related infections between October 2006 and December 2008. Sex ratio was 1/1 and mean age was 54 years [range 21–68]. Median delay between DBS implantation and infection diagnosis was 28 days [range 8–820]. Infection sites included pulse generator (n=8), retroauricular sites requiring total hardware removal (n=3), partial removal (pulse generator and electrode extender, n=7), or wound debridement (n=1). Surgical samples yielded Staphylococcus aureus (n=6), S. epidermidis (n=2), Propionibacterium acnes, and Micrococcus sp. (one patient each). Despite prolonged intravenous antibacterial treatment (median duration, 6 weeks), 3 patients initially managed with partial hardware removal ultimately required total hardware removal. All patients survived, and no disability was attributed to DBS-related infections.

Conclusions: DBS hardware-related infections limited to pulse generator can be successfully treated with partial hardware removal and prolonged antibacterial treatment, while infections involving frontal or retroauricular sites require total hardware removal.

High-level gentamicin resistant Enterococcus faecalis isolated from urinary tract infections and poultry in close contact with the patient

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Enterococci represent commensals of the human and animal gastrointestinal tract but also opportunistic pathogens causing e.g. urinary tract infections, endocarditis and sepsis. The role of Enterococcus spp. as nosocomial pathogens has increased but the sources of community-acquired and nosocomial infections are still unclear. In this study, species-specific polymerase chain reaction (PCR) was used to identify Enterococcus faecalis from patients suffering community-acquired urinary tract infection (UTI) in Vietnam and from poultry living in close contact with the patient. Isolates were characterized by multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) to investigate whether poultry serve as a reservoir for human E. faecalis infections. In addition, minimum inhibitory concentrations (MIC’s) were determined for all urine isolates for the following antimicrobials: ampicillin, amoxicillin, chloramphenicol, daptomycin, erythromycin, gentamicin, kanamycin, linezolid, moxifloxacin, penicillin, salinomycin, streptomycin, synergic, tetracycline, tigecycline and vancomycin.

E. faecalis was identified from 19% (n=57) of all UTI patients (n=300). In 54.4% (n=31) of these cases E. faecalis was also isolated from cloacal swabs from poultry raised at the patients household. In seven cases, the same MLST type (ST 16 (4 isolates), ST 93, ST 141 and a new type with the alleles; 9, 6, 7, 6, 11, 11, 8) was isolated from urine and poultry. PFGE typing showed identical patterns for urine and poultry isolates suggesting that poultry seem to serve as a reservoir of the human UTI E. faecalis infections. The majority of E. faecalis isolated from urine belonged to the MLST type ST 16 (51.8%, n=29), 17 (29.3%) of E. faecalis isolates demonstrated high-level gentamicin resistance (MIC ≥1024 μg/ml).

Demonstration of identical ST16 and PFGE types of E. faecalis from human UTI patients and poultry in close contact provides further evidence of the zoonotic potential and global spread of this clone which recently was reported associated with endocarditis patients and pigs in Denmark.

In conclusion: Poultry and pigs seem to represent a reservoir of human E. faecalis infections including high-level gentamicin resistant E. faecalis, the zoonotic potential of which should be further investigated.

Imported symptomatic Hantavirus infection in three family members travelling from Cuba

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Objectives: To monitor imported emerging and re-emerging viral infections in order to prevent potential local outbreaks.
Methods: Three imported cases of hantavirus pulmonary syndrome (HPS) in family members travelling from Cuba are reported. Etiologic diagnosis was based upon detection of specific IgM and IgG using ELISA and IFA commercial assays.

Results: From 13 to 30 August 2010 three family members (father, 59 yrs old, son, 29 yrs old and daughter, 28 yrs old) visited rural areas, natural reserves and caves in Cuba. Following their return to Italy, on September 13 the brother and the sister were hospitalized with high fever, mild dyspnea and bilateral diffuse interstitial infiltrates. Laboratory data, on admission, showed mononcytosis, slightly alteration of aspartate aminotransferase (AST), γ-glutamyl transferase (GGT), erythrocyte sedimentation rate (ESR) and C-reactive protein concentration. At the same time, the father showed milder symptoms, which worsened some days later, while the clinical picture of the two sons improved.

A 46-year-old male smoker with a history of chronic alcoholism was admitted for 39°C, sore throat, myalgia and headache. He had no sputum production, no dyspnea and no chest pain. Physical exam revealed no remarkable findings. Enzymatic and sedimentation tests were normal. On admission, CRP, sedimentation rate (ESR) and C-reactive protein concentration. At the same time, the father showed milder symptoms, which worsened some days later, while the clinical picture of the two sons improved.

Conclusions: Three imported hantavirus infection cases are reported. No information is available on hantavirus infections in Cuba in WHO, CDC and ECDC databases, while presence of hanta virus infection in Southern USA, Central and Southern America is known. Thus, this is the first report of human hantavirus infection imported from Cuba.

A severe H1N1 and Legionella co-infection necessitating extracorporeal membrane oxygenation treatment

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The most common co-pathogens occurring among patients with 2009 pandemic influenza A (H1N1) are Streptococcus pneumoniae, Staphylococcus aureus, Haemophilus influenzae, and occasionally other Gram-negative bacilli. Here we describe the first case of a co-infection with influenza A (H1N1) plus Legionella pneumophila in Austria.

A 46-year-old male smoker with a history of chronic alcoholism was admitted for 39°C, sore throat, myalgia and headache. He had no sputum production, no dyspnea and no chest pain. Physical exam revealed BMI 22 kg/m², 39.4 °C, HR 120 beats/min, BP 135/70 mm Hg, RR 28 breaths/min, fine basal crackles and was otherwise unremarkable. Leukocytes were 13 G/L, CRP 347 mg/L, procalcitonin 7.93 ng/mL. Other remarkable diagnostic laboratory results were hyponatraemia (131 mmol/L), and elevated creatine kinase (1869 U/L). The chest X-ray showed extensive infiltrates and a small pleural effusion on the left lung. Within hours after admission he was transferred to ICU for septic shock, and mechanical ventilation. Microbiological assessments, results and the clinical course are shown in the Figure.

This case highlights that also in the “influenza season” immediate testing for legionella is clinically useful in patients at risk.

P1002 Lymphogranuloma venereum variant L2b-specific PCR: insertion used to close an epidemiological gap

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Objective: After the outbreak of Lymphogranuloma venereum (LGV) in 2003 in The Netherlands, the disease appears to be endemic among men who have sex with men in many industrialized countries. In most cases, Chlamydia trachomatis serovariant L2b represents the causative organism. We developed a new L2b-specific real-time PCR based on the pmph gene to circumvent time-consuming and laborious ompA sequencing.

Methods: We sequenced the pmph gene (2952bp) of two L2b-containing clinical specimens. To our surprise several unique differences compared to C. trachomatis L2 were detected (Table 1). We identified a 9-bp insertion followed by a single mutation for L2b. These two heterogeneities were exploited to develop a new L2b-specific PCR. Specificity and sensitivity of the assay were then evaluated.

Additionally, one single-nucleotide polymorphism was found within the previously developed MGB-probe of a widely used LGV-specific real-time PCR assay.

Results: Our new L2b-specific real-time PCR using a MGB-probe was tested for specificity with all known C. trachomatis reference serovars, 31 micro-organisms found in the local body sites for sampling, 10 rectal swabs from healthy men, and 60 ompA-based L2b-positive clinical specimens from Switzerland, Italy and The Netherlands. Only the samples which had been previously diagnosed as L2b by ompA sequencing were identified using the new assay. The general C. trachomatis cryptic plasmid PCR was 10–50 times more sensitive than the L2b-specific PCR. Concerning LGV-specific PCR, we adjusted our previously published probe using degenerated bases. Compared to the old LGV-specific PCR, we observed a slightly increased analytical sensitivity of factor 2 to 20 for detection of L2b. But as compared to the previous version no additional positives were identified testing a collection of clinical samples with suspicion of LGV organisms.

Conclusions: Mapping the spread and prevalence of C. trachomatis variant L2b may answer important epidemiological questions needed for disease control and prevention. Therefore, a fast and highly accurate detection assay is a prerequisite. Our first L2b-specific test fulfills all these requirements omitting the laborious ompA sequencing step.

| Position | Nucleotide variation | Amino acid variation |
|----------|---------------------|---------------------|
| 404      | C to T              | Pro to Leu          |
| 754      | G to A              | Ala to Thr          |
| 1664     | G to A              | Arg to Lys          |
| 1271-1779| TCTGAAGTAGT (1669-1719) | Ser to Ser |
| 1780     | A to G              | Thr to Ala          |
| 2073     | C to T              | Met to Ala          |
| 2972     | C to T              | Ala to Val          |

Amplicon sequence variations of L2b (EF687158, EF812788) compared to L2 (EF161066)

Table 1: Sequence variations within the pmph gene of C. trachomatis L2 and L2b.
Infective endocarditis caused by *Nocardia cyriacigeorgica*, the first case reported

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*Nocardia cyriacigeorgica* was first reported in 2001, and has been described as an emerging pathogen in the United States. We present a case of infective endocarditis (IE) caused by *N. cyriacigeorgica* in idiopathic thrombocytopenic purpura (ITP) patient with prolonged corticosteroid therapy.

**Case report:** A 62-year-old Thai female was admitted to a tertiary-care university hospital with a 3-week history of high grade fever without other symptoms. She was previously diagnosed of ITP for 1 year. The patient was on prednisolone and had a reducing dose to 10 mg/day since 4 weeks after improvement of her condition. On examination, the patient was alert with temperature 39°C, heart rate 78/min, blood pressure 82/57 mmHg and respiratory rate 20/min. Heart sounds revealed a pansystolic murmur grade III/VI over the apex radiate to axilla. No vascular or immunological phenomenon was present. The rest of physical examination was unremarkable. Transthoracic echocardiography (TTE) revealed a 9.27*6.6 mm oscillating mass attach to anterior mitral valve leaflet. Four sets of blood cultures revealed *Nocardia spp.* with subsequently identified as *N. cyriacigeorgica* by using 16S ribosomal DNA and secA1 gene sequencing. IE was diagnosed.

From recent study in Thailand, *N. cyriacigeorgica* was the third most common species (13.5%) after *N. farcinica* (35.4%) and *N. beijingensis* (18.8%) by using 16S ribosomal DNA sequencing. The organism has been reported from cases of septicaemia, brain abscesses, pleuropulmonary infection and keratitis. *Nocardia spp.* are a rare cause of endocarditis. Only 17 cases of *Nocardia endocarditis* have been reported in the literature. There has been a reported case of *N. cyriacigeorgica* isolated from abscess of thigh and anterior chest wall in patient with suspected IE but negative blood culture. To our knowledge, this is the first case of definite IE caused by *N. cyriacigeorgica* that was successfully treated with antibiotics and valve replacement.

Q fever across the Dutch border in Limburg province, Belgium

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**Objective:** Recently the Netherlands experienced a large outbreak of human Q fever. We present the first data on Q fever in Limburg, the Belgian province which borders the outbreak area.

**Methods:** Data were compiled from three different sources. Firstly, data (Jan–Nov 2009) from the Belgian Q fever reference laboratory were reviewed. Ratios of samples with elevated levels of IgM phase II antibodies were determined. Secondly, a multi-centre prospective survey was started in Limburg in April 2010. Five clinical hospital laboratories each consecutively collected 20 serum samples from patients for whom clinicians requested *Mycoplasma pneumoniae* but not Q fever serology. Only samples with negative *Mycoplasma* IgM results were included. Methods to detect C. burnetii exposure depended on the symptom duration.

Thirdly, data (Nov 2009–Sept 2010) from the Belgian livestock screening program, which started in winter 2009–2010, were analyzed. The program 1. Investigated all compulsory reported abortions (RT-PCR); 2. Systematically tested tank milk on dairy sheep and goat farms of >10 animals (RT-PCR and serology); 3. Randomly performed serological screening of cattle farms.

**Results:** For 2003–2006, elevated IgM II antibody levels were found in 2%, 1%, 1%, and 1% of 1084, 1122, 1485, and 1447 human clinical samples from the national reference laboratory respectively. In 2007, an increase to 60% (4%) positive samples out of 1656 was notified. In Limburg, 5% (4 out of 75) of samples were positive in that year. The positive ratio dropped in 2008–2010 to 1% for both Belgium and Limburg.

In the prospective provincial study, evidence of an acute *C. burnetii* infection (positive PCR) was found in 3 out of 100 human clinical samples. Livestock screening in Limburg revealed a seroprevalence of 10% in randomly selected cows; 42% of 45 tested cattle farms contained seropositive cows. *C. burnetii* was detected in 17% of 129 cattle abortions. In contrast, no Coxiella was found in the 4 goat and 3 sheep abortions that were reported. Tank milk showed evidence for *C. burnetii* in 0 out of 1 dairy sheep, and 1 out of 9 dairy goat farms.

**Conclusion:** *C. burnetii* is highly prevalent in Limburgian livestock, especially in cattle, and is also causing human infections. The ratio of positive human samples from the national reference centre has remained stable over 7 years (1–2%), with just a temporary increase to 4–5% in 2007, the year of onset of the notorious outbreak in the Netherlands.

Prevalence of *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum, Rickettsia spp.* and *Babesia spp.* in ticks removed from humans in the area of Belluno, Italy

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**Objectives:** To investigate the prevalence of 4 human pathogens in *Ixodes ricinus* hard ticks removed from humans, in order to assess the potential risk of transmission of human pathogens vectored by ticks resident in the mountain area of Belluno, close to Dolomites.

**Methods:** A total of 275 ticks were collected in 2009 (n=102) and 2010 (n=173) removed from patients at the emergency ambulatory care of 3 hospitals in the area of Belluno, located in Belluno city, Agordo and Pieve di Cadore. Ticks were collected during spring-summer and counted 35% of adults, 65% of nynphae and 2% of larvae. Each tick was individually processed for DNA extraction with bioMérieux® NucliSens® miniMAG system. Extracts were preliminarily checked with specific primers targeting *Ixodes ricinus* mitochondrial DNA. Each sample was screened by Real-Time TaqMan PCR for *Borrelia burgdorferi* sensu lato (fla gene), *Anaplasma phagocytophilum* (msp2 gene) and *Rickettsia spp.* (gltA gene), all performed on an Applied Biosystems® 7300 Real-Time PCR system. A conventional PCR was adopted for detection of *Babesia spp.*

**Results:** 19 of 102 of ticks collected in 2009 resulted positive for *B. burgdorferi* sl, 4 for *A. phagocytophilum*, 8 for *Rickettsia spp.* and 3 for *Babesia spp.* In 2010 positive samples counted 14% of *B. burgdorferi* sl (24/173), 3% of *A. phagocytophilum*, 16% of *Rickettsia spp.* and 9% of *Babesia spp.* Four tick result co-infected with *B. burgdorferi* sl and *A. phagocytophilum*, 3 with *B. burgdorferi* sl and *Rickettsia spp.*, one with *B. burgdorferi* sl and *Babesia spp.* and one with *A. phagocytophilum* and *Babesia spp.* No sierological screening was included in the study but one case of borreliosis acquired after the bite of a tick resulted positive for *Borrelia burgdorferi* sl was documented clinically and sierologically.

**Conclusion:** Residents of Belluno area are often exposed to ticks and thus are at risk of acquiring infection with multiple tick-borne pathogens. Epidemiological reports are mainly focused on Tick Borne Encephalitis virus infection and borreliosis, endemic in the area of Belluno. Infections caused by tick-vectored *A. phagocytophilum, Rickettsia spp.* and *Babesia spp.* could be misdiagnosed because serological test are performed only in reference centres and clinical diagnosis could be critical. Data obtained from the study suggest that borreliosis is not the only diagnosis to be considered along that could by transmitted to humans by hard tick bite.
**P1006 Intracellular growth and survival of Vibrio cholerae in human macrophages**

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**Objectives:** Vibrio cholerae is Gram-negative bacteria found in water and it can be carried by sea living animals, such as shellfishes. V. cholerae O1 and V cholerae O139 produce cholera toxin and cause cholera in humans. Despite that cholera infects millions and kills many thousands of people worldwide. The combination of increased water temperature and salinity may contribute to increased association rates of the bacteria with sea living animals or protozoa. Author doctoral thesis showed that V. cholerae O1 and O139 grew and survived inside the protozoa Acanthamoeba castellanii. The current project is financed by the Swedish Civil Contingencies Agency (MSB), and is aimed to study the intracellular growth and survival of V. cholerae.

**Methods and Microorganisms:** Vibrio cholerae O1 classical, V. cholerae El Tor wild type, V. cholerae El Tor outer membrane mutant, V. cholerae O139 wild type, V. cholerae O139 capsule mutant, V. cholerae O139 capsule/LPS O-side chain double mutant, human macrophage THP-1 and human macrophage U937. An antibiotic assay used to differentiate between extracellular and intracellular V. cholerae. Vibrio species cultivated with human macrophages. Gentamicin used to kill extracellular bacteria. Intracellular growth and survival examined by viable count, fluorescent microscopy and RNA detection. Intracellular localising visualized by confocal- and electron microscopy. Roles of bacterial capsule, lipopolysaccharide O-side chain and outer membrane protein A on the intracellular growth and survival of V. cholerae.

**Conclusions:** The intracellular behavior of V. cholerae may induce cell mediated and humoral immunity and explains complexity of V cholerae and aids to new strategies for vaccination as well as treatment against cholera.

**P1007 Clinical and epidemiological features of Chikungunya fever in returning travellers**

P. Papineni*, M. Armstrong, T. Doherty (London, UK)

**Objectives:** Data on the epidemiology and clinical course of chikungunya virus infection in returning travellers to the UK is scarce. The Indian Ocean Islands, where outbreaks have been identified, are a popular holiday destination for British travellers, and there is a large immigrant population from the Indian sub-continent in London, who visit friends and relatives in endemic areas. Here we present a case series from ten years experience of managing patients diagnosed with chikungunya fever at the Hospital for Tropical Diseases (HTD), London.

**Methods:** Patients were identified from a database of patients presenting to the HTD inpatient and outpatient services from 2000 to 2009. Retrospective analysis of case notes to identify clinical and epidemiological features was conducted. Serological testing was performed by the Health Protection Agency’s (HPA) Special Pathogens Reference Unit.

**Results:** 17 patients were identified. The majority of patients had visited Mauritius (56%). 4 had visited India and 3 patients had travelled to Sri Lanka. All patients reported symptoms of fever and arthralgia. In 44% of patients, the presentation included headache and 56% reported macular erythematous rash. Only 2 patients had documented pyrexia (greater than 38 degrees celsius) at presentation to HTD. 13 patients had full blood count performed and 15 patients had serological testing. Thrombocytopenia (platelet count less than 150,000/microL) was only present in one patient. Lymphopenia (lymphocyte count less than 1.3/microL) was only observed in 2 patients; these were both laboratory confirmed cases by polymerase chain reaction (PCR). 11 patients had acute serology positive for chikungunya. 4 patients had negative acute serology, but were confirmed chikungunya by PCR. Convalescent serology on 3 of these patients then demonstrated seroconversion with chikungunya IgG detected.

**Conclusions:** There is a risk to British travellers to chikungunya-endemic areas, particularly Mauritius. The absence of thrombocytopenia and lymphopenia may help to distinguish chikungunya infection from dengue and serological testing is useful in suspected cases.

**P1008 Crimean-Congo haemorrhagic fever: six-year experience of a secondary care hospital in the epidemic region**

C. Kader*, A. Erbay, S. Bukić Özhay (Kastamonu, Yozgat, TR)

**Objectives:** Crimean-Congo hemorrhagic fever virus (CCHFV) is the most widespread tick-borne virus that causes disease in humans. Since 2002, a rapid emergence of CCHF in the central, northern and eastern regions of Turkey has occurred and CCHF has become a public health problem in Turkey. The aim this study was to evaluate the patients characteristics followed at a secondary care hospital at the CCHF epidemic region.

**Methods:** Retrospective cohort study. Among the patients who were followed up during the spring and summer of 2005–2010, patients with IgM antibodies or PCR results positive for CCHFV in blood were included in the study. The demographics, clinical and laboratory findings were examined for each patient from the medical records.

**Results:** 281 patients were followed up between 2005 and 2010. Male patients accounted for 121 (43.1%) of the patients and the mean age was 48.8 ±19.8 years. 251 (90%) of the patients were living in rural regions. Husbandry and raising livestock were the most common occupations (73%). Mean duration of symptoms was 1.6 ±1.2 (range 1–7) days before hospital admission. Initial complaints were myalgia (79.7%), headache (59.8%), nausea (57.1%), fever (38%), vomiting (12.5%) and bleeding (2.1%). History of tick bite was present in 262 (93.6%) cases. Time between tick bite and clinical symptoms were 3.9 ±1.6 days. Clinical findings were as follows: fever >38°C (38.8%), epistaxis (2.1%), splanomegaly (1.4%), somnolence (1.1%), melaena (1.1%), gingival bleeding (0.7%) and ecchymosis (0.7%). Almost all of the patients had leukopenia (99.6%), thrombocytopenia (100%), and elevated AST (93.6%), ALT (93.6%), LDH (60.9%) and CPK (96.8%) levels at admission. Thrombocyte level was <20000/mm3 in 20.6% of the patients. Ribavirin was initiated 84.7% of the patients at the admission day. 61.9% of the patients received platelets and 30.6% erythrocyte infusions. 142 (50.5%) patients transferred to tertiary care facilities. The main reason for transfer was deterioration of the clinical status (85.9%). Overall fatality rate was 3.6%, mortality was seen only in transferred patients.

**Conclusion:** Most of the reports about CCHF were from tertiary care centers. Patient profile, clinical symptoms, laboratory findings and case fatality rate were much more milder in secondary care compared to tertiary care centers.

**P1009 All-cause mortality among hospitalised CDI patients is associated with PCR-ribotype 027 but independent of the presence of binary toxin**

M.P. Hensgens*, A. Goorhuis, O.M. Dekkers, E. Kuipers (Leiden, NL)

**Objectives:** Incidence and mortality of patients with Clostridium difficile infections (CDI) have increased since the hypervirulent PCR ribotype 027
027 emerged in 2002. CDI due to this type is associated with mortality up to 25% after 30 days. This is often due to underlying comorbidities, but estimated attributable mortality still accounts for about 4% or all deaths. Since reliable mortality data are scarce, we performed a large cohort study in an endemic setting in the Netherlands. A second aim of this study was to determine the association of 30-day mortality with *C. difficile* PCR-ribotype.

**Methods:** From July 2006 to the end of April 2009, 13 Dutch hospitals participated in a surveillance study investigating the incidence of CDI, clinical course and outcome. All hospitalized patients with a first episode of diarrhea and a positive assay for the toxin of *Clostridium difficile* were included. Survival status was obtained of all patients via the Dutch Civil Registration System. We used a Cox-regression analysis to identify determinants associated with death within 30 days.

**Results:** We identified 1367 patients with CDI, corresponding with an incidence rate of 13 per 10,000 admissions. The cumulative all cause mortality was 13% after 30 days, 30% after 6 months and 37% after one year. Data on PCR-ribotype and the presence of binary toxin were available for 685 patients (50%). 22% of the 55 patients with CDI due to type 027 died within 30 days, compared to 15% of the 98 patients with non-027/binary toxin positive CDI and 11% of the 399 patients with non-027/binary toxin positive CDI and 11% of the 399 patients with non-027/binary toxin negative CDI. Death within 30 days was significantly associated with age, Charlson comorbidity index and PCR ribotype 027 and remained associated after adjustment for age. The presence of binary toxin in non-027 strains, was not significantly associated with mortality.

**Conclusion:** Mortality among CDI patients is high, even in an endemic situation. All cause mortality after 30 days was independent of the presence of binary toxin, but depended on age, underlying diseases and PCR ribotype 027.

**Emerging infectious diseases**

**0101** Characteristics of microsporidial keratoconjunctivitis in an eastern Indian cohort: a case series
S. Saha*, A. Khetan, D. Banerjee, J. Sengupta (Midnapore West, Kolkata, IN)

To determine the characteristics of microsporidial keratoconjunctivitis in an otherwise immunocompetent group of patients attending the cornea care unit of Kolkata (eastern India) based tertiary care eye hospital. A retrospective, noncomparative, observational case series involving patients with microsporidial keratoconjunctivitis from June 2009 to September 2009. Of the 24 patients identified, microbiological treatment received before presentation, clinical characteristics, treatment offered and resolution time with sequel. The management consisted of simple debridement and application of chloramphenicol ointment (1%) 2 times a day till healing of epithelial defect. Mean age of onset was 18.7 yrs (95% CI 15.7–21.7; range 11–36 years). All patients gave history of wetting in the rains prior to episode. Predisposing activities included playing soccer (54.5%), cricket (18.2%), golf (13.6%) and rugby in 1 patient. Antecedent treatment comprised of Acyclovir eye ointment (45.4%), antibiotic eye drop (27.3%) most commonly. Microsporidia was identified in Grams stain (81.8%), KOH (72.7%), Modified ZN staining (36.4%) and Giemsa (18.2%). Majority presented as unilateral superficial keratoconjunctivitis with punctate epithelial keratitis except one with bilateral disease. Mean resolution time was 9 days (95%CI 7.9–10.2).

Microsporidial keratoconjunctivitis can occur in normal patients with exposure to rain and mud, related to outdoor activity often misdiagnosed as viral ocular infections. Strong clinical suspicion with proper microbiological evaluation helps to diagnose this commonly missed condition.

**0102** Rapid recognition of *Clostridium difficile* PCR ribotypes 027 and 078 using MALDI-TOF mass spectrometry
C.W. Knetesch, J. Corver, E. Kuijper* (Leiden, NL)

**Objective:** In the past decade, incidence of *Clostridium difficile* infections (CDI) with more severe disease increased and coincided with the emergence of hypervirulent PCR ribotype 027. Recently, a new virulent strain has been noticed in Europe which was characterized as
Invasive disease caused by *Haemophilus influenzae* serotypes e and f in England and Wales

A. Vickers*, C. Crawford, D. Litt, S. Ladhani, M. Slack (London, UK)

**Objectives:** To analyse epidemiological data on invasive *H. influenzae* disease caused by serotypes e and f (Hie and Hif) between 2000 and 2010, and to characterise the clinical bacterial isolates using multilocus sequence typing (MLST).

**Methods:** The UK Health Protection Agency routinely collects epidemiological data (patient’s age, sex and broad clinical presentation, and the serotype of the bacterial isolate) for all cases of invasive *H. influenzae* disease. After 2008, clinicians were asked to complete a questionnaire for all invasive *H. influenzae* cases and provide detailed clinical information relating to underlying co-morbidities, specific clinical presentation, case fatality and cause of death. This study analysed data on invasive Hie and Hif cases diagnosed between 2000 and 2010 inclusive. Clinical isolates were collected from the majority of cases. MLST was performed using standard methods.

**Results and Conclusions:** The number of cases of invasive disease caused by serotypes e and f (Hie and Hif) between 2000 and 2010, and to characterise the clinical bacterial isolates using multilocus sequence typing (MLST).

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**P1016** Comparative genetic characterisation of human and animal isolates of methicillin-resistant *Staphylococcus aureus* ST398

D. Jamroz*, M. Sharma, M. Fielder, A.E. Kelly, P. Butaye, R. Ehrlich, S. Monene, N. Coldham (New Haw, London, UK; Brussels, BE; Jena, Dresden, DE)

**Objectives:** The recently emerged livestock-associated methicillin-resistant *Staphylococcus aureus* ST398 poses a potentially significant risk to both farming industry as well as persons in contact with food-producing animals. In this study a panel of MRSA ST398 strains were subjected to comparative genetic characterisation to identify markers that distinguish the strain from other lineages of *S. aureus*.

**Methods:** Fifty six strains were characterised consisting of: MRSA ST398 (N = 18) and non-ST398 *S. aureus*: MSSA derived from cattle (N = 18), MSSA and MRSA derived from humans (N = 20). All isolates were subjected to PFGE, spa typing, and sequence type/clonal complex assignment. Strains were characterised by Identibac MRSA isolates were subjected to PFGE, spa typing, and sequence type/clonal complex assignment. Strains were characterised by Identibac MRSA array tube (version pm5.5), which allows detection of *S. aureus*-associated virulence and antimicrobial resistance genes. In addition MRSA strains only were SCCmec typed, screened by PCR for presence of additional antimicrobial resistance genes (ermT, dfrK, tetL) and subjected to antimicrobial susceptibility testing by broth microdilution method according to CLSI.

**Results:** Strains belonging to ST398 were found to harbour no lineage-specific virulence determinants. The only genes detected in MRSA ST398 panel were those found in all or majority of characterised strains: hla, hld, hlgA, lukS, lukF, enoX (SAV1601), entY and sarA. All MRSA strains were found to carry at least one additional resistance gene other than mecA. Previously described novel resistance genes such as dfrK were detected among MRSA ST398 strains only. Erythromycin resistance was common among all MRSA strains. All strains belonging to ST398 were resistant to tetracycline, with resistance to clindamycin (N = 14), trimethoprin (N = 13) and gentamicin (N = 9) also being common. Resistance to tiamulin (N = 4) and florfenicol (N = 3) was also detected. Human MRSA strains belonged to CC22 and CC30, with all showing resistance to ciprofloxacin and the majority being susceptible to the remaining antimicrobials tested.

**Conclusions:** MRSA ST398 strains appear to lack major virulence factors in comparison to other lineages of both animal and human derived *S. aureus* strains. MRSA ST398 also displays high frequency of antimicrobial resistance elements including presence of novel resistance genes. While such multi-drug resistance would pose a considerable therapeutic challenge the apparent lack of significant virulence genes would suggest low pathogenic potential.

**P1017** Essentiality of dUTPase mediated by its Mycobacterium-specific structural motif identifies a novel TB drug target

I. Peci*, R. Hrmondo, A.C. Brown, A. Lopata, T. Parish, V.G. Beata, J. Toth (Budapest, HU; London, UK)

**Objectives:** Thymidine biosynthesis is an essential metabolic pathway in all cells. dTTP is synthesized via the uracil-containing nucleosides dUTP and dUMP. To fulfill the task of dTTP synthesis, three major pathways exist in humans, two de novo and one salvage pathway. In mycobacteria, only one of these is present; this involves the action of the enzyme dUTPase (dut) whose role is to eliminate excess dUTP. dUTPase has been implicated as a potential drug target due to its central role in mycobacterial thymidylate synthesis and to its essentiality in *E. coli* and *S. cerevisiae*. All known mycobacterium genomes contain dUTPases of 85% sequence identity which possess a mycobacterium-specific motif that distinguishes them from the human dUTPase. The purpose of this study was to obtain formal genetic proof of essentiality of dut in mycobacteria and to assess the physiological role of the insert present solely in mycobacterial genomes.

**Methods:** Experiments were performed in *M. smegmatis* to disrupt the dut gene via two-step homologous recombination. Merodiploid strains were generated to assay functional complementation of the wild-type or mutant dut. The mutant contained a five-amino-acid deletion of the mycobacterium-specific insert.

**Results:** Initial attempts to obtain mutant double crossover strains (DCO) with the disrupted dut were unsuccessful. This provided preliminary evidence that the gene might be essential. To prove this, we introduced a functional copy of dut into a well-defined chromosomal site and screened for DCOs in this merodiploid background. Southern blot analysis confirmed that we could obtain the disrupted chromosomal copy of dut in this background. Then we attempted to complement the lethal mutant phenotype with the mutant dut gene lacking the mycobacterium-specific insert. The screening procedures resulted in a wild type and a spontaneous sucR single cross-over cell line; the remaining 86 colonies were nonviable in the applied selection conditions.

**Conclusions:** These results provide formal genetic proof of dut essentiality in *M. smegmatis*. Additionally, the mycobacterium-specific motif exposed on the protein surface is necessary for complementation of the dut function. As this genus-specific structural motif is not present in the human dUTPase and is required for mycobacterium viability we propose that targeting this site will potentially yield an efficient and specific antimycobacterial treatment.

**P1018** West Nile fever outbreak in humans in northern Greece

D. Karabaxoglou*, A. Karabournitis, G. Kagalou, E. Vassiliadou, F. Kamaria, D. Chrysogios, A. Bakas, A. Kansozidou (Thessaloniki, GR)

West Nile Fever (WNV) is a viral disease transmitted by mosquitoes and is distributed worldwide. Sporadic human cases, clusters or outbreaks have been reported from European countries. WNF usually is a febrile illness characterized by fever, headache, manulopapular or roseolar rash, lymphadenopathy and many other symptoms. Occasionally, aseptic meningitis or encephalitis occurs.

**Aims:** The aim of the report is the recording and study of the WNV outbreak that has been observed in Northern Greece, during the period July-September 2010. Patients with febrile-rash illness (followed by leucopenia and thrombocytopenia) or with Central Nervous System infection were included in the study.

**Methods:** A total of 119 patients, 112 adults (56 males, 56 females) aged 18–93 years old and 7 children (5 males, 2 females) aged 7–12 years old, were examined. Of the total patients, 35 were hospitalized with central nervous system infection (34 adults, 1 child), 73 with febrile-rash illness (67 adults, 6 children) and 11 with febrile illness (all adults). In patients with suspected WNV infection serum samples were tested for the presence of IgM and IgG antibodies against WNV. In 14 patients a second serum sample was available. WNV specific antibodies were detected with an indirect qualitative ELISA (Focus Diagnostics, USA). The diagnosis of WNF was based on positive titers of IgM or/and IgG specific antibodies.

**Results:** WNV neuroinvasive disease was confirmed in 23 adult patients and in 1 child (rate 68.6%). In these patients specific IgM and/or IgG antibodies were detected in serum specimens. WNV neuroinvasive infection was confirmed in 17 patients older than 50 years and 8 of them died (case-fatality rate 34.8%). In 33 patients (29 adults, 4 children) out of the 73 patients with febrile-rash illness and in 5 out of 11 adult patients with febrile illness specific WNV antibodies were detected, rate 45.2% and 45.6% respectively. In these patients specific IgM antibodies were mainly detected in serum samples. WNV febrile-rash disease was documented in 25 patients aged less than 50 years old. In patients in which a second serum sample was examined both IgM and IgG WNV antibodies were detected.

**Conclusions:** This is the first time that WNV outbreak has been documented in humans in Greece. Infection of CNS occurred mainly in patients older than 50 years and febrile-rash disease in patients aged less than 50 years. Most fatal cases have been observed in patients older than 50 years.
Using real-time PCR for detection of Yersinia pestis in field samples from Mountain Altai natural focus of plague

M. Afanas’ev*, E. Chipanin, V. Shestakov, A. Denisov, L. Fomina, A. Oystyak, S. Balakhonov (Irkutsk, RU)

Objectives: Plague is an extremely dangerous infection disease caused by Yersinia pestis. Presence in the Russian territory (particularly, in the Siberia) active natural foci of plague needs effective monitoring measures that based on modern diagnostic methods. The main goal of this study was to construct and testing of real-time PCR (RT-PCR) for fast and robust Y. pestis detection in field laboratory condition during examination of natural foci of plague.

Methods: For detection of Y. pestis in field samples we constructed SYBR green-based RT-PCR assay followed by melting curve analysis for reaction’s specificity estimation adapted for programmed real-time machine “Smart Cycler” (Cepheid, USA). Samples of ectoparasites (fleas and ticks) (n=1206) and rodents and lagomorphs (n=264) were collected in September 2010 in Kuray and Sailugem range’s sectors of Mountain Altai natural plague focus. Ectoparasites were obtained from small mammals and its borrows and nests. Bacteriological testing of samples was performed according to WHO recommendation. DNA extraction from suspension of ectoparasites was carried out by boiling. Same procedure for homogenate of small mammals organs was performed by DNA-sorb Extraction Kit (ILS Ltd., Russia). Strain Y. pestis EU was used as a control for all microbiological and genetic procedures. Y. kristensenii, Y. intermedia, Y. pseudotuberculosis, Y. enterocolitica, Y. frederiksenii were used for specificity control of suggested assay.

Results: RT-PCR assay was constructed and tested in field conditions. Among ectoparasites included in the study Dermacentor nutalli, Paramonosyllis scolanae, Paradoxopsyllus scodoumoci and Clethryphillus hirticus were prevalent (24.2%, 292/1206; 22.8%, 275/1206; 18.4%, 222/1206 and 16.5%, 199/1206). Ochotona pricei was a dominating species among studied small mammals (77.3%, 204/264). Eight Y. pestis strains were isolated by traditional microbiological methods: six (66.7%, 6/9) — from fleas and three (33.4%, 3/9) — from rodents and lagomorphs. Eleven positive results were obtained by RT-PCR assay. These positive samples included eight that were identified as Y. pestis positive by microbiological methods.

Conclusion: Proposed RT-PCR assay demonstrated excellent sensitivity and specificity and may be consider as a good, robust and simple method for field screening studies in natural foci of plague.

Clonal distribution of Streptococcus pneumoniae serotype 19A isolated in Malaysian hospitals

R Md. Yasin*, K.N. Mohd Khaled, R. Issa, N. Ahmad (Kuala Lumpur, MY)

Objectives: Streptococcus pneumoniae serotype 19A has emerged all over the world in recent years. In 2008—2009 we see a sudden rise of this serotype in Malaysia representing second most common (9.68%) serotype of all invasive isolate. The aim of this study was to characterize pneumococcal strains of the 19A serotype received from various hospitals.

Methods: Multilocus sequence typing (MLST) based on 7 housekeeping genes areE, gdh, gki, recP, spa, xpt and dsl were carried out to determine the population structure of pneumococcal strains serotype 19A. Penicillin, amoxiclavulanic acid, ceftriaxone, erythromycin, meropenem and levofloxacin MICs were determined using the E-test method (AB Biodisk, Solna, Sweden). MICs were interpreted using CLSI guidelines. Serotyping was performed by latex agglutination (pool latex antisera and factor sera; Statens Serum Institut, Copenhagen, Denmark).

Results: From a total of 31 pneumococcal isolates of serotype 19A received at our laboratory between 2008 and 2009, 21 (67.4%) were invasive isolates (bacteremia, meningitis, and pleural effusion). Only 25 including 17 invasive isolates were included in this study. Penicillin MIC of the 25 strains ranged from 0.016–2 μg/ml and 17 (68%) were less susceptible to penicillin including 2 resistant strains (MICs 2 μg/ml). None of the isolates were resistant to levofloxacin while resistant (intermediate) to meropenem was 28% (n=7), ceftriaxone 16% (n=4), and amoxiclavulanic acid 20% (n=5); and resistant to erythromycin was 40% (n=10). Five isolates belonged to multiresistant clones related to sequence type ST320. The other penicillin less-susceptible strains are related to sequence type ST172 (n=4) and one of each ST15, ST166, ST5817, ST5822, ST5818, ST276, ST2013 and ST2855. Penicillin less-susceptible strains belonged to sequence type ST3781 (n=2) and one of each ST5819, ST217, ST1848, ST5820, ST5821 and ST771. Ten macrolide resistant strains related to sequence type ST320 (n=5) and one each ST15, ST1848, ST166, ST5820 and ST276.

Conclusions: Multiple clones were associated with serotype 19A. The worldwide-disseminated multiresistant clones ST30, ST166 and ST276 were also present among our local strains. The clonality of the penicillin less-susceptible strains also differs from the penicillin-sensitive strains.

Coinfection of ticks with Borrelia burgdorferi sensu lato and Rickettsia spp. in densely populated areas of the Po River valley

M. Pajoro*, S. Epis, F. Comandatore, M. Montagna, N. Vicari, M. Fabbi, D. Pistone, P. Marone, C. Bandi (Pavia, Milan, IT)

Objective: Several tick-borne rickettsiae cause diseases in humans and among them Borrelia burgdorferi sensu lato and transovarially and transstadially transmitted microorganisms. The aim of this study was to construct and testing of real-time PCR (RT-PCR) for fast and invasive isolates (bacteremia, meningitis, and pleural effusion). Only 25

Methods: For detection of Y. pestis in field samples we constructed SYBR green-based RT-PCR assay followed by melting curve analysis for reaction’s specificity estimation adapted for programmed real-time machine “Smart Cycler” (Cepheid, USA). Samples of ectoparasites (fleas and ticks) (n=1206) and rodents and lagomorphs (n=264) were collected in September 2010 in Kuray and Sailugem range’s sectors of Mountain Altai natural plague focus. Ectoparasites were obtained from small mammals and its borrows and nests. Bacteriological testing of samples was performed according to WHO recommendation. DNA extraction from suspension of ectoparasites was carried out by boiling. Same procedure for homogenate of small mammals organs was performed by DNA-sorb Extraction Kit (ILS Ltd., Russia). Strain Y. pestis EU was used as a control for all microbiological and genetic procedures. Y. kristensenii, Y. intermedia, Y. pseudotuberculosis, Y. enterocolitica, Y. frederiksenii were used for specificity control of suggested assay.

Results: RT-PCR assay was constructed and tested in field conditions. Among ectoparasites included in the study Dermacentor nutalli, Paramonosyllis scolanae, Paradoxopsyllus scodoumoci and Clethryphillus hirticus were prevalent (24.2%, 292/1206; 22.8%, 275/1206; 18.4%, 222/1206 and 16.5%, 199/1206). Ochotona pricei was a dominating species among studied small mammals (77.3%, 204/264). Eight Y. pestis strains were isolated by traditional microbiological methods: six (66.7%, 6/9) — from fleas and three (33.4%, 3/9) — from rodents and lagomorphs. Eleven positive results were obtained by RT-PCR assay. These positive samples included eight that were identified as Y. pestis positive by microbiological methods.

Conclusion: Proposed RT-PCR assay demonstrated excellent sensitivity and specificity and may be consider as a good, robust and simple method for field screening studies in natural foci of plague.

Pacemaker-related endocarditis: clinical features and microbiological spectrum of 43 consecutive cases

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Introduction-aim: Pacemaker (PM) related endocarditis is a rare but serious complication of permanent transvenous pacing. The reported
incidence varies in the literature from 0.13% to 19.9%. The aim of this study was to evaluate clinical features, microbiological spectrum, echocardiographic findings and outcome of PM endocarditis in two regional hospitals.

Material and methods: We studied retrospectively all the patients who suffered a PM endocarditis in two hospitals between 2001 and 2009. All the patients underwent clinical evaluation, blood and leads cultures and transthoracic or/and transesophageal echocardiography. The diagnosis of endocarditis was considered according to the Duke modified criteria for PM endocarditis.

Results: 43 patients, 31 (72.1%) male and 12 (27.9%) female with mean age of 64 ± 13 years, were included in this study. The presentation was acute in 11 (25.6%) patients (onset of symptoms < 6 weeks after implantation) and chronic in 32 (74.4%) patients (onset of symptoms > 6 weeks). Main comorbid conditions were: chronic heart failure (67.4%), coronary artery disease (62.8%), diabetes mellitus (48.8%), chronic obstructive pulmonary disease (37.2%), hemodialysis (20.9%) and malignancy (9.3%). Most frequent clinical signs were fever (95.3%), malaise (83.7%), anorexia (53.5%) and symptoms of heart failure (48.8%). Local symptoms (erythema, pain, tenderness, skin ulceration) were presented in 16 (37.2%) cases. A positive culture was obtained in 39 out of 43 cases (90.7%), 32 times on blood culture, 27 times on lead culture and 14 times on blood and lead culture. Staphylococcus epidermidis in 24 (61.5%) cases, Staphylococcus aureus in 8 (20.5%), Streptococcus viridans in 3 (7.7%), Enterobacter cloacae in 2 (5.1%), Klebsiella oxytoca in 1 (2.6%) and Candida albicans in 1 (2.6%) cases. 54.5% of staphylococci isolates in acute group were S. aureus and 68.75% in chronic group were S. epidermidis. We found mixed infections in 3 cases. 37.5% of the S aureus isolates and 12.5% of the S. epidermidis isolates were methicillin resistant. TTE showed vegetation in 13 (30.2%) cases while TEE showed vegetation in 39 (90.7%) cases. In all patients removal of the pacemaker and antibiotic treatment was performed. Overall mortality was 18.6%.

Conclusion: Staphylococci were responsible for the vast majority of PM endocarditis in our study, especially S. epidermidis in chronic group and S. aureus in acute group. TTE was required for the diagnosis of vegetations. Mortality was high.

P1023 H1N1 influenza pandemic from June 2010 to November 2010 at a single nodal centre in Hyderabad, India

F Mohammadi*, R Paul, L Natankulco, S Narreddy (Hyderabad, IN)

Objectives: H1N1 influenza began in spring/summer 2009 and spread to all the continents. In India the second wave was between May and November 2010. Here we report the characteristics and outcome of patients at one tertiary care hospital. From June to November 2010, H1N1 Influenza Pandemic emerged in Hyderabad and here we describe the clinical characteristics, the progression, severity and the outcome of 87 Positive patients at one of the Nodal center in Hyderabad, India.

Methods: All the patients who presented with Flu like symptoms in an outpatient as well as Inpatient setting were tested for H1N1 with the use of reverse transcriptase polymerase chain reaction assay.

Results: There were 87 Positive patients with H1N1 Influenza, 62% were in 20–40 year range, of which 67% were males. Twenty seven percent were above 40 years of age. Out of the 84 patients, 93% had symptoms of cough with only 30% showing chest Xray abnormalities. All the patients had Pneumonia or Acute lung injury required Non-invasive Ventilation and 10% of them needing Invasive Ventilation. Of 8 patients who were mechanically intubated, 2 died of ARDS. There were 11% patients who were health care providers from the hospital. Use of statins and steroids along with anti-viral drugs were quite helpful in patients with severe respiratory insufficiency.

Conclusions: Most of the patients presented with mild symptoms. Patients with respiratory distress were admitted in isolation rooms. The length of morbidity was reduced with early use of Anti-viral drugs. Statins and Steroids remarkably decreased the duration of ventilation and length of ICU Care. Non Invasive ventilation was sufficient in majority. The role of statins and steroids needs to be further studied.

is also strongly advised for the health care providers to take preventive vaccinations.

| Characteristic                  | Number | Percent |
|---------------------------------|--------|---------|
| Fever ≥ 38°C                    |        |         |
| Cough                           | 67/84  | 79.3%   |
| CVD                             | 51/84  | 60.7%   |
| MSB                             | 36/84  | 42.5%   |
| Septicarct                      | 44/84  | 52.3%   |
| Contact with confirmed cases    | 31/84  | 37.1%   |
| Chest X Ray Abnormalities       | 25/84  | 29.7%   |
| Admitted                        | 31/84  | 37.1%   |
| Non-invasive Ventilation        | 25/84  | 29.7%   |
| Invasive Ventilation            | 8/84   | 9.5%    |
| Days of Invasive Ventilation    |        |         |
| Mean ± SD days                  | 11 ± 7.1 days |
| Prior Vaccite                   | 2/84   | 2.4%    |
| Shingles                        | 25/84  | 29.7%   |
| Pneumonia                       | 25/84  | 29.7%   |
| Anti Virals                     |        |         |
| HIV                              | 82/84  | 97.5%   |
| AIDS                             | 8/84   | 10.0%   |
| Outcome (mortality)             |        |         |
|                       | 2/84   | 2.4%    |

Bulgaria (7.5 million inhabitants) is known to be an endemic area for Lyme borreliosis and Mediterranean spotted fever (MSF) with about 1000 cases reported per year. Less common are two other tick-borne infections – Q-fever and Crimean-Congo hemorrhagic fever (CCHF). Tick-borne encephalitis (TBE) has not been reported in Bulgaria. In 2008–2010, we have conducted a hospital-based surveillance survey for acute febrile illness (AFI) in 5 different geographical regions in Bulgaria. Acute and convalescent blood samples were collected from 422 AFI patients. Serum samples were tested by commercial ELISA kits for specific IgM and IgG antibodies. Anti-TBE IgM antibodies were found in acute serum samples from 5 patients by Euroimmun ELISA (1.2%). Only one case appeared as meningitis, the other were febrile diseases without neurological manifestation. In addition, 104 (24.6%) cases had serologic evidence of other tick-borne etiologies, including 10 cases of CCHF (2.4%), 36 cases of Q-fever (8.5%) and 53 cases of MSF (12.6%). Five (4.8%) of these 104 established cases of tick-borne infections showed serologic evidence for co-infection with MSF and Q-fiver. Our results highlighted that tick-borne infections are more widespread in Bulgaria than previously thought, especially rickettsioses. Although TBE is believed to be common in North and Central part of Europe, it is obviously not uncommon in Bulgaria, but most probably often presents as unspecified febrile disease only. To our knowledge, this study is the first to provide serologic evidence of TBE in Bulgaria. Further studies are ongoing to evaluate the disease burden of tick-borne disease in Bulgaria using both clinical and other laboratory procedures.
**P1025** Strong relationship between total thiol status and thrombocytopenia in patients with Crimean-Congo haemorrhagic fever

R. Guner, M.A. Tasyaran*, S. Keske, I. Hasangolu, D. Yapı, A. Kaya Kalem, T. Arslan Gulen, S. Isikoglu, S. Neseloghlu, O. Erel (Ankara, TR)

**Objectives:** To determine serum total thiol levels and total oxidant status (TOS) in patients with Crimean-Congo hemorrhagic fever (CCHF) and to investigate relationships of these parameters with thrombocytopenia.

**Methods:** Eighty three patients and 21 controls were enrolled in the study. Serum thiol levels were measured by DTNB method and total oxidant status (TOS) was measured by Erel's method. Thrombocytes were counted by automated hemocounter. Obtained results were compared and relationships among the parameters were investigated by correlation analysis.

**Results:** Serum thiol levels were significantly lower in the patients than those of the controls (220±78 mmol/L and 542±51 mmol/L, p < 0.0001, respectively). Area under curve was 1.0 and, sensitivity and specificity was 100% at 423 mmol/L level. Serum TOS level was significantly higher in the patients (16.63±11.32 mmol H2O2 equiv/L and 7.86±3.59 mmol H2O2 equiv/L, p < 0.001, respectively). There was an important correlation between serum total thiol levels and platelet counts (r = 0.82, p < 0.0001).

**Conclusion:** Serum thiol levels are importantly decreased in the patients with CCHF. This situation, which may be developed as primary or secondary to the disease, may have a significant role in the etiopathogenesis of the thrombocyte dysfunction and bleeding in CCHF. Thiol replacement might be helpful for treatment of CCHF.

**P1027**  

**Tularaemia outbreak in central Anatolia**

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G. Gülten, F. Sezen, I. Sencan (Ankara, TR)

**Objective:** An increase in the number of tularaemia cases was observed in Central Anatolia at the end of the year 2009. This study was aimed to evaluate the clinical characteristics and the efficacy of antibiotic therapy in tularaemia cases occurred in six different provinces of Central Anatolia in the last year.

**Methods:** Specific antibodies were screened by microagglutination test (MAT). Throat swab and lymph node aspirate cultures were obtained and PCR was performed from these specimens.

**Results:** A total of 63 cases were evaluated. Age of the patients ranged from 6–83 (average 41.9), thirty-six of them (54.1%) were females. 80.9% of cases were using spring water. Patients had clinical presentations compatible with oropharyngeal (n=45, 71%), glandular (n=12, 19%) and ocuolocular (4, 6.3%) tularaemia mostly. A patient was presented with pneumonic form and another with laryngeal mass. Initiating symptoms were sore throat (49.3%), fever (31.8%), swollen neck (6.3%) and eye redness (6.3%). Cervical lymphadenopathy (92.1%) and fever (77.2%) were the most common findings at admission. MAT titers ranged from 1:160 to 1:5120. Francisella tularensis holarctica was identified, providing a definitive diagnosis in 50% of the cases who had tested by PCR. Causative agent recovered from cultures in 5 clinical samples of 28 patients (17%). All the patients were treated with antibiotics considered effective against F. tularensis; 45 with aminoglycosides (streptomycin, gentamicin, amikacin) and 18 with quinolones (ciprofloxacin, levofloxacin). However, therapeutic failure was observed in 29% of the cases, which was found to be related to the delay in the initiation of antibiotics. Although the success rate in patients with early treatment (within 21 days after the onset of symptoms) was significantly better, no difference was found between the effectiveness of aminoglycoside or quinolone treatments (p = 0.001 and p > 0.005 respectively).

**Conclusion:** This was the first time emergence of tularaemia epidemic in Central Anatolia although small clusters of cases have been reported before. Treatment was unsuccessful in patients with delayed diagnosis. Tularaemia is an emerging disease spreading to nonendemic regions representing a significant threat for public health.

**P1028**  

**Short-course high-dose rifaximin rescue treatment in CDI – a single-centre experience**

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**Objective:** Clostridium (C.) difficile infection (CDI) is commonly encountered in hospitalized patients undergoing antimicrobial treatments. Recent studies note an increase in disease severity and more often worse outcomes than in earlier years. Metronidazole and/or local vancomycin are still the drugs of choice, failures are increasingly seen in severely compromised patients. Several antimicrobials are recommended in this setting. Rifaximin as a non-absorbable ansamycin is probably useful because of its effect on high stool concentrations and lack of resistance in C. difficile. Goal of this analysis was to examine efficacy and tolerability of HD-rifaximin (HD-RFX) in patients failing standard treatment regimes with metronidazole or vancomycin.

**Patients and Methods:** All patients attending the infectious diseases wards suffering from CDI in the years 2009 and 2010 were analyzed for the presence of treatment failure while using metronidazole or vancomycin with subsequent use of HD-RFX. CDI risk score as well as stool frequency and consistency, vital signs and base labo values were
estimated at presentation and treatment switch. Rifaximin was given after individual informed consent orally 400 mgs TID for one week. Patient's individual outcomes and overall in-hospital mortality were analyzed.

Results: During the observation period 107 patients presented with CDI, 69 were treated with metronidazole, 31 using vancomycin. HD-RFX was given to seven (6.5%). Complete resolution (clinically and microbiologically) of CDI was seen in 7/7. HD-RFX failed in none patient. Crude in-hospital mortality for the HD-RFX treated patients was 12.15% (metronidazole 14.5%, vancomycin (6.5%).

Conclusions: Rifaximin given at high dosages for one week is safe and efficacious in the rescue treatment of CDI. Results from this and other case series warrant larger trials regarding first line treatment with HD-RFX compared to standard medications.

**P1029** Pyosalpinx and pelvic pain due to *Brucella melitensis*

*A. Poulov*, F. Markou, G. Vrioni, A. Tsakris (Serres, Athens, GR)

**Introduction:** Brucellosis remains a worldwide health problem. In Mediterranean countries, it is endemic and is caused primarily by *B. melitensis*. Although there a lot of complications related to brucellosis, soft tissue involvement is rare. In this case report a patient with pyosalpinx caused by *B. melitensis* was presented.

**Case report:** A 21-year-old female patient was admitted to the hospital complaining of abdominal pain and low-grade fever. Physical examination confirmed rebound tenderness in the right abdomen. Laboratory examination revealed white cell count of 12500 with 78% polymorphonuclear while C-reactive protein (CRP) was 13.5 mg/dL. There were no history of systemic complaints or other organ involvement. A distended, pus-filled, right fallopian tube was detected at ultrasonographic examination. The patient underwent an emergency laparotomy. Fluid was sent to the laboratory and inoculated into the standard aerobic and anaerobic Bactec bottles. Three days later the sample of the positive bottles was subcultured and a Gram-negative coccobacillus was grown on blood agar plates that was catalase- and oxidase-positive. The microorganism was positive for urease production and negative for carbohydrates fermentation, motility and H2S production. Susceptibility testing was performed with E-test method. The standard tube agglutination test was positive at a titer of 1:80 while the blood cultures were negative. The patient was treated with oral doxycycline and rifampicin for 6 weeks. No history of contact with infected animals or consumption of unpasteurized dairy products was reported.

**Conclusion:** Although few cases of ovarian abscess due to *B. melitensis* have been reported, this is the first case of pyosalpinx due to this pathogen. Brucellosis should be considered in the differential diagnosis of pelvic pain and abscess especially in endemic areas.

**Detection of resistance in Gram-positive bacteria**

**P1030** Validation of the new inducible clindamycin resistance test on Vitek2 cards for staphylococci (AST-P610)

*B. Van Meensel*, M. Lontie (Louvain, BE)

**Objectives:** For the detection of inducible macrolide-lincosamide-streptogramin B (MLSB) resistance in *Staphylococcus* spp., the advanced expert system (AES) of Vitek 2 (bioMérieux) uses a recently developed ‘Inducible Clindamycin Resistance’ (ICR)-test. Our aim was to validate the ICR-test on AST-P610 cards, using the CLSI double disc method (D-test) as reference test.

**Methods:** 95 consecutive patient samples with *Staphylococcus* spp. (54 *S. aureus* and 41 coagulase-negative staphylococci) were collected in our laboratory (MCH Leuven). The strains were identified with the GP card and tested for inducible clindamycin resistance by Vitek 2 ICR-test on the AST-P610 card. Disk diffusion using the D-zone test was used as reference method (CLSI M100-S20, M02-A10). Based on Cumitech 31 A, we used following validation criteria: very major errors (VME) (D-test +, Vitek 2 ICR −) ⩽3%; major errors (ME) (D-test +, Vitek 2 ICR +) ⩽3%; categorical agreement (CA) ⩾90%.

**Results:** We noted a sensitivity of 100%, a specificity of 98.7% and a categorical agreement of 98.9%. One major error (D-test −, Vitek 2 ICR +) was noted but fell within our validation criteria (ME ⩽3%).

**Conclusion:** The Vitek 2 ICR-test on the AST-P610 card provided a reliable method to detect iMLSB resistance in staphylococci and was implemented in the daily routine of our laboratory.

| n=95 | D-test + | D-test - |
|------|--------|--------|
| Vitek 2 ICR+ | 17 1 | |
| Vitek 2 ICR− | 0 77 | |

**P1031** Assessment of heteroresistance to vancomycin and other antibiotics by broth macrodilution in *Staphylococcus aureus* strains

*FF. Arhin*, J. Sarmiento, G. Moock (Ville Saint Laurent, CA)

**Objective:** Heteroresistance (HR) to vancomycin (VAN), defined as a small subpopulation with elevated VAN MIC (>2 mg/L) in a culture, is becoming increasingly prevalent and has been linked to clinical therapeutic failure. The most reliable procedure to detect VAN HR is the agar-based population analysis profile-area under curve (PAP-AUC) method. This procedure is labour-intensive and cannot be applied to agents for which agar-based assays are unsuitable. We developed a broth macrodilution assay (Bmac) to assess HR to vancomycin. The Bmac, with an increased number of cells relative to broth microdilution assay (Bmic), increases the probability of identifying rare resistant subpopulations while respecting the inoculum density recommended by CLSI. This assay was used to assess HR to daptomycin (DAP), teicoplanin (TEI), telavancin (TEL) and oritavancin (ORI) in heterogeneous VAN-intermediate *Staphylococcus aureus* (SA) (hVISA) strains.

**Methods:** 5 SA clinical strains (from NARSA or Eurofins Medinet) used in this study exhibited VAN HR by PAP-AUC. MICs were determined by Bmac and broth microdilution assay (Bmic) following CLSI M7-A8. SA strains ATCC 29213 (VAN-susceptible) ATCC 700699 (VAN intermediate SA [VISA]) and NRS2 (Mu3; hVISA) were used as reference strains. Bmac was performed in 50 mL polypropylene screw cap tubes containing 5×10^5 CFU/mL in a final assay volume of 20 mL (total of 107 CFU). Bmac was read after 24 h at 37°C. All experiments were performed at least twice.

**Results:** By PAP-AUC, the 5 *S. aureus* strains were VAN HR. These isolates, along with NRS2 (hVISA reference strain), had VAN MICs of 1 or 2 mg/L by Bmic and 4mg/L by Bmac, indicating growth of subpopulations at a VAN concentration of 2 mg/mL in Bmac. In contrast, the VSSA and VISA control strains showed MIC values that were identical for both Bmic and Bmac with an increased number of cells relative to broth microdilution assay (Bmic), increases the probability of identifying rare resistant subpopulations while respecting the inoculum density recommended by CLSI. This assay was used to assess HR to daptomycin (DAP), teicoplanin (TEI), telavancin (TEL) and oritavancin (ORI) in heterogeneous VAN-intermediate *Staphylococcus aureus* (SA) (hVISA) strains.

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**Conclusion:** The Bmac assay described here provides a simple means to identify the presence of rare HR isolates. The procedure is useful for agents such as ORI for which agar-based assays are not possible. Two of the 5 VAN HR strains of SA were identified by Bmac assay as possibly HR to TEL and ORI. Clinical significance of HR as identified by Bmac remains to be determined.
Evaluation of a rapid detection method for methicillin-resistant coagulase-negative staphylococci, the Clearview® Exact PBP2a test

E. De Witte, M. Ieven* (Edegem, BE)

Objectives: Conventional susceptibility methods take 24 hours and identification of the mecA gene is expensive and time-consuming. The Clearview® Exact PBP2a test is a rapid immunochromatographic assay that detects the penicillin binding protein 2a present in methicillin resistant Staphylococcus aureus and has already been evaluated in these isolates. However, methicillin resistant coagulase negative staphylococci (MR-CNS) can also be an important cause of infection, especially of bloodstream infections. As the performance of this test has not been evaluated for this indication, we evaluated the test on CNS isolates.

Methods: The Clearview® Exact PBP2a test was evaluated on 200 CNS isolates out of a large collection stored at −80°C in the University Hospital of Antwerp (Ieven M et al. J Clin Microbiol 1995; 33:1060). These isolates were selected taking into account the relative prevalence of different CNS species in routine clinical samples. Methicillin resistance was confirmed by mecA detection with an in-house PCR. Among the mecA positive 56% were Staphylococcus epidermidis isolates and 44% non-S. epidermidis isolates. After thawing, cultures were isolated on blood agars and incubated for 24 hours, which was repeated the next day for again 24 hours. The third day cultures could be used to perform the PBP2a test according to the instructions of the manufacturer. We used 10 methicillin sensitive (MSSA) and 15 methicillin resistant Staphylococcus aureus (MRSA) isolates as controls. The presence of the mecA gene using PCR was considered as the gold standard.

Results: Of the 200 isolates tested, 125 (63%) were methicillin resistant confirmed by PCR and 108 (64.4%) of these were correctly detected by the PBP2a test. Sensitivity and specificity results for the PBP2a test on CNS were 85.6% and 98.7% respectively. Within the most important CNS species sensitivities were 80.0% for S. epidermidis, 94.9% for S. haemolyticus and 85.7% for S. hominis (table 1). These differences were not statistically significant (P > 0.05).

Conclusion: Compared to the detection of PBP2a in MRSA, the presence of PBP2a in MR-CNS is detected with a lower sensitivity by the Clearview® Exact PBP2a test. Consequently, this rapid test has to be used in combination with the conventional susceptibility methods. However, the major advantage of this test for CNS is that, in case of a positive result, it provides faster results to improve the patients’ outcome and hospital costs.

| Clinical isolates | mecA+ | PBP2a detection |
|-------------------|-------|-----------------|
| S. capitis esp. capitis | 4 | 0 |
| S. capitis esp. urealyticus | 4 | 0 |
| S. cohnii | 2 | 1 |
| S. epidermidis | 87 | 70 |
| S. haemolyticus | 46 | 39 |
| S. hominis | 32 | 14 |
| S. lactis | 1 | 0 |
| S. lugdunensis | 5 | 0 |
| S. saprophyticus | 1 | 1 |
| S. schleiferi | 4 | 0 |
| S. simulans | 1 | 1 |
| S. warneri | 13 | 10 |
| S. xylosus | 1 | 0 |

Total: 200 125 108

Table 1: Results for the evaluation of the Clearview® Exact PBP2a test in comparison to the presence of the mecA gene detected by PCR.

Evaluation of a rapid detection method for methicillin-resistant coagulase-negative staphylococci, the Clearview® Exact PBP2a test

B. Van Meerssaele*, J. Frans, W. Lafrat, D. Van Kerckhoven, A. Lemmens, J. Van Schaeren, E. De Luere (Louvain, Bonheiden, Lie, Sint-Truiden, Mechelen, Antwerp, Roeeleare-Men) BE)

Objectives: To validate the Clearview® Exact PBP2a test, a qualitative in vitro immunochromatographic assay for the detection of penicillin-binding protein 2a (PBP2a) in isolates identified as Staphylococcus aureus.

Methods: Seven Belgian laboratories (MCH Leuven, Imelda ziekenhuis Bonheiden, H. Hartziekenhuis Lier, LKO-LMC Sint-Truiden, AZ Sint-Maarten Mechelen, GZA Antwerpen, H. Hartziekenhuis Roeselare-Men) analysed a total of 151 S. aureus strains. Both clinical isolates (n = 136) and QC-strains (n = 15) were cultured and identified following local standard procedures in each lab. The Clearview® Exact PBP2a test was performed in duplo with the currently used kit for PBP2a detection, all requiring a boiling and centrifugation step (SlideX® MRSA detection bioMérieux, MRSA-screen Denka Seiken, PBP2 Oxoid). Discrepancies were resolved using molecular testing.

Results: The S. aureus colonies were tested from different culture media (blood agar, chromogenic SA agar, chromogenic MRSA agar, Mueller Hinton agar). On a total of 151 S. aureus strains, only one discrepant result was obtained. It concerned a S. aureus isolate positive with Clearview® Exact PBP2a but negative with MRSA-screen (Denka Seiken). The strain was confirmed as MRSA using molecular testing (Tuf, Nuc and MecA positive). In total, 116 MRSA-strains and 35 MSSA-strains were tested. All the labs mentioned the problem of weak control lines, especially with positive isolates. When a smaller amount of isolate was used, the strength of the control line increased.

Conclusion: The Clearview® Exact PBP2a test provides an accurate, easy to use (no centrifugation or boiling stages required) and quick method to detect the PBP2a protein in S. aureus isolates. However, attention should be paid to over-inoculation of isolates positive for PBP2a, resulting in weak or even negative (and therefore invalid) control lines.

Evaluation of CHROMagar MRSA agar (E&O) and brillianc MRSA 2 agar (Thermo Fisher Scientific)

D. Bangura* (Salisbury, UK)

Objectives: The need for accurate meticillin-resistant Staphylococcus aureus (MRSA) screening methods is essential to a busy clinical microbiology laboratory. This study evaluates the efficacy of two MRSA chromogenic media, CHROMagar MRSA Agar (E&O) and Brilliant™ MRSA (Thermo Fisher Scientific) for screening for MRSA from patient samples.

Methods: Three hundred and eighty five patient samples collected for routine MRSA screening (mostly nasal and groin swabs) were inoculated onto Brilliance MRSA 2 Agar and CHROMagar MRSA Agar. Plates were inoculated at 36±2°C for 22–24 hr. Any presumptive MRSA colonies were confirmed using DNase Agar, staphylococcus latex, PBP2 latex and cefoxitin and oxacillin antibiotic sensitivity testing.

Results: Both chromogenic media successfully isolated MRSA from twelve samples, giving a prevalence of 3% However, CHROMagar MRSA Agar incorrectly identified three meticillin-sensitive Staphylococcus aureus (MSSA) as MRSA, reducing the positive predictive value (PPV) of the plate to 80% compared to 100% for Brilliance MRSA 2 Agar. The negative predictive value (NPV) for both chromogenic media was 100%.

Conclusions: A high PPV and NPV are essential for any MRSA screening method. Brilliance MRSA 2 Agar accurately detected MRSA from patient samples, showing 100% PPV and NPV. Brilliance MRSA 2 Agar can be relied upon to correctly identify patients with MRSA while remaining a highly specific chromogenic medium. Whilst the CHROMagar MRSA Agar had a high NPV it misidentified three MSSAs as MRSA as bringing its PPV down to 80%. This misidentification resulted in extra work being undertaken and therefore delaying the true negative
Methicillin-resistant *Staphylococcus aureus* screening using Brilliance MRSA 2 Agar

C. Sander*, W. Kalka-Moll, E. Scopes (Mönchengladbach, DE; Basingstoke, UK)

**Objectives:** To evaluate the performance of the newly formulated Brilliance MRSA 2 Agar (Thermo Fisher Scientific) and chromID MRSA Agar (BioMérieux) for screening for methicillin-resistant *Staphylococcus aureus* (MRSA) from patient samples.

**Methods:** One thousand and five patient samples collected for routine MRSA screening, including nasal, perineum and wound swabs, plus tracheal secretions, were inoculated onto Brilliance MRSA 2 Agar and chromID MRSA Agar. Plates were incubated for 18 to 24h. Any presumptive MRSA colonies were confirmed using a coagulase test, automated identification and antimicrobial susceptibility testing, plus an in-house PCR method detecting mecA, Sa442 and IS431 genes.

**Results:** One hundred and seven confirmed MRSA were isolated on one or both of the chromogenic media, giving a prevalence of 11%. Sensitivity of Brilliance MRSA 2 Agar was statistically significantly higher (P=0.04) than chromID MRSA Agar (96.3% and 85.5% respectively). Specificity, positive predictive value (PPV) and negative predictive value (NPV) of Brilliance MRSA 2 Agar were equal to or higher than that of chromID MRSA Agar. chromID MRSA Agar showed notably varied presumptive MRSA colony colours, ranging from dark to light and transparent green. However, not all of these were confirmed as MRSA. In comparison, Brilliance MRSA 2 Agar showed a more consistent blue MRSA colony colour.

**Conclusions:** Brilliance MRSA 2 Agar detected considerably more MRSA than chromID MRSA Agar while still showing excellent specificity and NPV. Brilliance MRSA 2 Agar showed a more uniform, distinctly blue MRSA colony colour compared to chromID, making it reliable and straightforward to identify MRSA from patient samples.

| Performance of Brilliance MRSA 2 Agar and chromID MRSA Agar |
|-------------------------------------------------------------|
| **Percentage of isolates identified**                         |
| **Sensitivity**                                             |
| Brilliance MRSA 2 Agar                                      |
| 96.3                                                        |
| (95.9-96.7)                                                 |
| ChromID MRSA Agar                                           |
| 95.6                                                        |
| (95.1-96.1)                                                 |
| **Specificity**                                             |
| Brilliance MRSA 2 Agar                                      |
| 96.3                                                        |
| (95.9-96.7)                                                 |
| ChromID MRSA Agar                                           |
| 96.3                                                        |
| (95.9-96.7)                                                 |
| **PPV**                                                     |
| Brilliance MRSA 2 Agar                                      |
| 96.3                                                        |
| (95.9-96.7)                                                 |
| ChromID MRSA Agar                                           |
| 96.3                                                        |
| (95.9-96.7)                                                 |
| **NPV**                                                     |
| Brilliance MRSA 2 Agar                                      |
| 96.3                                                        |
| (95.9-96.7)                                                 |
| ChromID MRSA Agar                                           |
| 96.3                                                        |
| (95.9-96.7)                                                 |

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P1036 Prospective evaluation of Brilliance VRE® and VRESelect® Chromogenic Agars for detection of vancomycin-resistant enterococci from surveillance specimens

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**Objectives:** Prospective evaluations of VRE selective agars have limitations due to the small number of distinct clones in circulation at one time. Using highly-characterized genetically diverse VRE and non-VRE isolates, this study evaluated multiple lots of 2 chromogenic agars, comparing them to 2 common non-chromogenic agars. **Methods:** A total of 137 unique strains, selected for chromosomal and VRE-genotype variation, were used to evaluate Brilliance VRE (BRIL, Oxoid), VRESelect (SLCT, Bio-Rad), Bile Esculin Azide Vancomycin (BEAV, Oxoid), and mEnterococcus with 6mg/L vancomycin (Delco BD, prep in-house). The strains included: 49 *E. faecium* (EFE: 43 VRE – 23 vanA, 1 vanA-like, 12 vanB1, 5 vanB2/3, 1 vanD; 6 vancomycin-susceptible (VSE)), 44 *E. faecalis* (EFC: 26 VRE – 2 vanA, 17 vanB1/vanB2/3, 6 vanE, 1 vanL; 18 VSE), 15 *E. gallinarum* (GAL), 8 *E. casseliflavus* (CAS), 10 Leuconostoc, 7 Peptococcus, and 4 *Lactobacillus*. Each lot was inoculated with 100µl of a 0.5 McFarland STD equivalent suspension of each organism. Plates were streaked by IsoPlater, incubated simultaneously at 37°C, and independently read at 24h and 48h; mEv was also read at 72h. The number, size and colour of colonies at each time point were noted. As an uneven number of lots were tested, group inoculation of isolates at time point for all agar lots was pooled and converted to % to be comparable.

**Results:** Ultimately, 548 isolates were tested on 4 BRIL lots, 411 isolates on 3 lots ea of mEv and BEAV, and 274 isolates on 2 SLCT lots. The
Table summarizing overall % growth (95% CI) shows that no agar grew 100% of VRE at 24h or inhibited all non-VRE. While BRIL grew all but 2 vanB EFc by 24h, it failed to grow vanL, and 2 lots failed to grow 1 vanE. SLCT grew 108/120 vanA/B VRE by 24h, 11 at 48h, but 1 vanA EFE and 1 vanL EFc did not grow, and only 3/12 vanE EFc grew overall. For BEAV, 168/180 vanA/B grew by 24h, 9 vanA/B and 3 vanE grew by 48h, but 3 VRE (1 ea vanA EFC, vanE BFE, vanB EFc) failed to grow, as did 2 vanE and the vanL EFC. While mEV isolated 100% vanA/B VRE, only 120/180 grew by 24h, and grew at 72h, and it grew the least non-vanA/B VRE, missing 14/18 vanE and 2/3 vanL EFC. For non-VRE, BRIL inhibited 261/276, SLCT 84/138, mEV 134/207, and BEAV, 102/207 isolates tested.

Conclusions: This study found BRIL had a 24h sensitivity and specificity that exceeded all other agars combined. However, even BRIL, as with other VRE selective agars, required 48h to grow all VRE isolates.

**P1038**

External quality assessment of culture-based detection of methicillin-resistant *Staphylococcus aureus* by a network of European laboratories

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**Objectives:** An external quality assessment (EQA) was carried out to assess the culture-based methods utilized by 17 hospital laboratories in 8 European countries and their proficiency in detecting methicillin-resistant *S. aureus* (MRSA).

**Methods:**

- The EQA panel included 2 MRSA harbouring SCCmec IV and I, and one strain each of MRCoNS (methicillin-resistant coaguлагative negative *S. epidermidis*), MSSA (methicillin-sensitive *S. aureus*), and *Escherichia coli* as pure strains or in mixtures at varying concentrations (Table). All 17 participants analyzed the panel utilizing their in-house culture protocol (IHP) for MRSA detection, and a standardized protocol (SP) implemented as a diagnostic intervention in clinical trials as part of the EU-FP6 project, MOSAR. SP included sample inoculation on a chromogenic medium (BBL CHROMagar, BD Diagnostics) and confirmation of putative MRSA by 2 of the 3 suggested tests (slide agglutination, coagulase test, and mannitol salt agar).

**Results:** Of the 17 participants, 16 (94%) could detect up to 10 colony forming units (cfu) of absolute MRSA loads using both protocols (Table). At 1 cfu absolute load, SCCmec I MRSA could be detected by a larger no. of participants (82%, n = 14, and 88%, n = 15) by SP and IHP, respectively than SCCmec IV MRSA (53%, n = 9 and 76%, n = 13 by SP and IHP, respectively). SP and IHP results showed 96.7% concordance (kappa: 0.65, 95%CI: 0.44 to 0.86). One participant reported false-negative results with 6 MRSA samples (pure strains: 1–100 cfu, and mixtures) using both SP and IHP (Columbia agar with 6 μg/ml oxacillin; confirmatory tests: coagulase, slide agglutination, and Vitek). Another participant reported false-positive results with the MRCoNS as a pure strain and mixed with MSSA using IHP (mannitol-salt agar; confirmatory test: not described). As IHP, 71% (n = 12) participants utilized chromogenic media either alone (41%, n = 7) or combined with conventional media (29%, n = 5). ChronID (BioMerieux) was the most commonly employed chromogenic medium (35%, n = 6) followed by BBL CHROMagar (29%, n = 5), and MRSASelect (Bio-Rad, 6%, n = 1). Coagulase was the most commonly performed confirmatory test (71%, n = 12). ChronID combined with coagulase test was the preferred IHP for 29% (n = 5) participants.

**Conclusions:** This is the first EQA programme assessing culture-based detection of MRSA. Performance of the participating laboratories was generally high with the majority implementing ‘rapid’ chromogenic media as IHP for MRSA detection.

**Table:** Characteristics of the external quality assessment panel and MRSA detection by laboratories using both standardized MOSAR and in-house protocols

| Sample characteristics | Absolute bacterial loads inoculated on media* (CFU) | MRSA confirmed by standardized MOSAR protocol (SP) | No. of laboratories (%) | MRSA confirmed by in-house (IHP) protocol | No. of laboratories (%) |
|------------------------|-----------------------------------------------|-----------------------------------------------|--------------------------|------------------------------------------|--------------------------|
| Pure strains           |                                               |                                               |                          |                                          |                          |
| MRSA 1 (SCCmec IV, pvI) | 1000                                          | 17 (100%)                                     | 17 (100%)                |                                          |                          |
| MRSA 1 (SCCmec IV, pvI) | 100                                           | 17 (100%)                                     | 17 (100%)                |                                          |                          |
| MRSA 1 (duplicate) (SCCmec IV, pvI) | 100 | 17 (100%) | 17 (100%) | | |
| MRSA 1 (SCCmec IV, pvI) | 10                                            | 17 (100%)                                     | 17 (100%)                |                                          |                          |
| MRSA 1 (SCCmec IV, pvI) | 1                                              | 9 (83%)                                       | 13 (76%)                 |                                          |                          |
| MRSA 2 (SCCmec I)      | 1000                                          | 17 (100%)                                     | 17 (100%)                |                                          |                          |
| MRSA 2 (SCCmec I)      | 10                                            | 16 (84%)                                      | 16 (84%)                 |                                          |                          |
| MRSA 2 (SCCmec I)      | 1                                              | 16 (82%)                                      | 16 (82%)                 |                                          |                          |
| Mixtures               |                                               |                                               |                          |                                          |                          |
| MRSA 1 + MSSA (SCCmec IV, pvI) | 1 + 100 | 16 (94%) | 17 (100%) | | |
| MRSA 1 + MRCoNS (SCCmec IV, pvI) | 1 + 10 | 17 (100%) | 17 (100%) | | |
| MRSA 2 + MSSA (SCCmec I) | 10 + 10 | 16 (94%) | 15 (88%) | | |
| MRSA 2 + MRCoNS (SCCmec I) | 10 + 100 | 16 (94%) | 15 (88%) | | |
| Negative controls      |                                               |                                               |                          |                                          |                          |
| MRCoNS                 | 1000                                          | 0 (100%)                                      | 1 (88%)                  |                                          |                          |
| *Bacterial load in 100 μl of sample inoculated on culture plates as part of SP and IHP. MRSA: methicillin-resistant *S. aureus*; MSSA: methicillin-sensitive *S. aureus*; MRCoNS: methicillin-resistant coagulase negative *S. epidermidis*; CFU: colony forming units

**P1039**

Improved sensitivity and reduction in MRSA surveillance costs by using MRSA-B enrichment broth

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**Objectives:** Multiple body sites are swabbed when screening a patient for MRSA colonization. Each swab is directly inoculated onto a selective and differential agar plate, usually a Chromogenic-based plate, specific for MRSA. A significant portion of the laboratory budget is utilized for this purpose, yet the sensitivity of direct plate inoculation is suboptimal compared to the broth culture method.

The objectives of the study are as follows:

1. To determine whether MRSA-B broth [Copan, Italia SpA, Brescia Italy], has superior sensitivity to conventional direct plate inoculation method for isolation of MRSA.
2. To reduce laboratory costs by pooling multiple body site swabs and inoculate into 1 tube of MRSA-B broth per patient.

**Method:** Using Amies® Transport Swab with charcoal [Copan, Italia SpA, Brescia Italy], 372 swabs (multiple body sites) were collected from 190 patients. As per clinical laboratory standard procedure, each swab was directly inoculated onto CHROMagar MRSA® [CHROM agar Microbiology, Paris, France] for isolation of MRSA. All swabs from each patient were pooled and inoculated into a single tube of MRSA-B broth and incubated for 5 hours at 37ºC and vortexed. 50 μL of broth was plated onto a CHROMagar MRSA® plate. Plates were incubated at 37ºC for 24 hours and examined. Presumptive MRSA colonies were confirmed by PCR for nuclease, PVL, and mecA.

**Results:** The positive rate of MRSA was 11.05% (21/190 patients) using the standard clinical laboratory method of direct plate inoculation of
Antimicrobial resistance

Validation of new BinaxNow Staphylococcus aureus and PBP2a tests performed directly from blood cultures

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Background: Rapid detection of both Methicillin-sensitive (SA) and Methicillin-Resistant (MRSA) Staphylococcus aureus significantly improves patient outcome. Two rapid immunochromatographic test devices using polyclonal antibodies to detect a SA specific protein and penicillin binding protein 2a (BPBP) are now available. This study compared the two tests with the routine in-house protocol of direct tube coagulase (DTC) and modified direct PBP2a test (PB) from blood cultures (BC) with Gram positive cocci resembling staphylococci in the Gram stain.

Method: From October to December 2010, 85 BACTEC BC with GPC resembling staphylococci in the Gram stain were included. Only the first positive bottle for each patient was tested. DTC, BSA, BPBP and PB were done. There were 30 Staphylococcus aureus (STAU) of which 7 were methicillin resistant (MRSA) and 55 Coagulase negative Staphylococci (CNS). All isolates were confirmed by routine identification and susceptibility testing.

S. aureus test (SAT): 1.0 mL of solution A was mixed with 1.0 mL of BC broth, and centrifuged at 1500g for 5 minutes, supernatant removed and washing repeated. 5 drops of Reagent B were added to the deposit and mixed, 25mL of Reagent C was mixed in. 50mL of supernatant and one drop of Reagent D was added to the device. Four drops of Reagent D was then added to the left side of the device. The result was read at 10 minutes.

PBP2a test (PB): 1.5 mL of solution 1 was mixed with 0.5 mL of BC broth and centrifuged at 1500g for 5 minutes. The supernatant was removed, 200mL of Reagent 2 was added, mixed thoroughly, 100mL of Reagent 3 was added and mixed then centrifuged at 1500g for 5 minutes. 75mL of supernatant was added to the device and read at 10 minutes.

Results: Twenty two of 23 STAU and all seven MRSA were SAT positive. All CNS were SAT negative. All seven MRSA were BPBP positive and all SA negative. The sensitivity (SN) and specificity, (SP), Positive Predictive Value (PPV) and Negative predictive value (NPV) of the SAT was 97%, 100%, 100% and 98% respectively. The SN, SP, PPV and NPV of the PB test were all 100%.

Conclusion: The SAT and BBPB are fast and reliable alternatives for the direct detection of SA and MRSA from BC. It is particularly useful for those laboratories without molecular facilities for rapid detection. The study should be expanded to include more patients, particularly those with MRSA.

Detection of oxacillin resistance of S. aureus by whole cell matrix assisted laser desorption time of flight mass spectrometry: the time has not yet come

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Objectives: Several studies have been published that claimed to identify oxacillin resistance of Staphylococcus aureus by whole cell matrix assisted laser desorption time of flight (MALDI-TOF) mass spectrometry. In these studies selected methicillin susceptible S. aureus isolates were compared to selected methicillin resistant S. aureus (MRSA) isolates. Nevertheless, the study design in all of those studies was not suitable to achieve this goal, because only mecA/SCCmec “isogenic” strains could discriminate between strain specific- and “mecA/SCCmec”-dependent peak variations.

It has been also described that strains specific peaks exist within the species of S. aureus. We identified in our study mecA/SCCmec “isogenic” strains of S. aureus and analysed their peak profiles by MALDI-TOF MS.

Methods: A large MRSA strain collection was used containing 120 strains with molecular divergent backgrounds. The mecA gene was found in only 104 out of 120 of these isolates after culturing the freezing culture. The loss of the mecA gene during freezing culture has been previously described. Single colonies of those isolates were identified by the cefoxitin resistance phenotype and mecA PCR results. The bacteria from freezing culture were submitted to LB media containing increasing oxacillin concentrations in order to select the corresponding mecA containing strain. In two out of 16 samples we could select a mecA containing strain and a corresponding “isogenic” strain lacking the mecA gene. These two pairs of molecularly characterised strains were submitted for whole cell MALDI-TOF MS. In order to increase the expression of the PBP2a, the oxacillin resistant strain was cultured in LB medium containing Nal and oxacillin. The corresponding oxacillin susceptible strain was cultured in LB medium only.

Results: We did not found evidence for a difference in the peak profiles in a mass-to-charge-ratio (Da) of 2000 to 15000 Da in two mecA-“isogenic” pairs of S. aureus strains cultured on blood agar plates. The protein peak profiles of the mecA positive strains, cultured on oxacillin containing media, were also virtually identical compared to the mecA negative corresponding isolates.

Conclusion: In contrast to several reports in literature, we could show for the first time that whole cell MALDI-TOF MS-derived peak profiles are not suitable for the discrimination of mecA positive S. aureus strains in the mass-to-charge-ratio of 2000 to 15000 Da.

Antimicrobial resistance

Antimicrobial resistance of H. pylori in Smolensk, Russia

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Objectives: To evaluate the susceptibility to antimicrobials of H. pylori isolated from patients with H. pylori-associated gastrointestinal disorders in Smolensk (Russia).

Methods: Overall 210 adult patients with H. pylori-associated gastrointestinal disorders were included in the study in 2009-2010. From each patient 2 antral and 2 corpus gastric biopsy samples were taken for culture and placed in a phosphate buffer (Sigma, USA) or Portagerm pylori (BioMerieux, France) for transportation to the laboratory. Pylori agar (BioMerieux, France) plates were incubated for 3–5 days at 37°C under microaerophilic conditions (9% CO2, 80% N2, 11% O2). Antimicrobial susceptibility testing was performed on Mueller-Hinton agar (BBL, USA) supplemented with 5% sheep blood by the dilution method to amoxicillin, erythromycin, clarithromycin, levofloxacin, ciprofloxacin, tetracycline and metronidazole.

Results: H. pylori were isolated in 64% (n=135) patients. Among them 18% were resistant to one antimicrobial agent, 3% were resistant to more than one antimicrobial agent. Seven (5.3%) isolates were resistant to clarithromycin (MIC >1 mg/L), 10 (7.6%) – to erythromycin (MIC >4 mg/L). The resistance rate of H. pylori to metronidazole was 3.8% (n=5) (MIC >4 mg/L). No strains resistant to amoxicillin and tetracycline were found. Resistance to ciprofloxacin was detected in 12.8% (n=17) (MIC >1 mg/L) of strains, to levofloxacin in – in 8.3% (n=11) (MIC >1 mg/L).

Conclusion: Our study highlights the importance of antibiotic resistance surveillance to guide test and treat policies. In Smolensk we can use current recommendations of the European H. pylori study group (proton pump inhibitor + clarithromycin + amoxicillin or metronidazole) as
the initial treatment for *H. pylori* infection. Quadruple therapy (proton pump inhibitor + bismuth + metronidazole + tetracycline) could be an alternative initial therapy.

**P1043** Resistance and virulence characterisation of CTX-M15 and KPC-3 *Klebsiella pneumoniae* clinical isolates  
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**Objectives:** The aim of this study was to evaluate the relationship between the type of ESBL produced and the resistance and virulence determinants in *Klebsiella pneumoniae* clinical isolates recovered at the 1070-bed Hospital in Lisbon, Portugal.

**Methods:** This study included 41 representative clinical isolates of *K. pneumoniae* ESBL producers that were collected between 2008 and 2010 in a tertiary care hospital, Lisbon. It was included representatives of CTX-M-15 (n = 24) as well as the novel carbapenemase KPC-3 *K. pneumoniae* (n = 17). The isolates were selected according to antimicrobial and genetic profile by disk diffusion disk and RAPID M13 DNA fingerprint, respectively. Plasmid replicons were determined using the PCR-based replicon typing scheme described by Carattoli et al. (2005) with specific primers for 18 plasmid replicons. The isolates were screened by PCR amplification for gene markers of 8 virulence factors genes: k2A, rpmA, magA, fimH, mkd2, mrdk3, khe and iucC.

**Results:** The most frequent virulence genes were the fimbrial adhesins fimH (89%), mrdk2 (93%), and mrdk3 (68%). The gene khe, that encodes a haemolysin, was found in 54% of the isolates and only 4% of the isolates of had showed the iucC gene of the aerobactin system. 8% of the isolates of *K. pneumoniae* belong to capsular serotypes K2. No rpmA and magA genes were detected. The prevalence of the virulence factors was comparable among all genetic groups, namely isolates with endemic CTX-M15 enzyme and those with KPC-3 carbapenemase ESBLs. In *K. pneumoniae* isolates producing CTX-M15 were found only the IncH1 plasmid incompatibility group although all the KPC-3 producers had plasmids belong to the Inc F incompatibility group.

**Conclusions:** Our results indicate a distribution of *K. pneumoniae* isolates producing ESBLs, with abilities to simultaneously express several virulence factors regardless the strain background. However, fimbrial adhesions seems to be an important virulence factor. The *K. pneumoniae* CTX-M-15 ESBL isolates presented the plasmid incompatibility group IncH1 and none belonged to the IncF group. Overall, it is important to note a high degree of variability in plasmid profile compared the recent KPC-3 isolates that showed the Inc F group suggesting that these ESBL resistance genes cannot be readily transmitted indistinctly between *K. pneumoniae* isolates.

**P1044** In vitro assessment of the core/shell/extra-shell nanosystem influence on the antibiofilm effect of usnic acid  
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**Objective:** To investigate the influence of core/shell/extra shell – Fe3O4/oleic acid/usnic acid nanosystem on the antibiofilm effect of usnic acid (UA), known for its antimicrobial and especially its quorum-sensing inhibitory activity, on the in vitro *Staphylococcus aureus* biofilm development.

**Methods:** Microscopic slides were coated with UA (1/2/3 layers), as free substance and respectively, as extrashell of biocompatible nanostructures. The Fe3O4/oleic acid-core/shell of 5–20 nm range have been synthesized by Massart adapted method, under microwave conditions. Two controls, represented by uncoated microscopical slides and respectively coated with core/shell were used in the study. Each slide was immersed in 3 ml of liquid nutrient broth distributed in six-well polystyrene plates and inoculated with 300μl of 0.5 McFarland bacterial suspension prepared from *S. aureus* ATCC 25923. The plates were incubated for 72 hrs under stirring conditions (150 rpm). The temporal dynamic of *S. aureus* biofilm was studied by harvesting the slides at 24/48/72 hrs, washing them for removing the non-adherent bacteria and discarding the adherent ones by rigorously shaking for 30 min in 10 ml phosphate buffered saline. The obtained suspensions were further further ten-fold diluted and 5 μl of each dilution were seeded in triplicates on brain heart agar, incubated for 24 hrs at 37°C in order to perform the viable cell counts (VBCs) and appreciate the number of bacterial cells embedded in biofilms. The biofilm architecture was examined by CLSM.

**Results:** The free UA pelliculised on the microscopic slides exhibited an antibiofilm activity, evidenced by the drastic decrease of VBCs recovered from the coated slides at 24 and 48 hrs, comparatively with the biofilm developed on the uncoated glass support. At 72 hrs, the number of cells was similar comparatively with the control. A similar biofilm dynamic was observed when *S. aureus* adhered to microscopic slides coated with core/shell/adsorption-shell nanosystem. The core/shell exhibited an antibiofilm activity evidenced only in the first 24 hours of incubation, thereafter the number of adherent bacterial cells being similar with the control.

**Conclusions:** Our results are demonstrating that Fe3O4/oleic acid nanoparticles could be used as successful coating agents for obtaining antibiofilm pellicles on different devices, exhibiting the great advantage of not affecting the antimicrobial properties of the absorbed substance.

**P1045** Major genetic plasticity found in NDM-1 plasmids grown under different conditions  
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**Objectives:** Initially these experiments were set up in order to determine how stable NDM-1 plasmids are in their host clinical isolates without selection over a two week period and also the effect of the SOS system on the mobilisation of NDM-1.

**Methods:** Nine clinical isolates that harboured NDM-1 on different sized plasmids and different incompatibility groups were grown with daily subculture at different temperatures (30°C and 37°C) without selection and also at 37°C with sub MIC amounts of ciprofloxacin (a known SOS response inducer). Each day samples were taken and pluses were prepared for pulsed field electrophoresis (PFGE). At the end of the 14 days the pluses were digested with S1 and the genomic DNA separated by PFGE. Gels were then probed with 32P labelled blaNDM-1 to determine the presence and location of NDM-1.

**Results:** All cultures were positive for NDM-1 throughout the two weeks of growth without selection. The genomic location of the NDM-1 gene varied markedly during the course of these experiments. The NDM-1 gene moved onto the chromosome in four isolates (IR25, IR26, IR18K and IR29). For IR25 this was under all conditions and continued being both on the chromosome and on a 350kb plasmid throughout the experiment, in addition at day 12 NDM-1 also appeared on a plasmid of ~100kb. However, for IR26 this movement occurred at day 1 at 30°C and at day 14 at 37°C. In one *Klebsiella* isolate collected from the north of India, the NDM-1 gene moved from being on a single plasmid of 100kb to being on four plasmids of different sizes ranging from 50kb to 300kb in size when grown at 37°C but not when ciprofloxacin was present. In general the majority of the movement events appeared to occur preferentially at 30°C.

**Conclusions:** NDM-1 is a stable resistance gene being inherited over a two week period without selection in 100% of the clinical isolates tested in this study. NDM-1 also appears to be remarkably mobile and able to move from plasmids to the chromosome of its host organism in approaching 50% of the isolates over a 14 day period. Movement and amplification of NDM-1 copy number were common events during the course of these experiments being observed in all isolates studied. Interestingly growth at 30°C appears to have the most dramatic effect on these events and may indicate that movement of NDM-1 occurs rapidly in the environment.
New insights on mechanisms of resistance in Staphylococcus aureus

P1046 Carbapenemase-2 producing Klebsiella pneumoniae transmission in an Italian university hospital
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Objective: To describe the emergence of carbapenemase-2 (KPC-2)-producing Klebsiella pneumoniae in an university hospital in Italy, focusing on the epidemiological, microbiological, and clinical characteristics of the KPC-2 spread and transmission.

Methods: All data and records for patients with a KPC-2-producing K. pneumoniae infection were prospectively collected. All available clinical KPC-2-producing K. pneumoniae strain were genotyped by repPCR and PFGE. Species identification of isolated bacteria and MIC determinants were performed using an automated system (Vitek-2 bioMérieux); MICs of meropenem, colistin, gentamicin, tigecycline and cotrimoxazole were evaluated with E test and were classified according to Clinical and Laboratory Standards Institute (CLSI) breakpoints and guidelines.

Results: From December 2009 through 20 October 2010, 50 patients (40% female; mean age 70.2 years; range 19–94 years) were infected by KPC-2-producing K. pneumoniae. Fingerprinting analysis showed a cluster of 49 strains. 1 strain was not correlated. Eleven patients were hospitalized in the ICU when the KPC-producing isolate was detected, 29 in medical ward and 10 in surgical ward. The total mean length of stay in hospital before infection was 25.98 days (range 0–100 days). Twenty five (50%) of the infected patients were receiving piperacillin/tazobactam containing antimicrobial regimen when infection was diagnosed, 6 (12%) were receiving meropenem. The prevalent strain tested was susceptible to colistin, gentamicin, tigecycline and cotrimoxazole.

Sites of infection: 21 urinary tract infections, 16 lower tract respiratory infections, 5 bacteremia, 7 surgical site infections, and 1 post-surgical meningitis. Fifteen (30%) patients did not receive antimicrobial treatment for KPC-2-producing K. pneumoniae. Twenty five (50%) of the infected patients were receiving piperacillin/tazobactam containing antimicrobial regimen when infection was diagnosed, 6 (12%) were receiving meropenem. The prevalent strain tested was susceptible to colistin, gentamicin, tigecycline and cotrimoxazole.

Conclusion: The emergence of KPC-2-producing K. pneumoniae create an important challenge for clinicians and hospital epidemiologists.

P1047 A significant impact on the rate of ESBL-producing Klebsiella pneumoniae by changing the antibiotic policy and consumption
J. Knudsen*, S.E. Andersen for the Bispebjerg Intervention Group

Background: In a 600-bed university hospital in Copenhagen, Denmark, the extended spectrum β-lactamase (ESBL) producing Klebsiella pneumoniae became a major problem, and the resistance rate among patients with K. pneumoniae reached more than 40%, in spite of numerous infection control initiatives as reintroducing choric cleaning and focus on isolations precautions. The effect of an intervention focusing on optimising diagnostics and a totally changed antimicrobial policy was evaluated.

Methods: During 2009 all possible interventions were studied, and an intervention, involving many different initiatives, was started in January 2010. The antimicrobial policy was changed, and it was only possible to use cephaplorin for surgical prophylaxis and in the treatment of meningitis. The use of quinolone was diminished to only certain indications. Focus was set on diagnostic initiatives and isolation precautions and use of small spectrum antibiotics when possible. Numerous teaching lectures were given, and written informations and guidelines were distributed to all clinical working employees in brochures and electronically.

Results: The rate of patients with ESBL producing K. pneumoniae decreased from 45% in January 2010 to 16% in November 2010 (p<0.007), and the rate for ESBL-producing E. coli patients were unchanged app. 12%. The number of bed-days with patients under isolation precautions for patients with ESBL producing K. pneumoniae and E. coli were reduced from more than 260 per month to less than 50 (p < 0.001) The compliance to the new guidelines was almost complete, the cephaplorin consumption decreased from 2409 to 581 DDD/month (76%), and quinolones from 2859 to 2298 DDD/month (20%), from 2009 to 2010.

Conclusions: An intervention, involving many different initiatives including changing antibiotic policy, reduced the rate of patients with ESBL-producing Klebsiella pneumoniae significantly. The number of bed-days with patients under isolation precautions was also reduced significantly.

P1048 Chromosome-encoded extended-spectrum class A β-lactamase MIN-1 from Minibacterium massiliensis
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Objectives: Minibacterium massiliensis is a newly discovered water-borne motile bacterium that has been isolated in Marseille from a 0.22-μm filtered water used for patients in the hospital. The genome of the corresponding strain has been recently sequenced, and a putative β-lactamase gene was identified. The aim of our study was to characterize this novel β-lactamase.

Methods: MICs were determined by Etest method. The blaMIN-1 gene was amplified by PCR and then cloned into PCR-blunt vector (Invitrogen). The resulting plasmid was sequenced and expressed in Escherichia coli, and the MIN-1 protein was purified and kinetic parameters determined by UV spectrophotometry.

Results: The M. massiliensis isolate was resistant to narrow-spectrum β-lactams. The blaMIN-1 gene encoded MIN-1, a 300 amino-acid Ambler class A β-lactamase with a pl value of 8.5 and a molecular mass of ca. 29 kDa. MIN-1 shared 56%, 54% and 51% amino acid identity respectively with the intrinsic extended spectrum β-lactamase (ESBL) LUT-1 of Pseudomonas luteola and with the plasmid-mediated β-lactamases KPC-2 and CTX-M-2. The hydrolysis spectrum of the clavulanic acid-inhibited MIN-1 included expanded-spectrum cephalosporins such as cefotaxime, ceftiraxone and aztreonam, but not cefazidime. The blaMIN-1 gene was chromosomally-located, as indicated by analysis of the whole genome sequence. No putative regulatory gene was identified upstream of the blaMIN-1 gene, in accordance with the lack of induction of the β-lactamase expression in M. massiliensis.

Conclusions: This work further illustrates the diversity of chromosome-encoded Ambler class A ESBLs. This study further indicates that water-living bacterial species are common sources of antibiotic resistance genes.

New insights on mechanisms of resistance in Staphylococcus aureus

P1049 Loss of activity of the GdpP protein leads to β-lactam tolerance in Staphylococcus aureus
J. Griffiths*, A.J. O’Neill (Leeds, UK)

Objectives: Tolerance (TOL) describes a strain-specific phenomenon in which the killing activity of a bactericidal antibiotic becomes attenuated. It is most commonly observed in Gram-positive bacteria in response to cell-wall active antibiotics. The presence of TOL Staphylococcus aureus in deep-seated infections is reported to negatively impact treatment with cell-wall active antibiotics and can lead to treatment failure. Despite this, the genetic basis of TOL in S. aureus remains obscure. The objective
of this study was to generate B-lactam-tolerant strains and use them to elucidate the genetic basis of TOL.  

**Methods:** Strains exhibiting tolerance to B-lactams were generated by exposing *S. aureus* SH1000 to cycles of oxacillin (OXA) selection (>100× minimum inhibitory concentration (MIC)). Genetic changes were identified by comparative genome sequencing (CGS), and verified by PCR and DNA sequencing. Disruption of a putative TOL locus was performed using plasmid pMUTIN4 and complementation was carried out using expression plasmid pEPSA5.  

**Results:** TOL, defined here as a >90% drop in viability after 6h challenge with 12.5mg OXA/L, was observed in SH1000 after 10–20 cycles of OXA exposure without a concomitant increase in OXA MIC. CGS revealed that two independently-recovered TOL strains carried two and five mutations per genome, respectively, with the majority lying within, or in the vicinity of, genes of unknown function. Both TOL strains harboured different missense mutations in gdpP, a gene encoding a putative membrane-located signalling protein containing a GDP-binding domain. The role of this gene in tolerance was further examined by insertional inactivation, which resulted in a stronger tolerance phenotype compared with that observed in the TOL strains (<50% loss of viability after 6h of OXA exposure vs. ~80%). Complementation of the gdpP-disrupted mutant by providing wild-type gdpP in trans resulted in loss of the TOL phenotype.  

**Conclusion:** Two independently-selected TOL mutants both carried missense mutations in gdpP. Since insertional inactivation of this gene also resulted in TOL, we conclude that it is loss or reduction of GdpP activity that underlies this phenotype. Bioinformatic analysis suggests that GdpP is a signalling protein, potentially implicating altered transduction of environmental signals in the reduced B-lactam killing observed in TOL strains.  

**P1050** A novel mechanism of reduced susceptibility to vancomycin in *Staphylococcus aureus*  

A.Y. Peleg*, D. Cameron, D. Ward, R. Moellinger, G.M. Eloupoulos (Melbourne, AU; Boston, US)  

**Objectives:** Staphylococcal strains with reduced susceptibility to vancomycin (Vn) are being reported. This study aimed to elucidate the genetic mechanisms of resistance.  

**Methods:** Next generation whole genome sequencing was performed on a unique collection of isogenic, clinical and laboratory derived pairs that had been exposed to Vn and developed Vn non-susceptibility. Four clinical pairs or series (11 strains) and 1 laboratory derived series (3 strains) underwent comparative genomics. pKOR1 allelic exchange was used for targeted mutagenesis and pL150 for complementation. Gene expression analysis was performed using TIGR microarray chips. Biofilms on plastic surfaces, electron microscopy and population analysis profiles (PAP) were also performed.  

**Results:** An average of 5 single nucleotide polymorphisms, 2 deletions and 1 insertion were identified in each Vn non-susceptible strain compared to its Vn susceptible parent strain. Mutations in previously described genes that control cell wall biosynthesis and autolysis were identified including agr and sarA and saeA. However, mutations in new genes were identified including an sensor kinase, walK, and a serine/threonine phosphatase, stp1, which also regulate cell wall metabolism. A clean deletion of the stp1 gene confirmed its importance to vancomycin susceptibility, with an increase in MIC to vancomycin (from 1.5 μg/mL to 2 μg/mL) and an nVISA phenotype on PAP compared to its isogenic, Vn susceptible parent strain. Cell wall thickness was also increased (from 26.32 nm to 30.34 nm, P < 0.05) and biofilm formation was reduced 1.5 fold in the stp1 mutant (P < 0.05). These features returned to wild-type with complementation of the stp1 gene. Apart from cell wall related genes, microarray analysis showed that stp1 regulates virulence factors, including hemolysins and phenol soluble modulins.  

**Conclusions:** Mutation in stp1, a recently described serine/threonine phosphatase, appears to be a novel genetic mechanism for reduced susceptibility to vancomycin in *S. aureus*. Interestingly, this gene also appears important for staphylococcal virulence.
The effect of ermTR gene in isogenic conditions on antimicrobial activity of daptomycin tested against clinical strains of Staphylococcus aureus. We examined the ultrastructural characteristics of clinical GM-resistant S. aureus strains by transmission electron microscopy.

Methods: Fourteen S. aureus clinical isolates used in this study were obtained from the Department of Laboratory Medicine, Kawasaki Medical School Hospital, Japan. The minimal inhibitory concentrations (MICs) were evaluated according to the standard agar dilution method recommended by the Japan Society of Chemotherapy. It was recognized that seven isolates were resistant and seven isolates were sensitive to GM. Transmission electron microscopic analysis of each strain involved measurement of cell wall thickness in more than 10 cells with equatorial cut surfaces. We have genetically characterized these isolates using polymerase chain reaction, targeting three genes of the aminoglycoside-modifying enzyme, AAC(6′)-APH(2′), APH(3′)-III and ANT(4′)-I.

Results: Electron microscopy indicated that the cell wall was significantly (P<0.001) thicker in clinical GM-resistant strains (32.2±5.99 nm) than in GM-sensitive strains (19.02±2.72 nm). Although these ultrastructural characteristics were found in all GM-resistant isolates, there was no clear correlation between these isolates and the three known GM-resistance genes of S. aureus. Thus, the mechanism of cell wall thickening does not appear to be dependent on a particular GM-resistant gene. We also demonstrated that GM-resistant mutant strains, derived in vitro from a GM-sensitive S. aureus parent strain (209P), had a thickened cell wall.

Conclusion: These results strongly suggest that clinical GM-resistant S. aureus strains have a thicker cell wall as a common ultrastructural characteristic.

Conclusions: In this work we show that linezolid decreases exotoxin expression in S. aureus by promoting early reduction of PVL and HLA mRNA levels. Our results show that linezolid represses three major positive regulators of exotoxin expression: agr, sae and sarA. Contrarily, the expression of rot “repressor of toxins” was not modified. Therefore linezolid treatment induces an impaired expression of virulence regulators. Repressive regulators prevail thus leading to decreased expression of exotoxins.

**New insights on mechanisms of resistance in Staphylococcus aureus**

**P1053**

The effect of ermTR gene in isogenic conditions on erythromycin and lincomycin susceptibilities of *S. aureus* RN4220

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**Introduction:** ermTR is one of the most common gene that confers resistance to macrolides especially in *Streptococcus*. The effect of ermTR was never shown in isogenic conditions. The purpose of the present study was to show the effect of ermTR in isogenic conditions by cloning ermTR and transferring this gene to a Gram positive bacteria.

**Material and Methods:** Total DNA from *S. pyogenes* C1 was extracted and used for amplification of ermTR gene. DNA extraction was done using Instagene Matrix as described by manufacturer. To simplify cloning modified primers with addition of restriction sites was used. Amplicons as well as pUC18 were restricted with appropriate enzymes, were ligated and transformants were selected after electrotransformation of *E. coli* DH10B. Recombinant plasmids were purified and restricted and subcloned in a shuttle plasmid pJM2246 and were used for transformation of *E. coli* DH10B. Recombinant pJM2246 with ermTR gene was introduced to *S. aureus* RN4220 by electroporation. MICs for *E. coli* and *S. aureus* transformed with ermTR gene. The construct contained no regulatory region to see the effect of ErmTR methylase continuously synthesized.

**Results:** The initial erythromycin MICs of the recipient strains *E. coli* DH10B and *S. aureus* RN4220 were 32 and 1 mg/L, respectively. After transformation MICs were increased 8 fold for *E. coli* and 32 fold for *S. aureus* RN4220. The increase in lincomycin MIC was more important in *S. aureus* and >128 fold increase was reached. The MICs obtained are summarized in Table 1.

**Conclusions:** This study showed two main differences between ErmTR and other methylases: i) Level of erythromycin resistance conferred by ErmTR remains low. ii) Lincomycin resistance level conferred by ErmTR methylation is higher than macrolide. Other methylases ermA, ermB confer high level of macrolide resistance and high level lincomycin resistance if the synthesis is permanent. These methylases methylates adenosine at position 2058 of 23S RNA. Also mutations at that position confer high level resistance. Further studies are necessary to determine methylation site of ErmTR. ErmTR may methylate an other site than 2058 at 23S rRNA.

**TABLE 1.** MICs of transformants with ermTR gene.

| Lincosycin | Erythromycin |
|------------|--------------|
| E. coli DH10B | ND | 32 |
| ermTR-pUC18+pJM2246 in DH10B | ND | 256 |
| S. aureus RN4220 | 2 | 1 |
| ermTR-pUC18+pJM2246 in RN4220 | >324 | 32 |

**Conclusion:** These results strongly suggest that clinical GM-resistant *S. aureus* strains have a thicker cell wall as a common ultrastructural characteristic.

**P1055**

Antimicrobial activity of daptomycin tested against *S. aureus* collected from ostearthritis infections

H. Sader*, D. Farrell, R. Jones (North Liberty, US)

**Objectives:** To evaluate the antimicrobial activity of daptomycin and comparator agents tested against *S. aureus* from ostearthritis infections. *S. aureus* is a major cause of ostearthritis infections and those caused by oxacillin-resistant strains (MRSA) are particularly difficult to treat due to limited therapeutic options. MRSA ostearthritis infections are a primary indication for outpatient parenteral antimicrobial therapy.

**Methods:** As part of the Daptomycin Surveillance Program, 606 *S. aureus* causing ostearthritis infections were collected from 56 medical centers located in North America (NA); 29, Europe (EU); 17 and Latin America (LA); 10 in 2002-2009. Isolates were tested for susceptibility (S) against daptomycin and several comparators by CLSI broth microdilution method. Calcium content of the broth was adjusted (50 mg/L) for testing daptomycin. S breakpoint approved by the USA-FDA, CLSI and EUCAST (<1 mg/L) was applied.

**Results:** S to oxacillin was higher in EU (80.5%) and LA (76.0%) compared to NA (51.7%). Daptomycin was very active against *S. aureus* from ostearthritis infections independent of geographic region. Daptomycin activity was not adversely affected by resistance to oxacillin or other antimicrobials (Table). All isolates were S to daptomycin (MIC<2 mg/L) and 98.5% of strains were inhibited at ≤0.5 mg/L of daptomycin. Vancomycin (MIC<1 mg/L) and linezolid (MIC<2 mg/L) were also very active (both 100.0% S), but were four- to eight-fold less potent than daptomycin. Among MRSA, S to levofloxacin (LEV; 27.1% overall) ranged from only 16.0% in EU to 32.5% in NA; while S to clindamycin (CLI; 47.4% overall) varied from 19.2% in LA to 62.7% in NA and 72.0% in EU.

**Conclusion:** Daptomycin was highly active against a large collection of *S. aureus* from ostearthritis infections. Due to its excellent anti-*S. aureus* spectrum, high potency and rapid bactericidal activity,
Correlation of consumption and resistance in *Staphylococcus aureus* in Lebanon

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**Background:** In this study, we attempt to correlate resistance of MRSA and antibiotic consumption data and identify any association between consumption and resistance within participating hospitals in Lebanon.

**Methods:** We determined antimicrobial susceptibility testing for *S. aureus* isolated in the laboratories of 10 Lebanese hospitals. Oxacillin susceptibility was determined by using an oxacillin screening plate at 6 mg/L, an oxacillin (1 mg) disc and a cefoxitin (10 mg) disc, non-susceptible isolates were confirmed through oxacillin MIC. Susceptibility testing of other antibiotics was also performed. Consumption data were aggregated at the level of the active substance, in accordance with the WHO's ATC classification and estimated in DDD/100BD. Hospitals were sorted in an ascending order by resistance proportions and the median was established. The sorted list was divided into above and below median groups for MRSA. Mann Whitney analysis was performed for the total levels of antibiotic consumption of the respective two different clusters. Univariate regression analysis was performed for each individual antibiotic group in order to identify individual correlations between any one class and respective MRSA.

**Results:** A total of 855 isolates were studied for the 24 month study period (Jan 2008-Jan 2010), by 10 hospital laboratories spread over the Lebanese territories. The overall median MRSA proportion was 36%. Over the 2-year duration of data collection, a statistically significant increasing trend (P < 0.05) was observed in 6 participating hospitals, while significant decreases were seen in the remaining 4 hospitals. Resistance proportions were not related to the type or size of the hospital. We also examined the distribution of single methicillin resistance and multiresistance of *S. aureus*. The highest rate of multiresistance was found in Beirut, reaching levels of 42% of all MRSA isolates reported.

| Antibiotic class | Correlation coefficient |
|------------------|------------------------|
| Extended spectrum penicillins (J01C) | 0.02 |
| β-Lactamase sensitive penicillins (J01CE) | 0.41 * |
| β-Lactamase resistant penicillins (J01C) | 0.11 |
| Combination penicillins (J01CR) | 0.08 |
| 1st generation cephalosporins (J01DB) | 0.05 |
| 2nd generation cephalosporins (J01DC) | 0.02 |
| 3rd generation cephalosporins (J01DD) | 0.22 |
| Carbapenems (J01D01) | 0.41 * |
| Macrolides (J01HA) | -0.24 |
| Aminoglycosides (J01GB) | 0.26 |
| Fluoroquinolones (J01MA) | -0.1 |

* Statistically significant

Hospitals reporting lower MRSA proportions showed a median value for total antibiotic use of 0.5 DDD/100BD, as compared to 1.3 DDD/100BD in above median MRSA group. There were no evident differences in the consumption of cephalosporins (ATC classes: J01DB – J01DE) by hospitals in the below and above MRSA median arms.

**Discussion:** Our findings suggest that Lebanese hospitals with above-median MRSA proportions tended to be associated with higher levels of antibiotic consumption.

Susceptibility of *S. aureus* from inpatient vs outpatient settings – a global analysis 2009–2010

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**Objective:** *Staphylococcus aureus* is a common cause of nosocomial and community-acquired infections. Strains isolated from inpatients usually exhibit higher resistance than community-acquired strains. In this study, data from the Tigecycline Evaluation Surveillance Trial (T.E.S.T.) were used to compare the in vitro activity of several antimicrobial agents against MSSA and MRSA from inpatient and outpatient settings.

**Methods:** In 2009 and 2010, 5,124 *S. aureus* isolates from multiple specimen sources were collected in 48 countries. MICs were performed at each site following CLSI guidelines using commercially-prepared microbroth panels. Results were interpreted according to CLSI breakpoints (FDA breakpoints for tigecycline). % susceptible for several antimicrobials, as well as p values comparing isolates from inpatient and outpatient settings.

**Results:** 2,740 and 658 MSSA and 1,424 and 302 MRSA from inpatients and outpatients, respectively, were collected. The following table shows the MIC50, geometric mean (GM) MIC, and % susceptible for several antimicrobials between inpatients and outpatients were tested using the Fisher exact test, while geometric mean MICs were compared by the non-parametric Wilcoxon rank sum test.

| Antibiotic | Inpatient | Outpatient | p | % Susceptible |
|------------|-----------|------------|---|--------------|
| **MSSA**   |           |            |   |              |
| Amoxicillin | 0.5 ± 0.25 | 0.5 ± 0.25 | >0.05 | 100 |
| Ceftriaxone | 2 ± 1.94  | 2 ± 1.94  | >0.05 | 100 |
| Cefotaxime | 0.12 ± 0.05 | 0.12 ± 0.05 | >0.05 | 94.3 |
| Cefoperazone| 1.2 ± 0.62 | 1.2 ± 0.62 | >0.05 | 92.3 |
| Vancomycin | 1 ± 0.06  | 1 ± 0.06  | >0.05 | 100 |

**MRSA**

| Antibiotic | Inpatient | Outpatient | p | % Susceptible |
|------------|-----------|------------|---|--------------|
| Tigecycline| > 8       | > 8        | >0.05 | 100 |
| Vancomycin | 1 ± 0.08  | 1 ± 0.08  | >0.05 | 97.9 |

**Conclusions:** In 2009/10, amoxicillin-clavulanic acid, levofloxacin, meroxepen, and minocycline exhibited significantly higher MICs (p < 0.05) in inpatient isolates than in outpatient specimens, mostly for MRSA. Examining the GM MIC and testing for significant differences is often more sensitive for detecting changes in in vitro activity than the MIC50, geometric mean (GM) MIC, and % susceptible for several antimicrobials, as well as p values comparing isolates from inpatient and outpatient settings.

**Susceptibilities of antibiotics with activity against methicillin-resistant *Staphylococcus aureus* from 2004–2008

T. Crawford*, L. Danziger, K. Rodzold, W. Janda (Chicago, US)

**Objective:** Presence of subtle changes in vancomycin minimum inhibitory concentrations (MICs) against *Staphylococcus aureus* (i.e., MIC creep) has not been conclusively shown in scientific literature. At the University of Illinois Medical Center at Chicago, vancomycin MICs for methicillin-resistant *Staphylococcus aureus* (MRSA) obtained by automated systems [Vitek Legacy (2004–2007)] and Vitek 2 (2007–2008)] decreased from 2004–2008. Objectives of this study are to determine vancomycin MICs against MRSA utilizing E-test strips,

| Antibiotic | Inpatient | Outpatient | p | % Susceptible |
|------------|-----------|------------|---|--------------|
| Vancomycin | 1 ± 0.06  | 1 ± 0.06  | >0.05 | 97.9 |

* Statistically significant
compare vancomycin MICs obtained by Vitek 2 and E-test, and establish daptomycin, trimethoprim/sulfamethoxazole, rifampin, linezolid, and tigecycline MICs against MRSA.

Methods: 103 blood isolates were collected and stored at −70°C. Identification of MRSA and initial vancomycin sensitivity testing were performed by Vitek Legacy and Vitek 2. Blood isolates were tested for susceptibility to six antibiotic agents (vancomycin, rifampin, daptomycin, linezolid, tigecycline, and trimethoprim/sulfamethoxazole) by E-tests, using Mueller Hinton agar plates (MHA) inoculated with inoculum suspension with a turbidity equivalent to 0.5 McFarland standard. MHA plates were incubated at 35°C for 24 hours and read by a single reviewer.

Results: 31 isolates in 2004, 30 isolates in 2005, 19 isolates in 2006, 22 isolates in 2007, and 10 isolates in 2008 were identified. 18 isolates in 2004 and 2 isolates in 2008 reported a vancomycin MIC=2 obtained by Vitek 2. Vancomycin MICs obtained by E-test report 3 isolates with MIC=2 in both 2004 and 2008. All isolates were susceptible to daptomycin during 2004–2006 however, 2 isolates in 2007 and 3 isolates in 2008 were resistant. 1 isolate in 2005 and 3 isolates in 2007 were resistant to trimethoprim/sulfamethoxazole. 1 isolate in both 2004 and 2006 and 2 isolates in both 2007 and 2008 were resistant to rifampin. All isolates were susceptible to linezolid and tigecycline.

Conclusion: Vancomycin MICs obtained by E-test did not reflect a declining vancomycin MICs as reported by Vitek 2. Vancomycin MICs remained fairly constant based on MIC reports obtained by E-test. Small changes in the susceptibility of daptomycin, trimethoprim/ sulfamethoxazole and rifampin were reported while isolates remained susceptible to linezolid and tigecycline.

**[P1050]** Antimicrobial susceptibility phenotypes of vancomycin intermediate *Staphylococcus aureus* isolates

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Objectives: The purpose of this study was to determine if evidence of telavancin non-susceptibility existed among vancomycin intermediate *Staphylococcus aureus* (VISA) isolates in our medical center.

Methods: *S. aureus* isolates that grew on a novel 3 mg/L vancomycin screening agar were subsequently tested for vancomycin susceptibility by broth microdilution and VISA (MIC 4–8 μg/mL) isolates were frozen at −80°C for future analysis. A collection of 148 consecutive VISA isolates were then retrieved for characterization of telavancin susceptibility, and repeat vancomycin and daptomycin MIC testing by broth microdilution. Clonality of VISA isolates was determined by repPCR. A selection of isolates was subjected to microdilution population analysis profile (PAP) and BACcel analyses to determine hVISA status.

Results: 141 of 148 isolates (95.2%) failed to maintain their VISA phenotype, by MIC, upon retesting under non-selective conditions. 16 of these isolates were tested by population analysis (PAP) and BACcel – a novel real-time growth analysis system, with concordant findings between the two methods that 14/16 were hVISA. The remaining 2 isolates were identified as VSSA by PAP and hVISA by BACcel. RepPCR analysis revealed that the 7 isolates that remained vancomycin intermediate after thawing were not clonal, but fell into 5 distinct clusters. 46% of *S. aureus* isolates that initially tested vancomycin intermediate had also tested daptomycin non-susceptible. Upon retesting after thawing, only 6% of isolates were daptomycin non-susceptible. 69% of the initial VISA isolates were methicillin resistant, while 31% were methicillin susceptible. No isolates were telavancin non-susceptible (MIC >1 mg/L).

Conclusion: This study demonstrates that phenotypic expression of vancomycin-intermediate status in *S. aureus* is unstable and likely to be lost when selective pressure is removed. Retrospective studies of stocked organisms to determine vancomycin susceptibility prevalence may yield inaccurate results. The use of vancomycin screening agar likely identifies hVISA/VISA that would have been missed if broth microdilution had been done directly from blood agar plates. There is a correlation between reduced susceptibility to vancomycin and daptomycin. Additionally, a significant proportion of hVISA/VISA isolates are methicillin susceptible. No evidence of telavancin non-susceptibility was detected.

**[P1060]** Present situation of antimicrobial resistance of *Staphylococcus* in Spain (2010): seventh nationwide prevalence study and emerging resistance to linezolid

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Objective: Data regarding the evolution of *Staphylococcus* resistance in a whole country have a definite influence in the design of empirical treatment regimens. For the last 25 years we have been performing prevalence studies in order to ascertain the situation of the antimicrobial resistance of *Staphylococcus* in our country. In this study we present the results of the seventh point prevalence study performed in 2010.

Methods: In a selected day of June 2010, we collected all staphylococci isolated in 152 Spanish hospitals. All microorganisms were sent to a coordinating laboratory where identification and antimicrobial susceptibility testing was performed against 15 antimicrobial agents using and automated microdilution method (MicroScan). Additional E-test susceptibility testing was performed for vancomycin (VAN), linezolid (LIN), daptomycin (DAP) and tigecycline (TG).

Results: We collected 542/438 *S. aureus/coagulase negative staphylococci (CoNS) isolates*. Percentages of resistance of *S. aureus*/CoNS against selected antimicrobials (PEN = penicillin, OXA = oxacillin, ERY = erythromycin, CLI = clindamycin, GEN = gentamicin, TOB = tobramycin, CIP = ciprofloxacin, RIF = rifampin, T/S = trimethoprim/ sulfamethoxazole) in comparison with the results obtained in two previous studies (2002 and 2006) are shown in the table. No resistance to VAN, LIN, DAP, and TG was found among *S. aureus*. However, among CoNS, resistance to LIN was 4.3%, and one isolate showed decreased susceptibility to DAP (MIC 1.5 mg/L). All strains were susceptible to TG. The most frequent phenotypes of resistance among methicillin-resistant *S. aureus* were ERY+CIP+TOB (21%); ERY+CIP (17.5%); CIP (17.5%); and TOB+CIP (11.7%).

Conclusions: Methicillin-resistance of *S. aureus* and CoNS in Spain seems to be stabilized (28%, and 60%, respectively) and strains are more susceptible to GEN. However, emerging resistance of CoNS to linezolid (4.3%) is a cause for concern.

| Year | PEN | OXA | ERY | CLI | GEN | TOB | CIP | RIF | T/S | VAN |
|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 2002 | 88/97 | 31/83 | 33/84 | 20/35 | 13/85 | 9/30 | 27/22 | 0/0 | 0/0 |
| 2006 | 86/87 | 20/70 | 32/86 | 20/46 | 8/44 | 28/50 | 31/54 | 1/7 | 1/31 | 0/0.5 |
| 2010 | 87/109 | 28/90 | 24/100 | 20/60 | 7/26 | 15/92 | 31/50 | 1/9 | 0/33 | 0/0 |

Table. Percentages of resistance of *S. aureus*/CoNS to selected antimicrobials.

**[P1065]** Thioridazine increases susceptibility to oxacillin in methicillin-sensitive *Staphylococcus aureus*

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Objectives: The psycho-active drug, thioridazine, has been shown to increase oxacillin (methicillin) susceptibility in MRSA. The underlying mechanism is still unclear but seems to involve down regulation of resistance and cell wall biosynthesis genes, while a number of surveyed virulence factors are not induced by this drug combination. Now we find that thioridazine also can boost the susceptibility of Methicillin-sensitive *Staphylococcus aureus* (MSSA) strains toward oxacillin and make them even more sensitive to this β-lactam antibiotic. Although MSSAs are in principle sensitive to oxacillin, recurring infections is a big issue with staphylococcal infections. Subsequently, any increase in susceptibility of the MSSA toward antibiotic treatment will possibly ease treatment. Furthermore, we examined the effect of thioridazine with or without oxacillin on the cell wall stimulum, vraSR and on a number of virulence factors to verify that the treatment does not complicate the infection.
**Methods:** Growth of four MSSA strains (ATCC 25923, Newman, UAMS-1 and 8325–4) was examined in liquid media in the presence of the methicillin analogue, oxacillin and the non-antibiotic, thioridazine alone and in combination. We also used phase contrast microscopy to examine the cells and analyzed transcription of vraSR and RNAIII by northern blot and primer extension under the same conditions.

**Results:** We observed an increased susceptibility of all four MSSA strains toward oxacillin in the presence of thioridazine compared to bacteria grown with oxacillin or thioridazine alone. Phase contrast microscopy reveals how cells treated with both oxacillin and thioridazine become deformed and misshaped, their membranes are clearly affected and many cells have lysed within 10 hours. We also observed a concentration-dependent induction of vraSR by thioridazine alone indicating that the cell wall is damaged by the treatment, which was also noticed by microscopy. Notably, we do not find an increase in expression of the virulence marker, RNAIII, indicating that thioridazine does not induce virulence.

**Conclusion:** Our study shows that addition of thioridazine can increase oxacillin susceptibility in MSSAs. This important observation allows treatment of recurring MSSA infections with lower concentrations of oxacillin, which is of utterly importance in times where the world consumption of antibiotics is alarmingly high leading to corresponding high development of resistance.

**P1062** Secondary metabolites from lichens: drug interaction of usnic acid, lobaric acid and protolichesterinic acid with antibiotics against MRSA clinical isolates

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**Objectives:** The objectives of this work were:
- to characterise the potential antibacterial activity of usnic acid, protolichesterinic acid and lobaric acid secondary metabolites from lichens against methicillin-resistant Staphylococcus aureus clinical isolates;
- to investigate the interaction of the bioactive compounds from lichens in combination with the antibiotics which susceptibility against MRSA is reduced.

**Methods:** MICs of secondary metabolites from lichens were characterised by microdilution test in Mueller-Hinton broth, where bacteria were grown at 37°C 18 hours. MBCs were calculated by plating 100 μL of bacterial culture on MH agar medium from the determined MIC value of each compounds up to eightfold the MIC value. The compounds were tested in combination with erythromycin, clindamycin, gentamicin, levofloxacin and oxacillin. All tested clinical isolates showed resistance to those antibacterial agents. Drug interactions were assessed by broth microdilution assays. The growth of each well was quantified by microplate reader at 600 nm. FICI was calculated. Synergy was defined as FICI < 0.5, antagonism as FICI > 4, no-interaction for values FICI between 0.5 and 4.

**Results:** MIC performed on 10 clinical isolates of MRSA showed a value ranging from 2–4 μg/mL for usnic acid, 4–8 μg/mL for protolichesterinic acid, 16–32 μg/mL for lobaric acid. MBC for each compounds was ranging from the MIC value to twofold the MIC value calculated for each isolate. The checkerboard assay showed synergy between each natural compound in combination with LVX and GEN (FIC ≤ 0.5), while for ERY, CLI and OXA no-interactions were observed (FICI between 0.5 and 4). No antagonism has been observed. Beside the antibacterial activity the cytotoxic effects of the secondary metabolites were evaluated in human tumor cell lines (MCF-7, human breast adenocarcinoma; HCT-116, human colon carcinoma; HeLa, human cervix carcinoma. Usnic acid and protolichesterinic acid inhibited significantly the growth of HeLa and HCT-116 cells.

**Conclusion:** Although the antimicrobial activity of usnic acid against Gram-positive bacteria is known, the interaction between secondary metabolites from lichens in combination with antibiotics has been investigated for the first time in this study. This would pave the way for the introduction of new classes of molecules in clinical practice from natural sources. The potential biological activity of those compounds might be extended to eukaryotic cells.

**P1063** Genetic analysis of bacteriophages encoding virulence factors among methicillin-resistant Staphylococcus aureus isolated from clinical, animal and environmental samples in Iran

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**Introduction:** Most of the Staphylococcus aureus isolates contain lysoytic phages are responsible for production of various virulence factors. All isolates are classified in 6 groups according to their sensitivity to 27 known lysoytic phages. This study is done since 2008, to detect genetic analysis of different bacteriophages encoding virulence factors among S. aureus isolated from clinical, animal and environmental samples.

**Material and Methods:** Totally 300 isolates of MRSA from human, animal and sewage samples were collected from hospitals, farms and sewage treatment plants in Tehran. All isolates were identified at the species level using specific primers and typed using PGE and Phene-Plate system. Susceptibility to eighteen antibiotics was determined using disc diffusion method and MIC of resistant isolates was also done using Etest according to CLSI recommendation. Primers for identification of 6 classes of bacteriophages were used in Multiplex-PCR assay. Different virulence factor genes were detected using PCR. All results were confirmed by sequencing.

**Results:** Using PCR all isolates were confirmed as MRSA. 100%, 100%, 89%, 94%, 83%, 2%, 1%, 94%, 87%, 94%, 95%, 65%, 49%, 59%, 32%, 0% and 0% of isolates were resistant to oxacillin, penicillin, clindamycin, tobramycin, tetracycline, nitrofurantoin, fusidic acid, kanamycin, amikacin, erythromycin, ciprofloxacin, SXT, rifampin, gentamycin, nimicyncline, synergic, linezolid and chloramphenicol respectively. 90% of isolates were highly resistant to oxacillin. All MRSA isolates contain at least one phage. Out of the total MRSA isolates, 95% and 93% of isolates contained 4 and 5 different classes of phages, respectively. Only 2% of the total MRSA isolates had one class of phage. 100%, 0%, 1.5%, 0%, 0%, 14%, 0%, 18%, 0%, 100%, 2%, 3%, 2%, 1%, 100%, 100%, 92%, 0%, 0% and 1% of isolates contained seA, seB, seC, seD, seE, seF, seG, seH, seI, seJ, seK, seL, seM, seN, seO, seP, seQ, hIB, sak, et and tis respectively. 6 single types and common types were seen.

**Conclusion:** More diversity in the type of phages was found in human MRSA isolated than in animals and environment. Prophage segments from Phi-77-like phages were common in the isolates indicating the ability of these isolates to produce enterotoxins and other virulence factors. High prevalence of different classes of phages and also presence of broad spectrum virulence factors and high oxacillin resistance provide an important role of phages in the evolutionary development of virulence factors and also diversity in methicillin resistance cassette in MRSA isolates.

**P1064** Comparison of MIC values of vancomycin, teicoplanin, linezolid and daptomycin in methicillin-resistant Staphylococcus aureus strains between 2007–2010

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**Objectives:** Methicillin-resistant Staphylococcus aureus (MRSA) has been emerging worldwide as one of the most important problems in communities and hospitals. Vancomycin has been considered the Standard for the treatment of MRSA infections. An increase of vancomycin minimum inhibitory concentration (MIC) values in MRSA strains has been noted in several reports. This increase is a concern, since a growing number of studies have suggested that patients with infections caused by MRSA with vancomycin MICs at the higher end of susceptibility range are less responsive to vancomycin. Clinicians must
have alternative therapies for such patients. Teicoplanin has demonstrated similar efficacy to vancomycin against S. aureus strains and is less toxic than vancomycin. Linezolid is especially approved for the treatment of nosocomial pneumonia caused by MRSA. Daptomycin is a new lipopeptide antibiotic against MRSA and rare instances of resistance have occurred. It is unclear whether the increase in vancomycin MICs for MRSA is associated with similar shifts in MICs of other anti MRSA agents. We aimed to examine the correlation between vancomycin MICs and the MICs of teicoplanin, linezolid and daptomycin in MRSA strains.

**Methods:** A total of 200 MRSA strains isolated from clinical samples were included in this study. The MICs for vancomycin, teicoplanin, linezolid and daptomycin were determined with E test strips (AB Biodisk, Sweden). The statistical analysis of the study was performed by \( \chi^2 \) test.

**Results:** All the MRSA strains were susceptible to the four antimicrobials. Table 1 shows the MIC range, MIC50 and MIC90 values for vancomycin, teicoplanin linezolid and daptomycin. Although there were not statistically significant differences among MIC values of teicoplanin linezolid and daptomycin, there was a statistically significant difference in the MIC values of vancomycin between 2007 and 2010 (p = 0.05).

**Conclusion:** It is supported with our data that, the most common anti-MRSA agents maintained their full spectrum of in vitro activity against MRSA strains in our hospital. There are limited data on the association between the MIC values of vancomycin and other anti-MRSA agents. In our study, although there is an increase in vancomycin MIC values between 2007 to 2010, there is no increase in MICs of other anti- MRSA agents like vancomycin. In order to prevent treatment failure with vancomycin, MIC values must be determined during vancomycin therapy.

**Table:**

| Site | No. N | Error Rates |
|------|-------|-------------|
|      |       | % CA | % VM | % MA | % MI |
| 1    | 40    | 85.0 | 7.5 | 7.5  | 5.0  |
| 2    | 38    | 86.8 | 2.6 | 10.5 | 7.9  |
| 3    | 40    | 90.0 | 0   | 10.0 | 0    |
| 4    | 40    | 80.0 | 17.5| 2.5  | 7.5  |
| 5    | 60    | 86.7 | 5.0 | 8.3  | 5.0  |

New insights on mechanisms of resistance in Staphylococcus aureus

**Table:**

| MIC Range (g/mL) | MIC90 (g/mL) | MIC50 (g/mL) | MIC90 (g/mL) |
|------------------|--------------|--------------|--------------|
| VANCOMYCIN       | 0.02-8.0     | 1.5          | 2.0          |
| TEICOPLANIN      | 1.0          | 2.0          | 4.0          |
| LINEZOLID        | 0.25-24.0    | 0.75         | 1.5          |
| DAPTOMYCIN       | 0.007-15.5   | 0.125        | 0.25         |

An in vitro multisite study of vancomycin and daptomycin

**Objective:** The purpose of this study was to determine daptomycin and vancomycin MIC and MBC results for recent Staphylococcus aureus clinical isolates at various medical centers in Spain.

**Methods:** A total of 6 medical centers from 4 distinct cities in Spain tested 628 contemporary, clinically-significant isolates MRSA (n = 516) and MSSA (n = 112) isolates from various specimen sources. All isolates were tested to determine MIC and MBC using dried broth microdilution panels containing daptomycin and vancomycin (TREK Diagnostics, West Sussex, England). With exception of dried panels, MIC procedures followed EUCAST guidelines and MBC procedures followed CLSI and ASP guidelines. MIC quality control was performed with S. aureus ATCC 29213. MIC interpretative criteria were based on EUCAST clinical breakpoints. No testing was performed to detect VRSA.

**Results:** The daptomycin MIC90 for all S. aureus was 0.5 mg/L, the range of MICs was 0.12–2 mg/L (MIC for 3 MRSA was 2 mg/L). The vancomycin MIC90 for all S. aureus was 1 mg/L, the range of MICs was 0.25–2 mg/L (MIC for 17 MRSA and 3 MSSA was 2 mg/L). The percentage of vancomycin tolerant strains for all sites was 6.8% (43/628), 86% of which were MRSA. In comparison, 0.3% (2/628) of strains (both MRSA) were considered daptomycin tolerant (See Table).

**Conclusion:** There were no VISA/VRSA strains detected and >99% were susceptible to daptomycin. The higher level of vancomycin tolerant strains compared to daptomycin suggests superior in vitro bactericidal activity of daptomycin.

**Table:**

| City       | n MRSA/ MSSA | Daptomycin | Vancomycin | % Vancomycin Tolerant* |
|------------|--------------|------------|------------|------------------------|
|            |              | 50%        | 50%        | 50%                    |
| Madrid     | 840          | 0.5        | 1.0        | 0.5                    | 11.5 |
| Barcelona  | 138/63       | 0.5        | 0.5        | 1                      | 6.1  |
| Barcelona  | 800          | 0.25       | 0.5        | 0.5                    | 11.25|
| Donostia   | 98/19        | 0.5        | 0.5        | 1                      | 0    |
| Madrid     | 75/0         | 0.5        | 0.5        | 1                      | 6.7  |
| Palma de Mallorca | 43/0       | 0.5    | 0.5        | 1                      | 4.6  |

**All Sites**

| 516/112    | 0.5        | 0.5        | 1                      | 6.8  |

* Tolerance defined as MBC/MIC ratio ≥ 16.
**P1062** Influence of telavancin sub-minimum inhibitory concentration on methicillin-resistant *Staphylococcus aureus* virulence factor expression

C. Teles*, C. Gemmell, S. Lang (Glasgow, St Andrews, UK)

**Objectives:** Telavancin is semi-synthetic lipoglycopeptide antibiotic currently under review by the European Medicines Agency for treatment of nosocomial pneumonia and complicated skin and soft tissue infections caused by Gram positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA). Despite the bactericidal activity of this agent, evidence suggests that exposure of bacterial cells to antibiotic sub-minimum inhibitory concentrations (sub-MICs) can modulate virulence factors expression in a growth stage dependent manner. This study aims to evaluate the temporal expression of virulence factors by clinical isolates of hospital-acquired MRSA (HA-) and community-acquired MRSA (CA-) after exposure to 0.25× MIC of telavancin.

**Methods:** HA-MRSA 784 and CA-MRSA USA300 suspensions grown in Mueller-Hinton broth at 37°C were exposed to 0.25× MIC of telavancin for 1h prior to harvesting in the mid-exponential, early- and late-stationary growth phases, at which point bacterial cells RNA was extracted and reverse transcribed. The effect of the antibiotic on the expression of toxins (pvl and tst), adhesins (clfB and cna) as well as the quorum-sensing intracellular effector molecule RNAIII, was determined through qRT-PCR. The expression data were analysed using the 2−ddCt method and the gene modulation threshold was defined as a 2-fold change.

**Results:** Exposure of CA-MRSA USA300 to 0.25× MIC of telavancin did not influence the expression of the virulence genes assessed at any of the growth stages analysed. In contrast, the tst and clfB genes of HA-MRSA 784 were down-regulated at the late-stationary phase of growth, whilst the expression of the remaining genes examined was not subjected to modulation. Additionally, the expression of the RNAIII encoding gene of both CA- and HA-MRSA was not altered due to antibiotic exposure.

**Conclusion:** This study has demonstrated that CA- and HA-MRSA respond differently to a sub-MIC of telavancin, however, the effect of this antibiotic is independent of the quorum-sensing system of these organisms, as demonstrated by the lack of modulation of the RNAIII molecule. The suppression of the tst and clfB genes of the HA-MRSA isolate is indicative of the positive effects of telavancin in the management of MRSA infections.

**Experimental treatments in vivo**

**P1068** Ceftobiprole efficacy in vitro on Panton-Valentine leukocidin production and in vivo in a rabbit community-associated methicillin-resistant *Staphylococcus aureus* osteomyelitis model

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**Objectives:** Community-Associated Methicillin-Resistant *Staphylococcus aureus* (CA-MRSA) can cause osteomyelitis with severe sepsis and/or local complications in which a Panton-Valentine Leukocidin (PVL) role is suspected. In vitro Ceftobiprole (CEF) efficacy against 3 major PVL+ CA-MRSA clones (USA300 (LAC), -400 and -1000) was tested in time-kill analyses and for PVL production. In a rabbit CA-MRSA osteomyelitis model, efficacies of CEF (40 mg/kg sc qd) or vancomycin (VAN) (60 mg/kg im bid) alone or combined with rifampin (RIF) (10 mg/kg bid) were compared.

**Methods:** Eleven CA-MRSA strains were cultured for 18 h in CCM broth with SubMIC CEF. In parallel, CEF at its MIC time-kill analyses were run with LAC, starting at 107 CFU/mL. A specific ELISA measured PVL production. Intramedullary injection of a searing agent into the tibia, followed by inoculation of 4×10⁷ LAC CFU (respective CEF, VAN and RIF MIC: 0.75, 1.5 and 0.012 mg/L) induced osteomyelitis. Treatment was started 14 days post inoculation and lasted 14 days; 7 days later, bacteria in crushed tibial bones were counted.

**Results:** PVL production by strains cultured with subMIC CEF was 1.6–4.8-fold above the control level for 6/11 strains tested, with no link to specific clones. Time-kill analyses at the MIC showed a transient PVL production rise at 6 and 8 h. In vivo, CEF had significantly lower mean log10 CFU/g of bone (1.44±0.40) vs controls (4.33±0.91) (p < 0.01) and sterilized 7/10 bones vs 0/6 controls. CEF was more effective than VAN (2.37±1.22 CFU/g of bone (p < 0.05), 5/11 sterile bones). CEF+RIF (1.16±0.04 CFU/g of bone, 11/11 sterile bones) was more effective than CEF alone (p < 0.05).

**Conclusions:** In this CA-MRSA (USA300) model, in vivo CEF efficacy was enhanced by RIF adjunction. CEF bactericidal activity and the known RIF anti-PVL effect could partly explain these findings, which should be of interest for treating osteomyelitis.

**P1069** Efficacy of daptomycin versus vancomycin in an experimental model of foreign body infection by biofilm producer *Staphylococcus epidermidis*

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**Objectives:** Coagulase-negative staphylococci (CoNS) are frequent causes of infections of prosthetic devices (75% of cases). In addition, CoNS cause more than 50% of infections in ventricle-peritoneal shunts and 40% of prosthetic endocarditis. In 2009 (data from our hospital), 75% of 224 bacteremic strains of *S. epidermidis* were resistant to methicillin (MRSE). On the other hand, the emergence of strains with reduced susceptibility to vancomycin (VAN) makes necessary to know other therapeutic alternatives for this kind of infections. The aim of this study is to compare the efficacy of daptomycin (DAP) versus VAN in an experimental model of foreign body infection caused by biofilm-producer MRSE strains, with reduced susceptibility to vancomycin.

**Methods:** In vitro studies: MICs of DAP and VAN were determined in 2 isolates of MRSE (SE284 and SE385). Biofilm production was assessed by genotypic (ica genes) and phenotypic (Congo red and crystal violet) methods. Bactericidal activity was assayed by time-kill curves (1xMIC and Cmax). In vivo studies: a murine model of foreign body infection (intraperitoneal catheter) in C57BL/6 neutropenic (cyclophosphamide 200 mg/kg) and bacterial inoculum of 8 to 8.5 Log CFU/mL was performed. Pharmacokinetic/Pharmacodynamic (PK/ PD) studies (Cmax [mg/L], AUC [mgol/L], t1/2 [h], t<--MIC [h], AUC/MIC) in serum after single dose of DAP (50 mg/kg) and VAN (110 mg/kg) in healthy mice. Therapeutic groups: untreated (CON), DAP (once daily) and VAN (four times daily). Later, viable bacteria in liver (Log CFU/g) and catheter (Log CFU/mL), survival (%), and qualitative blood culture (%) were measured. Statistical analysis: ANOVA, post hoc test, and χ².

**Results:** MIC (mg/L): SE284: DAP = 1, VAN = 4; SE385: DAP = 1, VAN = 2. Both strains were positive for biofilm production. Bactericidal activity: SE284, DAP (Cmax), VAN (1xMIC and Cmax);
SE385, DAP (Cmax), VAN (1xMIC and Cmax), PK/PD parameters for each antimicrobial are shown in the attached table. The results of the experimental model are shown in the attached table. Statistical significance (p < 0.05): [a] vs. the control group; [b] vs. vancomycin group.

**Conclusion:** Daptomycin was more effective than vancomycin in the treatment of experimental foreign body infection caused by MRSE with reduced susceptibility to vancomycin and biofilm-producer, in terms of bacterial burden reduction in catheter and liver.

**[P1070] Meropenem treatment of experimental thigh infections caused by meropenem-heteroresistant Acinetobacter baumannii clinical isolates**

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**Objectives:** Meropenem heteroresistance was previously characterized in Acinetobacter baumannii clinical isolates. The current study investigated the efficiency of meropenem treatment in experimental infections caused by meropenem-heteroresistant A. baumannii clinical isolates.

**Materials:** The study included 12 strains: native populations of five meropenem-susceptible A. baumannii strains and the respective five meropenem-heterogeneous subpopulations (colonies grown at highest meropenem concentrations in population analyses), a non-heteroresistant meropenem-susceptible isolate and Escherichia coli ATCC 25922 as controls. Thighs of mice were infected in triplicate by approximately 7log10 CFU of each strain, animals were treated for 24h with meropenem at 20 mg/kg/8h, 100 mg/kg/12h or 400 mg/kg/8h and equal numbers of animals remained untreated as controls. Mice died from infection or sacrificed at 24h, thighs excised, homogenized and cultured serially diluted to enumerate bacterial counts. Growth curves were performed for all study strains.

**Results:** All 15 untreated mice infected by the 5 native populations died in <24h. Untreated mice infected by heterogeneous populations of 2 strains survived 24h in contrast with mice infected by 3 heterogeneous populations. Mice infected by the non-heteroresistant and the E. coli ATCC 25922 control strains survived 24h regardless of treatment. Using 20 mg/kg and 100 mg/kg meropenem treatment 27/30 mice infected by the 5 native populations died in <24h; no significant decrease in colonies grown was observed for all 30 treated mice compared with untreated ones (P > 0.05). In contrast, 24/30 mice infected with heterogeneous populations survived for 24h receiving 20 and 100 mg/kg meropenem, although colony counts were similar in treated and untreated animals (P > 0.05). With 400 mg/kg meropenem regimen 28/30 infected mice survived 24h; a significant decrease (P < 0.05) in colony counts was observed for 12/15 mice infected by native populations and all 15 mice infected by heterogeneous populations, compared with untreated mice. The control strains responded sufficiently to 100 and 400 mg/kg meropenem regimens. Considerably lower growth rates were observed among 4/5 heterogeneous populations compared with the native ones.

**Conclusions:** The results of the present study indicate that A. baumannii isolates exhibiting heteroresistance are responsive to high meropenem dosages. Heterogeneous seem to be less virulent than native populations.

**[P1071] In vivo activity of 22β-hydroxyoleanonic acid, against Streptococcus pneumoniae in a mouse model**

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**Objective:** The 22β-hydroxyoleanonic acid is a congenor of pentacyclic triterpenoid Lantadene A with potent activity against Gram-positive pathogens. The in vivo activity of 22β-hydroxyoleanonic acid against Streptococcus pneumoniae was compared with those of gatifloxacin and ciprofloxacin in a mouse model.

**Methods:** For study, two strains of S. pneumoniae were used [penicillin-susceptible S. pneumoniae (PPSP) and penicillin-resistant S. pneumoniae (PRSP)]. Five-week-old male CBA/J specific-pathogen-free mice (body weight, 20 g) were used for in vivo studies. The MICs of antibiotics were determined by a broth dilution method with Mueller-Hinton broth.

Mortality was recorded for 14 days, and the 50% effective dose (ED50) of each drug was calculated by the probit method. For bacteriological examination, the lungs (n = 7 for each group) were dissected under aseptic conditions and suspended in saline (1ml). Lung tissue for histological examination (n = 3 for each group) was fixed in 10% buffered formalin and stained with hematoxylin-eosin.

**Results:** The survival rates of mice infected with PPSP and PRSP at 14 days after infection were 80% in the 22β-hydroxyoleanonic acid-treated group and 0 to 10% in the other three groups. In murine infections caused by PPSP, the 50% effective doses (ED50) of 22β-hydroxyoleanonic acid, gatifloxacin, and ciprofloxacin were 26.4, 40.3, and 136.2 mg/kg, respectively. Against PRSP-caused pneumonia in mice, the ED50 of 22β-hydroxyoleanonic acid, gatifloxacin, and ciprofloxacin were 37.4, 62.5, and 124.2 mg/kg, respectively. Compared with the other drugs, 22β-hydroxyoleanonic acid showed excellent therapeutic efficacy and eradicated viable bacteria in both PPSP- and PRSP-infected mice. The means±standard errors of the means of viable bacterium counts in the lungs of gatifloxacin-treated, ciprofloxacin-treated, and untreated control mice infected with PPSP were 2.90±0.34, 3.10±0.48 and 3.80±0.80 log10 CFU/ml, respectively. The same counts in mice infected with PRSP treated with the same three agents were 6.50±0.99, 6.52±0.40, and 7.12±0.42 log10 CFU/ml, respectively. 22β-Hydroxyoleanonic acid significantly decreased the number of viable bacteria in the lungs compared with gatifloxacin and ciprofloxacin.

**Conclusion:** From results it is inferred that 22β-hydroxyoleanonic acid has potent in vivo efficacy against both PPSP and PRSP.

**[P1072] Evaluation of combinational antimicrobial treatment using rifampicin and gentamicin for Escherichia coli O157:H7 in vitro and in vivo in BALB/c mice**

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**Objectives:** Escherichia coli O157:H7 is the most commonly encountered member of the Enterohemorrhagic E. coli (EHEC) group. Symptoms of infection with this organism include the potentially fatal hemolytic uraemic syndrome (HUS). Treatment of E. coli O157:H7 infections with antimicrobial agents is controversial due to an association with inducing HUS. The production of Shiga-like toxins is believed to be central to the pathogenesis of this organism. Therefore, decreasing the expression and release of these toxins prior to bacterial eradication may provide a safer course of therapy. In this study, the utility of decreasing toxin expression with rifampicin prior to eradication with gentamicin was evaluated.

**Methods:** The in vitro effect of rifampicin and gentamicin on the expression of stx1 and stx2 genes, which code for SLT-I and SLT-II respectively, was assessed using real time reverse transcriptase polymerase chain reaction (RT-PCR). This was performed on samples of each drug was calculated by the probit method. For bacteriological examination, the lungs (n=7 for each group) were dissected under aseptic conditions and suspended in saline (1ml). Lung tissue for histological examination (n=3 for each group) was fixed in 10% buffered formalin and stained with hematoxylin-eosin.

**Results:** Shiga-like toxin genes was considerably decreased as an effect of treatment E. coli O157:H7 in vitro with the minimum inhibitory concentration (MIC) of rifampicin followed by the minimum bactericidal concentration (MBC) of gentamicin (>99% decrease) compared to treatment with gentamicin alone (50−75% decrease). RPLA-based detection of Shiga-like toxins released from E. coli O157:H7 incubated with the MIC of rifampicin followed by addition of the MBC of gentamicin confirmed a decrease in toxin release as well. The highest rate of survival in BALB/c mice infected with E. coli O157:H7 was observed in those treated with the in vivo MIC equivalent dose of rifampicin followed by the in vivo MBC equivalent dose of gentamicin (50% survival) compared to mice treated with gentamicin (0% survival) or rifampicin (25% survival) only.

**Conclusions:** The use of expression-inhibitory antimicrobial agents prior to bactericidal doses in treating E. coli O157:H7 infection is effective and may potentially be useful in human infections with this agent.
**Efficacy of high doses of daptomycin in combination with cloxacinil in an experimental foreign-body infection by methicillin-resistant Staphylococcus aureus**

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**Objectives:** Clinical failures and emergence of resistance when using daptomycin (D) in monotherapy against high bacterial inoculums and foreign-body infection (FBI) could be avoided with high doses of D alone or in combination. D plus cloxacinil (C) has shown in vitro synergism and limited efficacy in experimental endocarditis by D-nonsusceptible MRSA. Herein, we studied the efficacy of D (equivalent to 10 mg/kg/d in humans) in combination with C and compared with that of D plus rifampin (R) in a FBI model by methicillin-resistant S. aureus (MRSA).

**Methods:** In vitro studies: MICs (mg/L): 1 (D), >256 (C), 0.03 (R). MBCs and 24 h kill-curves were performed in the log-phase at inoculum 10^6 CFU/ml (5LP) and at high inoculum 10^7 CFU/ml (7LP), and in the stationary-phase (SP). Animal studies: Two Teflon tissue-cages with a cover-slip (CV) were subcutaneously implanted in rats; after 3 weeks, tissue-cage fluid (TCF) was infected with MRSA-HUSA 304 and, 72 h later, therapy was given for 7 days. Criteria of efficacy: Decreases in TCF bacterial counts between the beginning and the end of treatment (at 1 and 4 days after the end: d8 and d11, respectively); and bacterial counts from CV at d11. Therapeutic groups (mg/kg/h): D (100/24), C (200/12), D+C, D+R (25/12) and controls (CO).

**Results:** MBCs (mg/L) 5LP/7LP/SP: 4/16/24 (D), >256/>256/>256 (C), 0.5/>8/>8 (R). Kill-curves, the combination D+C achieved synergistic effect especially in LP where it was bactericidal with D at 0.5 mg/L (5LP) and with D at 4 mg/L (7LP); in SP, D (16 mg/L) + C was bactericidal. In vivo, all groups were better than CO (n=10) (decreases in TCF bacterial counts at d8 and d11, log CFU/ml: +0.80, +0.62, respectively, p<0.05). D+R (n=11) (−4.99,−5.35) was significantly the most active treatment (p<0.05), D+C (n=19) (−3.27,−3.10) was also better (p=0.06) than D (n=8) (−2.45,−2.51). Eradicating adherent bacteria from CV (bacterial counts, log CFU/ml), all therapeutic groups were better than CO (4.65); D+R (0.91) was significantly the most effective treatment (p<0.05) vs D (1.62) and D+C (1.48).

**Conclusions:** D+C combination achieved higher bactericidal and synergistic in vitro activity especially in the LP. D+C improved the efficacy of D against FBI but it was less effective than D+R. The potential benefits of adding C to anti-MRSA therapy with D alone could be in the setting of infections with high bacterial inoculums mainly in the log-phase or in FBI by R-resistant MRSA strains.

**Comparative efficacy of omadacycline (PTK796) in a lethal Streptococcus pneumoniae and Staphylococcus aureus pneumonia model**

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**Objectives:** Omadacycline is a novel aminomethylcyclin with excellent activity against pulmonary pathogens and overrides tetracycline resistance. The objective of these studies was to evaluate omadacycline as a clinical candidate to treat Gram-positive pneumonias.

**Methods:** Neutropenic (cyclophosphamide treated) male CD-1 mice were infected intranasally with 50 micro liters containing approximately 8×10^7 CFU of Staphylococcus aureus USA300 (SA) or 7.5×10^8 of Streptococcus pneumoniae PPS1339 (SP) diluted in sterile PBS. Animals were treated with omadacycline, tigecycline, vancomycin or saline at 2 hours post-infection (pi) with a single intravenous dose and survival determined out to 7 days pi.

**Results:** Omadacycline was more effective than tigecycline and vancomycin in preventing death in both the SA and SP pneumonia models at all time points tested (Table 1). Omadacycline was over 3 fold more effective than tigecycline at the early end points tested for both SA and SP, and 1.5–2 fold more effective by 7 days pi. Vancomycin was ineffective at any of the doses tested and at either of the time points for both SA and SP infections. In addition, doxycycline was tested against SA and daptomycin, linezolid, ceftriaxone, and levofloxacin were tested against SP. All these comparators failed by 7 days pi even at the highest doses tested (>18 mg/kg or higher).

**Conclusion:** Omadacycline was successful in protecting mice against a lethal SA or SP pneumonia and was more effective than either tigecycline or vancomycin. This data suggests that omadacycline may be considered as a potential candidate for the treatment of Gram-positive pneumonias.

**Invasive pneumococcal disease in adults, Poland, 2006–2009**

A. Skoczynska*, A. Kuch, A. Gołebiowska, I. Wasko, W. Hryniewicz (Warsaw, PL)

**Objectives:** Streptococcus pneumoniae is a major causative agent of severe infections especially in children and elderly. Pneumococcal conjugate vaccines (PCVs) offer a new possibility to reduce the incidence of pneumococcal infections and there are plans to use them also in adult population. Therefore, the aim of this study was to describe the characteristics of invasive pneumococcal disease (IPD) among Polish adults and to determine the coverage of the serotypes by different pneumococcal vaccines.

**Methods:** The study was performed on all invasive S. pneumoniae isolated from adults older than 54 in Poland between 2006 and 2009, and collected in the National Reference Centre for Bacterial Meningitis (NRCBM). All the strains were identified based on typical morphology, Gram stain, susceptibility to optochin and bile solubility. Serotypes of S. pneumoniae were determined by the Pneumotest-Latex kit, a PCR or the the Quellung reaction. MICs were determined by the Etest method.

**Results:** Among 725 invasive pneumococcal isolates collected during the study period, 260 (35.9%) were isolated from patients older than 54 years. Among them 37 different serotypes were identified along with 3 nontypeable isolates. The most common were isolates of serotype 3 (14.3%), 12F and 14 (8.9% each). Together with 1, 23F, 9V, 4 and 9N they accounted for 65.3% of all serotypes. The overall coverage rates of PCV7, PCV10, PCV13 and PPV23 of invasive isolates in adults over 54 years of age were 38.2%, 48.3%, 64.9%, and 87.6% respectively. When the studied patients were divided into three age groups 55–64, 65–74 and 75plus, the coverage rates of PCV7/PCV10/PCV13/PPV23 were 36.1/44.3/67.0/88.7%, 34.2/48.7/61.8/86.8%, and 44.2/52.3/65.1/87.3%, respectively. The case fatality ratios in these age groups were 15.5%, 26.3%, and 33.3%, respectively. Penicillin MICs higher than 0.06 mg/L were found in 42 isolates (16.2%) of which 12 were isolated from cerebro-spinal fluid. Such phenotypes were the most prevalent among isolates of serotype 14 (78.3%) and 9V (72.2%). PCV7, PCV10 and PCV13 covered 85.7, 88.1 and 95.2% of isolates with elevated MICs of penicillin, respectively. Thirty-one (12%) isolates had MIC of cefotaxime higher than 0.5 mg/L.

**Conclusion:** The registration of PCV for prophylaxis in adults could have a considerable effect on limitation of IPD-associated morbidity and mortality among Polish adults, especially of cases caused by bacteria with decreased susceptibility to antibiotics.
**P1076** Incidence estimate of invasive pneumococcal disease in Belgium in 2009 using the capture-recapture method

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**Objectives:** An ongoing prospective active IPD (Invasive Pneumococcal Disease) surveillance network was set up in Belgium to estimate the annual incidence of IPD in hospitalized adults older than 50 years of age.

**Methods:** Cases were defined as isolation of *S. pneumoniae* from culture of a normally sterile site by hospital microbiological laboratories. The network consisted of a convenience sample of 49 acute care hospitals. The denominators are obtained from publicly available data sources on the number of acute care hospital/hospital beds and the Belgian population older than 50 (mid-year 2009). The surveillance time frame covers January to December 2009.

The annual incidence is estimated by the capture-recapture method, which combines the information obtained through different existing data sources. Data sources consist of the active adult IPD surveillance network and the national reference laboratory for *S. pneumoniae*. Cases are matched source by source (by age, gender, sampling date, hospital code and postal code) and missing numbers are estimated using the Lincoln-Petersen estimate.

**Results:** A total of 551 patients older than 50 (mean age 71.7 range 50–88) with IPD were detected in 1 year in the prospective IPD surveillance in 49 acute care hospitals. The National reference laboratory received 1,172 samples from 99 laboratories. After matching cases from both sources, the total number of IPD cases was estimated at about 1,200 (Lincoln-Petersen estimate). This corresponds with an estimated incidence just above 30 per 100,000 inhabitants older than 50 years of age.

**Conclusion:** The capture-recapture method based on two data sources estimated the incidence of IPD at just over 30/100,000 in persons older than 50 in Belgium. Since both data sources showed a strong interdependence, the incidence is likely to be underestimated; adding the information from a third, less dependent, data source could lead to a more precise incidence estimate.

**P1077** Evolution of meningococcal disease incidence on Navarre’s population (north of Spain)

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**Introduction:** The meningococcal disease is a clinical feature of low incidence but with a very important epidemiologic weight, because its high morbimortality.

**Objectives:**
- Analysis about evolution of invasive meningococcal disease’s (IMD) incidence on Navarre’s population.
- Stratified analysis about evolution of *Neisseria meningitidis* serogroups.
- Evaluate changes on the IMD’s incidence on their different serogroups and on different population groups after the introduction of the systematic vaccination against meningococcal C at the last months on 2000 year.

**Methods:** Population data was obtained from Official Census of Navarre. Meningococcal disease data was obtained from the Navarre’s Institute for Public Health through obligatory declaration diseases epidemiology surveillance’s system, from January of 1987 to December of 2009. Statistical analysis by SPSS 17.0 for Windows. We used independent samples Student T test, χ2 test and binary logistic regression analysis.

**Results:** We observed a global decrease in IMD’s incidence on our population from prevaccination period (1987–2000) to postvaccinal (2001–2009), from 4.84 to 2.98 c/105 (OR: 0.749; CI95%: 0.617–0.908). This fall was due to the drop in cases of Serogroup C (SGC) from 1.13 to 0.42 c/105; OR: 0.376; CI95%; 0.234–0.6) and in non-grouped cases. On the contrary, the cases of serogroup B (SGB) increased on the postvaccination period (from 1.32 to 2.33 c/105; OR: 1.78; CI95%: 1.37–2.33). When we stratified by ages and serogroups, we observed an increase of SGB on pediatric population (<15 years, OR: 1.57; IC: 1.13–2.19) and also on adult population (≥15 years, OR: 2.78; IC: 1.75–4.40). This rise was more significant on teenagers and young adults.

**Conclusions:** After the introduction of vaccination, there was a decrease in IMD’s incidence on general population. However, the important fall of SGC cases on little children has been disguised by the increase of SGB cases on olderer groups.

**P1078** Increase of invasive meningococcal disease caused by serogroup Y in Finland, 2010

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**Objectives:** Since a serogroup A epidemic in the 1970’s, there have been no major meningococcal epidemics or outbreaks in Finland. The incidence of invasive meningococcal disease (IMD) in Finland has fluctuated at low levels between 0.6 to 1.5 per 100,000 inhabitants (29–79 notified cases annually) in 1995–2009. Most cases (73%) have been due to group B, followed by group C (17%), and group Y (8%). Here we report an increase of IMD caused by serogroup Y in Finland in 2010.

**Methods:** Reporting of IMD in Finland is obligatory and based on notifications from clinical microbiology laboratories reporting all positive CSF/blood culture, antigen detection and/or PCR findings and clinicians reporting all laboratory-confirmed cases. All case isolates are requested to be sent to National Institute for Health and Welfare (THL), for confirmation and typing.

**Results:** In 2010, the overall number of IMD cases was comparable to previous years, but both the number and the proportion of cases caused by serogroup Y increased significantly from an average of 4 cases (8%) in 1995–2009 to 11 cases (36%) in 2010. Thus far, no epidemic link between the cases has been recognized. The phenotype and genotype distribution of the isolates will be presented.

**Conclusion:** Both the number and the proportion of IMD caused by serogroup Y in Finland have increased during 2010. The possible clonality of the serogroup Y isolates will be analysed by the use of molecular typing methods, which have been introduced in 2010 in Finland.
Epidemiological characteristics of Q-fever in Vojvodina Province, Serbia, from 1985 to 2009

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Objective: The aim of this study was to give some data of Q-fever in the Autonomous Province of Vojvodina, Serbia, which is known as an area with a high degree of agricultural activity and cattle-breeding.

Materials and Methods: From 1985 to 2009 surveillance reports of the serologically confirmed Q-fever cases to Institute of Public Health of Vojvodina were analyzed on demographic (age, sex, current residence), seasonal and regional distribution of disease, clinical signs of the disease and contact exposures, using standard statistical methods. For comparative analysis of frequency, $\chi^2$ test was used.

Results: In a 25-year period a total of 2134 serologically confirmed cases of Q-fever were reported, with an incidence rate ranged from 0.0 to 20.5/100,000 population in different local districts of Vojvodina Province. Most of the cases were registered in outbreaks (76.6%). The mean annual incidence rate significantly decreased from 12.8/100,000 in 1980’s, 3.7/100,000 in 1990’s to 0.8/100,000 population in the last decade ($p<0.01$).

The male:female sex ratio of cases was 2.3:1.

The frequency of disease differed significantly among age groups; it was highest for the group from 30 to 39 years of age ($p<0.01$).

Seasonal distribution indicated the lowest incidence rate from June to December (range 0.6 to 6.3/100,000), while from January to May incidence rate was 12.6 to 23.3/100,000 population ($p<0.01$).

Overall, 75.4% patients had clinical symptoms of pneumonia. Among cases with reported exposure risks, 77.1% were related to individual household cattle handlers, and 23.9% to occupational exposure in food industry (live stock farms, slaughterhouses, research facilities).

Conclusion: Vojvodina Province is an endemic area of Q fever. The highest incidence rate in last 25 years was in 1988 (20.5/100,000 population), while it decreased significantly further on with mean rate of 0.8/100,000 population in the last decade ($p<0.01$).

Decreased incidence of Q-fever from 1985 to 2009 can be attributed to changes in farming practices from the nomadic cattle-breeding to live stock farms and to overall reduction of the cattle herds.

Seroprevalence of whooping cough among Danish adults

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Objective: Several studies around the world on whooping cough seroprevalence among adults have shown large discrepancies between population based seroprevalence data and the number of diagnosed whooping cough cases. The prevalence of whooping cough among adults in the general Danish population was estimated by measurements of Bordetella pertussis specific antibodies in sera.

Methods: Sera from the “Helbred 2006” population study conducted in the vicinity of Copenhagen from 2006 to 2008 were studied for the presence of pertussis toxin specific IgG antibodies. Sera were available from 3440 individuals, 19 to 72 years old. The study population is considered representative of the general adult Danish population.

Results: When using a diagnostic cut-off value of 75 IU/ml (IU/ml according to the WHO international standard serum, NIBSC: 06/140), the seroprevalence of whooping cough among the participants in the study was 3.0% and the median concentration of pertussis toxin IgG antibodies was 8.7 IU/ml. The serological results from each individual are combined with data from a questionnaire on general health topics.

Conclusion: The seroprevalence of whooping cough among adults 19–72 years old in Denmark is estimated to be 3%. The prevalence of laboratory-confirmed whooping cough for the 19–72 age-group was 3.3 per 100,000 in 2008. Therefore, the true incidence of whooping cough among adults in Denmark is probably a thousand fold higher than what is currently detected in the laboratories.
was lower at 14.8% (4/27) (Fisher's exact test, p = 0.63, not statistically significant).

There were no deaths in those who were vaccinated and who had a recorded dose of TIG given.

Conclusion:
- Individuals can get clinical tetanus following minor injuries despite having one or more tetanus vaccinations previously
- Clinicians should ensure that they ascertain patient’s tetanus vaccination history following all types of injuries
- Clinicians should take vaccination histories at every opportunity e.g. in every health service or primary care visit
- England and Wales should consider adopting the World Health Organisation’s recommendations for vaccination against tetanus

**P1082** Epidemiological survey of diabetic foot infections in Lisbon

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**Objectives**: Epidemiological survey of the infectious microbiota of the diabetic foot in Lisbon Clinical Centers, stratifying it in relation to the anamnestic data of patients, characteristics of the diabetic foot (using the PEDIS classification), time of evolution and current and previous (<3 months) antibiotic therapy.

**Methods**: Transversal observational study, with clinical data collection using a structured questionnaire and microbiological products (swabs collected by the Levine method, biopsies or aspirates) of clinically infected diabetic feet ulcers of patients followed in Lisbon Clinical Centers. Microbiological diagnosis (including aerobic and anaerobic quantitative bacterial cultures) and antibiotic sensitivity profiles were carried out and analyzed using standard procedures.

**Results**: 49 hospitalized and ambulatory patients were enrolled in this study and 147 microbial isolates were cultured, comprising 43 species. Systematized results are presented in the table. *Staphylococcus* spp. was the main genus identified, with *Staphylococcus aureus* being present in 51% of the samples, in 96.6% of cases in quantities $\geq 10^5$ CFU/g or mL. In the clinical samples collected under antibiotic therapy, corresponding mainly to hospitalized patients with osteomyelitis, 93% of the antibiotic regimens were considered inadequate based on the antibiotic susceptibility test results. Regarding these samples, qualitative and quantitative differences were found, with less microorganisms being identified (2.1 vs 3.4), however with a higher prevalence of multi-drug resistant (MDR) organisms (66.7% vs 26.5%). The average time of ulcer evolution for the appearance of MDR organisms was 29 days, being previous treatment with ciprofloxacin the only statistically significant risk factor. Methicillin-resistant *Staphylococcus aureus* (MRSA) was identified in 24.5% of cases.

**Conclusions**: 1. *Staphylococcus aureus* is the most common cause of diabetic foot infections in our area. 2. MDR organisms are frequent, being MRSA the main pathogen. 3. Standard practice not to collect microbiological examination in diabetic foot infections, as advised by many clinical guidelines, should be discouraged because of the high likelihood of infection by MDR not covered by the recommended antibiotic regimens.

**P1083** Rectal carriage and pyogenic liver abscess associated CPS genotyping of *Klebsiella pneumoniae* in asymptomatic diabetic patients in Taiwan

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**Background**: Pyogenic liver abscess (PLA) caused by *Klebsiella pneumoniae* (KP) is an emerging community acquired infectious disease in Taiwan and certain serotypes are predominated in the causative isolates. Diabetes mellitus (DM) has been identified as a risk factor of KP related PLA infection. Rectal carriage of the PLA causing serotypes in DM patients remained undetermined.

**Methods**: We prospectively followed rectal swab for KP culture in asymptomatic DM patients attended for regular medication from March 2008 to June 2009. Patients with antibiotics usage or hospital admission three months earlier were excluded. Diabetic control was evaluated by their fasting blood glucose and glycosylated hemoglobin (HbA1C). The swab was cultured on EMB agar and suspected isolates were identified by Enterotube (Becton Dickinson, MD). Capsular serotype was determined by polymerase chain reaction cps genotyping using primers specific for most commonly encountered (>80%) capsular types associated with PLA (K1, K2, K5, K20, K54, K57 and a new capsular type [N1]) as previously described.

**Results**: There were 102 male and 64 female DM patients were enrolled with a mean age of 56.7 year-old (S.D = 9.08). Eighty patients (48.2%) had rectal KP colonization. The colonizer were elder than the non-colonizer (58.4 vs 55.1 y/o; p = 0.022). Gender, fasting blood glucose and HbA1C were not different statistically significant between colonizer and non-colonizer (p = 0.364, 0.176, and 0.183, respectively). The results of cps-genotyping for the 65 available isolates revealed that 22 isolates (33.8%) belong to the 7 common serotypes identified in PLA. Serotype K57 was the most commonly identified serotype (N = 7, 10.8%) followed by K1 (N = 6, 9.2%), K2 (N = 3, 4.6%), K20 (N = 3, 4.6%), K54 (N = 2, 3.1%) and K5 (N = 1, 1.5%). Serotypes of the remaining 43 isolates (66.2%) could not be identified by the PCR cps genotyping.

**Conclusions**: KP strains of PLA associated capsular type could be found in asymptomatic diabetic patients. Further follow up is mandated whether these patients developed subsequent PLA.

**P1084** Fever of unknown origin: a retrospective study of 52 cases with evaluation of the diagnostic utility of PET/CT

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**Objectives**: Fever of unknown origin (FUO) is a challenge to the clinician. The diseases which comprise FUO may change in a given geographical setting as a consequence of naturally occurring shifts in disease epidemiology or as a consequence of the implementation of disease curbing health care measures. Knowledge of the prevalent spectrum of diseases and consequently use of the most rational diagnostic approach is important. PET/CT is a non-invasive diagnostic tool which may be of great benefit in establishing a diagnosis in patients with FUO.

**Methods**: Retrospective study of 52 cases of FUO. FUO was defined as fever higher than 38.3°C recorded on several occasions and lasting at least 3 weeks with no diagnosis in spite of at least 3 days of investigation. Patients admitted during a five year period 2005–2010 and fulfilling these criteria were included. Patients with known immunodeficiency, HIV or nosocomial FUO were excluded. Clinical records, laboratory data and radiographic findings were assessed. Follow-up on patients was performed using a national registry database in order to identify patients with recurrence of febrile episodes or death. Finally, the utility of PET/CT in establishing a final diagnosis was evaluated.
Results: 52 patients fulfilled the criteria for FUO, 36 males and 16 females. Median age was 48 years and median duration of fever before referral was 6 weeks. A final diagnosis was achieved in 60% (31/52) of the cases, and the most frequent diagnosis was non-infectious inflammatory disease 55% (17/31) followed by infections 32% (10/31) and malignancy 13% (4/31). Adult-onset Still’s disease and CMV infection alone accounted for 29% of all cases. A PET/CT scanning was performed for 24 patients. In 22 of these cases it was possible to evaluate the diagnostic utility of PET/CT. PET/CT was considered helpful in attaining a final diagnosis in 10 of the cases (45%; 10/22). 

Conclusion: In this study non-infectious inflammatory diseases comprised the majority of final diagnoses in patients with fever of unknown origin. PET/CT scanning proved to be a valuable aid in establishing the diagnosis with a PPV of 0.83.

**P1085** Risk factors and outcome of treatment in patients with psoas abscesses

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Objectives: Although psoas abscesses (PAs) have not been reported frequently in the literature, they remain a significant therapeutic challenge. We retrospectively evaluated demographic characteristics, symptomatology, predisposing factors, microbiology and treatment outcomes of patients with PAs in a large tertiary care university hospital.

Methods: We have electronically searched Hacettepe University Hospital database. There were 83 patients who were diagnosed clinically and radiologically with PA between 2003 and 2009. Individual patient files were searched for detailed patient characteristics, possible risk factors and underlying diseases, infecting microorganisms and treatment outcomes.

Results: 37 (44.6%) of 83 patients were female. Median age was 51 years (range: 18–83). Mean abscess size was 6.4 centimeters (range: 1–25). Fever and pain were the most common symptoms. In 37 patients, abscesses were localized at right psoas, in 40 patients at left and 6 patients had bilaterally. Multiple abscess formation were determined in 10 patients. The most common underlying diseases were malignancies (16.9%) (4 with bladder carcinoma, 3 with colon cancer, 2 with multiple myeloma and one of each with lung, breast, endometrium, ovarian and prostate cancer), diabetes mellitus (13.2%), Crohn's disease (4.8%), and rheumatological diseases (4.8%). 13 patients (15.7%) had no underlying disease and 46 (55.4%) of patients had a history of an abdominal operation. 19 (22.9%) patients had previously and incompletely been treated for PA. The most common isolated microorganisms were Staphylococcus aureus (18 MSSA, 5 MRSA), Escherichia coli (n = 13; 3 of which with ESBL production) and Mycobacterium tuberculosis (n = 11). 68 patients (81.9%) were treated successfully with percutaneous drainage and with appropriate antimicrobial therapy. Four patients (4.8%) died due to complications related to PA.

Conclusion: We report a high-rate of previous surgical intervention as a predisposing factor for PAs. M. tuberculosis was also a frequent cause of PAs which might be a consequence of intermediate-high prevalence of tuberculosis in Turkey. High rates of treatment success were achieved by a combination of drainage and antimicrobial treatment.

**P1086** Clinical features and outcome of cardiovascular implantable electronic device infections due to staphylococci

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Objective: To define the clinical features and outcome of staphylococcal cardiovascular implantable electronic device (CIED) infections.

Methods: We retrospectively reviewed all cases of CIED infection seen at Mayo Clinic Rochester between 1991 and 2008. Clinical features including host and device characteristics, presenting symptoms/signs, and laboratory parameters were identified using logistic regression. Mortality was examined at 30-day and 1-year.

Results: Of the 416 patients with CIED infection, 129 (31.0%) were due to Staphylococcus aureus, and 159 (38.2%) due to coagulase-negative staphylococci (CoNS). S. aureus pocket infection generally occurred closer to time of device implantation (<1 year); in contrast, S. aureus bloodstream infection (BSI) and CIED-related infective endocarditis (CIED-IE) often presented later (>1 year). In the multivariate setting, S. aureus pocket infection was associated with high frequency of device

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**Patient** | **Age (years)** | **Sex** | **FDG-PET/CT** | **Final diagnosis** | **Verified by**
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5 | 60 M | Glandular uptake in the neck, thorax and mediastinum (TP) | Malignant lymphoma | Malignant lymphoma | Lymph node biopsy
19 | 65 M | Multiple foci in the lungs (TP) | Malignant lymphoma | Malignant lymphoma | Lung biopsy
20 | 67 F | Foci in the thyroid gland (TP) | Autoimmune thyroiditis | Autoimmune thyroiditis | Serology
24 | 61 F | Multiple foci in the lungs (TP) | Microscopic polyangitis | Microscopic polyangitis | Renal biopsy
34 | 36 F | Uptake in the peripheral joints (TP) | Systemic lupus erythematosus | Systemic lupus erythematosus | Filling up the diagnostic criteria for systemic lupus erythematosus
35 | 56 F | Uptake in the aorta, arteriae subclavia and iliaca (TP) | Large vessel vasculitis | Large vessel vasculitis | PET/CT
38 | 62 F | Uptake in the large arteries (TP) | Large vessel vasculitis | Large vessel vasculitis | PET/CT
42 | 17 M | Uptake in the spleen (TP) | Rheumatologic disease | Rheumatologic disease | Spleen biopsy with inflammation no malignancy
44 | 33 F | Uptake in the mediastinum, liver, retroperitoneum, lungs and bones (TP) | Metastatic adenocarcinoma | Metastatic adenocarcinoma | Liver biopsy
28 | 28 F | Glandular uptake behind esophagus (TP) | Bartonella infection | Bartonella infection | Serology
43 | 31 F | Sub-hepatic uptake (FP) | Fever subsided spontaneously | Fever subsided spontaneously | Clinical course
33 | 87 F | Uptake in the lumbar spinal vertebra (FP) | Fever subsided spontaneously | Fever subsided spontaneously | Clinical course
12 | 78 M | Negative (FN) | Died during hospitalization with continued fever | Died during hospitalization with continued fever | Autopsy without any particular findings
15 | 68 F | Negative (FN) | Necrotizing vasculitis | Necrotizing vasculitis | Renal biopsy
29 | 42 M | Negative (FN) | Treated in rheumatological department with prednisolone and TNF-alpha inhibitor | Treated in rheumatological department with prednisolone and TNF-alpha inhibitor | Clinical course
36 | 69 M | Negative (FN) | Continued to have fever following discharge, died 1½ years later, no autopsy was performed | Continued to have fever following discharge, died 1½ years later, no autopsy was performed | Clinical course
51 | 39 M | Negative (FN) | Adult-onset Mb Still | Adult-onset Mb Still | Clinical course
8 | 65 M | Negative (TN) | ND. Fever subsided spontaneously | ND. Fever subsided spontaneously | Clinical course
11 | 66 M | Negative (TN) | ND. Fever subsided spontaneously | ND. Fever subsided spontaneously | Clinical course
31 | 33 M | Negative (TN) | ND. Fever subsided spontaneously | ND. Fever subsided spontaneously | Clinical course
41 | 65 F | Negative (TN) | ND. Fever subsided spontaneously | ND. Fever subsided spontaneously | Clinical course
52 | 34 M | Negative (TN) | ND. Fever subsided spontaneously | ND. Fever subsided spontaneously | Clinical course
manipulation (OR 3.00, [1.30, 6.91]). However, S. aureus BSI or CIED-IE more frequently involved originally implanted devices (OR 5.24 [1.73, 15.87]), patients receiving corticosteroid therapy (OR 7.95 [3.08, 20.53]), or patients who had remote sources of infection (OR 4.48 [2.06, 9.71]). They often presented with fever (OR 5.03 [1.79, 14.11]) or hypotension (OR 2.55 [1.07, 6.05]), and had leukocytosis (OR 2.47 [1.23, 4.98]) or elevated serum creatinine (OR 1.37 [1.09, 1.77]). S. aureus BSI or CIED-IE were associated with increased mortality at 30-day (OR 5.68 [2.10, 15.37]) and 1-year (OR 4.98 [2.41, 10.26]). CoNS pocket infection was more frequent in patients with prior lead revision (OR 3.30 [1.49, 7.30]) or prior device infections (OR 2.09 [1.10, 3.97]). CoNS BSI or CIED-IE was more common in patients with implanted vascular grafts (OR 5.10 [1.08, 24.08]). There was no increased mortality among patients with CoNS CIED infection.

Conclusion: CIED infections due to staphylococci have divergent clinical features and mortality risks based on coagulase designation. Recognition of the distinct characteristics that these two groups of organisms manifest in patients with CIED infection could dramatically impact management decisions.

Mortality risk factors in patients with prosthetic vascular graft infection: a prospective cohort study

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Introduction: Prosthesis vascular graft infection is a devastating complication with a significant mortality up to 75%, especially in intracavitary graft infection and mycotic aneurysm. The purpose of this study was to evaluate factors associated to peri-operative mortality in patients with definite graft infection.

Material and Methods: We reviewed the medical records of 85 patients treated for early and late graft infection defined as a positive bacterial culture on intra-operative or blood culture and clinical, biological or radiological signs of infection. Operative mortality was defined as death occurring within the same hospitalization as surgery and, cure as the absence of evidence of infection during the follow-up period (minimum one year).

Results: 85 patients with 54 intracavitary and 31 extracavitary graft infection and treated with removal infected prosthesis (n=41), surgical debridement (n=34) or medical treatment (n=10) were included. Operative mortality rate was 16.5%. Three variables were independently associated to mortality: Age >70 years (HR 2.32; CI95%; 0.87–7.01; p=0.008), intracavitary graft infection (HR 2.30; CI95%; 0.86–7.11; p=0.0007) and admission in intensive care unit (HR 1.98; CI95%; 0.89–4.86; p=0.02). Cure was observed in 94% and 90% of evaluable patients treated with removal prosthesis and replaced in situ and surgical debridement, respectively. In patients with graft infection due to Gram positive Cocci, rifampin use as adapted treatment is associated with a significantly better prognosis; p = 0.05.

Conclusion: Age >70 years, intracavitary graft infection and admission in intensive care unit were associated to high operative mortality. To limit the mortality risk, and for a better outcome, patients with graft infection should be managed in referral center.

Spread of Corynebacterium diphtheriae in Ukraine during post-epidemic period

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Objective: The recent diphtheria epidemic in eastern Europe are a warning that diphtheria can make a comeback in susceptible populations. The aim of this study was to evaluate the diphtheria epidemiological surveillance conducted in Ukraine based on an analysis of morbidity and carriers by Corynebacterium diphtheriae (C.d.) in the population and to characterize circulating human clinical isolates of C.d. in postepidemic period.

Methods: The 898 cases of diphtheria, 11312 cases of C.d. carriages during 2002-2009 in Ukraine were analyzed. Production of diphtheria toxin in isolates of C.d. was assessed by Elek test. Carriage of the diphtheria toxin gene tox in the 2058 isolates of non toxigenic C.d. was assessed by the polymerase chain reaction (PCR). Isolates were assigned to biotypes based on biochemical characteristics.

Results: In postepidemic period incidence of diphtheria in Ukraine, ranged from 0.57 (2002) to 0.05 (2009) per 100000 population. Toxigenic strains of C.d. from patients were isolated in 52.0% of cases, there were in 26.5 times more toxigenic C.d. biotype gravis than C.d. biotype mitis. Nontoxigenic strains of C.d. from patients with diphtheria were isolated in 28.5% of cases, there were in 1.6 times more C.d. biotype mitis than C.d. biotype gravis. Among C.d. isolates from carriers of C.d. there were 931 (8,2%) toxigenic C.d. and 10381 nontoxigenic C.d.

Conclusion: Although vaccination has resulted in a low incidence of diphtheria in Ukraine, isolates of C.d. which harbor and produce the diphtheria toxin remain in circulation. It is necessary to enhance the microbiological monitoring using molecular genetic techniques to identify the nontoxigenic strains of C.d. The continued circulation of toxigenic strains of C.d. and nontoxigenic strains of C.d. carried the toxin genes but not express them highlights the importance of continuing vaccination programs against diphtheria.

Measles among healthcare workers in Bulgaria

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Vaccine-preventable measles is highly infectious via airborne droplets. Health care workers (HCWs) are at a higher risk of the disease than the adult population. They may introduce measles in healthcare facilities, leading to nosocomial spread and dire consequences. In 2009/2010, 8 years after the last indigenous cases, Bulgaria experienced a large measles outbreak as a result of virus importation from Germany. Thirty-eight HCWs at community hospitals and primary-care medical facilities contracted the disease.

Objective: To assess the occupational risk of measles among HCWs and describe the epidemiological characteristics of these cases.

Methods: A clinical case definition was defined, using the revised WHO/EURO criteria (2008). Cases were considered confirmed if they met clinical and laboratory criteria (positive ELISA IgM test) and probable if they met clinical criteria and had epidemiological link. A short questionnaire recording occupation, age, history of the present disease and immunization status was distributed to the outpatients participants. The same information was collected from the medical records of hospitalized ones.

Results: Until mid April 2010 thirty-eight measles cases (age range 24–48 years) were notified among HCWs from 7 different regions in Bulgaria. Twenty cases were defined as confirmed and eighteen as probable. The largest groups of HCWs measles were physicians (n=21, 55.5%) and nurses (n=7, 19.4%). Thirty-seven HCWs acquired the infection from patients and 1 physician from a colleague. Although 32 were eligible for vaccine all but one had no written evidence of appropriate immunization. None transmitted measles to susceptible patients or family members. Ten developed pneumonia but all recovered uneventfully.

Conclusion: Prevention of future measles outbreaks will require maintaining a high coverage with 2 doses measles-containing vaccine, including HCWs and a high index of suspicion for measles among travelers with unexplained rash.
**P1090** Transient bradycardia in patients with Crimean-Congo haemorrhagic fever

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**Objective:** Crimean-Congo haemorrhagic fever (CCHF) is a fatal viral infection described in parts of Africa, Asia, eastern Europe, and the middle east. There is a variety of potential clinical manifestations following infection, and not all patients develop the classic form of the disease. The typical course of CCHF has been noted as progressing through four distinct phases, i.e. incubation, prehemorrhagic, hemorrhagic, and convalescence. Duration and associated symptoms of the phases can vary greatly. Although some cardiovascular changes – eg, bradycardia and low blood pressure were emphasised in earlier publications, these have not been reported in recent studies. To our knowledge, there is no study detailing bradycardia in this patient group. The main objective of this study was to determine the frequency and clinical course of bradycardia in the patients with CCHF.

**Patients and Methods:** Between May 2002 and September 2010 a total of 380 patients with CCHF were followed at the Department of Infectious Diseases and Clinical Microbiology, Atatürk University Medical School. All patients who had a transient bradycardia episode during this period were included in this study. All CCHF patients who had cardiovascular symptoms or signs were investigated by electrocardiogram, and in some case by echography. Bradycardia (sinus bradycardia) was defined as a heart rate less than 60 beats per minute.

**Results:** During the study period, 14 patients (3 female, 11 male) had an episode of bradycardia. The mean age was 46.2 ± 19.8 years (range: 18–76). All patients were in sinus rhythm by electrocardiogram. Three patients had clinical symptoms of bradycardia (dizziness, shortness of breath, hypotension), and intraventricular atropin was administered to them. The patients were responsive to atropin. In the other patients, bradycardia resolved spontaneously. No patient had underlying condition for bradycardia such as electrolyte disorders, drug use, toxic exposure, hypoglycemia, hypothyroidism, previous cardiac disorder, and increased intracranial pressure. The onset of bradycardia appeared 7.0 ± 1.7 days (range: 5–10) after first clinical symptoms. In all patients, bradycardia continued for 5.1 ± 1.6 days (range: 2–8), and resolved without any additional problem or sequelae. All patients had good prognosis.

**Conclusions:** Transient sinus bradycardia is a rare and benign clinical manifestation of CCHF. This is the first study detailing bradycardia on this patient group.

**P1091** Evaluation of cutaneous anthrax patients in eastern Anatolia, Turkey

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**Objectives:** Anthrax is a zoonotic infectious disease that caused by *Bacillus anthracis*. The human is incidental host, they are infected with contaminated animals and animal products directly or indirectly. Anthrax infectes human by means of three major ways: Inhalation, gastrointestinal and cutaneous. Cutaneous anthrax (CA) is the most common naturally occurring form.

Sporadic cases and outbreaks of anthrax are still present worldwide although they are relatively rare in developed countries. Anthrax is an endemic infectious disease in the eastern part of Turkey. We investigated epidemiologic properties of the cases of anthrax in our clinics for four years period. In this study, we also evaluated the clinical histories, clinical features, therapies and outcomes of 44 patients with cutaneous anthrax followed up in our clinics in Atatürk University Faculty of Medicine.

**Methods:** The patients, who were thought as CA clinically and applied to the clinic of infectious diseases in Atatürk University Faculty of Medicine from January 2005 to December 2008, were included in our study. The diagnosis of CA was based on a typical anthrax skin lesion (dermatologic findings, including painless, pruritic ulcers covered by a characteristic black eschar) and/or microbiologic methods, including Gram stain and culture of samples obtained from the lesions.

**Results:** Twenty-four patients (54.5%) were male and 20 (45.5%) were female. All of patients almost were living in rural areas of Erzurum and Kars province in eastern Anatolia, Turkey. The highest number (%) of presentations was seen in period of August-October. All patients with a diagnosis of CA were followed up. Malignant oedema was observed in 11 patients (25%) and malignant pustules in 33 (75%). All of patients almost always had a history of close contact with sick cows or sheep; slaughtering 34 (%) (77.3), skin peeling 7 (%) (15.9), cutting of meat 36 (%) (81.8), carrying contaminated packet 5 (%) (11.4). The distribution of the lesions by site is: hand 24 (%) (54.5), finger 11 (%) (25), arm 18 (%) (40.9), eyelids 3 (%) (6.8), lips and surrounding area 3 (%) (6.8), neck 4 (%) (9.1) and face 5 (%) (11.4). There were 11 (%) (25%) patients with a total of two lesions and 5 (%) (11.4) patients with a total of three lesions from different body site.

**Conclusion:** There were no any complication (like anthrax sepsis and respiratory tract obstruction) occur in our patients. All of the patients, were discharged from the hospital after a full recovery.

**P1092** Epidemiology and clinical aspects of murine typhus in central Tunisia

*A. Toumi*, **A. Joussouf**, *C. Loussaief*, **H. Ben Brahim**, **F. Ben Romdhane**, *M. Chakroun* (Monastir, TN)

**Objective:** Generally unrecognized, murine typhus continues to confound physicians because of its nonspecific clinical manifestations. In an effort to update our knowledge on this poorly known disease we describe the epidemiological, clinical, laboratory and treatment features of murine typhus.

**Patient and Methods:** Retrospective study of 73 adult patients hospitalized for murine typhus, in the department of infectious diseases, university hospital Monastir, Tunisia, during the period 2005–2010. Diagnosis was confirmed by a four-fold rise in antibody titer, by a single high titer ⩾ 128 or by seroconversion to typhus group antigen by indirect immunofluorescent antibody assay (IFA). All sera cross-reacted to *Rickettsia conorii* antigens were at lower titer.

**Results:** Mean age was 33 years (range, 13–68 years). Forty-seven of the 73 patients were male (64%), and 26 were female (36%). A seasonal pattern (87.6% from June to September) was observed. Thirty-eight patients (52%) were from rural or suburban areas; neither fleas bites nor exposure to rats were mentioned. The most common clinical symptoms were fever in all patients, headache in 63 (86.3%), and myalgia in 49 (67.1%). A maculopapular and non-confluent rash was noted in 47 cases (64.4%). The eschar wasn’t found in any patient. There were 16 cases of pneumonia, with interstitial infiltrates on chest X-ray film in 8 cases. Meningitis was diagnosed in two cases and cerebrospinal fluid showed lymphocytic meningitis. Routine laboratory tests showed thrombocytopenia was found in 38 cases (52%). C-reactive protein was higher than 20 mg/ml in 62 cases (mean = 67 mg/l). Liver enzyme levels were elevated in 60 cases (82.2%). Diagnosis was confirmed by indirect fluorescence assay detecting specific *R. typhi* antibodies in all cases. A single titer of these antibodies higher than 128 was found in 62 (84.9%) cases. The other 11 cases were diagnosed by a seroconversion. All patients received antibiotics: 32 (43.8%) received tetracycline, 25 (34.2%) doxycycline, 13 fluoroquinolone and 3 azithromycine. The 2 patients having meningitis were treated with fluoroquinolone. The outcome was favourable for all patients and no relapse was observed.

**Conclusion:** In our region, murine typhus is endemic. It is characterized by benign prognosis. Doxycycline or tetracycline are considered the drugs of choice against *R. typhi*.

**P1093** A nosocomial outbreak of red mite (*Dermanyssus gallinae*) dermatitis in Italy

*D. Galante*, **M. Lomuto**, **G. Totaro**, **G. Mancini**, **C. Nardella La Porta**, **M.A. Caffero** (Foggia, IT)

**Objectives:** The poultry red mite, *Dermanyssus gallinae*, is a widespread blood-sucking ectoparasite of poultry and other birds. It can also bite
mammals, including humans and cause a non-specific dermatitis with intense itching. As a nest parasite it only comes to the host to feed. The source of red mite infestation and its zoonotic role in urban environment are presented.

Methods: In October 2010 the Istituto Zootrofisitico Sperimentale della Puglia e della Basilicata (IZSPB) was contacted about an outbreak of pruriginous dermatitis in patients/personnel in the maternity unit of a hospital located in Apulia Region (Italy). For about four months, a not reported number of employees and hospitalized women complained of a pruriginous dermatitis in different body areas and referred to feel like if they had been “bitten or stung”, but no definitive diagnosis had been established. The symptoms disappeared or attenuated when employees and patients left the hospital. In September, the health staff of the hospital inspected the rooms and collected parasites initially identified as probable ticks. The area was evacuated and three different interventions of sanitation were effected using deltamethrin, but the infestation was not solved. On the 6th of October other parasites were collected and sent to IZSPB for a further identification.

Results: The mites collected were identified as *Dermanyssus gallinae* by the IZSPB, following morphological keys. Remains of a pigeon nest were later found on the outside ledge, near a window of the infested rooms. Removal of the nest and careful disinfection using pyrethroids led to the complete regression of the symptoms, and no more evidence of mites or dermatitis was observed during the follow-up period.

Conclusions: RMD is underestimated and often misdiagnosed even though the growing number of synanthropic birds in our cities, first of all pigeons. We believe that the true incidence of this epizoonosis in humans living in urban areas is much greater than that suggested by the scarce number of reports in literature and can be considered an emerging public health problem. A prompt identification of the mite, accurate disinfections, nests removal and counter-measures to prevent nests building are vital for the diagnosis and the management of the RMD. So, it is important to always consider this infestation in case of single or massive episodes of non-specific dermatitis in places such as schools, hospitals, offices or private habitations.

**P1094** Increased incidence of invasive meningococcal disease in Switzerland due to serogroup Y strains: a molecular characterisation

*B. Nimet*, R. Born, J. Schrenzel (Geneva, CH)

Objectives: The invasive meningococcal diseases (IMD) remain one of the more severe infections due to *Neisseria meningitidis* of different serogroups. In Europe, the majority of strains belong to the serogroups B and C, strains; other serogroups are rarely isolated. Switzerland is a country with very low IMD endemicity and the incidence continues to decline since 2000s. However strains of serogroup Y have significantly emerged in the last two years. To improve the understanding of serogroup Y IMD in Switzerland, we conducted a systematic typing of isolates.

Methods: All strains submitted to the Swiss National Center for Meningococci in 2009 and 2010 (n = 98) were characterized by serological assays, antimicrobial susceptibility testing and molecular markers. The serogroup was defined both by agglutination and by specific PCR targets (siaD gene). The isolates were tested for antimicrobial susceptibility profile by the Minimal Inhibitory Concentration (MIC) method. Genotyping was performed by multilocus sequence typing (MLST), sequencing of the *porA* gene, and *fetA* allele determination.

Results: Among the ninety-three strains analyzed, 43 were of serogroup B (46%) and 27 of serogroup C (28%) and 20 of serogroup Y (21.5%). The additional strains were of serogroup W135 (n = 2) or non determined serogroup (n = 1). The majority of serogroup Y strains (90%) belong to the sequence type 23 (ST-23) clonal complex. One additional strain was ST-1627 and one of ST-174. Among the 20 Y isolates, more than half belonged the clone ST-23/P1.5−1.0−1 known to be commonly distributed across all continents and correlated with invasiveness. No specific resistance was detected for these strains. Fifty five percent of infected patients (n = 13) were older than 40 years which is very rare for IMD due to other serogroups.

Conclusion: An increase of the serogroup Y strains has been already reported in the North America where one third of IMD cases are now caused by this serogroup. In Europe, the proportion of IMD cases caused by Y strains remains low (overall 5.8%). In the contrary, the percentage is clearly increasing in Switzerland compared to other neighboring country like in France (3% in 2009). The results of this study allow us to draw the profile of the invasive serogroup Y strains. The knowledge about these emerging endemic strains will be helpful to follow the epidemiology and pathology of IMD and can be an alarm for other European countries.

**P1095** Epidemiologic study of bacterial gastroenteritis in Greek children (2003–2009)

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Objectives: Bacterial gastroenteritis is a common illness worldwide. A seven-year study was conducted in a pediatric hospital in Athens, in order to assess the isolation incidence and antimicrobial resistance of pathogens involved in acute diarrhea in children younger than 14 years.

Methods: We retrospectively reviewed a total of 9,667 stool cultures performed in our lab from children with acute gastroenteritis from Jan 2003 to Dec 2009.

Results: In total, 1783 samples from an equal number of children yielded one or more enteropathogenic bacteria. During a seven-year period, from 2003 to 2009, on a sequential, yearly basis starting in 2003, the following bacteria, associated with acute diarrhea, were isolated: *Salmonella* spp n = 708 (119,48.3% - 177,50.5% - 117,40.4% - 10063.7% - 82,31.2% - 67,29.5% - 46,23.8%), *Campylobacter jejuni* n = 435 (17,6.9% - 62,17.7% - 42,14.6% - 58,13.2% - 81,30.9% - 82,31.2% - 93,48.1%), enteropathogenic *Escherichia coli* n = 449 (69,28.1% - 76,21.7% - 71,24.5% - 80,29.4% - 67,25.5% - 52,22.9% - 34,17.6%), *Aeromonas hydrophila* n = 95 (16,6.5% - 13,3.7% - 16,6.5% - 12,4.8% - 17,6.5% - 14,6.2% - 7,3.6%), *Shigella* spp n = 74 (18,7.3% - 15,4.3% - 7,2.4% - 12,4.4% - 10,3.8% - 7,3.1% - 5,6.7%), *Listeria monocytogenes* n = 57 (7,2.8% - 7,2.8% - 11,3.8% - 10,3.8% - 5,9.1% - 5,2.2% - 8,4.1%). The predominant serovars among *Salmonella* isolates were: *Enteritidis* (n = 525, 74.1%) and *Typhimurium* (n = 111, 15.8%). Among *Shigella* isolates, the majority were *S. sonnei* (n = 32, 43.2%) and *S. flexneri* (n = 31, 41.9%). All but one, *Y enterocolitica* isolates belonged to O:3 (n = 52, 98.1%). A significant increase was observed in the resistance rates for *Salmonella* isolates to ampicillin (2.5% to 23.9%) and to cotrimoxazole (1.7% to 10.9%), as well as a dramatic increase in the resistance for *C. jejuni* to ciprofloxacin (0% to 63%) and to tetracycline (0% to 45.7%). All *Salmonella* isolates were found susceptible to cefotaxime, and all *C. jejuni* strains were found susceptible to erythromycin.

Conclusion: *Salmonella* spp and *C. jejuni* remain the most frequent enteropathogens among children in Greece. It is of concern, that their isolation rates have been reversed since 2008. A worrisome increase in drug resistance was found for *Salmonella* spp and *C. jejuni*. This phenomenon emphasize the need for a more restrictive policy on the use of antibiotics in humans and animals.

**P1096** Communicable diseases among Roma population in selected district of Slovakia, 1997–2006

*K. Rimarcova*, M. Feckova, M. Rimar (Kosice, Bardejov, Bratislava, SK)

Objectives: The main aim of the study was to find out and compare the frequency of selected communicable diseases among Roma population in comparison with non-Roma majority population in Bardejov district (eastern Slovakia) in the period of 1997–2006.

Methods: Data about proportion of Roma population and majority population demography in Bardejov district were obtained from regional municipality statistics and from Statistical Office of Slovak Republic. Data about frequency of diseases were obtained through an information system database EPIS from Regional Public Health Authority Bardejov.
Miscellaneous antimicrobial resistance

**P1097** Susceptibility to antibacterial agents in Germany amongst ocular isolates, 2004-2009: results of two laboratory surveillance studies

M. Kresken*, B. Körber-Irrgang on behalf of the German Ophthalmologic Study Group

**Objectives:** Antimicrobial resistance has increased over the past two decades in Germany, as in many other countries. Data on the in vitro susceptibility of pathogens isolated from ocular infections, however, are scarce. Two multi-centre studies were carried out in order to assess the occurrence of resistance amongst ocular isolates against antibacterial agents used topically in the treatment of superficial eye infections.

**Methods:** Bacterial isolates were prospectively collected in two surveillance studies conducted in cooperation with the same 23 medical laboratories in 2004 and 2009. Minimal inhibitory concentrations were determined in a central laboratory using the broth microdilution method according to the German DIN standard (now DIN-EN-ISO 20776-1:2006). Interpretive criteria applied were EUCAST clinical breakpoints for systemic use of antibacterial agents as well as EUCAST epidemiological cut-off values (ECOFFs) where available. Susceptibility data of nine antibacterial agents were analysed: levofloxacin (LVX), moxifloxacin (MFX), ofloxacin (OXF), gentamicin (GEN), kanamycin (KAN), azithromycin (AZI), erythromycin (ERY), chloramphenicol (CHL) and oxytetracycline (OTE). Additionally, the susceptibility of staphylococci to oxacillin (OXA) was determined.

**Results:** A total of 2,006 ocular isolates (2004, n = 933; 2009, n = 1,073) primarily recovered from conjunctival swabs were tested. *S. aureus* was the predominant pathogen in either study (28–34%), followed by *H. influenzae* (13–18%) and *S. pneumoniae* (15–16%). Patient related data were comparable in both studies, with two third of the isolates derived from out-patients and more than half of the patients being <10 years old. Of the *S. aureus* isolates collected in 2004 and 2009, 10.3% and 9.8%, respectively, were OXA-resistant (i.e. MRSA). Resistance to CHL, GEN and KAN among *S. aureus* decreased between 2004 and 2009, while resistance to ERY and the fluoroquinolones (FQ) remained unchanged (see Table). No significant changes in the rates of resistance were observed for *S. pneumoniae* and *H. influenzae*. In contrast, isolates with reduced susceptibility to LVX and OFX among Enterobacteriaceae increased from 0% and 1% in 2004 (n = 103) to 1.9% and 5.8% in 2009 (n = 104), respectively.

**Conclusion:** Overall, studied aminoglycosides and CHL displayed lower resistance rates in 2009 as compared to 2004, while resistance rates of FQ remained either unchanged or slightly increased.

**Table:** Proportion of resistant isolates (%)

| Year  | S. aureus | S. pneumoniae | H. influenzae |
|-------|-----------|---------------|--------------|
| No. tested | 2004 | 2009 | 2004 | 2009 | 2004 | 2009 |
| Levofloxacin | 13.3 | 15.4 | 0.7 | 0 | 0 | 0 |
| Moxifloxacin | n.t. | 15.1 | n.t. | 0 | n.t. | 0 |
| Ofloxacin | 14.1 | 15.4 | 0.7 | 0 | 1 | 0.0 |
| Kanamycin | 14.1 | 8.5 | n.a. | n.a. | n.a. | n.a. |
| Gentamicin | 15.4 | 4.3 | n.a. | n.a. | 0 | 0 |
| Azithromycin | n.t. | 23.0 | n.t. | 11.6 | n.t. | 1.0 |
| Erythromycin | 27.9 | 23.0 | 18.4 | 13.3 | 0 | 1.0 |
| Chloramphenicol | 6.3 | 1.0 | 0 | 0 | 0 | 2.1 |
| Oxytetracycline | n.t. | 3.9 | n.t. | 7.5 | n.t. | 1.0 |

Resistance rates determined by ECOFFs are given in italic, n.t.: not tested; n.a.: no clinical or epidemiological breakpoint available; Significant difference (P < 0.05)

**P1098** Detection of antibiotic residues in pork and chicken meat using the STAR test

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**Objectives:** The aim of the present study was the assessment of antibiotic residues in pork and chicken meat using the STAR (Screening Test for Antibiotic Resistance) test suggested by AFSSA Fougères Community Reference Laboratory for Antimicrobial Residues in Food.

**Methods:** There were examined 59 chicken carcasses, from which muscle and liver tissue was collected and 50 swine carcasses from which muscle, liver and kidney tissue was collected. Tissue samples of specified diameter were placed on solid agar and were inoculated with the following microorganisms: *Bacillus subtilis* BGA (for testing susceptibility to aminoglycosides), *Kocuria varians* ATCC 9341 (for testing susceptibility to macrolides), *Bacillus cereus* ATCC 11778 (for testing susceptibility to tetracyclines), *Escherichia coli* ATCC 11303 (for testing susceptibility to quinolones) and *Bacillus steatotheyphophilus* ATCC 7953 (for testing susceptibility to sulfonamides and β-lactams).

The appearance of an inhibition zone radically to the sample indicates the presence of antibiotic residues.

**Results:** 33.9% of the chicken samples and 26% of the pork samples were found positive in one or more antibiotics. Of the positive chicken and the pork samples 95% and 53.85% were found positive in the detection of sulfonamides and β-lactams respectively, while 5% and 23.07% of the chicken and pork samples were found positive in the detection of tetracyclines respectively. Concerning the detection of the aminoglycosides and macrolides, only the pork samples were found positive in 7.7% and 15.3% of the examined samples respectively, while quinolone residues were not detected in any of the examined samples.

**Conclusion:** The STAR-Test is considered a cost-effective, easily applied method, which can be used for screening potential antibiotic residues in foods of animal origin. However, STAR test is neither a qualitative or sensitive method for the detection of antibiotic residues and further analytical methods (HPLC, GC-MS) are needed for confirmation of results. Nevertheless, the detection of positive samples for the presence of antibiotic residues suggests the necessity of a continuous surveillance in order to ensure the quality of the consumed foods.
Surveillance of Aspergillus section Nigri in a general hospital during two decades

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Background: Aspergillus fumigatus, A. flavus and A. niger cause >95% of invasive & non-invasive aspergillosis. However, antifungal susceptibilities for most species within the section Nigri have been poorly investigated; their identification and susceptibility profiles have clinical interest since 7 pts had invasive A. section Nigri aspergillosis at our institution.

Methods: A total 211 Aspergillus section Nigri strains (collection of the Hospital General Universitario Gregorio Marañón) were found since 1988 by reviewing clinical data. MICs were determined by CLSI M38-A method with amphotericin B (AMB), itraconazole (IZ), voriconazole (VZ), posaconazole (POS), terbinafine (TB), caspofungin (CAS) and micafungin (MF). Modal MICs & epidemiological cut-off values (ECVs & (% of isolates encompassed) were: AMB (0.5–4/1), IZ (0.125–32/1), VZ (0.06–1/0.06), POS (0.125–2/0.5), TB (0.03–0.5/0.03), CAS (0.06–1/(0.06) and MF (0.06–0.125/0.06). MICs & ECVs & (% of isolates encompassed) were: AMB 2 (96.7%), IZ 2 (95.1%), VZ 2 (99.2%), POS 2 (99.2%), TB 0.25 (97.6), CAS 0.5 (97.6%) and MF (99.4%). Azole values were slightly higher than those for other Aspergillus spp. One strain of A. fumigatus was highly resistant to azole drugs, it was isolated from one patient with chronic obstructive pulmonary disease.

Conclusions: During the study period, there was a significant increase of patients with Aspergillus of the Nigri group in our institution. These isolates exhibited lower susceptibility to azoles than other Aspergillus spp., but their susceptibilities were good to amphotericin B, echinocandins and terbinafine.

Twelve years of resistance monitoring in Salmonella from healthy chickens in two EU regions

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Objective: Non-typhoidal Salmonella is a main cause of food-borne illness in man. For severe salmonellosis, antimicrobial treatment is used with fluoroquinolones (FQs) and 3rd generation cephalosporins (CPs) as drugs of choice. A susceptibility survey with focus on FQs and CPs was conducted in chickens, a major source of human Salmonella infections, from 1998 to 2009. Both epidemiological cut-off values (ECVs) and clinical breakpoints were applied to categorize antimicrobial susceptibility.

Methods: Random, not-repetitive sampling was conducted in two surveys. One programme, caecal samples were taken at Belgian abattoirs. In another, carcass samples were collected in various abattoirs across Germany. Susceptibility testing to ciprofloxacin (CIP) and cefotaxime (CTX), and various older molecules was done by agar dilution (CLSI; M31-A3, 2008). E. coli ATCC was used as control strain. The 211 Aspergillus section Nigri isolates were recovered between 1988–2009 from 184 patients (respiratory tract [102], ear [41], cutaneous [38], other sites [30]). MICs/MECs ranges/modes (μg/ml) were: AMB (0.5–4/1), IZ (0.125–32/0.5), VZ (0.125–32/0.5), POS (0.125–2/0.5), TB (0.03–0.5/0.03), CAS (0.06–1/(0.06) and MF (0.06–0.125/0.06). ECVs & (% of isolates encompassed) were: AMB 2 (96.7%), IZ 2 (95.1%), VZ 2 (99.2%), POS 2 (99.2%), TB 0.25 (97.6), CAS 0.5 (97.6%) and MF (99.4%). Azole values were slightly higher than those for other Aspergillus spp. One strain of A. fumigatus was highly resistant to azole drugs, it was isolated from one patient with chronic obstructive pulmonary disease.

Conclusions: During the study period, there was an increase of patients with Aspergillus of the Nigri group in our institution. These isolates exhibited lower susceptibility to azoles than other Aspergillus spp., but their susceptibilities were good to amphotericin B, echinocandins and terbinafine.

Phenotypic and molecular-genetic characterisation of antimicrobial resistance of Neisseria meningitidis isolates from Belarus

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Invasive meningococcal disease (IMD) incidence in Belarus remains quit high – in 2008 was 1.4/100000, in 2009 – 2.2 per 100000 population. Mainly three serogroups of meningococci (MC) circulate in Belarus: A, B and C, the leading serogroup is B. Improving of the diagnostics, as well as identification and susceptibility testing of N. meningitidis is indispensable.
as treatment are crucial for medical science and practice. Antimicrobial resistance monitoring is an important part of the disease surveillance system.

Objectives: To characterize antimicrobial resistance of Neisseria meningitidis isolates from patients and contact persons at the phenotypic and molecular-genetic levels.

Methods: A total of 19 MC strains were isolated from patients with IMD and contacts in hospitals of Belarus. All isolates were grown on GC agar in 5% CO2 at 37°C. Determination of minimum inhibitory concentrations (MICs) by agar dilution test was performed. The following antimicrobials and range of concentrations were used: ceftriaxone (0.008–0.0005 mg/l), ciprofloxacin (0.5–0.06 mg/l), ampicillin (0.5–0.015 mg/l), doxycycline (4.0–0.25 mg/l), meropenem (0.06–0.004 mg/l), levomecetin (8.0–0.5 mg/l) and rifampicin (0.5–0.008 mg/l). Amplification of penA and rpoB genes was performed.

Results: All meningococcal strains had reduced susceptibility or/and resistance to penicillins according to disc diffusion tests performed directly in the hospitals, where the strains were isolated. No meningococcal strains with high-level resistance to antibiotics were found by agar dilution method. The following results for MIC 90% obtained: ceftriaxone > 0.04 mg/l, ampicillin > 0.125 mg/l, doxycycline > 2.0 mg/l, meropenem > 0.003 mg/l, levomecetin > 4.0 mg/l, rifampicin > 0.03 mg/l. Tested strains were susceptible to all used concentrations of ciprofloxacin. PenA and rpoB genes were detected by PCR. Fragments of these genes were isolated and prepared for further sequencing and point mutation analysis. The emergence of MC with reduced susceptibility and/or resistance to penicillins is of particular concern in regard to the management of patients with IMD and asymptomatic carriers or contacts.

Conclusions: Resistance of the MC to antimicrobials is a great problem for public health in the world. According to the data obtained, an increase of reduced susceptibility and/or resistance level to penicillins, widely used in IMD treatment and chemoprophylaxis in Belarus, is observed in recent years, and should be taken into account.

**P1104 Routine susceptibility testing: selection bias, sensitivity, specificity, and performance related to MIC**

R. Reynolds*, R. Hope, K. Maher on behalf of BSAC Working Party on Resistance Surveillance

Objective: Routine susceptibility testing influences treatment choice, and contributes to large-scale surveillance studies. We compared its performance with centralised MIC testing, as standard, over a wide range of organisms and antibiotics tested in the BSAC Resistance Surveillance Project.

Methods: Laboratories in the UK and Ireland provided varied isolates from blood (N= 5,628; 28 centres) and respiratory infections (RTI, N= 2,418; 22 centres) in 2008–2009, with their local routine (categorical) susceptibility results as available. Isolates were tested by the BSAC agar dilution MIC method at two central laboratories and categorised by BSAC/EUCAST breakpoints, which did not change for relevant organism-agent combinations in this period. Analysis covered 94 organism-agent combinations in this period. Analysis covered 94 organism-agent combinations in this period. Agreement between routine and central tests was poorest for isolates with MICs above but close to the breakpoint, contributing to low local test sensitivity and approximately 20% probability of misclassifying a non-susceptible isolate as susceptible. Nonetheless, local reports may overestimate the prevalence of NS when it is rare, as then false NS results may outnumber false S.

**P1105 Does the long-term use of a triclosan toothpaste lead to resistant organisms?**

M.P. Cullinan*, P.S. Bird, N.C. Heng, G.J. Seymour (Dunedin, NZ; Brisbane, AU)

Objectives: To determine whether long-term use of a triclosan toothpaste selects for triclosan-resistant bacteria within the oral biofilm.

Methods: Dental plaque samples were collected from thirty-nine subjects at the final visit of a 5-year longitudinal clinical study examining the effectiveness of a toothpaste containing triclosan (TCS; 2,4,4′-trichloro-2-hydroxydiphenylether) in slowing the progression of chronic periodontitis in a population with cardiovascular disease. Participants had been randomly allocated to receive either a triclosan-containing (0.3% [wt/vol] TCS) or placebo toothpaste. Plaque samples were collected with sterile curettes, placed in sterile Wilkens Chalgren broth (WCB) containing 10% glycerol and processed immediately. Plaque samples (50 µl of a 10−1 dilution in WCB) were plated onto Wilkens Chalgren agar plates supplemented with 5% laked sheep red blood and TCS (concentration gradient from 25–150 µg/ml), and were then incubated under both aerobic and anaerobic conditions. Plates were examined for bacterial growth and colony diversity. Isolates were identified by partial 16S rDNA sequencing and their MIC (Minimal Inhibitory Concentration) of triclosan determined.

Results: There were 22 and 17 participants in the TCS and placebo groups, respectively. No growth, in both aerobic or anaerobic conditions, was observed in either group with 0.3% TCS. Without 0.3% TCS, >200 colonies of mixed growth with abundant black-pigmented colonies occurred in both groups. The lowest level where growth was not inhibited in both groups was at a TCS concentration of 150 µg/ml, however there was very little growth and very little diversity at this concentration. The MIC of TCS for isolates growing at 100 µg/ml TCS was similar in both groups and ranged from 125–1000 µg/ml. Species isolated most frequently were Veillonella parvula (MIC 500–1000 µg/ml), Streptococcus anginosus (MIC 125–250 µg/ml), Streptococcus mutans (MIC 125–250 µg/ml) and Campylobacter gracilis (MIC 1000 µg/ml).

Conclusion: Continuous use of a 0.3% (wt/vol) triclosan-containing toothpaste over a 5-year period did not result in the growth of resistant organisms under either aerobic or anaerobic conditions.

Acknowledgement: Funding for this study was provided by an unrestricted grant from Colgate Palmolive USA.
**Reporting nosocomial infections – Where are we?**

**P1106** Antibiotic susceptibility pattern of nosocomial and community-acquired *Clostridium difficile* in Hungary

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**Objectives:** The rising incidence of *C. difficile* infection is getting more and more important, this has been partly attributed to the increased consumption of broad-spectrum antibiotics among hospitalized patients and in the community. The increased antibiotic consumption may have impact on the emergence of multiple resistant *C. difficile* strains too; therefore our aims were to continue our previous investigations, in which changes in the antibiotic susceptibility patterns of toxigenic *C. difficile* isolated from human diarrhoeal faeces were determined.

**Methods:** Up to this time, 171 toxigenic *C. difficile* strains isolated between 2008 and 2010 in three Hungarian laboratories represented West, East and South Hungary from diarrhoeal faecal samples of both inpatients and outpatients were analyzed. Antibiotic susceptibility of the isolated toxin-positive strains was determined by MIC Test Strip (Loeflich, Italy) for metronidazole, moxifloxacin, clindamycin, erythromycin and rifampicin. The results were compared with our earlier findings given in 2007.

**Results and Conclusion:** All of the tested isolates were susceptible to metronidazole during the recent study period. 39 (22.8%) moxifloxacin resistant isolates were detected between 2008 and 2010. The prevalence of erythromycin resistant isolates was 28.7%, while 19.9% of tested strains proved to be resistant to clindamycin. 12.3% of the isolates were resistant to rifampicin. In comparison of the given results with our data from a previous study, in Hungary, all of the tested isolates were sensitive to metronidazole, the prevalence of isolates resistant to moxifloxacin, or erythromycin, or clindamycin has not been increased, while the prevalence of rifampicin resistant isolates was higher between 2008 and 2010. This work was supported by ESCMID/bioMérieux grant 2010.

**P1107** Antibiotic susceptibility, class I integrons and sporulation activity of *Clostridium difficile* strains from antibiotic-associated diarrhoea patients in two different years in Estonia and Norway

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**Objectives:** To investigate (1) fenotype- and genotypic characteristics of antibiotic susceptibility; (2) presence of integrons and (3) sporulation activity of *Clostridium difficile* (Cd) strains isolated in patients with antibiotic associated diarrhea (AAD).

**Material and Methods:** Altogether 119 Cd isolates – 22 from Estonia in 1994 (EE1) and 38 in 2008 (EE2) and 59 from Norway in 2008 (NO) from hospitalized patients were included. The values of minimal inhibitory concentrations (MIC) to metronidazol (MTR), vancomycin (VAN), erythromycin (ERY), clindamycin (CLI), moxifloxacin (MXF) and imipenem (IMP) were measured by E-tests. Heteroresistance (HR) was defined if colonies were growing inside the E-test inhibition zone. The presence of class I integrons and metallo-β-lactamase (MBL) genes (IMP, VIM, SPM, GIM, SIM) were studied using PCR. Cd sporulation level (SL) was tested at room temperature applying serial dilution starting with the concentration of 8×10⁷ CFU/ml and plating.

**Results:** All Cd isolates from AAD patients were susceptible to MTR and VAN. All antibiotic susceptibilities in 1994 and 2008 were similar, except of median MIC to ERY that had significantly decreased. Median MIC values to MTR, VAN, IMP of EE2 strains were higher whereas those to ERY, CLI, MXF were lower than in NO strains. 32/56 strains had HR to IP. Cd isolated before the introduction of carbapenem treatment (1994) exhibited less frequently IP HR than those isolated after (2008) (3/21 vs 29/35, respectively; p < 0.001). No MBL genes were detected. Altogether 29/82 strains possessed class I integrons (200–600 bp) which were present more often in EE2 than in EE1 or NO strains (27/42 vs 1/21 or 1/17, respectively; p < 0.001). SL varied from 6.1 to 10.7 log10 CFU/ml whereas EE2 strains exhibited higher levels than NO strains (median 10 vs 8 log10 CFU/ml, respectively; p < 0.001). Integron-positive strains showed higher SL than integron-negative ones (median 9.7 vs 8.3 log10 CFU/ml, p = 0.02).

**Conclusions:** In Estonia and Norway MTR and VAN are still equally effective for the treatment of AAD. Introduction of carbapenems might be associated with the development of HR in Cd but mechanisms other than MBL production ought to be suggested.

**P1109** Comprehensive reporting of nosocomial infection incidences

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**Objectives:** The incidence of nosocomial infections is often reported by means of the incidence density (i.e., the number of observed infections divided by the sample-time at risk) or the incidence proportion (i.e., the number of observed infections divided by the disease-free sample at baseline). However, results based on both quantities do not always agree. A reason for this is that not all patients experience a nosocomial infection, but may instead leave the critical phase without infection. We illustrate the need to report incidence densities for all observed outcomes besides reporting the incidence proportion. New results will be shown.
from the ongoing German multicenter surveillance project ONKO-KISS on pneumonia during neutropenia in adult patients who have undergone blood stem cell transplantation.

**Methods:** We introduce a new comprehensive multi-state graphic (see Figure) that clearly displays the complete study situation by incorporating all relevant incidence densities. In addition, we illustrate how incidence density and incidence proportion are connected in a competing risks setting, and how the cumulative incidence function can be derived.

**Results:** Allogeneic transplant is seen to have hardly any effect on the pneumonia incidence density compared to autologous transplant type, while a doubling of pneumonia incidence proportion is seen for the former. This seemingly contradictory result can be explained by considering the competing outcome state of leaving neutropenia without infection. As seen from the Figure, generally, the incidence density for leaving neutropenia without getting infected is much more pronounced than the pneumonia incidence density. However, autologous transplant even doubles the former compared to allogeneic transplant. Therefore, more patients that received allogeneic transplants develop pneumonia during neutropenia.

**Conclusion:** When reporting infection incidence, all potential competing events must be considered. This is facilitated by the newly proposed comprehensive multi-state graphic. The competing risks perspective also provides deeper insight with respect to the infection incidence proportion. For clinicians, the graphic provides comprehensive surveillance data feedback at a glance. This is particularly helpful in situations with apparently conflicting results on incidence densities and incidence proportions.

**P1110 The concordance of European (HELICS/IPSE) and US (CDC/NHSN) definitions for healthcare-associated infections**

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**Objective:** Across Europe comparison of infection rates from surveillance of healthcare-associated infections (HAI) is restricted since some countries are using Centers for Disease Control and Prevention (CDC/NHSN) definitions while others use Hospitals in Europe Link for Infection Control through Surveillance (HELICS/IPSE) definitions. As part of the harmonization process of the European surveillance of HAI, the European Centre for Disease Prevention and Control (ECDC) outsourced a study to analyze the concordance between the two definition types for HAI.

**Methods:** A working group with experts from 7 European countries was set up in order to identify definitions’ differences and to realize the surveillance during a 3-months period (1 March until 31 May 2010). To estimate the agreement between two case definitions Cohen’s kappa statistic was chosen.

**Results:** Differences in HAI definitions were found for bloodstream infection (BSI), pneumonia (PN), urinary tract infections (UTI) and the two key terms “ICU-acquired infection” and “mechanical ventilation”. Agreement for all of these definitions except UTI was analyzed. The study was performed on 47 ICUs and 6506 patients, 180 PN and 123 BSI cases. Agreement for PN was k = 0.99 (C195 0.98;1.00) when all cases were considered. When cases were divided in clinically and microbiologically defined PN, kappa values were 0.90 (C195 0.86;0.94) and 0.72 (C195 0.63;0.82) respectively.

Agreement for BSI was k= 0.73 (C195 0.66;0.80), but since all BSI cases secondary to another infection site (42% of all BSI) were missed by the CDC/NHSN definitions, BSI concordance was perfect (k= 1.00) when only primary BSI cases (HELICS/IPSE BSI with origin “catherer” or “unknown” and CDC/NHSN “Laboratory confirmed BSI” (LCBI)) were analyzed.

**Conclusion:** Countries using CDC/NHSN or HELICS/IPSE definitions for HAI surveillance can compare PN and primary BSI cases as long as the following considerations are taken into account: Data on PN should always be compared in total but not as subcategories. For BSI the source should always be reported since all “secondary” cases according to HELICS/IPSE definitions ought to be excluded in the comparison with CDC/NHSN defined BSI. Although other methodological differences exist between HELICS/IPSE and CDC/NHSN surveillance protocols, HAI case definitions per se do not compromise comparability of results between the two protocols and should therefore not be an obstacle for harmonization of European surveillance.

**P1111 The ECDC Pilot Point-Prevalence Survey of healthcare-associated infections and antimicrobial use: main results**

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**Objectives:** The ECDC pilot Point Prevalence Survey of healthcare-associated infections (HAI) and antimicrobial use (AU) in European acute care hospitals (ECDC-pPPS) was coordinated by ECDC and outsourced to a consortium led by the University of Antwerp (UA) in collaboration with the French Institute for Public Health Surveillance (InVS) and the Belgian Scientific Institute of Public Health (WIV-ISP). The objectives were to test and finalize a European protocol allowing to: estimate the prevalence of HAI and AU in participating hospitals; stratify estimates by patients’ characteristics and invasive procedures and; provide a standardized tool for hospitals within Europe to identify targets for quality improvement.

**Methods:** All patients on ward by 8:00 am and not discharged at the time of survey were included. Each ward was surveyed in one day. Two protocols – a patient-based with risk factors collected for each patient and a unit-based with aggregated denominator data at ward level – were tested.

**Results:** Fifty hospitals using the patient-based protocol and 16 hospitals using the unit-based protocol contributed a total of 19888 patients from 23 countries from May until October 2010. The overall prevalence of patients with HAI (n = 1408) was 7.1% [mean of hospitals 7.0%; median 7.6%, IQR 4.2–9.4%] and varied between 0.2% in psychiatric wards to 26.2% in intensive care units. Pneumonia represented 22%, surgical site infections 19%, urinary tract infections 17% and bloodstream infections 12%. The 3 most frequently reported microorganisms were *E. coli* (15%), *S. aureus* (12%) and *Pseudomonas* spp. (12%). The prevalence of AU was 34.6% (n = 6875) [IQR 28.0–44.7%] and varied between 2.2% in psychiatric wards to 61.3% in intensive care units. Patients with antimicrobials received on average 1.4 prescriptions. Combinations of penicillins and β-lactamase inhibitors were the most frequently prescribed drugs (16%), followed by fluoroquinolones (14%), second- (9%) and third- (7%) generation cephalosporins. Indication was treatment intention for 66% (community infection 41%, hospital infection 24%, other healthcare-associated infection 1%) and prophylaxis in 31% (surgical 17%; medical 14%).

**Conclusions:** The successful accomplishment of the pPPS enabled making adjustments to the final ECDC-pPPS protocol in view of its implementation in all EU Member States in 2011–2012. However, the results of this pilot survey should be interpreted with caution (no representative sample).
**Risk factors for nosocomial infections: how many are modifiable?**

**[P1112] Reducing nosocomial infections in neutropenic patients through participation in the multicentre surveillance project ONKO-KISS**

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**Objectives:** Surveillance of nosocomial infections reduces their incidence after initiation of such a program. However, these data were generated primarily from immunocompetent patients. Only few studies focused on the effect of surveillance in immunocompromised hosts. We evaluated the extent of risk reduction achieved by participation in ONKO-KISS, a German national nosocomial surveillance project focusing on immunosuppressed patients only. It prospectively collects data on nosocomial bloodstream infections (BSIs) and pneumonia during neutropenia in patients undergoing haematopoietic stem cell transplantation (HCT).

**Methods:** Participating centres included adult patients (age ≥16 years) undergoing allogeneic or autologous HCT during neutropenia. Cases of BSI were determined using Centers for Disease Control and Prevention (CDC) definitions for laboratory confirmed BSI. Cases of pneumonia were determined using modified CDC criteria adapted for neutropenic patients (www.nrz-hygiene.de/surveillance/kiss/onko-kiss). Data of centres participating for at least 3 consecutive years were included into the analysis. Relative risks (RR) were calculated (in univariate analyses) for comparison of incidences and incidence densities in the first year of participation with those in 2nd and 3rd year. Multivariate analysis was performed using a generalized linear Poisson regression model including year of participation in ONKO-KISS and variables related to clinical characteristics and demographic data.

**Results:** Data from 23 centres were included into the analysis, representing 8,561 patients, 126,924 neutropenic days, 1,417 cases of BSI and 724 cases of pneumonia (for details on HCT types see table). Regression analysis showed a statistically significant risk reduction for BSI in allogeneic HCT patients in the third year of participation compared to the first year with an incidence rate ratio (IRR) of 0.62, 95% confidence interval (CI95): [0.52–0.75], p < 0.001. For pneumonia in allogeneic HCT patients and for BSI and pneumonia in autologous HCT patients a risk reduction was observed (crude IRRs [CI95]: 0.80 [0.6–1.06], 0.85 [0.64–1.14] and 0.85 [0.53–1.37], respectively), but did not reach statistical significance.

**Conclusion:** This study clearly shows that participation in the active surveillance program ONKO-KISS is associated with significant reduction of BSI rates in neutropenic patients undergoing allogeneic haematopoietic stem cell transplantation.

| Autologous HCT | total | 3,548 |
|---------------|-------|-------|
| early disease status | 1,851 |
| advanced disease status | 1,697 |
| Allogenic HCT | total | 5,913 |
| early disease status | 2,516 |
| advanced disease status (related donor) | 2,497 |
| (unrelated donor) | 1,757 |
| All patients | total | 8,561 |

**[P1113] Epidemiological trends of emerging organisms and antimicrobial resistance in Italian ICUs: risk-adjusted rates from the SPIN-UTI project**

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**Objectives:** The main aims of our study were to compare risk adjusted rates from the first two editions of the SPIN-UTI, Italian Nosocomial Infections (NIs) Surveillance in Intensive Care Units (ICUs), project and to follow up epidemiological time-trends of nosocomial pathogens, emerging organisms and their antimicrobial resistance patterns.

**Methods:** The SPIN-UTI methodology is based on the HELICS-ICU protocol (version 6.1, 2004) (Agodi et al., 2010). Prospective patient-based surveillance was implemented from October 2006 to March 2007 by 49 ICUs in the first edition and from October 2008 to March 2009 in the second one by 28 ICUs.

**Results:** In the first study, the cumulative incidence of ICU-acquired infections for all sites was 19.8 per 100 patients and the incidence density 17.1 per 1000 patient-days. The most frequently reported NI type was pneumonia (PN, 53.6%) followed by bloodstream infections (BSIs, 23.4%), urinary tract infections (UTIs, 16.7%) and catheter-related infections (6.3%). In the second study, the cumulative incidence and the incidence density were 19.9 per 100 patients and 19.0 per 1000 patient-days, respectively. Comparing the proportion of infections by type, a decrease of PN (from 53.6% to 47.3%) and a significant increase of UTIs (from 16.7% to 22.3%; p < 0.000) were shown. In the first edition, bacterial species most frequently detected were: *Pseudomonas aeruginosa* (19.0%), *Staphylococcus aureus* (9.4%) and *Acinetobacter baumannii* (7.5%) (Agodi et al., 2010). In the second, *P. aeruginosa* remained the most frequently reported micro-organism (16.9%), while the proportion of *A. baumannii* (14.2%) and *K. pneumoniae* (9.6%) significantly increased. Trend of resistance rates were as follows: imipenem-resistant *A. baumannii* revealed no change (76.9% and 77.3%), imipenem-resistant *K. pneumoniae* increased (from 8.3% to 13.9%), and imipenem-resistant or ciprofloxacin-resistant *P. aeruginosa* revealed an increase from 40.5% to 48.1% and from 42.7% to 50.6% respectively.

**Conclusion:** Our risk estimates of ICU-acquired PN and BSI reflect the European scenario, as reported by the BURDEN project (Lambert et al., 2010). Furthermore, our study revealed an increasing risk of UTIs as a target for infection control and highlighted the emerging role of *A. baumannii* and *K. pneumoniae* in Italy as well as an increasing trend in time of specific resistance patterns. National and international cooperative efforts are needed to prevent NIs in ICUs.

**Risk factors for nosocomial infections: how many are modifiable?**

**[P1114] Identification of widespread seasonal variations in the occurrence of healthcare-associated infections: role of climatic and host factors**

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**Objective:** Seasonal variations have been detected for some health care-associated infections (HCAI); however, the extent of the phenomenon is not known. Therefore, we sought to assess the presence of seasonal variations in the occurrence of HCAI and the role of climatic factors in those variations.

**Methods:** Assessment of seasonal variations from 2006 to 2008 in the incidence rates per 1000 patient-days of various by autocorrelation analysis, principal component analysis, time series analysis and linear regression analysis at a teaching tertiary care reference centers in Marseille, France.

**Results:** 9,989 HCAI were included. Seasonal variations were detected for the overall rate of HCAI, bacteremia, deep SWI, methicillin-susceptible *S. aureus*, *S. epidermidis*, *K. pneumoniae*, and *P. aeruginosa*. Time series analysis showed that high temperature was an independent predictor of seasonal variations for the overall incidence of HCAI, deep surgical wound infections, third generation cephalosporin-resistant Enterobacteriaceae, ceftazidime-resistant *P. aeruginosa* and *K. pneumoniae* infections. Seasonal variations were stronger for males and persons aged 0 to 16 than in the other age groups. No significant seasonal variations of HCAI were observed among ICU patients hospitalized in units with air conditioning systems and by principal component analysis no correlation was found between infections and climatic factors. These findings were confirmed by a times series analysis predicting a decrease in the number of HCAI for a decrease in temperature.
Conclusions: Seasonal variations are more widespread than expected and HCAI, as a whole, are subject to seasonal variations. Unexpected, was the detection of seasonal variations in infections caused by Gram-positive organisms, in some age groups and male gender. Comparison between ICU and non-ICU patients confirmed the influence of climatic factor in seasonal variations and controlling temperature and humidity could serve as a prevention method of HCAI.

**P1115** Identifying and exploring core group dynamics in healthcare-associated methicillin-resistant Staphylococcus aureus transmission

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Objectives: Patients admitted to hospital colonized with MRSA have an increased risk of MRSA infection and are potential sources of onward transmission. The probability of a patient being colonized on admission with healthcare-associated MRSA depends on the frequency of previous hospital admissions and also the duration of MRSA carriage and the time between admissions. The objectives of this study were to quantify the impact of a patient’s history of exposure to hospital care on their potential for MRSA colonization and transmission and to identify the role of a core group of frequently readmitted patients.

Methods: Data were collected from a 1000 bed teaching hospital during a 7 year period and parameters calculated governing patient’s length of stay in hospital, probability of readmission and length of time spent away from the hospital. These parameters were used to simulate intra-hospital MRSA transmission with a stochastic individual based model (IBM).

Results: A mean of 58% patient admissions had prior admission in the previous 7 years. Of these 36% had been discharged from hospital fewer than 100 days previously. On a patient’s first discharge there is a 56% probability that they will be readmitted; using an estimated mean MRSA carriage duration of one year (and a negative exponential distribution), a patient who is MRSA colonized when discharged has a 91% probability colonization will persist at readmission. Although only 2% of admissions were admitted at least 10 times within the 7 year period, 93% of these patients had less than a year between consecutive admissions. If MRSA colonization prevalence was 5% in patients admitted for the first time, the model showed it would be approximately 10% in patients admitted for the sixth time, assuming patients differed only in their frequency of admission. The model indicated that a core group of patients admitted three times or more account for up to 60% of MRSA colonized admissions in one year.

Conclusion: Even in the absence of other risk factors for colonization, those in a core group of frequently readmitted patients have an increased risk of becoming colonized with MRSA due to frequent exposure to the hospital environment. If colonized, they are more likely to still be colonized on subsequent admissions, due to shorter times between admissions, and may account for up to 60% of MRSA admissions. These results suggest MRSA control policies could make substantial efficiency savings by targeting this core group.

**P1116** Colistin resistance in Gram-negative bacteria during prophylactic colistin use in intensive care units

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Objectives: Colistin is increasingly used in Intensive Care Units (ICU) patients as a last resort antibiotic to treat infections caused by multi-resistant Gram-negative bacteria (GNB). Although reported sparsely, colistin use may lead to colistin resistance. Selective Digestive Tract Decontamination (SDD) and Selective Oropharyngeal Decontamination (SOD) are prophylactic regimens for ICU-patients consisting of topical application of tobramycin (TOB), colistin (COL) and amphotericin B applied every 6hr throughout ICU stay. As SDD and SOD are almost universally used in Dutch ICUs, we quantified the conversion rate from COL susceptibility to resistance (S-R conversion) among GNB in a Dutch tertiary care ICU.

Methods: Using all microbiological data from a tertiary care ICU in the Netherlands we identified all S-R conversions of colistin in Escherichia coli (EC), Klebsiella spp (KS) or Enterobacter spp (EB), occurring within a single bactericidal species, during a single ICU-admission, between Jan 2008 and Oct 2010. In this ICU, SDD was used from Jan 2008 until Aug 2009 and SOD from Sep 2009 to Oct 2010. As part of SDD and SOD rectal and respiratory cultures were obtained at admission and twice weekly. For each COL S-R conversion, the time between the first COL susceptible and resistant isolate was calculated.

Results: In total 41,597 cultures were obtained in 6049 patients. 2103 of 6049 patients had an ICU stay >48 hours comprising 23,179 patient days. In 18 ICU-patients (0.86%) COL S-R conversion occurred: 11 (61%) EC, 3 (17%) KP and 4 (22%) EB. 14 Conversions occurred during 20 months of SDD, and 4 during 9 months of SOD. In 14 episodes COL resistance was associated with TOB resistance and in 4 of these episodes there also was a TOB S-R conversion. Median length of ICU stay of COL S-R converters was 29 days (range 8–96, IQR 29.8) and median time to conversion was 11 days (range 4–35, IQR 10). Conversion rate was 0.79 per 1,000 ICU-patient days at risk. Two patients had ICU-acquired bacteremia with the same species after COL S-R conversion, yet the bacteremia isolate was COL susceptible. Based on epidemiological linkage there was no evidence of cross-transmission of colistin resistant GNB.

Conclusion: In an ICU with low endemicity of antibiotic resistance and continuous exposure to topical COL the COL S-R conversion rate in GNB was 0.78 per 1,000 patient days at risk.

**P1117** Nosocomial infections in critically ill patients: when should multidrug-resistant Pseudomonas aeruginosa be suspected?

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Objective: In critically ill patients, multidrug resistant (MDR) Pseudomonas aeruginosa (PA) is a leading cause of nosocomial infections, accounting for a high attributable mortality. Indeed, in this setting MDRPA represents the main reason of inappropriate of empiric antibiotic therapy. Knowledge of predisposing factors to MDRPA acquisition could aid to anticipate an effective antimicrobial therapy. The aim of this study was to identify risk factors for MDRPA in critically ill patients.

Methods: Patients consecutively admitted to the adult medical-surgical 16-bed ICU of our University Hospital between 1 October 2007 and 30 September 2009 were enrolled. Patients with either MDRPA colonization/infection on admission or a ICU stay <72 hours were excluded. A PA isolate was defined as MDRPA when resistant to at least three of the following antipseudomonal classes of antimicrobial agents: carbapenems, anti-pseudomonal cephalosporins, fluoroquinolones, aminoglycosides. Routine tracheal and urinary screening specimens (on admission and twice weekly), as well as clinical specimens were analyzed. MDRPA acquisition was defined as a positive culture after 48 h of ICU stay. Antibiotic exposure was defined by at least 72 h of treatment in the previous 2 weeks for MDRPA group and all over the ICU stay for the control group. A binomial analysis, followed by a stepwise logistic regression procedure, was used to select the best predictors of MDRPA acquisition. Only those variables with a p value <0.10 and a number of responses defining the outcome >10 in the univariate analysis were entered into the logistic model.

Results: Out of 251 eligible patients, 83 patients (33%) acquired MDRPA. Primary sites of isolation were: lung (79.5%), urine (10.8%), blood (7.2%), and cerebrospinal fluid (2.5%). Clinical characteristics of the study population, as well as risk factors for MDRPA acquisition are illustrated in table 1. In the stepwise analysis, total parenteral nutrition (p = 0.0001) and previous exposure to glycopeptides (p = 0.008) were the best independent promoting factors of MDRPA acquisition.

Conclusions: A prolonged ICU length of stay, receipt of total parenteral nutrition, previous exposure to glycopeptides and previous prolonged exposure to non-antipseudomonal agents should rise the suspicion of MDRPA implication, when an empiric antibiotic regimen must be planned in a critically ill septic patient.
Table 3: Clinical characteristics of the study groups and risk factors for MSSA infections

| MODP group (n=98) | Control group (n=160) | Univariate p value | Multivariate p value |
|-------------------|-----------------------|-------------------|---------------------|
| Age in y (M±SD)   | 63±10                 | 63±10             | 0.28                |
| Male sex (%)      | 54(56)                | 139(86.3)         | 0.28                |
| Previous UTI (%)  | 22(22.4)              | 31(19.4)          | 0.38                |
| Source of infection (%) |                   |                   |                     |
| Community         | 38(38.8)              | 60 (35.7)         | 0.16                |
| Hospital setting   | 16(16.3)              | 8 (5.6)           | 0.06                |
| Charlson co-morbidity index, n=90 | 1.5±1.7               | 1.6±1.7           | 0.16                |
| Prior antimicrobial therapy (%) |                       |                   |                     |
| No therapy        | 62(62.6)              | 121 (75.6)        | 0.05                |
| One or more drugs  | 36(36.6)              | 39 (24.4)         | 0.02                |
| Total percent positive (%): |                      |                   |                     |
| Gram-positive (%)  | 62(62.6)              | 112 (70.0)        | 0.05                |
| Gram-negative (%)  | 36(36.6)              | 48 (29.4)         | 0.02                |
| Duration, days (M±SD) | 0.5±0.8               | 9.5±10.6          | 0.15                |
| Outcome, n=98     | 80(81.6)              | 151 (94.4)        | 0.06                |
| Treatment duration (n=98) | 15.6±15.6             | 14.5±15.9         | 0.06                |
| Kick-out, n=98    | 82(83.7)              | 148 (92.7)        | 0.06                |
| Time at risk, days (n=98) | 10±11.2               | 11±11.2           | 0.008               |

*Results are given as median (25–75% range) unless otherwise stated. p values are calculated using the Mann–Whitney U test.*

### P1119 Assessing risk factors for acquiring extended-spectrum β-lactamase-producing Gram-negative infections in long-term care facilities: a case-control study

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**Background:** Extended-spectrum β-lactamase-producing Gram-negative (ESBL+Gn) are emerging pathogens among health-care associated infections. However, to our knowledge, risk factors for ESBL+Gn infections in long term care facilities (LTCFs) were rarely investigated.

**Methods:** A prospective case-control study. This approach was chosen because allows to compare the relative contribution of the ESBL production over and above simply having the Gn infection. To homogenize the comparisons, urinary tract infection was chosen as common indicator of infections in LTCFs. ESBL determinants were characterized by hybridization and confirmed by PCR and sequencing. In the first study, cases were defined as patients harbouring the ESBL+Gn while controls, similar to the first approach, were those patients harboring the ESBL-Gn. For ESBL-Gn infections, nospecific risk factors were identified. For ESBL+Gn infections, the following risk factors were identified: age ≥ 65 years (OR, 4.9, p = 0.02), previous hospitalization (OR, 6.7, p = 0.02), and 3rd gen. cephalosporins (OR, 3.2, p = 0.04). In the second study, cases were instead defined as those patients harboring the ESBL+Gn while controls were those without Gn infections (random sampling). In the second study, cases were instead defined as those patients harboring the ESBL+Gn while controls, similar to the first approach, were those patients without Gn infections.

**Results:** The study involved 279 patients in a LTCF. Case (1st group) developed UTIs due to the following ESBL+Gn: *E. coli* (71%), *K. pneumoniae* (11%), and *P. mirabilis* (17%). CTX-M-type enzymes (CTX-M-1 and CTX-M-15) were prevalent (84%). TEM-type were produced by 10% (TEM-92). All *K. pneumoniae* co-produced CTX-M group 1, SHV and TEM enzymes. Patients with ESBL+Gn UTIs were more likely to have been hospitalised in the previous year (p = 0.04), to suffer from cirrhosis (p = 0.04) and to have permanent urinary catheter (p = 0.02). Compared with controls, these patients had a longer duration of hospital stay before UTI developed (17 vs. 9 days, OR 1.1 per 1-day longer, p = 0.04) and were more likely to have been hospitalised in the previous year (OR 9, p < 0.01). The risk was higher in patients with at least 7 days of antibiotic exposure (OR 11). In the case–case comparison using a multinomial logistic regression model, after adjusting for demographic and clinical risk factors, we found a statistically significant risk difference for previous use of quinolones (OR, 11.5, p = 0.01) and 3rd gen. cephalosporins (OR, 3.7, p = 0.02), in patients with ESBL+Gn infections. For ESBL-Gn infections, no specific antibiotic remained a significant risk after adjusted analysis.

**Conclusions:** Exposure to quinolones and 3rd gen cephalosporins was associated with subsequent UTIs due to ESBL+Gn in a LTCF. Interventions aimed to the reduction of antimicrobial usage in LTCFs should be further developed and implemented.

### P1120 Trends in antimicrobial susceptibility in European Gram-positive anaerobes: the Tigecycline European Surveillance Trial, 2007–2010

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**Objectives:** Antimicrobial resistance in anaerobic isolates is of increasing concern in clinical settings, with empiric treatment often incorrect. The Tigecycline European Surveillance Trial (TEST) has been monitoring susceptibility of anaerobes in Europe since 2007. This report compares the trends in susceptibility levels of Gram positive anaerobes from 2007 to 2010.

**Methods:** 2,338 Gram-positive anaerobic pathogens, including *Anaerococcus* spp., *Clostridium* spp., *Finegoldia magna*, *Peptostreptococcus* spp., and *Prevotella shredderi* spp., were collected and identified from 44 sites in 7 countries in Europe, and sent to a central laboratory where MICs of tigecycline and five comparators were determined using CLSI agar methods. When the WHO/FAO guidelines were the first to draw a specific safety list for testing of probiotic microorganisms which included antimicrobial resistance profile testing, we decided to test selected, most common probiotic strains used in Poland to daptomycin and tigecycline, which are considered as salvage therapy in life-threatening infections due to multiresistant strains.

**Results:** We isolated 12 strains including 11 lactobacilli and 1 *Bifidobacterium* strain. MIC values for daptomycin were surprisingly high, only two strains had MIC values of 1 μg/ml and all the other strains had MIC values above that (up to 48 μg/ml). For Tigecycline MIC values were low, except for 5 strains with MIC values between 1.5–48 μg/ml. The highest MIC values for both daptomycin and Tigecycline were shown for the strains originating from the most popular probiotic in Poland (used by 66.5–93.7% of persons using probiotics, depending on the region).

**Conclusions:** The most popular probiotic products were found to have very high MIC values to daptomycin and tigecycline. There were reports, including our previous studies, about infections with strains contained in probiotic products. The empirical therapy of infections with probiotic strains in Poland should not include these two novel drugs, especially daptomycin, owing to high MIC values. Data for other European markets are not fully described and joint cooperation is necessary in the years to come. As an additional conclusion, we think it would be wise for the producers of the oldest probiotic preparation available in Poland (used from 1960's) to revise their product formulation or even consider withdrawing it from the market.

**Tigecycline**

P. Kochan*, A. Drzewiecki, W. Wąs, G. Jastrzebski, T. Gosiewski, K. Pakosz, P. Hezko (Cracow, Katowice, PL)

**Objectives:** Most probiotics used as dietary supplements and food products have a long history of safe use and generally recognized as safe status (GRAS), meaning they are regarded as food-grade microorganisms with no imposed health risk for consumers or the environment. There are however documented studies that describe resistance patterns in these bacteria. The WHO/FAO guidelines were the first to draw a specific safety list for testing of probiotic microorganisms which included antimicrobial resistance profile testing. We decided to test selected, most common probiotic strains used in Poland to daptomycin and tigecycline, which are considered as salvage therapy in life-threatening infections due to multiresistant strains.

**Methods:** Selected probiotic products (n=6) of different composition were selected and purchased in the local pharmacies. After initial quantitative culture on appropriate media, the colonies were identified by microscopic observation of stained specimens and by API 50CH. Next step was setting up Etstes for Tigecycline determination for daptomycin and tigecycline.

**Results:** We isolated 12 strains including 11 lactobacilli and 1 *Bifidobacterium* strain. MIC values for daptomycin were surprisingly high, only two strains had MIC values of 1 μg/ml and all the other strains had MIC values above that (up to 48 μg/ml). For Tigecycline MIC values were low, except for 5 strains with MIC values between 1.5–48 μg/ml. The highest MIC values for both daptomycin and Tigecycline were shown for the strains originating from the most popular probiotic in Poland (used by 66.5–93.7% of persons using probiotics, depending on the region).

**Conclusions:** The most popular probiotic products were found to have very high MIC values to daptomycin and tigecycline. There were reports, including our previous studies, about infections with strains contained in probiotic products. The empirical therapy of infections with probiotic strains in Poland should not include these two novel drugs, especially daptomycin, owing to high MIC values. Data for other European markets are not fully described and joint cooperation is necessary in the years to come. As an additional conclusion, we think it would be wise for the producers of the oldest probiotic preparation available in Poland (used from 1960's) to revise their product formulation or even consider withdrawing it from the market.
Conclusions: Tigecycline, metronidazole, meropenem and piperacillin tazobactam showed excellent in vitro activity against Gram-positive anaerobic bacteria isolated from European hospitals from 2007 to 2010, inhibiting nearly 100% of all isolates. Clindamycin and penicillin generally inhibited less than 80% of Gram-positive isolates. No significant decreases in susceptibilities were noted over the four years surveyed.

Susceptibility of Gram positive anaerobes from Europe, 2007-2010

Objective: Tigecycline is a novel glycylcycline demonstrating a broad range activity against numerous Gram-positive, Gram-negative, anaerobic and “atypical” bacteria. The worldwide advent of multiresistant bacteria support its role as an important adjunct in the antimicrobial armamentarium. The goal of this study was to analyze the activity of tigecycline against a collection of well-defined clinical pathogenic isolates of a large tertiary care institution.

Methods: The University of Saarland Hospital is a 1300-bed (150 ICU-beds) tertiary care facility serving all medical specialties including transplant and oncologic units. From January until June 2009, 751 isolates preferentially recovered from the blood and other primarily sterile sites were collected. Copy strains were excluded. All isolates were analyzed according to current CLSI standards for agar dilution susceptibility testing (AST). Mueller-Hinton Agar plates with serial dilutions of tigecycline (0.0625 to 8 mg/l) were prepared freshly the day before analysis.

Results: We confirmed that tigecycline is active against a broad array of Gram-negative and -positive bacteria. Increased MICs were found for Morganella, Proteus, Providencia and Serratia spp. Higher MICs were also found when comparing E. coli with and without ESBL. For multiresistant Gram-positive bacteria (MRSA and VRE) the MIC was not increased. Interestingly, MIC50 and MIC90 values were found to be superior to those previously reported for tigecycline (Table 1), typically determined using broth microdilution AST. It has been reported that tigecycline susceptibility testing may be sensitive to the freshness of the media preparation due to inactivation by oxygen. Accordingly, in a smaller series of AST, an antioxidant available for human use has been added to the agar dilution media preparations in order to preserve tigecycline function. 81/751 clinical isolates have been additionally tested in parallel in, and in 59/81 isolates, the MIC was found to be reduced by a factor of 2–16.

Conclusion: Agar dilution techniques may yield elevated MIC values for tigecycline against a large spectrum of clinical isolates when compared to broth microdilution assay. The reduction of MIC values as a result of supplementation with an antioxidant suggests a role of oxidative inactivation of tigecycline. Whether or not oxidative inactivation is of relevance in clinical application should be subject of testing in appropriate models.
Tigecycline susceptibility trends in Germany among Gram-positive and Gram-negative pathogens recovered from wounds, the peritoneal cavity and blood

M. Kresken*, K. Becker, H. Seifert, B. Körber-Irrgang, C. von Eiff, P.-A. Löschmann on behalf of the German Tigecycline Study Group

Objectives: Following regulatory approval for use in complicated skin and skin structure infections and complicated intra-abdominal infections, tigecycline (TGC) was introduced in Germany in 2006. Data of the German Tigecycline Evaluation Surveillance Trial (G-TEST) conducted between 2005 and 2009 were analyzed for the susceptibility of most important aerobic Gram-pos. and Gram-neg. pathogens to TGC and comparators.

Methods: The susceptibility of isolates of four Gram-pos. and seven Gram-neg. species consecutively collected in three surveillance studies conducted in cooperation with the same 13 laboratories in 2005, 2007, and 2009 (G-TEST I-III) were tested. Each laboratory collected approx. 250 pathogens from hospitalized patients. MICs were determined in a central laboratory using the microdilution method according to the standard ISO 20776–1:2006. EUCAST breakpoints were applied to all antibiotics for interpretation. ESBL-producing organisms were identified by CLSI criteria. E. coli isolates expressing an ESBL phenotype were further characterized by PCR and sequencing.

Results: A total of 3,248 isolates recovered from wounds (n=1,573; 48.4%), the peritoneal cavity (n=567; 17.5%), and blood (n=1,108; 34.1%) were tested. Based on MIC-50/90 values, TGC demonstrated unchanged (within +/− one dilution) in vitro activity against all species tested (see Table). In contrast, a considerable increase of resistance (R) for β-lactams and fluoroquinolones (FQ) was noted for members of the Enterobacteriaceae family. Between 2004 and 2009, R to FQ in E. coli, E. cloacae and K. pneumoniae increased from 20% to 27%, 6% to 11%, and 7% to 23%, respectively. The increase of R to cefotaxime in E. coli and K. pneumoniae went along with a rise of ESBL-producing strains, from 6% to 11% and from 5% to 14%, respectively. In all three studies, most common ESBLS found in E. coli were CTX-M-1 and CTX-M-15. R to imipenem in A. baumannii group isolates was not observed in 2005, but was 18% and 8% in 2007 and 2009, respectively. The rate of vancomycin-resistant strains among Enterococcus faecium isolates varied between 9% and 19%.

Conclusion: TGC retained its very good in vitro activity against all Gram-pos. and Gram-neg. organisms tested after the introduction in Germany. Against a background of pathogens that are frequently resistant to various antibiotic classes, TGC remains an important treatment option.

P1124 In vitro activity of tigecycline and comparators against Enterococcus faecalis and Enterococcus faecium isolates collected in Germany

F.-J. Schmitz* (Minden, DE)

Objectives: Tigecycline is a new broadspectrum antibiotic against Gram-positive and Gram-negative pathogens including multi-drug-resistant strains, licenced for complicated intra-abdominal infections (cIAI) and skin and soft tissue infections (cSSTI) in Germany. Enterococcal pathogens are one of the most important bacterial species responsible for cIAI (e.g. peritonitis) and cSSTI (e.g. deep wound infections). Aim of the present study was to compare the in vitro activities of tigecycline and comparators.

Methods: In total 600 bacterial strains were tested (200 Enterococcus faecium and 400 Enterococcus faecalis) mostly collected from patients with cIAI or cSSTI in the year 2009. MICs were determined by the broth microdilution according to the guidelines of the CLSI. The accuracy of susceptibility testing was evaluated by MIC testing of quality control organisms.

Results: According to MIC ranges as well as to MIC50/90 values, tigecycline demonstrated excellent in vitro activity against all 600 enterococcal isolates tested. No difference could be observed between E. faecalis and E. faecium, although E. faecium isolates are very often multiresistant. In addition, tigecycline was active against vancomycin-resistant E. faecium isolates. None of the isolates tested showed resistance to tigecycline, although tigecycline has now been used for a couple of years. In comparison to other compounds with activity only against Gram-positive isolates (e.g. daptomycin, vancomycin and linezolid) tigecycline displayed a MIC90 value of 0.125 mg/L against E. faecalis isolates, while the other comparators displayed a value of 2 mg/L. Against E. faecium isolates MIC90 values for tigecycline, linezolid, daptomycin and vancomycin were 0.125, 2, 4 and 16 mg/L, respectively. Both species (E. faecalis and E. faecium including VRE) were 100% susceptible to tigecycline and linezolid.

Conclusion: In summary, tigecycline displayed an excellent in vitro activity against all enterococcal isolates tested and no tigecycline resistant isolate was found.
Comparison of tigecycline activity tested against multidrug-resistant bacteria isolated from European medical centres in two time periods: 2003–2005 and 2008–2010

H. Sader*, D. Farrell, P. Romberg, R. Jones (North Liberty, US)

Objectives: To compare the activities of tigecycline (TIG) and comparators tested against bacteria with clinically important resistance (R) phenotypes isolated in Europe (EU) in 2003–2005 (before TIG was approved for clinical use) with those isolated in 2008–2010 (contemporary strains).

Methods: 6,345 clinical isolates, including 3,881 oxacillin-R S. aureus (MRSA), 501 vancomycin (VAN)-R enterococci (VRE), 581 E. coli with ESBL phenotype (ESBL-EC), 647 K. pneumoniae with ESBL phenotype (ESBL-KPN), 112 meropenem (MER)-R KPN and 466 imipenem-R Acinetobacter spp. (IMI-R-ASP), were collected from 48 hospitals in 18 EU countries and Israel. The isolates were tested for susceptibility (S) by the CLSI microdilution broth method and EUCAST breakpoint criteria were used for MIC result interpretations.

Results: Overall, 96.6% of strains were TIG-S and TIG MIC distributions were very similar in the two time periods evaluated. Two-thirds of TIG non-S strains were IMI-R-ASP with TIG MIC at >1mg/L (EUCAST breakpoint for enterics). MRSA showed low S to levofloxacin (LEV; 8.1–11.4%), and clindamycin (50.2–64.7%), and high S to TIG (>99.9%), VAN (100.0%), daptomycin (DAP; 100.0%), linezolid (LZD; 99.9–100.0%) and cefotaxime (93.4–97.6%) in both periods. Against VRE, TIG (MIC<50, 0.06–0.12/0.25 mg/L), DAP (MIC<50, 2–4/4–8 mg/L) and LZD (MIC<50, 1/2 mg/L) were the most active compounds (>99% S). 99.5% of ESBL-EC were S (MIC, ≤1 mg/L) to TIG (MIC<50, 0.25/0.5 mg/L). ESBL-EC exhibited low S to LEV (24.5–31.7%) and gentamicin (GEN; 54.2–59.9%) in both time periods, but >99% of strains were S to MER. TIG inhibited 89.7–91.7% of ESBL-KPN and 88.1–88.6% of MER-R-KPN at ≤1 mg/L. Only 29.6–35.8% of ESBL-KPN was S to GEN, and S to LEV and MER dropped from 55.2 and 93.9% in 2003–2005 to 26.7 and 86.0% in 2008–2010, respectively. Among MER-R-KPN, S to amikacin and LEV fell from 59.5 and 42.9% in 2003–2005 to 28.6 and 8.6% in 2008–2010, respectively. Only 67.1% of MER-R-KPN isolated in 2008–2010 were S to colistin. TIG (MIC<50, 1 mg/L; 66.7–73.1% inhibited at ≤1 mg/L) and the polymyxins (>99% S) were the most active compounds tested against IMI-R-ASP.

Conclusion: TIG was very active against this large collection of multidrug-R organisms and no trend toward decreased TIG activity over time was observed for any of the organisms or R subsets. TIG demonstrated sustained potent in vitro activity and a broad-spectrum against clinically important R organisms from EU hospitals.

Objective: Stenotrophomonas maltophilia is intrinsically resistant to a plethora of antimicrobial agents that severely limit commonly used empiric standard antimicrobial therapies. In this study we investigated the in vitro activity of tigecycline against clinical isolates of S. maltophilia by comparing Etest to the broth microdilution method (BMD).

Methods: In total, 105 S. maltophilia isolates were obtained from serious infections sites in a tertiary single center in Germany in 2008 and 2009. Multi-copy strains were excluded from the analysis by molecular fingerprinting using a repetitive-sequence-based polymere chain reaction. Minimal inhibitory concentration values were determined based on the Clinical Laboratory Standards Institute (CLSI) BMD method. A tigecycline breakpoint of minor or equal to 2 mg/L was used according to most published studies.

Results: None of the S. maltophilia isolates was tested resistant against tigecycline. MICs ranged from 0.19 to 2.0 mg/L with a MICc50/MIC90 of 1.0/2.0 mg/L by BMD. Agreement with one twofold dilution between the Etest and the BMD method was 85.3%.

Conclusion: In this single center study tigecycline was highly effective against noncolonial S. maltophilia isolates from serious infections sites.

Comparative MIC and MPC results for tigecycline repeatedly tested against American Type Culture Collection control strains: suggested MPC quality control value ranges

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Objectives: The minimum inhibitory concentration (MIC) determines the minimum drug concentration inhibiting 100 cfu/ml of bacteria in vitro whereas the mutant prevention concentration (MPC) determines the minimum drug concentration required to block the growth of the least susceptible cell of a ≥10^9 cfu inoculum applied to drug containing agar plates. While MIC testing is standardized and controlled using American Type Culture Collection (ATCC) strains, similar standardized protocols are not yet available for MPC testing. We compared MIC and MPC results with 2 ATCC strains repeatedly tested against tigecycline to document MIC and MPC quality control (QC) ranges.

Methods: MIC testing was based on current Clinical and Laboratory Standards Institute procedures using a 10^5 cfu/ml inoculum and for MIC testing ≥10^9 CFU were inoculated to drug containing media in 2-fold concentration increments. Optimal media, incubation in established atmospheres and temperatures were used. For each organism type, the lowest drug concentration preventing growth was recorded as either the MIC or MPC. ATCC strains included S. aureus (SA) 29213 and E. coli (EC) 25922 tested against tigecycline.

Results: Following repeat MIC assays, MICs were within acceptable QC limits: EC 25922 0.016–0.031, SA 29213 0.031–0.063. Following repeat MPC testing, the following QC organism MPC results (µg/ml) were noted against ATCC strains SA 29213, EC 25922 respectively: 0.5 and 1; 0.25 and 0.5. MPC values for the QC strains were within one doubling dilution.

Conclusion: As with MIC testing, MPC testing provides reproducible QC organism results that are consistent over narrow drug concentration ranges with MPC values being within one doubling dilution of the ATCC control strains tested. The MPC drug concentration ranges presented may serve as a useful guide to insuring MSC assay accuracy for clinical isolates of EC and SA tested against tigecycline.
New antimicrobials in vitro activity

Dalbavancin surveillance results for European Gram-positive species in a contemporary (2006–2009) sample of 23,825 strains

R. Jones*, D. Farrell, H. Sader (North Liberty, US)

Objectives: To assess the in vitro activity of dalbavancin (DALB) for in vitro potency and breadth of spectrum against Gram-positive cocci (3 genera) isolated in European medical centers between 2006 and 2009. Reference MIC susceptibility (S) testing methods (CLSI, M07-A8, 2009) and EUCAST breakpoints were applied to comparator antimicrobials.

Methods: A total of 23,825 strains were tested as follows: S. aureus (SA: 50,271 strains; 44.5% MRSA), and DALB potency remained stable across the monitored time interval (2006–2009). All SA and 99.8% of CoNS were inhibited at ≤0.5 mg/L, only 30 CoNS had DALB MIC values at 1 or 2 mg/L (a number comparable to daptomycin, data not shown). DALB was 16- and four-fold more potent than vancomycin and daptomycin, respectively. The susceptibility rates of comparator agents were not superior to DALB against staphylococci e.g. daptomycin (99.8–99.9%), vancomycin (>99.9%), linezolid (99.3–99.9%), teicoplanin (70.8–99.4%) and cotrimoxazole (61.3–95.4%). No variations in DALB activity by geographic region were observed.

Conclusions: DALB activity updated with contemporary staphylococcal strains worldwide through 2009 shows sustained potent inhibition and a modal MIC value at only 0.06 mg/L. This level of potency was many-fold greater than currently available glycopeptides or lipopeptide-class agents, thus warranting renewed clinical investigations for several indications where multidrug-R staphylococci may be prevalent.

Dalbavancin activity and spectrum evaluated against a contemporary (2007–2009) worldwide collection of staphylococci (62,590 strains)

R. Jones*, H. Sader, R. Mendes, D. Farrell (North Liberty, US)

Objectives: To update the in vitro profile of dalbavancin (DALB), an investigational lipoglycopeptide, for its anti-staphylococcal potency and spectrum via the testing of a collection of clinical isolates from 2006–2009. A total of 62,590 staphylococci were evaluated (14,492–17,664/year) from the Asia-Pacific region (11,692 strains), Europe (16,001), Latin America (6,711) and North America (28,186).

Methods: All organisms were susceptibility (S) tested by CLSI (M07-A8, 2009) reference MIC methods in a central laboratory design. Staphylococcus species from 21 countries (201 medical centers) were sampled as follows: S. aureus (SA: 50,271 strains; 44.5% MRSA), and DALB MIC results were determined in validated panels equivalent to reference polysorbate-80 (0.002%) containing broth media. All QC results were within published ranges (CLSI M100-S20-U, 2010). Most isolates came from blood (63.0%), lower respiratory or acute bacterial skin and skin structure infection (ABSSSI) sources.

Results: DALB was highly active against SA (MIC50/90, 0.06/0.12 mg/L). Methicillin S or R did not influence DALB activity (Table) and DALB potency remained stable across the monitored time interval (2006–2009). All SA and 99.8% of CoNS were inhibited at <0.5 mg/L, only 30 CoNS had DALB MIC values at 1 or 2 mg/L (a number comparable to daptomycin, data not shown). DALB was 16- and four-fold more potent than vancomycin and daptomycin, respectively. The susceptibility rates of comparator agents were not superior to DALB against staphylococci e.g. daptomycin (99.8–99.9%), vancomycin (>99.9%), linezolid (99.3–99.9%), teicoplanin (70.8–99.4%) and cotrimoxazole (61.3–95.4%). No variations in DALB activity by geographic region were observed.

Conclusions: DALB activity updated with contemporary staphylococcal strains worldwide through 2009 shows sustained potent inhibition and a modal MIC value at only 0.06 mg/L. This level of potency was many-fold greater than currently available glycopeptides or lipopeptide-class agents, thus warranting renewed clinical investigations for several indications where multidrug-R staphylococci may be prevalent.
Analysis of oritavancin activity tested against a challenge set of Staphylococcus aureus from Europe (2008–2010)

R. Mendes*, H. Sader, D. Farrell, R. Jones (North Liberty, US)

Objectives: To assess the activities of oritavancin (ORI) and comparator agents against S. aureus (SA) from Europe (EU). In addition, this analysis includes categorization of strains with decreased susceptibility (S) to vancomycin (VA), teicoplanin (TE) and daptomycin (DA). ORI has demonstrated potent activity against Gram-positive isolates, including VA-resistant staphylococcal and enterococcal strains. Moreover, it has been demonstrated that ORI possesses multiple mechanisms of actions.

Methods: 7,053 consecutive, non-duplicate SA were collected from 29 hospitals in 13 EU countries, including Turkey and Israel, as part of the SENTRY Antimicrobial Surveillance Program. Isolates were submitted to a central monitoring laboratory and species identification confirmed by reference methods against a collection of linezolid-non-susceptible (NS) Gram-positive cocci having genetically defined mechanisms of oxazolidinone resistances.

Results: A total of 90 linezolid-NS Gram-positive cocci, obtained from toxin-positive stools, were quinolone-resistant, some with MICs to metronidazole ≥16 mcg/mL and elevated MICs to vancomycin. Most isolates were from bacteremia (38.5%) and skin and skin structure infections (37.8%). ORI (MIC90/90, 0.03/0.06 mcg/mL) was eight-fold more potent than DA (MIC90/90, 0.25/0.5 mcg/mL) and 16- to 32-fold more active than VA (MIC90/90, 1/2 mcg/mL) and linezolid (LZ; MIC90/90, 1/2 mcg/mL) when tested against all SA. ORI exhibited similar potency when tested against meticillin-susceptible SA (MSSA) with decreased S to VA (MIC90/90, 0.03/0.06 mcg/mL), TE (MIC90/90, 0.03/0.06 mcg/mL) or DA (MIC90/90, 0.03/0.12 mcg/mL) compared to their respective counterparts with lower MIC results (all MIC90/90, 0.03/0.06 mcg/mL; Table). When ORI was tested against a challenge set of methicillin-resistant SA (MRSA), slightly higher (two- to four-fold) MIC50/90 values were noted for those strains with elevated MIC results for VA (MIC50/90, 0.03/0.12 mcg/mL), TE (MIC50/90, 0.03/0.12 mcg/mL) and DA (MIC50/90, 0.06/0.25 mcg/mL).

Conclusions: ORI exhibited overall greater potency (>eight-fold) than four comparators against all SA. When tested against a challenge set of MSSA clinical isolates, ORI sustained high activity. ORI showed higher MIC values against MRSA strains with elevated MIC results for VA, TE and DA, yet inhibiting all SA at ≤0.25 mcg/mL. The ORI multiple mechanisms of actions likely provide advantageous in vitro activity even against isolates with decreased S to same class agents.

Activity of a novel cyclic lipopeptide, CB-183,315 against Gram-positive aerobic and anaerobic enteric isolates, including vancomycin-resistant enterococci and C. difficile strains with elevated MICs to metronidazole, vancomycin and fluoroquinolones

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Objective: To evaluate in vitro activity of CB-183,315, a novel cyclic lipopeptide, vs recent clinical aerobic and anaerobic Gram-positive intestinal isolates, including Clostridium difficile, Clostridium species, Peptostreptococci, Gram-positive-non-spore-forming-anaerobic bacilli (GPNSF), enterococci and S. aureus.

Methods: The isolates were obtained from patients at Tufts Medical Center. Resistant strains were included. The MICs vs 199 isolates were determined by agar dilution following CLSI recommendations. The activities of CB-183,315 vs the anaerobes were compared to those of amoxicillin-clavulanate, clindamycin, linezolid, metronidazole, meropenem, minocycline, vancomycin, moxifloxacin and piperacillin-tazobactam. Against enterococci, CB-183,315 was compared to daptomycin, ampicillin, levofloxacin, minocycline, erythromycin and linezolid. For staphylococci, amoxicillin-clavulanate, meropenem, cefoxitine and trimethoprim:sulfamethoxazole were added. Forty percent of C. difficile, all from toxin positive stools, were quinolone-resistant, some with MICs to metronidazole ≥16 mcg/mL and elevated MICs to vancomycin.

Results: See table.

Summary: CB-183,315 showed very good activity vs enteric Gram-positive aerobic and aerobic pathogens. CB-183,315 showed excellent activity vs 16 quinolone-resistant C. difficile as well as 9 C. difficile with MICs to metronidazole ≥16 mcg/mL and 6 isolates with vancomycin MICs of 4 mcg/mL. CB-183,315 also showed good activity against peptostreptococci. CB-183,315 exhibited good activity against enterococci including 21 vancomycin-resistant strains as well as against oxacillin-resistant enteric S. aureus pathogens.

Conclusion: Our results showed that CB-183,315 is a potential agent for the treatment of enteric infections in particular those caused by antimicrobial resistant strains, including C. difficile.

Results: Activities of CB-183,315 vs Enteric Gram Positive Pathogens

Bacterial Group | MIC Range (mcg/mL) | MIC90 (mcg/mL)
--- | --- | ---
C. difficile (40) | <-0.12 - 1 | <-0.12
Clostridium spp. (33) | <-0.12 - 4 | 2
Peptostreptococci (45) | <-0.12 - 2 | 0.5
Anaerobic GPNSF (6) | 1 – 16 | NA
Enterococci (60) | <-0.12 - 2 | 2
Enteric S. aureus (15) | <-0.12 - 2 | 1

Potency of radezolid (RX-1741) and torezolid (DA-7157) tested against a collection of linezolid-non-susceptible strains with genetically defined resistance mechanisms

D. Farrell*, R. Mendes, H. Sader, R. Jones (North Liberty, US)

Objectives: To provide a potency evaluation of the investigational oxazolidinone radezolid (RX-1741), against another investigational oxazolidinone, torezolid (DA-7157), and linezolid when tested by reference methods against a collection of linezolid-non-susceptible (NS) Gram-positive cocci having genetically defined mechanisms of oxazolidinone resistance.

Methods: A total of 90 linezolid-NS Gram-positive cocci, obtained through the SENTRY Antimicrobial Surveillance Program, were tested for susceptibility by CLSI broth microdilution methods (M07-A8 and M100-S20-U). Linezolid, radezolid and torezolid were manufactured by Rib-X Pharmaceuticals, Inc. Torezolid could not be tested at
Conclusions: Radezolid and torezolid both demonstrated enhanced activity against this collection of 90 linezolid-NS strains. Radezolid showed at least two-fold greater potency when compared directly to torezolid against most strains. These investigational oxazolidinones demonstrate encouraging in vitro activity against contemporary linezolid-NS Gram-positive pathogens.

The investigation of antimicrobial activity of 5 heterocyclic compounds against some clinical isolates of oral streptococci

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Objectives: The aim of this work was to investigate the antimicrobial activity of newly synthesized heterocyclic compounds of the classes: 1,2,4-triazole, 1,3,4-thiaizazole and 1,3,4-oxadiazole (characterized by spectral and elemental analysis and recently reported by the last author of this study) against some oral streptococcal isolates of different species.

Methods: The minimum inhibitory concentrations (MIC) of the following 5 compounds: 4-(4-fluorophenyl)-1-(4-(phenylsulfonyl)phenyl)-2H-thiazole (C1), 4-(4-fluorophenyl)-5-(4-(phenylsulfonyl)phenyl)-2H-1,2,4-triazole-3(4H)-thione (C2), ethyl 2-[(4-[(4-fluorophenyl)-5-(4-(phenylsulfonyl)phenyl)]-4H-1,2,4-triazole-3-ylthio)acetate (C3), N-(4-fluorophenyl)-5-(4-(phenylsulfanyl)phenyl)-1,3,4-thiadiazol-2-amine (C4), and N-(4-fluorophenyl)-5-(4-(phenylsulfonyl)phenyl)-1,3,4-oxadiazol-2-amine (C5), were determined by the broth microdilution method against 64 oral streptococcal strains isolated from paediatric patients with respiratory infections at 3 hospitals in Bucharest, in 2009. In addition, the minimum bactericidal concentrations (MBC) of the 5 compounds were determined against the isolates belonging to the species S. oralis (33 strains), S. mitis (17 strains), S. sanguinis (9 strains), S. parasanguinis (2 strains), S. constellatus (2 strains) and S. anginosus (1 strain).

Results: The MIC values ranged between 32–256 mg/L for C1 and C3, 8–256 mg/L for C2, 128–256 mg/L for C4 and 64–256 mg/L for C5. C2 presented the best growth inhibition activity against all isolates except for the single strain of S. anginosus, when the same MIC value (256 mg/L) was obtained for all tested compounds. The MIC value of C1 and C3 which inhibited 50% of S. oralis, S. mitis and S. sanguinis isolates was 32 mg/L, while the MIC90 value of C4 and C5 was 128 mg/L. For C2, the median value of MIC was 16 mg/L against S. sanguinis and 32 mg/L against both S. oralis and S. mitis. The MIC/MBC ratios were less or equal to 4 in all cases.

Conclusion: Acylthiosemicarbazide (C1) and S-alkylated 1,2,4-triazole derivative (C2) showed a better growth inhibition action compared to thiazole (C4) and oxadiazole (C5) derivatives. The highest degree of antibacterial activity was found in the case of 1,2,4-triazole-3(4H)-thione (C2), which might be subjected to other chemical reactions in order to further improve its antimicrobial action. This work was supported by CNCSIS – UEFISCUG, project number 1136/12.01.2009 PNII – IDEI code 2625/2008.

Antimicrobial activity of solithromycin (CEM-101), a novel fluoroketolide, tested against isolates collected in Europe during 2010 surveillance

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Objective: To evaluate the potency and spectrum of solithromycin (SOL), a novel fluoroketolide, against a contemporary (2010) collection of European (EU) pathogens associated with community-acquired bacterial pneumonia (CABP) and acute bacterial skin and skin structure infections (ABSSSI), compared to erythromycin (ERY), azithromycin (AZI), clarithromycin (CLI) and telithromycin (TEL).

Methods: 6,378 isolates collected from 41 medical centers (18 EU countries) in 2010 were included. Species/group (number of isolates) were: Staphylococcus aureus (SA; 2,539), coagulase-negative staphylococci (CoNS; 610), enterococci (ENT; 934), Streptococcus pneumoniae (SPN; 764), viridans group streptococci (VGS; 274), β-haemolytic streptococci (BHS; 762), Haemophilus influenzae (HI; 326) and Moraxella catarrhalis (MCAT; 169). Consecutive isolates were susceptible (S) tested by CLSI broth microdilution methods and results were interpreted by CLSI and EUCAST breakpoints.

Results: SOL was eight-fold more active (MIC90, 0.25 mg/L) against SA compared to TEL (MIC90, 2 mg/L) with off-scale MIC90 values found for ERY (>4 mg/L) and CLI (>2 mg/L). SOL (MIC90, 0.03/≤0.5 mg/L) had a comparable activity to TEL against CoNS (74.3% S). SOL was only moderately active against ENT (MIC50/50, 0.5/2 mg/L), but was two-fold more potent than TEL (MIC50/50, 1/4 mg/L). SOL demonstrated greater potency against E. faecalis (EF) (MIC90, 0.06 mg/L) compared to E. faecium (EFM; MIC90, 1 mg/L). SOL was very active against SPN (MIC90, ≤0.03 mg/L), VGS and BHS (MIC90, both ≤0.03 mg/L) with 100.0% of all streptococcal isolates inhibited at <0.5 mg/L. The SPN isolates were only 72.8, 75.1 and 82.5% S to penicillin (PEN), ERY and CLI, respectively. SOL was very active against MCAT (MIC90, 0.06/≤0.5 mg/L) with lower activity against both β-lactamase-positive and -negative HI isolates (MIC90, 2 mg/L). SOL activity against HI was four-fold more active than ERY against MCAT. The EU collection sampled had 27.1% MRSA, 71.8% MR-CoNS, 1.2% vancomycin-resistant (VR)-EF, 18.1% VR-EM, 22.3% PEN non-S VGS and 15.3% of HI were β-lactamase-positive.

Conclusions: SOL clearly exhibited greater potency than currently available macrolide agents, CLI and TEL. Against contemporary (2010) EU pathogens commonly isolated in CABP or ABSSSI, this data supports clinical trial investigations of SOL for the treatment of these infections.

Activity of JNJ-Q2, a new fluoroquinolone, tested against contemporary (2010) European pathogens isolated from patients with community-acquired bacterial pneumonia

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Objectives: To determine the activity of JNJ-Q2 tested against contemporary (2010) European isolates of the most common bacterial species isolated from patients with community-acquired bacterial pneumonia (CABP). JNJ-Q2 is a broad-spectrum bactericidal fluoroquinolone (FQ) with potent activity against Gram-positive and -negative pathogens,
including methicillin-resistant *Staphylococcus aureus* (MRSA), and is in early clinical development for the treatment of CABP and acute bacterial skin and skin structure infection.

**Methods:** A total of 983 respiratory pathogens were collected from patients with CABP in 22 medical centres in 11 countries (including Turkey and Israel). Species (number of isolates tested) were: *Streptococcus pneumoniae* (SPN, 543), *Haemophilus influenzae* (HI, 308), and *Moraxella catarrhalis* (MC, 132). Isolates were tested for susceptibilities by CLSI broth microdilution methods (M07-A8). Susceptibility rates for comparator agents were determined using EUCAST breakpoints.

**Results:** The table shows the cumulative percentage MIC frequency against each species tested. JNJ-Q2 was highly active against all three species inhibiting >95% of all 983 isolates at a JNJ-Q2 MIC of ≤0.015 mg/L. Against SPN, resistances to penicillin, erythromycin, ciprofloxacin (CIP), levofloxacin (LEV) and MOX were 6.1, 27.4, 3.7, 1.1 and 1.1%, respectively, with JNJ-Q2 (MIC<sub>90</sub> = 0.015/0.015 mg/L) demonstrating at least four-fold higher activity compared to MOX (MIC<sub>90</sub> = 0.12/0.25 mg/L) and 32-fold higher activity than LEV (MIC<sub>90</sub> = 1.2 mg/L). 14.9% of HI were resistant to ampicillin. JNJ-Q2 (MIC<sub>90</sub> = 0.004/0.015 mg/L) was at least four-fold more active than MOX (MIC<sub>90</sub> = 0.015/0.03 mg/L) against HI. JNJ-Q2 (MIC<sub>90</sub> = 0.015/0.015 mg/L) was also four-fold more active than MOX (MIC<sub>90</sub> = 0.06/0.06 mg/L) against MC.

**Conclusions:** JNJ-Q2 demonstrated very potent activity against this collection of three common respiratory bacterial pathogens isolated from patients with CABP in European hospitals during 2010, and historically also covers MRSA. JNJ-Q2 also demonstrated four-fold or greater activity compared to CIP, LEV and MOX against all three species, including against strains resistant to these fluoroquinolone antimicrobial agents. These JNJ-Q2 in vitro results were very promising and support further clinical development of this new FQ for treatment of ABSSSI, including cases caused by MRSA.

**P1139** Activity of finafloxacin, a fluoroquinolone with enhanced activity at acid pH, against intracellular *Legionella pneumophila*: comparison with azithromycin, clarithromycin, telithromycin, ciprofloxacin and moxifloxacin

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**Background:** LP invades macrophages and multiplies in moderately acidic vacuoles (Sturgill-Koszycki & Swanson, J Exp Med 2000, 192:1261–72). Macrolides and quinolones are commonly recommended treatment for LP infections. In this context, our aim was to examine the activity FNX (a novel quinolone exhibiting increased activity under acidic conditions [Higgins et al., AAC 2010, 54:1613–5]) against intracellular LP.

**Methods:** *L. pneumophila* ATCC 33153 and THP-1 cells were used. MICs were measured in α-ketoglutarate buffered yeast extract pH 6.9). Infection of THP-1 cells was performed as described earlier (Lemaire et al. AAC 2009, 53:3734–3743). Briefly, cells were allowed to phagocytize bacteria at a 10:1 bacteria per cell ratio (2h). Non-phagocytized bacteria were eliminated by incubation in Phosphate Buffer Saline (PBS) supplemented with 50mg/L gentamicin (1h, MIC: 0.25 mg/L) and 4 successive washings with PBS. Infected cells were then transferred in fresh culture medium containing FNX and comparators covering a wide range of concentrations to obtain full concentration-dependent effects. After 48h, cells were harvested, washed with PBS, lysed and used for enumeration of CFUs and assay of cell protein. Data were used to determine the apparent static concentration (Cs) of each antibiotic and its activity (change in log cfu) at a concentration corresponding to the reported human Cmax.

**Results:** Cs and change in log cfu at Cmax are shown in the Table. Except for AZM, all antibiotics achieved a static effect at concentration similar to (CIP, MXF) or slightly above their MIC (CLR, TEL and FNX). At Cmax, AZM was unable to control intracellular bacterial growth, CLR and TEL were modestly and equally effective, whereas quinolones showed a clear ranking of efficacy (CIP < MXF < FNX), with FNX yielding an close to bactericidal effect (defined by a 3 log cfu decrease).

**S032** 21st ECCMID/27th ICC, Posters

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**S034** Antimicrobial activity of JNJ-Q2, a new fluoroquinolone, tested against contemporary (2010) European pathogens isolated from patients with acute bacterial skin and skin-structure infections

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**Objectives:** To determine the activity of JNJ-Q2 tested against contemporary (2010) European isolates of the most common bacterial species/isolates from patients with acute bacterial skin and skin-structure infections (ABSSSI). JNJ-Q2 is a broad-spectrum bactericidal fluoroquinolone (FQ) with potent activity against Gram-positive and-negative pathogens, including methicillin-resistant (MR) *Staphylococcus aureus* (SA), and is in early clinical development for the treatment of ABSSSI and community-acquired bacterial pneumonia.

**Methods:** A total of 750 pathogens were collected from patients with ABSSSI in 25 medical centres in 12 European countries (including Turkey and Israel) in 2010. Species/organism group (number of isolates tested) were: SA (635) and community-acquired *Staphylococcus aureus* including methicillin-resistant (MRSA), and is in early clinical development for the treatment of CABP and acute bacterial skin and skin structure infection.

**Results:** The table shows the cumulative percentage MIC frequency against each species/groups tested. Against 750 pathogens, JNJ-Q2 (MIC<sub>90</sub> = 0.008/0.25 mg/L) inhibited all isolates at a MIC ≤2 mg/L. Although activity was lower against MRSA (MIC<sub>90</sub> = 0.25 mg/L) compared to methicillin-susceptible (MS) SA (MIC<sub>90</sub> = 0.008 mg/L). 98.1% of MRSA were inhibited at a JNJ-Q2 MIC value of ≤0.5 mg/L. Against SA, JNJ-Q2 is four- to 16-fold more active than moxifloxacin (MOX; MIC<sub>90</sub> = 4/8 mg/L) and at least 32-fold more active than levofloxacin (LEV; MIC<sub>90</sub> = 8/16 mg/L) and ciprofloxacin (CIP; MIC<sub>90</sub> = 16/32 mg/L). JNJ-Q2 demonstrated excellent activity (MIC<sub>90</sub> = 0.008/0.015 mg/L) against BHS, inhibiting 100.0% of isolates at a MIC of ≤0.03 mg/L. JNJ-Q2 was 16-fold more active than MOX (MIC<sub>90</sub> = 0.12/0.25 mg/L) and 64-fold more active than CIP (MIC<sub>90</sub> = 0.5/1.0 mg/L) against BHS.

**Conclusions:** JNJ-Q2 demonstrated very potent activity against this collection of common bacterial pathogens isolated from patients with ABSSSI in European medical centers year 2010. JNJ-Q2 also exhibited four-fold or greater activity compared to CIP, LEV and MOX against these isolates. The JNJ-Q2 in vitro results were very promising and support further clinical development of this new FQ for treatment of ABSSSI, including cases caused by MRSA.
Conclusions: Compared to macrolides, quinolones appear more effective against intracellular Lp. Amongst them, FNX shows the greatest activity at clinically-relevant concentrations, perhaps in relation to its improved activity at acidic pH.

P1140 The activity of ozenoxacin (GF-001001–00) against atypical bacteria
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Objective: To determine the in vitro activity of Ozenoxacin (OZN), a novel non-fluorinated quinolone with no halogen substituent at 8-position currently in phase III clinical trials, against atypical bacteria.

Methods: MIC was determined for OZN and levofloxacin (LFX) against Chlamydia pneumoniae or Chlamydia trachomatis (McCoy cell culture line in a supplemented minimal growth medium), Mycoplasma spp or Ureaplasma urealyticum (broth microdilution in Mycoplasma Broth Base) and Legionella pneumophila (agar dilution in supplemented bacteriological agar). OZN and rifampicin (RIF) were tested against Mycobacterium tuberculosis (CLSI agar dilution).

Results: Summary MIC data are shown in the Table. OZN was more active than LFX against all atypical pathogens. OZN and RIF showed similar activity against M. tuberculosis. These results support the continued development of OZN as a new antibacterial agent.

| Pathogen (ID) | OZN (MIC) (μg/mL) | LFX (MIC) (μg/mL) |
|--------------|------------------|-----------------|
| C. pneumoniae (5) | 0.003 - 0.012 | 0.012 - 0.05 |
| C. trachomatis (14) | 0.006 - 0.012 | 0.012 - 0.05 |
| M. avium (22) | 0.003 - 0.012 | 0.012 - 0.05 |
| M. intracellulare (29) | 0.012 - 0.125 | 0.012 - 0.05 |
| U. urealyticum (12) | 0.012 - 0.125 | 0.012 - 0.05 |

P1141 In vitro activity of omadacycline (PTK796) in broth plus lung surfactant or human serum
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Objective: To determine the effects of lung surfactant and human serum on the in vitro activity of omadacycline (OMC).

Methods: MICs were performed in three systems. Cation-adjusted Mueller Hinton broth was used according to standard CLSI microdilution procedures, in addition to broth enriched with 1% bovine surfactant, and 25%-50% human serum. The strains included were clinical Gram-positive, S. aureus, S. pneumoniae, also Gram-negative, E. coli and H. influenzae, selected to include a range of susceptibilities, and appropriate ATCC controls. Doxycycline (DOXY) and daptomycin (DAPTO) were tested as comparators.

Results: MICs ranges in all three systems are shown in Table 1.

| Range MIC (μg/mL) | Organism (ID) | Condition | OMC | DOXY | DAPTO |
|------------------|---------------|-----------|------|------|-------|
| S. aureus (6) | broth | 0.125-0.25 | 0.006-0.125 | 0.125 |
| | surfactant | 0.125-0.25 | <0.006-0.125 | 0.25 |
| | serum (50%) | 0.125-0.25 | 0.006-0.125 | 0.25 |
| S. pneumoniae (3) | broth | 0.003-0.06 | 0.003-0.06 | 0.006-0.125 |
| | serum (50%) | 0.003-0.06 | 0.0003-0.06 | 0.006-0.125 |
| E. coli (6) | broth | 0.5-1 | 0.5-1 | 0.5-1 |
| | serum (100%) | 0.5-1 | 0.5-1 | 0.5-1 |
| H. influenzae (6) | broth | 0.5-1 | 0.25-1 | 0.5-1 |
| | serum (100%) | 0.5-1 | 0.25-1 | 0.5-1 |

Conclusions: MICs of omadacycline did not increase with the addition of surfactant or serum for either Gram positive or Gram negative organisms. The in vitro activity of DAPTO was markedly affected by surfactant, as well as serum reflecting its high protein binding character. DOXY activity was not affected by surfactant but exhibited some decreases in activity in the presence of serum. The inhibition of activity of daptomycin by surfactant has been suggested as the explanation for its lack of efficacy in pneumonia. (Silverman, JA et al., JID 191:2149–2152. 2005). In contrast, OMC was not affected by surfactant. This finding supports the potential use of OMC in treating pneumonia caused by susceptible bacteria.

P1142 In vitro activity of TP-2758 against panels of recent bacterial clinical isolates
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Background: TP-2758 is a novel, fully-synthetic IV/oral antibiotic based on the proven tetracycline core. TP-2758 was selected from more than 2000 analogs on the basis of its potent antibacterial activity, especially against multidrug-resistant (MDR) Gram-negative bacteria.

Methods: Using standard CLSI methodology, TP-2758 and clinical comparators were tested against recent clinical isolates. In vitro bactericidal activity over 24 hours was determined using standard time-kill assays and vigorous aeration.

Results: MICs were tested in Table. TP-2758 was bactericidal against some, but not all Gram-negative isolates, including Enterobacteriaceae producing extended-spectrum β-lactamases (ESBL+), Acinetobacter baumannii, and Proteus mirabilis. TP-2758 also had good antimicrobial potency against MDR Gram-positive pathogens, with representative MIC ranges of 0.5, 0.12, 2, and 0.12 mcg/mL for MRSA (n = 105), S. aureus (n = 50), Enterococcus faecalis (n = 59), Enterococcus faecium (n = 15), and Streptococcus pneumoniae (n = 111).

Conclusion: TP-2758 is 2- to 4-fold more potent than tigecycline against Gram-negative pathogens and 2- to 32-fold less active against Gram-positive pathogens. This novel tetracycline strongly targets MDR Gram-negative bacteria and its potential as a unique IV/oral therapy for treatment of ESBL-producing and carbapenem-resistant Enterobacteriaceae is being investigated.

P1143 E-101, a novel first-in-class topical anti-infective, has potent activity against clinical isolates of important pathogens in Europe collected from 2008–2010
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Objectives: E-101 is a novel topical antimicrobial developed in Europe for the prevention of surgical site infections (SSI). Its unique mechanism of action utilizes myeloperoxidase (MPO) to produce reactive oxygen species that kill bacteria locally. As with any new agent, it is important to understand its current activity profile and to monitor for changes in that profile that may indicate the emergence of resistance. This study reports the in vitro activity of E-101 against European isolates of SSI pathogens alongside key comparators (currently utilized agents and important phenotypic markers).
Methods: 260 non-duplicate clinical isolates of S. aureus, coagulase-negative staphylococci (CoNS), enterococci, enterics (E. coli, K. pneumoniae, P. mirabilis, and E. cloacae), and P. aeruginosa collected from 27 sites across 8 countries in Europe were evaluated. E-101 was evaluated using a modified broth microdilution method based on CLSI guidelines (M7) in which MPO was serially diluted and inocula were delivered in enzyme substrate. E-101 MICs represent mg/L of MPO. Comparator agents were evaluated in accordance with CLSI M7 and M100.

Results: E-101 had an MIC50 and MIC90 of 0.015 mg/L against S. aureus and CoNS. Among staphylococci, 18% of S. aureus and 77% of CoNS were methicillin resistant, while 100% were susceptible to vancomycin, linezolid, and daptomycin. E-101 had an MIC90/MIC90 of 0.06/0.12 mg/L against E. faecium (13% vancomycin resistant) and 0.25/0.25 mg/L against E. faecalis (no vancomycin resistant). Against enterics, E-101 had similar activity by MIC90/MIC90 (mg/L) across evaluated species (E. coli: 0.06/0.06, K. pneumoniae: 0.12/0.12, P. mirabilis: 0.03/0.06, E. cloacae: 0.06/0.12). Among enterics, resistance to imipenem was not observed and resistance to ceftazidime varied by species (3% for E. coli and P. mirabilis, 14% for K. pneumoniae, and 20% for E. cloacae). Of the evaluated P. aeruginosa, resistance to most comparators (gentamicin, imipenem, levofloxacin, piperacillin/tazobactam) was 10−20%, and E-101 had an MIC90/MIC90 of 0.03/0.06 mg/L.

Conclusions: Based on the in vitro activity profile, E-101 has potent activity against European clinical isolates of both Gram-positive and Gram-negative pathogens commonly associated with SSI. There is no apparent impact of current resistance common among these species on the activity profile of E-101. These data illustrate the potential of E-101 for the prevention of SSI.

E-101, a novel first-in-class topical anti-infective, maintains a high degree of potency in vitro against problematic resistant clinical pathogens (ESKAPE pathogens)

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Objective: E-101, a topical anti-infective which utilizes myeloperoxidase (MPO) for the generation of reactive oxygen species to kill bacteria, is being developed for the prevention of surgical site infections. Infections caused by antibiotic resistant pathogens have become common and are increasingly difficult to treat. This study evaluates the activity of E-101 against highly resistant “ESKAPE” pathogens (vancomycin resistant E. faecium [VRE], methicillin resistant S. aureus [MRSA]), extended spectrum b-lactamase [ESBL]/carbapenemase producing K. pneumoniae, multi-drug resistant [MDR] A. baumannii and P. aeruginosa, and AmpC cephalosporinase producing E. cloacae).

Methods: E-101 activity was evaluated using a modified broth microdilution method based on CLSI M7. Modifications included serial dilution of enzyme (MPO) and inocula delivery in solution containing enzyme substrate. E-101 MICs represent mg/L of MPO. Comparators (currently marketed agents and phenotypic markers) were tested in accordance with CLSI M7 and M100. 115 non-duplicate clinical isolates were pre-selected based on resistance phenotype for evaluation to include the “ESKAPE” phenotypes noted above.

Results: Against VRE, E-101 had an MIC90/MIC90 of 0.06/0.12 mg/L. Against S. aureus consisting of linezolid resistant isolates, daptomycin non-susceptible isolates, hospital and community acquired MRSA, VISA, and VRSA, E-101 had an MIC90/MIC90 of <0.008/0.015 mg/L with MICs not exceeding 0.06 mg/L. E-101 had an MIC90 and MIC90 of 0.12 mg/L against ESBL E. coli, ESBL K. pneumoniae, and KPC K. pneumoniae, with an MIC90 and MIC90 of 0.06 mg/L against E. cloacae/C. freundii with derepressed AmpC. Against MDR P. aeruginosa, E-101 had an MIC90/MIC90 of 0.03/0.06 mg/L with an MIC90 and MIC90 of 0.03 mg/L against MDR A. baumannii. E-101 activity against this subset of purely resistant isolates was equivalent to that observed for E-101 during recent surveillance where isolates with these phenotypes were infrequently or not encountered.

Conclusions: E-101 was potent in vitro against ESKAPE pathogens, which constitute clinically important pathogens with problematic resistance (multi-drug resistance, emerging resistance to commonly utilized agents). This attribute highlights the utility of E-101 for the treatment of surgical site infections where resistant organisms are likely to be encountered, and potential for the treatment of other superficial infections caused by resistant organisms.

Antibacterial activity of nanosized TiO2 against Escherichia coli

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Objectives: The photocatalytic disinfection property of TiO2 has been in particular interest from the first research work of Matsunaga et al., 1985 to nowadays. The bactericidal action of TiO2 photocatalytic reaction depends on the type and the source of TiO2 and may differ according to the kind of microorganism. The aim of the present study is to evaluate the efficiency of nanosized TiO2 synthesized by nonhydrolytic route against Escherichia coli as representative species of bacteria.

Methods: The synthesis of titanium oxide nanoparticles was carried out following the procedure described by Niederberger et al. (2002) which is based on the nonhydrolytic sol-gel reaction of benzyl alcohol and titanium tetrachloride. The reaction was performed at 80°C under vigorous stirring. The obtained white suspension was centrifuged and the collected material was dried and calcinated at 500°C. The particle size of thus prepared TiO2 (anatase) was about 10−20 nm and it was characterized by XRD, IR and SEM.

Antimicrobial activity measurements were performed by E. coli (ATCC 25922). Serial dilution of the E. coli-containing stock solution with PBS buffer was performed to yield starting concentration of approximately 107 colony forming units (CFU) ml-1. The bacteria growth was examined by the effect of UV light alone, in the presence of TiO2 at dark conditions and in the presence of both – TiO2 and UV radiation. The experiments were continued up to 3 hours at 25°C. The dynamics of antimicrobial action was assessed by killing curves determination.

Results: Our data showed that the antimicrobial activities of TiO2 and UV light alone were very similar – the number of E. coli cells was reduced about 50% for 60 min. In contrast, the combination of TiO2 and UV radiation led to complete killing of bacteria in 30 min. It can be concluded that the removal efficiency due to joint action of TiO2 and UV radiation was markedly increased in comparison to UV radiation or TiO2 alone.

Conclusion: The synthesized TiO2 (anatase) by nonhydrolytic method possesses strong bactericidal activity and could be used effectively for disinfection under UV illumination.

Activity of the novel sulfactam BAL30072, alone and in combination with meropenem, against Enterobacteriaceae harbouring the NDM-1 -b-lactamase

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Objectives: BAL30072 (manufactured by Basilea Pharmaceutica International Ltd.) is a new sulfated monocyclic -b-lactam not hydrolysed by metallo -b-lactamases (MBLs) and with activity against a broad spectrum of Gram-negative pathogens, including multidrug resistant P aeruginosa and A. baumannii. NDM-1 is a new MBL originating in India and recently isolated from pathogens infecting patients in Europe. We investigated the in-vitro activity of BAL30072 alone and in a 1:1 combination with meropenem (MEM) against recent clinical isolates of carbapenem-resistant Enterobacteriaceae harbouring the NDM-1MBL.

Methods: 78 Enterobacteriaceae comprising: E. coli (n=33), K. pneumoniae (n=43), K. oxytoca (n=1) and P. rettgeri (n=1) were collected from hospitalized patients in India during 2009. A sample of strains further characterised contained up to 7 b-lactamases
including TEM, OXA, SHV, DHA-1, CTX-M-15, CMY and NDM-1. MICs of BAL30072, MEM, ceftazidime (CAZ), aztreonam (ATM) and 1:1 combination of BAL30072:MEM were determined by broth microdilution according to CLSI standards. All isolates were screened using PCR for the presence of the NDM-1 gene immediately prior to MIC testing.

Results: The presence of NDM-1 was confirmed in all isolates. Susceptibility (presented as % strain inhibited per concentration) of the 78 NDM-1 harbouring isolates to the test drugs is summarised in the Figure.

Conclusion: BAL30072 alone was active against 60% of the strains at 4 mg/L, while ATM, CAZ and MEM covered <10%. Strains harbouring NDM-1 are known often to express multiple β-lactamases towards some of which BAL30072 may not be stable.

This data strongly suggests that BAL30072 is stable to hydrolysis by NDM-1, but that expression of other β-lactamases contributes to decreased susceptibility to BAL30072 alone. The addition of MEM protects BAL30072 against hydrolysis by these additional enzymes, resulting in >90% of isolates being inhibited at 4 mg/L. BAL30072 offers a potential therapeutic benefit for infections caused by NDM-1-positive Enterobacteriaceae. In addition, the activity BAL30072 is further enhanced in combination with MEM against clinical isolates carrying NDM-1 and multiple other β-lactamases gene loci.

Activity of ACHN-490 against MDR clinical isolates of Klebsiella pneumoniae, Escherichia coli and Enterobacter spp. from Athens, Greece

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Objectives: The in vitro activity of ACHN-490, a next-generation aminoglycoside, was evaluated against a total of 300 multidrug-resistant (MDR) organisms. Isolates [K. pneumoniae (n = 241), E. coli (n = 33) and Enterobacter spp. (n = 26)] were collected from 4 hospitals in Athens, an area where carbapenemase-producing organisms are endemic.

Methods: A total of 242 clinical isolates collected between 2008–2010 and 58 fecal carriage isolates were included in the study. Only one isolate per species per patient was permitted. Clinical isolates were derived primarily from blood (n = 197), while other sources included pus (n = 16), bronchial secretions (n = 5) and urine (n = 24). MIC determinations for most agents were performed with the BD Phoenix automated system. MICs of ACHN-490, tobramycin and fosfomycin were evaluated with agar dilution according to CLSI, whereas MICs of doripenem and tigecycline were determined using E-test, in accordance with the manufacturer’s instructions. All isolates were screened for MBL and KPC production with the BD Phoenix automated system. MICs of ACHN-490, tobramycin and fosfomycin were evaluated with the CLSI confirmatory test with and without carbapenemase inhibitors (EDTA, Boronic acid).

Results: ACHN-490 MICs were <4 mg/L against all 300 MDR isolates with MIC50 and MIC90 of 1 and 2 mg/L, respectively. Most of the isolates were resistant to the legacy aminoglycosides with the MIC50/MIC90 to tobramycin, amikacin and gentamicin being >32/≥32, ≥32/≥32 and ≥4/≥8 mg/L, respectively. No differences were observed in the activity of ACHN-490 against KPC (n = 151), VIM (n = 105), KPC-VIM (n = 15) or ESBL (n = 29) producing isolates or against K. pneumoniae, E. coli or Enterobacter spp. strains. Colistin was active, with 77.7% of the tested isolates displaying MICs ≤2 mg/L, while tigecycline’s MIC50 and MIC90 were 2 and 4 mg/L, respectively. Finally, fosfomycin demonstrated 84.7% susceptibility with an MIC50 of ≤16 and an MIC90 of 128 mg/L.

Conclusions: The novel aminoglycoside ACHN-490 retains activity against all isolates of K. pneumoniae, E. coli and Enterobacter spp tested, including MDR strains which are carbapenemase producers.
The novel broad-spectrum fluorocycline TP-434 is active against MDR Gram-negative pathogens

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Background: TP-434 is a novel broad-spectrum IV/oral fluorocycline antibiotic being developed by Tetraphase Pharmaceuticals. TP-434 has potent activity against multidrug-resistant (MDR) Gram-negative pathogens associated with complicated nosocomial and community infections, including Enterobacteriaceae such as Escherichia coli and Klebsiella spp. expressing extended-spectrum β-lactamases (ESBL) and/or carbapenemases, MDR Acinetobacter baumannii, and anaerobic bacteria such as Bacteroides fragilis.

Methods: Using standard CLSI methodology, TP-434 and clinical isolates from cystic fibrosis patients

Effects of BMAPs on bacterial morphology were also assessed by electron and confocal microscopy.

Results: MIC values showed that BMAP-27 and BMAP-28 exhibited comparable activity against P. aeruginosa (MIC90: 16 and 32 mcg/ml, respectively) and S. maltophilia (MIC90: 8 and 4 mcg/ml, respectively) isolates, while BMAP-28 exhibited higher activity than BMAP-27 against S. aureus (MIC90: 32 and >64 mcg/ml, respectively). Tobramycin resulted to be significantly less active than both BMAPs, regardless of species tested (MIC90: >64 mcg/ml for all species tested). Time-killing assays showed that both BMAPs exhibited a rapid (within 30–60 min) bactericidal activity generally leading to the complete eradication within 120 min. To the contrary, tobramycin showed bacteriostatic effect allowing some re-growth by 24h. BMAP-27 and BMAP-28 at sub-MICs significantly reduced the amount of biofilm formed on polystyrene by CF strains with comparable efficacy, although to a lesser extent than tobramycin (Figure).

Conclusion: Our results suggested that BMAP-27 and BMAP-28 may represent a potential therapeutic option for CF lung infections caused by P. aeruginosa, S. maltophilia, and S. aureus. Further work is warranted to evaluate their in vivo efficacy using a lung-infected animal model. (This work was supported by the Italian CF Research Foundation; grant FFC#12/2009)

**Objective:** Activity of POL7001 against *Pseudomonas aeruginosa* isolates from cystic fibrosis patients

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**Objectives:** POL7001 is a Protein Epitope Mimetic (PEM) antibiotic with potent antimicrobial activity against *Pseudomonas aeruginosa* (PA) (Srinivas et al., Science 2010). Chronic infection with PA is a primary driver of mortality in cystic fibrosis (CF) patients and resistance to multiple antibiotics develops over time. We have tested the activity of POL7001 against PA isolated from time of colonization for up to 16yrs post-colonization without a culture for PA and during regular visits (at least 4 times per year). All had confirmed clonal PA infections and harbored different PA clones. Isolates represent first subcultures of the original isolate obtained from sputum or throat swab. MICs were determined for POL7001, ciprofloxacin (CIP), meropenem (MER), ceftazidime (CAZ), colistin (COL), gentamicin (GEN), tobramycin (TOB), and imipenem (IMP). The multi-drug resistant isolate RP73 was used to set up a murine pneumonia model in order to test the in vivo efficacy of POL7001 in comparison to ciprofloxacin.

**Results:** MICs for POL7001 ranged between 0.015−0.5 mcg/mL with a median of 0.125 mcg/mL for all isolates. Over time, many of the PA isolates from patients became resistant to two or more antibiotics while remaining sensitive to POL7001. One isolate developed resistance to MER, IMP, COL, CAZ, and GEN 12.6 yrs post-colonization without a change in sensitivity to POL7001. There was no difference in activity of POL7001 against mucoid, non-mucoid or hypermutable isolates. The in vivo efficacy test of POL7001 is on-going.
Conclusions: Multiresistant PA isolated from CF patients shows sensitivity to the novel antibiotic POL7001. The development of resistance toward amnoglycoside and cephalosporin correlates with mortality in CF patients. POL7001 or a related PEm antibiotic may be a useful therapy for these patients, especially at later stages of disease.

P1152 The biology of environmental bacteriophages lytic to multidrug-resistant Klebsiella strains
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Objectives: The purpose of this study was to isolate and characterize active bacteriophages against Klebsiella pneumoniae ESBL(+) strains to apply alternative phage therapy in eradication of multidrug resistant strains.

Methods: The newly isolated phages were characterized by their morphology, plaque morphology and host range. All these phages were tested against 168 K. pneumoniae and 54 K. oxytoca multidrug resistant strains by spot-test method. Plaque morphology was determined by plaque assay using double-layer agar overlay technique. The morphological characterization of the bacteriophages was made by transmission electron microscopy (TEM) observations.

Results: In our research 27 Klebsiella lytic phages were isolated from environmental water samples and propagated on 10 ESBL-producing K. pneumoniae strains. Morphologically, they were assigned to three virus families: Myoviridae, Siphoviridae and Podoviridae. Significant differences in plaque morphology were observed. Results showed that phages belonging to the Myoviridae family had high efficacy and relatively broad spectrum of activity within Klebsiella sp. (activity against 20,2% K. pneumoniae strains and 35,2% K. oxytoca strains).

Conclusions: The isolated bacteriophages demonstrated lytic activity to multidrug resistant strains of K. pneumoniae and K. oxytoca. The characterization of this novel phages was helpful to allow the successful application of phages as therapeutic agents against bacterial infectious diseases.

New non-conventional antimicrobials (no drugs)

P1153 In vitro activity of wine compounds (resveratrol, methyl gallate and other phenolic compounds) against Helicobacter pylori clinical isolates
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Objective: The aim of this study was to determine the in vitro activity of different phenolic compounds against H. pylori clinical isolates by a disc diffusion method.

Methods: 28 H. pylori clinical isolates were obtained from gastric biopsies from patients suffering of gastric symptomatology. Biopsies were processed following standard methodology for these bacteria. The in vitro activity of different phenolic compounds was studied by a disc diffusion method: Epicatchin, gallic acid, resveratrol, coumaric acid, ferulic acid, methyl gallate, catechin, quercetin, vanillic acid and kaempferol. Compounds were diluted in ethanol:water (10:90v/v) at 25mM concentration. Ethanol was used in the same conditions to rule out its activity.

Blank disc were impregnated with 10μl of each compound and put in agar Columbia plus 7% sheep blood inoculated with a suspension of a 2 McFarland H. pylori. Plates were incubated for 3 to 5 days at 37°C in a 10% CO2 atmosphere. Inhibition zone around disc were measured.

RESULTS: Resveratrol produced inhibition in all the strains tested with a range of 17 to 35 mm. Kaempferol inhibited 10 strains with 10–23 mm inhibition zone. Epicatchin inhibited 3 strains with a range 12–25 mm, Catechin inhibited 7 strains with inhibition zone 10–20 mm. Quercetin inhibited 7 strains with 10–12 mm inhibition zone. Vanillic acid inhibited one strain with 8 mm inhibition zone.

Number of strains inhibited for each compound are included in the table 1. Gallic acid, coumaric acid and ferulic acid did not inhibit the strains tested. Ethanol produced not inhibition in the strains tested.

Somedifferenceswereobservedaccordingtoresistance to CLA or MTZ. Catechin was more active against CLA-R (6 out of 15, 40%) than CLA-S strains (1 out of 13, 7.7%) and against MTZ-R (4/7, 57,1%) than MTZ-S (3/21, 14,3%) strains. Moreover, quercitin was more active against MTZ-S (6/21, 28,6%) than MTZ-R (1/7, 14,3%) strains and Kaempferol more active against MTZ-S (9/19, 47,4%) than MTZ-R (1/5, 20%) strains.

Conclusions: Two of the phenolic compounds tested in this study (Resveratrol and methyl gallate) showed in vitro activity against all Helicobacter pylori clinical isolates tested by a disc diffusion method.

P1154 Fruit and vegetable extracts as source for anti-adhesion agents against oral bacteria
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Objectives: Anti-adhesion-based therapy of bacterial infections have been recognised as a promising alternative approach as resistance of bacteria to the commercial available antibiotics is constantly on the rise. This approach has been studied on oral bacteria using various food stuff as source to obtain fractions that inhibit bacterial inter-genera or inter-species interactions, also known as coaggragation, and the adhesion of oral bacteria to cells and teeth. In this study we tested extracts and fractions from fruits and vegetables for their ability to inhibit adhesion and coaggregation of oral bacteria.

Methods: Extracts prepared from frozen mushroom, frozen raspberry and fresh chicory were fractionated into low and high molecular mass fractions by ultrafiltration (mushroom and chicory, less and higher than 5000 Da) and by dialysis (raspberry, less and higher than 3500 Da). The extracts and fractions were tested for the ability to inhibit (i) coaggregation between pairs of Faunobacterium nucleatum (Fn) Streptococcus mutans (Sm) and Fn/Neisseria subflava; (ii) adhesion to and detachment from hydroxyapatite (HA) beads of Sm, Lactobacillus casei (Lc) and Streptococcus sanguinis; (iii) adherence of Actinomyces naeslundii (An) and Preotella intermedia (Pi) to cultured KB cells; (iv) biofilm formation by Sm, Lc, An and Pi.

Results: The mushroom extract inhibited the coaggregation of the test pairs of bacteria from 4+ score in the absence of extract to 0 score in the presence of 33% (V/V) extract diluted with saline buffer (pH 7.2), whereas the raspberry and chicory extracts were without effect. All

New non-conventional antimicrobials (no drugs)
extracts inhibited biofilm formation, reduced adhesion to HA and caused
detachment from the beads; raspberry and chicory, but not mushroom,
also inhibited adherence to KB cells. The coaggregation inhibition
activities were retained in the LMM fraction of the mushroom extract.
Although all fractions displayed some effect in inhibiting adherence
to HA and KB cells and biofilm formation, the LMM fractions were
generally the most effective.

Conclusion: The data taken together suggest that LMM fractions from
the tested extracts may be a useful source to identify specific anti
adhesion agent(s) to improve oral health care.

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[P1155] Evaluation of the probiotic potential of Weissella confusa
and Lactobacillus paracasei strains isolated from Nigerian
ditional dairy fermented foods and cow’s intestines

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Objective: Infectious diseases are still major causes of death in
developing countries especially Africa. Current chemotherapy rely
almost exclusively on the use of antibiotics, however this is becoming
ineffective due to global problem of antibiotic resistance. Hence the
need for alternative chemotherapy through the use of viable Lactic Acid
Bacteria (LAB) as probiotics. This study was carried out to characterize
the probiotic potential of selected LAB strains isolated in Nigeria from
traditional fermented dairy foods and cow’s intestines samples in order
to select strains for probiotic use in the gastrointestinal and urogenital
tracts that will lessen possible infections in the tracts.

Method: Antimicrobial potentials of 24 LAB had been previously
reported. Two Lactobacillus paracasei and three Weissella confusa
strains which have been previously selected from the 24 LAB due to their
high antimicrobial activities against uropathogens and enteropathogens
were used in this study. The survival of selected LAB and a probiotic
reference strain (Lactobacillus rhamnosus GG) in chemically simulated
human gastro-intestinal tract conditions, adherence to human intestinal
epithelial cell-lines (Caco-2, HT-29 and HT-29-MTX) and adherence to
epithelial vaginal cell line (HeLa) were all evaluated through counts of
surviving cells.

Result: Resistance of the selected LAB strains to simulated gastron-
testinal passage was satisfactory and better than that of the reference
L. rhamnosus GG strain. The highest survival after passage through
gastrointestinal conditions was observed with W. confusa UI021 (45.0%).
Lactobacillus paracasei UI014 and W. confusa UI007 strains adhered
better to HT-29-MTX cell line than the reference L. rhamnosus GG strain
while the two L. paracasei strains adhere better to HeLa vagina epithelial
cell line than the reference Lactobacillus rhamnosus GG. strain.

Conclusion: In general, the studied strains showed good survival in the
gastrointestinal transit and acceptable adhesion capability to intestinal
and vaginal epithelia that is comparable and better in some strains to the
tested reference probiotic strain. The strains are good candidates for
further studying of potential benefits that support their use as probiotics
in the gastrointestinal and urogenital tract. This is one of the few studies
reporting the characterization and the probiotic potential of Weissella
species.

[P1156] Antimicrobial effect of methanolic extract of ginger on
Helicobacter pylori

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Objective: Helicobacter pylori, a Gram negative microaerophilic bacterium, infection is now recognised as a worldwide problem. The
-growing problem of antibiotic resistance by the organism demands the
search for novel herbal compounds.

The present study is designed for evaluating the antimicrobial effect of methanol extract of this remedy on clinical isolates of H. pylori in
Kashan to identify potential sources of cheap staving materials for the
synthesis of new and more effective drugs.

Method: This study was taken from 20 Helicobacter pylori samples
isolated from patients with gastrentestinal disorders. The samples were
cultured on Columbia agar base plates (Merck) with supplements. Plates
were incubated at 37ºC for 3–5 days in a microaerophilic environment
(using anaerocult C, merck). The isolates that grown on plates were
identified by bacteriological tests.

The microdilution broth method was used to determine the susceptibility
of methanol extract of ginger on 20 isolates of H. pylori with a serial
dilution of 1000, 500, 250, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78 µg/ml.

Results: The MIC of extract ranged from 3.125–25 µg/ml for H. pylori,
the MIC of 12 (60%) isolates were 3.125 µg/g/m, 4 isolates (20%)
were 12.5 µg/ml, 3 isolates (15%) were 6.25 µg/ml, 1 isolate (5%) was
25 µg/ml.

Conclusion: The methanolic extract of ginger may contain compounds
with therapeutic activity, and the most effective MIC (60%) against
H. pylori were 3.125µg/ml. We also understand that H. pylori has a
high sensitivity against ginger.

[P1157] Photodynamic inactivation effect of methylene blue on
multidrug-resistant bacteria isolated from chronic diabetic
foot ulcers

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Objective: Photodynamic inactivation (PDI) has been investigated
to cope with the increasing incidence of multidrug-resistant (MDR)
pathogens. PDI employs nontoxic photosensitizers (PSs), which localize
in the microbial cells and are activated by a specific wavelength of visible
light. The excited-state PS reacts with in situ molecular oxygen and
transfers its energy to generate reactive oxygen species (ROS), such as
singlet oxygen and superoxide anion. These ROS can cause damage
to cell walls, proteins, nucleic acids, and membrane lipids, eventually
causing cell death.

Methylene blue (MB) is a widely known histological dye and belongs to
the phenothiazinium class of compounds. The main purpose of this study
was to explore the PDI effect of MB on multidrug-resistant Escherichia
coli and methicillin-resistant Staphylococcus aureus (MRSA) compared
to Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC
25923).

Methods: Effect of MB concentration (12.5, 25, 50 µg/ml) and laser
light dose (54.6, 109.2, 163.8 J/cm²) on lethal photosensitization was
investigated. The conditions tested were: 1) controls which contained
neither MB nor received irradiation, 2) incubation with MB in the dark,
3) irradiation in the absence of MB and 4) the test which was irradiated
in the presence of MB. To enumerate the surviving bacteria, serial 10-
fold dilutions were plated on nutrient agar.

Results: S. aureus (ATCC 25923) and clinical MRSA isolate were
susceptible to killing by photodynamic inactivation. With 50µg/ml MB
at 163.8 J/cm², 3 log killing was obtained for S. aureus (ATCC 25923)
and 2 log killing was obtained for MRSA (initial concentration 10⁴–10⁵
CFU/ml). Methylene blue (50 µg/ml) photosensitization using red laser
light (163.8 J/cm²) was able to achieve reductions of 53.1% and 37.6% in
the viable counts of Escherichia coli (ATCC 25922) and clinical isolate
of multidrug-resistant Escherichia coli (using starting concentrations
of 10⁴–10⁵ CFU/ml). The bactericidal effect was not dependent on the
concentration of MB; but it was dependent on the light dose. Under
the same MB concentration and light dose, ATCC reference strains
were consistently killed more when compared with the clinical isolates.

Conclusion: In our study, the selected MDR isolates were more resistant
to PDI-mediated killing than their ATCC reference strains. Therefore,
the PDI efficacy of MB may be affected by the antibiotic resistance
mechanisms that presented in MDR isolates.
Sterilisation efficacy of atmospheric plasma sources for biomedical applications

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Objectives: Gaseous non thermal plasma (NTP) has unique characteristics because it contains numerous biochemically active agents like UV photons, OH radicals, O atoms, etc. A distinguishing property of NTP is that all the foregoing agents mentioned can be generated in gas or liquid without heating, close to ambient temperatures.

Aim of work: To evaluate the efficacy of Gaseous non thermal plasma (NTP) on different microorganisms (vegetative cells, spores, fungi, biofilms).

Material and Methods: Light microscope (LM) and transmission electron microscope (TEM) were used to visually inspect the impact of plasma exposure on E. coli as a Gram-negative bacterium, Staphylococcus aureus as a Gram positive bacteria, Aspergillus-niger as a fungus, bacillus stearohermophilus immobilized spores on paper strip (biological indicator) and biofilm urinary catheter bacteria.

Results: Time dependant study allows us to distinguish between bacteriostatic and bacteriocidal action of NTP. Visually when the exposed colonies were used as source of new culture, showed nearly evacuated colonies. By inverted light microscope treated cells showed morphologically change in diameter and destruction in relation to time. Using transmission electron microscope (TEM) in observation of treated vegetative cells showed that cell wall remains but its central dissolved and after longer exposure total cell fragmentation and leakage occurred. As regard immobilized Bacillus stearohermophilus spores showed erosion of various spore layers. Also NTP were evaluated quantitatively, number colony forming represented by Log-vertical scale and versus time represented by Log-horizontal scale were plotted in semi-log scale. This revealed multislope or 3 phases survivor curve for vegetative cells and spores. Gram negative bacteria showed higher resistance against plasma treatment in comparison to Gram positive bacteria. Also spores are more resistant as to reach complete sterility, in which no growth after 7 days incubation needs 10 minutes exposure and addition of oxygen in plasma forming gas. NTP destroy biofilm by lower exposure time (hundreds of seconds).

Conclusion: Gaseous non thermal plasma (NTP) can destroy microorganisms but with different time of exposure and addition of oxygen.

New targets for new antimicrobials

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Objectives: Quorum sensing (QS) is a communication system mediated by chemical signals which depends on the population density of microbes. It has great significance in the formation of biofilms, the production of virulence factors, the development of intrinsic antibiotic resistance, the facilitation of growth in a competitive environment and the enhancement of motility. Antimicrobial effects of 30 trifluoromethyl ketones (TFs) have already been studied on various bacterial species. Some of TFs inhibited the growth and the motility of various Gram-positive and Gram-negative bacteria. Our aim was to study the quorum sensing inhibitory effect of 12 TF molecules.

Methods: The quorum sensing inhibitor activity of the twelve TF compounds were investigated in disc diffusion method with Chromobacterium violaceum 026 sensor strain. This strain produce a purple dust pigment only in the presence of N-acyl homoserine lactone (AHL) signal molecules. We used the EZF 10−17 AHL producer strain (a grapevine tumor isolate) to prove the signal molecules for the system, or we added the AHL-s externally. The quorum sensing inhibitor activity of the TF compounds was evaluated in a bioassay as a reduction of the diameter and the intensity of pigment production.

Results: The antibacterial effects of twelve TFs (1−12) were investigated at first. It was found that compounds 1, 2, 3 and 9 had remarkable antibacterial effect on the sensor strain at 20 μg/filter paper discs. Three TFs 2, 3 and 9 had antibacterial effect on the AHL producer strain. The QS inhibition was detected in case of six TFs 1, 2, 3, 4, 5 and 9 below
Activity of PF-1 and PF-2, novel siderophore conjugated β-lactams, tested against Enterobacteriaceae strains carrying emerging resistance mechanisms

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Objectives: To evaluate the activity of two novel conjugated siderophore β-lactam agents (PF-1 and PF-2) and comparator agents against Enterobacteriaceae (ENT) producing emerging β-lactamases, including CTX-M, NDM-1, KPC and OXA-48/-181 or carrying known plasmid-borne fluoroquinolone resistance (PMQR) genes, qnr and aac(6’)-Iib-cr.

Methods: PF-1 and -2 (with and without the iron chelator, 2,2’-dipyridyl) and comparators were tested for susceptibility using CLSI broth microdilution methods against 111 clinical strains of ENT (13 species [including 53 K. pneumoniae, 27 E. coli, 10 E. cloacae among others]) producing CTX-M-14 (12 strains), CTX-M-15 (12), KPC-2 to -4 (24), NDM-1 (12), OXA-48/181 (19), qnr (20), aac(6’)-Iib-cr (3). Isolates were collected from 2001–2009 from global surveillance programs.

Resistance mechanisms were identified by PCR and sequencing.

Results: PF-1 and -2 displayed potent activity against all groups tested (MIC50 results, ≤0.25 mg/L). Overall, in the presence of 2,2’-dipyridyl (a siderophore chelator; Table) MIC results were either identical or slightly lower (two- to four-fold) when compared to the compounds tested alone. PF-1 and -2 were the most active antimicrobial agent against NDM-1-producing strains, which were highly resistant to nearly all compounds tested except for tigecycline and polymyxin B (MIC50, 0.5 and ≤0.5 mg/L, respectively). Against KPC-producing strains, PF-1 and -2 MIC50 results (0.25 and 0.12 mg/L) were the lowest among all agents tested. Enterobacteriaceae strains producing CTX-M enzymes displayed the lowest MIC50 results for PF-1 and -2 (≤0.06 to <0.03 μg/mL) among all groups. PF-1 and -2 also demonstrated potent activity against all OXA-48/-181 strains and most PMQR strains tested. Among all comparator agents, tigecycline was the only antimicrobial agent demonstrating 100% susceptibility rates against these tested strains.

Conclusions: Overall, PF-1 and -2 inhibited >90% of all tested strains at ≤2 mg/L. PF-2 seems to be slightly more active compared to PF-1 against these selected resistant strains. The presence of 2,2’-dipyridyl did not appreciably impact MIC results. Both PF-1 and -2 retained activity (MIC50, ≤0.03 to 0.25 mg/L) against strains carrying resistance mechanisms (including NDM-1) that are rapidly disseminating among contemporary Gram-negative enteric bacilli.

P1162 Antimicrobial activity of a novel programme of protein synthesis inhibitors against clinical isolates containing KPC and NDM-1 carbapenemases

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Objectives: Multidrug-resistant Gram-negative bacteria are an increasing risk to public health. In these organisms, mobile genetic elements confer resistance to most antimicrobial agents currently used in the clinic. Of particular concern are those species of enteric pathogens containing extended spectrum β-lactamases and carbapenemases such as KPC and NDM-1. Clinical isolates with such resistance elements have emerged as a world-wide threat with few therapeutic options remaining for the infectious disease clinician. We tested representatives from three novel classes of antibiotics for activity against a number of these highly resistant Gram-negative organisms. These compounds inhibit protein synthesis and bind to the 50S ribosomal subunit, a molecular target not accessed by current drugs used to treat Gram-negative infections.

Methods: Clinical isolates were obtained from various laboratories and commercial repositories to include the Louis Stokes Cleveland Department of Veterans Affairs, Massachusetts General Hospital, Eurofins Medinet Inc., and the American Type Culture Collection. Susceptibility testing was performed according to CLSI broth microdilution methods (M07-A8 and M100-S20-U).

Results: Lead molecules from three unique scaffolds in this program were tested against contemporary clinical isolates of E. coli isolated from patients with urinary tract and wound infections, many having ESBL, AmpC, and quinoline resistance mechanisms. The compounds were highly potent, inhibiting growth of these organisms at concentrations in the range of 0.25 to 8 mg/L. Similar results were obtained against urine and respiratory isolates of K. pneumoniae with ESBL and KPC resistance mechanisms, MICs ranging from 0.125–4 mg/L. These same compounds, when tested against NDM-1 strains of K. pneumoniae and E. cloacae susceptible only to colistin and polymyxin B, gave MIC values ranging from 2–8 mg/L.

Conclusion: These protein synthesis inhibitors represent novel families of antimicrobial agents with promising in vitro activity against multidrug resistant Gram-negative pathogens, including isolates expressing the KPC and metallo-β-lactamases.

P1163 Interactome for essential genes as a prediction of antibacterial potency

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Phosphorodiimide morpholino oligomers (PMOs) are short synthetic DNA mimics that inhibit gene expression by binding specifically to mRNA with complementary base sequences. Previously, we synthesized PMOs targeted to essential genes of bacteria, and found that these PMOs can be potent bactericidal compounds. However, MIC values for PMOs can vary from 100 nM to over 256 μM indicating that the process of selecting the most potent targets from among the hundreds of essential genes remains empirical.

Objective: Studies were designed to evaluate the role of the interactome as a theoretical basis for selecting gene targets that would be most susceptible to inhibition by PMOs. Proteomics and high-throughput screening methods have shown that proteins tend to associate with other proteins that function in common pathways. Higher levels of organization called interactomes are formed when complexes of proteins link together to form networks that integrate diverse cellular processes. We hypothesized that the most susceptible targets for PMO antibacterials would be those whose gene product interacted with a high number of other proteins.

Methods: To test this hypothesis, we screened an interactome database (Bacteriome.org, http://www.compsybio.org/bacteriome/) for all 302 essential genes of Escherichia coli, as defined in “Profiling of the E. coli chromosome” (http://www.shigen.nig.ac.jp/ecoli/pec/index.jsp). Eighteen essential genes with core interactome scores above 24 were
selected for targeting, and PMOs were synthesized for each. The minimal inhibitory concentration (MIC) of each PMO was measured using the standard clinical method (Clinical Laboratory Standards Institute).

Results: The results show that there was no correlation between core interactome score and MIC in this study. However, further analyses using an extended experimental interactome score showed significant negative correlation.

Conclusions: We tested the hypothesis that interfering with protein-protein interactions, which establish the biological organization of cells as a network of stable protein-protein interactions, would correlate with the potency of antibacterial agents. There was a significant negative correlation between increased number of protein-protein interactions and MIC in this study.

**P1164** Inhibiting the activity of the DNA gyrase from *E. coli*

X. Vila-Farres*, J. Sanchez-Cespedes, E. Giralt, J. Vila (Barcelona, ES)

Objectives: The DNA gyrase is known to be one of the targets for quinolones. Mutants conferring resistance to quinolones have been detected, especially, in the QRDR (Quinolone Resistance Determining Region) region of the enzyme. These mutations in the amino acid codon ser83 and asp87 are related to the interaction between the enzyme and the quinolone. The objective of this work was to find peptides with inhibitory activity against DNA gyrase, that could overcome the resistance problem and provide an alternative to quinolones.

Methods: To find peptides with activity against the DNA gyrase four peptides were synthesized. The sequence of each peptide mimics the original sequence of the DNA gyrase from the residue 75 to the 130. Peptide (A) was the original sequence of the enzyme, while in the other peptides some mutations were introduced in the important residues, peptide (L) with the mutation S83L, peptide (N) with the mutation D87N and peptide (M) with both mutations. “In vitro” supercoiling assays were performed to study the activity of these peptides. This assay consists in testing the supercoiling of a linear plasmidic DNA (0.5 mg) in presence of the DNA gyrase (1 unit) and the peptides (250 μg/ml) with or without ciprofloxacin (0.25 μg/ml). The reaction was stopped with a stop buffer and run in a 1.5% agarose gel electrophoresis.

Results: Peptides A and N showed an inhibition of the activity of the DNA gyrase in the conditions described above, whereas this was not observed in peptides L and M. In addition, these inhibition was higher in the presence of ciprofloxacin, showing an additive effect between the specific peptide and ciprofloxacin.

Conclusion: The peptide mimicking the original sequence of the DNA gyrase and that with the mutation S83L have good inhibitory activity against the DNA gyrase. Moreover, these results also suggest that Ser83 plays a more important role than Asp87 in the interaction between quinolones or peptides and the DNA gyrase.

**P1165** Determination of the mechanism of action of three novel chemical scaffolds with broad-spectrum antibacterial activity, including multidrug-resistant Gram-negative pathogens

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Objectives: Three completely novel classes of protein synthesis inhibitors have been designed and prepared as broad-spectrum agents, focused on treating multidrug-resistant (MDR) Gram-negative pathogens. Each class evolved from a unique scaffold designed to target the large ribosomal subunit. The goal of this study was to demonstrate that the Gram-negative potency was attributable to protein synthesis inhibition.

Methods: MIC determinations followed Clinical and Laboratory Standards Institute (CLSI) guidelines. Compounds were examined at 2× MIC for their inhibitory effects on the synthesis of DNA, RNA, and protein in Escherichia coli ATCC25922 as the test organism. The assay exploited the incorporation of an appropriate radiolabeled precursor into a given macromolecule: [3H]-thymidine for DNA, [3H]-uridine for RNA and [35S]-methionine for protein. Effect of compounds on in vivo polysome formation was investigated by treating *E. coli* cells with different compound concentrations and analyzing polysome patterns by sucrose gradient centrifugation.

Results: Exemplar compounds from all three novel scaffolds inhibit protein synthesis in *E. coli* cells on a level comparable to chloramphenicol, a known protein synthesis inhibitor with a different mechanism of inhibition. However, like chloramphenicol, none of these novel antibiotics affected appreciably RNA or DNA synthesis, where the comparators were rifampin and ciprofloxacin, respectively. The mechanism of action was further corroborated in the polysome analysis experiments. In contrast to controls (with a pattern showing mostly monosomes and few polysomes), cells treated with exemplars from all three novel classes show a pattern that is highly enriched in stable polysomes (di- tri- tetra- and pentasomes). Furthermore, upon longer exposure the pattern shifts to one enriched in dissociated subunits and partially assembled ribosomal particles.

Conclusions: The three novel classes presented here clearly exert their antibacterial activity by interfering with protein synthesis. In addition, these compounds are also able to “freeze” or stabilize polysomes and eventually inhibit ribosome maturation, confirming that they have a direct impact on ribosome function.
Antibacterial activity of C10 and C12 fatty acids and their sucrose esters towards Escherichia coli and Clostridium perfringens

E. Skrivanová*, M. Marounek, S. Pražáková (Prague, CZ)

Antibacterial activity of C10 and C12 fatty acids and their sucrose esters against Escherichia coli ATCC 25922 and Clostridium perfringens CNCTC 5459 was evaluated. In our previous experiments, C2–C18 free fatty acids were tested for their antibacterial activity against a number of Gram-negative and Gram-positive bacteria. The most effective ones (C10 for E. coli and C12 for C. perfringens) were chosen to compare their effect with the corresponding sucrose monoesters.

Methods: Susceptibility of E. coli and C. perfringens to fatty acids and their sucrose esters was evaluated using the plating method. Bacterial cultures were incubated in glucose medium containing C10 or C12 free fatty acids or their sucrose esters (0.1, 0.2, 0.5, 1, 2, 3 and 5 mg/mL). After the incubation, samples were serially diluted and inoculated on agar plates (MacConkey or Wilkins-Chalgren). After the incubation, typical colonies were counted and means and SD of CFU/ml were calculated. Differences between treated samples and non-treated control were evaluated using the t-test.

Results: Incubation of E. coli with C10 fatty acid and its ester led to a significant decrease in numbers of viable bacterial cells from 9.76 to 7.91–5.13 log10 CFU/mL. Sucrose ester of C10 showed stronger antibacterial properties than the free form of acid. Incubation of E. coli with free C10 fatty acid at the concentration of 5 mg/mL led to the reduction of bacterial cells from 9.76 to 6.84 log10 CFU/mL, whereas the same concentration of the corresponding ester reduced bacterial cells from 9.76 to 5.13 log10 CFU/mL. Gram positive C. perfringens was more sensitive to both forms of C12 fatty acid. Incubation of sucrose ester of lauric acid led to the reduction of viable cells below the detection limit (2 log10 CFU/mL), free acid showed the same effect at the concentrations higher than 0.5 mg/mL.

Conclusion: Both C10 and C12 fatty acids and their sucrose esters were shown to be potent antimicrobial agents against E. coli and C. perfringens. As expected, Gram positive C. perfringens was more sensitive. Sucrose esters of fatty acids were shown to be more potent antimicrobials than free acids.

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In vitro activity profile of PMX30063 against recent clinical isolates of Gram-positive and Gram-negative pathogens

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Objectives: PMX30063 represents the first of a new class of antimicrobial agents, a non-peptidic compound that mimics the mechanism of action and function of antimicrobial peptides. PMX30063 is being developed for the treatment of acute bacterial skin and skin-structure infections and is currently in phase 2 clinical trial. The full spectrum of activity of PMX30063 against both target and non-target pathogens was evaluated in vitro against a diverse array of recent clinical isolates of Gram-positive and Gram-negative pathogens. In addition, the effect of human serum on bactericidal activity of PMX30063 was tested against MSSA (ATCC 29213 and ATCC 13709) and two MRSA (ATCC 33591 and 102469A) strains.

Methods: Isolates were selected from the Eurofins Medinet repository for MIC broth microdilution testing against PMX30063 and comparator agents (CLSI M7-A8 method). All isolates selected consisted of non-consecutive, non-duplicate clinical isolates from the US. Most recent isolates (2008 to 2009) were preferentially selected and were predominantly of skin and wound origin.

Results: PMX30063 was potent (MIC90 of 1 µg/mL) against staphylococci and maintained a consistent activity profile against methicillin-resistant isolates relative to methicillin-susceptible isolates. PMX30063 was more potent against β-hemolytic streptococci (MIC90 of 4–8 µg/mL) than S. pneumoniae and viridans group streptococci (MIC90 of 16 µg/mL). PMX-30063 was more active against E. faecium isolates (MIC90 of 2 µg/mL) relative to E. faecalis (MIC90 of 8 µg/mL). Among the evaluated Gram-negative isolates, PMX-30063 was more potent against E. cloacae and Citrobacter spp. (MIC90 of 4 µg/mL) than P. aeruginosa and Acinetobacter spp. (MIC90 of 16 µg/mL). Little activity was observed against P. mirabilis (MIC90 of 64 µg/mL).

The presence of 50% human serum had minimal effects on PMX30063 antimicrobial activity with only a 2–4 fold increase in MIC when compared to standard.

Conclusions: Overall, PMX30063 was potent against staphylococci and other Gram-positive cocci including β-hemolytic streptococci and E. faecium. PMX30063 was comparatively less potent against other evaluated Gram-positive isolates (S. pneumoniae, VGS, E. faecium and Gram-negative isolates (Enterobacteriaceae, P. aeruginosa and Acinetobacter spp.) though activity is maintained against E. cloacae and Citrobacter spp. The antimicrobial activity of PMX30063 was only marginally affected by the presence of human plasma.

Antimicrobial activity of different antibiotics alone or in combination against Gram-negative rods

Clinical and Laboratory Standards Institute (CLSI) has recommended that most of the members of Enterobacteriaceae should be screened for extended-spectrum β-lactamase (ESBL) enzyme and reported as resistant to cefalosporins when found positive since 1999. In January 2010, the CLSI published new breakpoints for some of the cefalosporins. The aim of this study is to determine the antimicrobial susceptibility of ESBL positive Escherichia coli and Klebsiella strains according to the new CLSI recommendations.

One hundred ESBL producing E. coli strains and 92 ESBL producing Klebsiella strains isolated from various clinical materials in the year 2010 were included in this study. The antimicrobial susceptibility was determined via disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) 2010 criteria.

Of 100 ESBL positive E. coli strains 3 (3%) were found to be susceptible to cefotaxime, 13 (13%) were susceptible to cefazidime, 16 (16%) were susceptible to cefepime. None of the E. coli isolates was susceptible to ceftiraxone, cefuroxime and cefazolin. Seven (7%) of the E. coli isolates were susceptible to amoxicillin-sulbactam, 11 (11%) were susceptible to amoxicillin-clavulunate and 45 (45%) were susceptible to piperacillin-tazobactam. Of the 92 Klebsiella spp. strains tested only one (1%) isolate was found to be susceptible to ceftriaxone, 5 (5.4%) were susceptible to cefazidime, 21 (22.8%) were susceptible to cefepime. None of the Klebsiella isolates was found to be susceptible to cefotaxime, cefuroxime and cefazolin. Five (5.4%) of the 92 Klebsiella strains were susceptible to amoxicillin-sulbactam, 3 (3.2%) were susceptible to amoxicillin-clavulanate and 19 (20.6%) were susceptible to piperacillin-tazobactam.

Antimicrobial treatment of the infections caused by ESBL producing bacteria has been a matter of debate for a longtime. Data indicating that all cefalosporins will fail in the treatment of ESBL positive isolates is not existent. On the other hand, determining the antimicrobial susceptibility solely by disc diffusion or minimal inhibitory concentration values, irrespective of the resistance mechanism, may recall false susceptible results. The results of this study shows that cefepime may be an alternative in approximately 15–20% of the infections caused by ESBL positive strains based solely on the inhibition zones. The clinical impact of these laboratory findings should be studied meticulously.
**Antimicrobial activity of different antibiotics alone or in combination against MDR Gram-negative rods**

**S313**

**P1170** Susceptibility to expanded-spectrum cephalosporins among ESBL-producing *Klebsiella pneumoniae* and *Escherichia coli* clinical isolates according to the new CLSI recommendations

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**Objectives:** Extended-spectrum β-lactamase (ESBL)-producing organisms have rapidly spread worldwide and limit treatment options because they hydrolyze efficiently expanded-spectrum cephalosporins and monobactams. In 2010 the CLSI has revised susceptibility breakpoints of these β-lactams eliminating the need for confirmatory phenotypic tests. We assessed the implications of these new recommendations on susceptibility reporting of ESBL-producing *Klebsiella pneumoniae* and *Escherichia coli* clinical isolates.

**Methods:** We studied 141 single-patient isolates of *K. pneumoniae* and *E. coli* that were collected during 2008–2010 and characterized as ESBL producers based on the CLSI confirmatory phenotypic test and molecular testing for ESBL genes. PCR and sequencing analysis were performed for the characterization of ESBL-type. Cefotaxime, ceftazidime and cefepime MICs were determined by agar dilution method and interpreted using the 2010 breakpoints issued by CLSI.

**Results:** 101 isolates (71.6%) were susceptible to at least one of the tested cephalosporins. Five (3.5%), 49 (34.8%) and 95 (67.4%) isolates were susceptible to cefotaxime, ceftazidime and cefepime, respectively. 82 of the 113 CTX-M-producing isolates (72.6%) showed susceptibility to at least one cephalosporin with 49 (43.5%) and 77 (68.1%) of isolates being susceptible to ceftazidime and cefepime, respectively. Among the 28 SHV-producing isolates, susceptibility only to cefepime was expressed in 18 isolates (64.3%). Of the 43 *K. pneumoniae* isolates, only one was susceptible to cefotaxime, two to ceftazidime and 25 (58.1%) were susceptible to cefepime, while 47 of 98 (48%) *E. coli* were susceptible to ceftazidime and 70 (71.4%) to cefepime.

**Conclusion:** According to the new CLSI susceptibility breakpoints for cephalosporins, a considerable percentage of isolates was interpreted as susceptible, mostly to cefepime and in a lesser extent to ceftazidime. In contrast, with previous recommendations, these ESBL producers should be reported as resistant to all cephalosporins irrespective to their MICs. The currently recommended approach would have important therapeutic implications. However, more clinical studies are required to support the use of third-generation cephalosporins against infections by cephalosporin-susceptible ESBL-producing Enterobacteriaceae.

**P1172** Expression analysis of DHA-1, AmpR, OmpK35 and OmpK36 genes in *Klebsiella pneumoniae* and *Escherichia coli* clinical isolates

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**Objectives:** Transferable AmpC-type cephalosporinases have been increasingly reported in recent years, becoming a significant source of their resistance to newer β-lactams in different geographic areas. We focused on the determination of AmpC gene (blaDHA-1) and its regulator (AmpR) together with the main porin genes (OmpK35 and OmpK36) in different subinhibitory doses of cephalosporins.

**Methods:** Previously well characterized DHA-1-producing strains of *Klebsiella pneumoniae* (ST11) with different MIC patterns were used for the experiments. Two strains with different PFGE patterns and MICs of cefotaxime and ceftazidime in susceptible/intermediate category and one strain with a high MICs of 3rd generation cephalosporins (>64mg/l) were included in the study. Different concentrations of cefotaxime were added to cultivation media and samples were collected to cover early and late exponential phase of the bacterial growth. The gene expressions were determined by RT-PCR. Inoculum effect was determined by a standard microdilution method.

**Results:** The expression of AmpR was found to be decreasing with increasing concentration of antibiotic and the expression of DHA-1 constantly increased with a high MICs, independent production of DHA-1 on expression of AmpR was found. In this strain, however, the expression of DHA-1 remained inducible by cefoxitin. After the addition of cefoxitin, the expression of DHA-1 in all strains significantly increased together with a decreasing of AmpR. Strong inoculum effect was found in all tested cephalosporins (cefotaxime, ceftazidime, and cefepime) with bacterial concentrations of $10^7$ and $10^8$ CFU/ml.

**Conclusion:** The results indicate that the expression of DHA-1 is also influenced and slightly inducible with different level of cephalosporins which are not strong inducers. These results could explain very strong inoculum effect observed in all tested strains and antibiotics. No changes in porins expression were found, although the expression in early exponential phase samples was non-detectable. Comparing the expression of both porins in highly resistant isolate with “susceptible” ones, no significant decreasing production was observed. In the strain with a high MICs, independent production of DHA-1 on expression of AmpR was found. In this strain, however, the expression of DHA-1 in all strains significantly increased together with a decreasing of AmpR. Strong inoculum effect was found in all tested cephalosporins (cefotaxime, ceftazidime, and cefepime) with bacterial concentrations of $10^7$ and $10^8$ CFU/ml.

**Conclusion:** The results indicate that the expression of DHA-1 is also influenced and slightly inducible with different level of cephalosporins which are not strong inducers. These results could explain very strong inoculum effect observed in all tested strains and antibiotics. No changes in highly resistant strains compared with “susceptible” ones were observed. These results indicate a presence of another resistance mechanism in this strain.

This work has been supported by the grants NS9717–4/2008 and MSM 0021620819.
**[P1173] Fosfomycin susceptibility testing in Enterobacteriaceae isolates producing KPC-2, KPC-3, OXA-48 and VIM-1 carbapenemases**

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**Objectives:** The prevalence of multidrug-resistant grammnegative pathogens is increasing worldwide. Especially the spread of carbapenemase producing Enterobacteriaceae is worrisome since those strains are usually also resistant to most other antibiotics. No new antibiotics with activity against grammnegative bacteria will be introduced in the next five years. Therefore, testing the activity of older and rarely used antibiotics is of high clinical impact.

**Methods:** Enterobacteriaceae with elevated carbapenem MICs were sent to the National Reference Laboratory for Multidrug-Resistant Gram-negative Bacteria from several laboratories all over Germany and investigated for the presence of carbapenemases by the modified Hodge test, synergy testing using EDTA and boronic acid, a microbiological assay using cell-free extracts and PCR followed by sequencing.

Resistance testing of fosfomycin was performed both by Etest as described by the manufacturer, agar dilution was performed on Glucose-6-phosphate containing Mueller-Hinton agar as described by CLSI. Resistance was defined as a MIC for K. pneumoniae (n=16), K. oxytoca (n=1), E. coli (n=1), E. cloacae (n=1) and S. marcescens (n=1) isolates were chosen for this study on the basis of the presence of a carbapenemase and diverse epidemiological background.

KPC-2 was found in two isolates, KPC-3 in six isolates, OXA-48 in eight isolates and VIM-1 in four isolates.

Using Etest the MIC range was between 1 mg/L and >1024 mg/L, the MIC50 was 48 mg/L and the MIC90 was >1024 mg/L. By agar dilution a MIC range between 0.25 mg/L and >1024 mg/L, a MIC50 of 4 mg/L and a MIC90 of 128 mg/L was found. Applying EUCAST criteria 65% of strains would be classified as resistant using Etest, while only 25% would be determined as resistant using agar dilution.

**Conclusion:** Compared to agar dilution, which is considered the reference method for susceptibility testing of fosfomycin, fosfomycin MICs determined by Etest tend to be considerably higher. The reason of this phenomenon remains unclear.

Using EUCAST criteria and agar dilution a diverse collection of carbapenemase producing Enterobacteriaceae strains showed fosfomycin susceptibility of 75%.

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**[P1174] Polymyxin heteroresistance in extremely drug-resistant Acinetobacter baumannii**

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**Objectives:** Polymyxin is an important treatment option for extremely-drug resistant (XDR) Acinetobacter baumannii, but the phenomenon of polymyxin hetero-resistance raises some concern about the continued effectiveness of this antibiotic. This study investigated the prevalence of polymyxin hetero-resistance in clinical isolates of XDR A. baumannii, and evaluated the effect of different Etest methods for detection of polymyxin hetero-resistance.

**Methods:** Population analysis profiles for polymyxin B were performed for 24 isolates of clonally unrelated, XDR isolates by plating bacterial suspensions containing 108 cfu/ml on Mueller-Hinton agar plates containing increasing concentrations of polymyxin B. Polymyxin hetero-resistance was defined as any isolate with polymyxin B MIC >2 mg/L, in which detectable subpopulations were able to grow in the presence of >2 mg/L. Confirmatory antimicrobial susceptibility testing was performed on any polymyxin B resistant isolates recovered from PAP studies. Polymyxin B time-kill testing was performed on a subset of non-heteroresistant and hetero-resistant strains to compare population kinetics of different strains to polymyxin B. Finally, the effectiveness of a hetero-resistant Etest screening protocol was evaluated by performing polymyxin Etests and varying testing parameters of inoculum density, media and incubation duration.

**Results:** Seven of 24 (29%) study isolates demonstrated polymyxin hetero-resistance, with populations of polymyxin-resistant isolates detected at concentrations of 101.0-102.2 cfu/mL. These polymyxin-resistant subpopulations were highly resistant to polymyxin B, but in contrast to the original isolates, also demonstrated susceptibility to multiple other antibiotics. Time-kill studies showed no difference in the activity of polymyxin B against hetero- and non-hetero-resistant strains. No combination of Etest parameters was effective at screening for polymyxin hetero-resistant isolates.

**Conclusion:** Polymyxin hetero-resistance is common in XDR A. baumannii. Polymyxin-resistant subpopulations demonstrated high-level resistance to polymyxin B, but unlike polymyxin-resistance that develops in-vivo, these subpopulations also demonstrated reverison of the XDR phenotype. None of the evaluated Etest parameters were suitable for as a screening test for detection of the hetero-resistant phenotype. Further work needs to be performed to determine the clinical relevance of polymyxin hetero-resistance in A. baumannii.

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**[P1175] Synergy of fosfomycin with carbapenems, colistin, netilmicin, and tigecycline against MDR Klebsiella pneumoniae, Escherichia coli, and Pseudomonas aeruginosa clinical isolates**

G. Samonis*, S. Maraki, D. Karageorgopoulos, E. Vouloumanou, M. Falagas (Heraklion, Athens, GR)

**Objectives:** Fosfomycin represents a potential last resort treatment option for infections caused by certain multidrug resistant (MDR) Gram-negative pathogens.

**Methods:** We evaluated double-drug combinations of fosfomycin with imipenem, meropenem, doripenem, colistin, netilmicin, and tigecycline for in vitro synergy against 100 MDR Klebsiella pneumoniae, Escherichia coli, and Pseudomonas aeruginosa clinical isolates, using the Etest method. Synergy was defined as a fractional inhibitory concentration index ≤0.5. The isolates were consecutively collected at a microbiological laboratory of a university hospital in Greece from various clinical specimens.

**Results:** Against 50 serine carbapenemase-producing K. pneumoniae isolates, synergy of fosfomycin with imipenem, meropenem, doripenem, colistin, netilmicin, and tigecycline was observed for 74.0%, 70.0%, 74.0%, 36.0%, 42.0%, and 30.0% of the isolates, respectively; against 1 metallo-ß-lactamase producing K. pneumoniae isolate fosfomycin was synergistic only with imipenem. Against 14 extended-spectrum ß-lactamase (ESBL) producing K. pneumoniae isolates, synergy of fosfomycin with imipenem, meropenem, doripenem, colistin, netilmicin, and tigecycline was observed for 78.6%, 42.9%, 42.9%, 7.1%, 42.9%, and 21.4%, respectively; for 20 ESBL-producing E. coli isolates the corresponding values were 55.0%, 25.0%, 30.0%, 15.0%, 25.0%, and 25.0%; and for 15 MDR P. aeruginosa isolates the corresponding values were 46.7%, 53.3%, 73.3%, 13.3%, 13.3%, and 13.3%. Antagonism was not observed for any of the combinations tested.

**Conclusion:** Fosfomycin was synergistic in combination with a carbapenem against the majority of the MDR pathogens studied, whereas synergy with other than carbapenems agents was less common. Further studies are needed to confirm the clinical relevance of the above findings.

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**[P1176] Antibiotic combinations for Klebsiella sp. harbouring New Delhi Metallo-ß-lactamase-1 in Singapore**

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**Objectives:** Klebsiella sp. (KS) with bla-New Delhi Metallo-ß-lactamase-1 (NDM-1) gene have emerged in Singapore. These easily transferrable genes pose a huge clinical threat, as they have the potential to spread rapidly. Effective Antibiotic (Abx) are scarce and we may have to resort to combination therapy until new antimicrobial agents are available. We aim to elucidate Abx combinations that may have a potential for clinical use against NDM-1 KS.
**Carbapenems**

Methods: 60 KS non-repeat isolates that were ertapenem resistant were collected from the largest hospital in Singapore in 2010. Clonal relatedness was determined by multiplex PCR strain typing. They were then screened for bla-NDM-1-like genes using RT-PCR. MICs of the test isolates were determined according to the reference CLSI broth-dilution method. Time-kill (TKS) were performed with approximately 5 log CFU/ml at baseline using maximally achievable clinical, unbound concentration (mg/L) of amikacin (A) (80), levofloxacin (L) (8), cefepime (C) (200), rifampicin (R) (2), tigecycline (T) (2) and polymyxin B (P) (2) alone & in combination against the test isolates.

Results: 4 non-clonal isolates harbouring NDM-1 were identified. Among the test isolates, they were multidrug-resistant with high carbapenem MICs (‡64 mg/L). MICs of P ranged from 0.5 to 4 mg/L. In single Abs TKS, most drugs did not exhibit any killing activity in all 4 strains although P alone exhibited bactericidal activity against all strains (>3 log decrease from baseline inocula) until 8h before regrowth occurred at 24h. In combination TKS at 24h, P+T was synergistic (>2 log decrease when compared to its most active antibiotic) against strain 1 while A+L, A+R, A+P, A+T, L+T and P+T were bactericidal against strain 2. Against strain 3, A+L, A+P, A+C, A+T, L+P were bactericidal while no Abs combinations were even synergistic against strain 4.

Conclusions: Combination therapy may be considered as a therapeutic option for NDM-1 KS infections. However, there was no antibiotic combination that reliably demonstrated bactericidal or synergistic activity against all isolates. The efficacy of combination therapy may be highly strain specific. The in-vivo relevance of the results warrants further investigation.

**Carbapenems**

**PI1177** Minimum inhibitory concentrations of meropenem and doripenem for *Pseudomonas aeruginosa* from the UK

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Objectives: Comparison of the minimum inhibitory concentrations (MICs) of meropenem and doripenem required for *Pseudomonas aeruginosa* from multiple hospitals in the UK.

Methods: Seven-hundred and forty-eight *P. aeruginosa* were selected from the British Society for Antimicrobial Chemotherapy (BSAC) bacterial surveillance programme and supplemented with an additional 216 meropenem- or doripenem-, non-susceptible *P. aeruginosa* isolates. MICs were determined using the BSAC agar dilution method.

Results: The table shows doripenem MICs (mg/L) cross tabulated against those for meropenem for the 964 *P. aeruginosa*. In 779/964 (80%) of cases MICs of meropenem were higher than those of doripenem with 30% of isolates >4 fold more susceptible to doripenem. The degree of difference between the two carbapenem’s MICs was not uniform across the MIC distribution. Rather, in general the differences in MICs were smallest at the extremes of the MIC distribution. Despite the MIC differential in favour of doripenem only 35 isolates that were non-susceptible to meropenem were susceptible to doripenem, where as 9 were susceptible to meropenem but not doripenem. This was mostly due to meropenem’s susceptible breakpoint being ≤2 mg/L vs. ≤1 mg/L for doripenem, thus off setting the fact that meropenem MICs are higher, in general, than those of doripenem.

Conclusions: Doripenem MICs for *P. aeruginosa* were lower than those of meropenem for 80% of *P. aeruginosa* isolates. However, due to doripenem having lower MIC breakpoints, than meropenem this does not manifest as a large difference in susceptibility. Doripenem’s relative advantage will only be exploitable if higher dosages can be shown as effective and safe, justifying a higher breakpoint.

**PI1178** Activity of doripenem versus imipenem, meropenem and ceftazidime in clinical isolates of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in Portugal

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Objective: The doripenem (DOR) is a new carbapenem with a broad spectrum of activity against aero and anaerobic bacteria, Gram negative and Gram positive, with approved indications for nosocomial pneumonia and complicated intra-abdominal and urinary tract infections. Studies point to improved activity relative to other carbapenems against bacteria considered problematic, particularly *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and ESBL-producing Enterobacteriaceae. The purpose of this study is to compare the activity of this antibiotic with imipenem, meropenem and ceftazidime in problematic strains isolated at Hospital Prof. Dr. Fernando Fonseca in Lisbon during 2010, namely *Pseudomonas aeruginosa* and *Acinetobacter baumannii* and to assess the benefit of introducing this antibiotic in the hospital as a better alternative to other carbapenems.

Method: Retrospective study of 52 strains of *A. baumannii* and 60 strains of *P. aeruginosa* from clinically significant samples of patients from the hospital. The identifications and MIC calculated from the breakpoint of the susceptibility card, to imipenem, meropenem and ceftazidime of these strains were obtained from the equipment VITEK2® (Biomerieux), using the GN and AST-N093 cards. The MIC determinations of doripenem were obtained with the Etest technique (Biomerieux) in Muller-Hinton medium (BioMerieux) with a suspension of 0.5 McFarland.

Results: Strains of *P. aeruginosa* revealed for doripenem a sensitivity of 67% and a resistance of 33%, for meropenem a sensitivity of 77%, a resistance of 21% and 2% of intermediate results, for imipenem a sensitivity of 83% and a resistance of 17% and for ceftazidime a sensitivity of 78%, a resistance of 10% and 12% of intermediate results. Strains of *A. baumannii* presented for doripenem and meropenem a sensitivity of 10% and a resistance of 90%, for imipenem a sensitivity of 8%, a resistance of 90% and 2% of intermediate results and for ceftazidime a sensitivity of 11%, a resistance of 79% and 10% of intermediate isolates. The MIC50 and MIC90 calculated based on graphs of cumulative percentages are very close for the several antibiotics and both strains.

Conclusions: The doripenem showed similar results to the other carbapenems for strains of *A. baumannii* and slightly lower sensitivity and greater resistance to *P. aeruginosa* tested. Doripenem, in a microbiological level, shows no advantages over other carbapenems and therefore has no advantages to being available at our hospital.

**PI1179** Antimicrobial activity and spectrum of doripenem against contemporary (2010) clinical bacterial pathogens from Europe

D. Farrell*, G. Moet, H. Sader, R. Jones (North Liberty, US)

Objective: To evaluate the in vitro antimicrobial activity of doripenem (DOR) tested against prevalent Gram-negative and -positive pathogens isolated across Europe (EU) during 2010. DOR is an approved carbapenem in EU for the treatment of nosocomial pneumonia (NP),
including ventilator-associated pneumonia (VAP), complicated intra-abdominal infections (cIAI) and complicated urinary tract infections (cUTI).

Methods: A total of 9,618 consecutive, non-duplicate isolates from a wide variety of infections were collected from 39 medical centers located in Europe, Turkey, and Israel during 2010. Species identification was confirmed by the central monitoring laboratory and all isolates were susceptibility (S) tested using reference CLSI broth microdilution methods (M7-A8, 2009) against DOR and numerous comparison agents.

Results: Doripenem was very active against Enterobacteriaceae, inhibiting 99% of isolates at 0.5 mg/L (MIC90, 0.12 mg/L). Cumulative percentage inhibition by DOR MIC for the major organism groups is shown in the Table. DOR exhibited good activity against P. aeruginosa (MIC90 0.5/4 mg/L) and some Acinetobacter spp., inhibiting 49% of isolates at ≤8 mg/L. Against Gram-positive pathogens, DOR had very high activity against methicillin-susceptible S. aureus (MSSA), MS-coagulase-negative staphylococci (CoNS), β-haemolytic streptococci, and S. pneumoniae with MIC90 values of ≤0.06, ≤0.06, ≤0.06, and 0.5 mg/L, respectively. DOR was less active against MRSA and MR-CoNS with both having a MIC90 of 2 mg/L, as well as E. faecalis (MIC90, 2 mg/L). DOR was not active against the vast majority of E. faecium (MIC90 >8 mg/L, range 2–32 mg/L).

Conclusions: DOR exhibited a wide-spectrum of antimicrobial activity against 9,618 contemporary EU pathogens and excellent activity against most Gram-positive pathogens except for MRSA, MR-CoNS and Enterococci. Against Gram-negative pathogens, DOR showed excellent activity against Enterobacteriaceae and against many multidrug-resistant P. aeruginosa and Acinetobacter spp. This data supports the use of DOR as therapy for hospitalized patients, in whom carbapenem therapy would be warranted to treat serious and typically difficult-to-treat infections, such as NP, VAP, cIAI, and cUTI in the European area.

### Results

| Organism (no. of strains) | ≤0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 |
|--------------------------|------|------|------|-----|---|---|---|---|
| Enterobacteriaceae (1,613) | 83 | 95 | 98 | 99 | 99 | 99 | 99 | 99 |
| Acinetobacter spp (136) | 3 | 16 | 27 | 31 | 41 | 44 | 44 | 49 |
| P. aeruginosa (539) | 6 | 22 | 40 | 50 | 70 | 78 | 90 | 95 |
| S. aureus (2,485) | 73 | 75 | 79 | 82 | 84 | 87 | 91 | 94 |
| MRSA (469) | 3 | 9 | 21 | 34 | 41 | 52 | 65 | 77 |
| MSSA (1,817) | 98 | 99 | 99 | 100 | - | - | - | - |
| CoNS (597) | 32 | 39 | 43 | 53 | 64 | 72 | 78 | 85 |
| MB-CNS (428) | 7 | 15 | 21 | 34 | 49 | 61 | 69 | 79 |
| MC-CNS (169) | 96 | 100 | 100 | 100 | - | - | - | - |
| Enterococci faecalis (564) | 1 | 1 | 1 | 1 | 2 | 52 | 94 | 99 |
| beta-haemolytic strep. (672) | 99 | 99 | 100 | - | - | - | - | - |
| S. pneumoniae (746) | 79 | 81 | 87 | 97 | 100 | - | - | - |

**P1180** In vitro activity of doripenem against multidrug-resistant clinical strains of *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*

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**Objectives:** Doripenem is an injectable carbapenem that gained approval for treatment of hospital acquired pneumonia, urinary tract infections and intra-abdominal infections. The objectives of the present study were to investigate the in vitro activity of doripenem against a range of multidrug resistant Gram(−) isolates and to compare its activity with that of meropenem, imipenem and ticaglycine.

**Methods:** A total of 113 different clinical isolates were tested. The isolates included 30 carbapenem resistant *A. baumannii* strains (non MBL or KPC), 30 *P. aeruginosa* MBL producing strains and 53 *K. pneumoniae* strains, of which 10 produced ESBL, 6 KPC, 2 MBL, 19 ESBL and KPC, 10 ESBL and MBL, 4 MBL and KPC and 2 ESBL, MBL and KPC. Susceptibility of the isolates was initially performed using the Wider system (Soria) and the results were confirmed using the E-test method following the manufacturer’s guidelines (bioMérieux, Sweden). Sensitivity and resistance breakpoints for doripenem, meropenem and imipenem were determined according to CLSI 2010 new interpretive criteria, while FDA guidelines determined susceptibility to ticaglycine. *E. coli* ATCC 25922 was used as QC. The phenotypic detection of the production of extended spectrum b-lactamasas, metallo-b-lactamasas MBL and carbapenemases KPC, was performed by the double disk synergy test, the combined disk test, the two-sided E-test and the modified Hodge Test on M. H. agar.

**Results:** The in vitro activity of doripenem, which is considered superior to meropenem against multidrug resistant *A. baumannii*, MBL producing *P. aeruginosa* and KPC producing *K. pneumoniae*, does not seem to support its use as monotherapy in the treatment of infections caused by those bacteria. On the other hand ticaglycine might provide an alternative therapeutic choice against infections caused by MBL producing *K. pneumoniae* strains.

### Results

| Organism | Meropenem | Imipenem | Ticaglycine |
|----------|-----------|----------|------------|
| *A. baumannii* (30) | 4 ± 32 | 8 ± 32 | 16 ± 32 | 5 ± 96 |
| *K. pneumoniae* (10) | 1 ± 32 | 2 ± 32 | 24 ± 32 | - |
| ESBL (9) | 0.03 ± 0.21 | 0.06 ± 0.75 | 0.05 ± 1.73 | 1.5 ± 3 |
| MBL (2) | 0.5 ± 32 | 0.5 ± 32 | 12 ± 32 | 1.5 ± 12 |
| E. coli ATCC 25922 | 0.75 ± 32 | 2 ± 32 | 32 ± 32 | 1 ± 6 |
| KPC (10) | 1.5 ± 32 | 2 ± 32 | 32 ± 32 | 1.5 ± 4 |
| MBL + KPC (4) | 0.5 ± 32 | 0.5 ± 32 | 32 ± 32 | 2 ± 3 |

**P1181** In vitro antibacterial activity of doripenem against multidrug-resistant *Acinetobacter baumannii* isolates from adult intensive care units

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**Objectives:** *Acinetobacter baumannii* is a frequent cause of serious hospital infections in intensive care units. Effective treatment options for these infections are limited. An alternative to current antimicrobial agents could be doripenem (DOR), a carbapenem with activity against Gram-negative bacteria, including *A. baumannii*, approved for the treatment of adults with complicated intra-abdominal (cIAI) and urinary tract infections (cUTI) in the United States, and cIAI, cUTI, and nosocomial pneumonia in Europe. The aim of the present study was to evaluate the in vitro activity of doripenem against multidrug resistant (MDR) *A. baumannii* isolates from hospitalised patients in the adult intensive care units (ICU) of AHEPA University Hospital.

**Material-Methods:** Between January and December 2009, 95 MDR *A. baumannii* were isolated from cultures of various clinical specimens, from inpatients of critical care units with a documented infection. Bacterial identification and susceptibility testing was performed using Vitek2 system (bioMérieux, France). Susceptibility testing of doripenem was performed with Etest strips (AB Biodisc, Sweden). Isolates were defined as susceptible (MIC ≤1 µg/ml) or resistant (MIC ≥4 µg/ml) to doripenem according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.

**Results:**

1. Most strains were isolated from cultures of bronchial secretions (38/95) 2) Overall, 51 (48%) strains were found to be resistant to >3 antibiotics belonging to different antimicrobial classes. Most strains were resistant mainly to cefazidime, ciprofloxacine, imipenem and piperacillin/tazobactam, moderately susceptible to meropenem, ceftazime, and susceptible to gentamicin and ticaglycine. 4) All strains were susceptible to colistin. 4) From 95 strains, only 4/95 (4%) were susceptible to doripenem, 42/95 (42%) moderately susceptible and 49/95 (54%) resistant. MIC90 of doripenem was ≥4 mg/ml.

**Conclusions:** *A. baumannii* strains from adult ICU showed high resistance rates against doripenem and in our hospital this agent doesn’t seem an alternative agent against MDR *A. baumannii* infections.
Comparative activity of doripenem, ertapenem and imipenem against clinical isolates of Klebsiella pneumoniae producing extended-spectrum β-lactamases, plasmid-mediated AmpC-type β-lactamases or both, associated or not with porin deficiency

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Objective: To evaluate the activity of doripenem (compared to ertapenem and imipenem) against clinical isolates of Klebsiella pneumoniae producing extended spectrum β-lactamases (ESBL), plasmid-mediated AmpC-type β-lactamases (pACBL) or both, associated or not with porin (POR) deficiency.

Methods: Fifty K. pneumoniae clinical strains were studied, including 2 ESBL+/pACBL+/POR+, 10 ESBL+/pACBL+/POR-, 12 ESBL-/pACBL+/POR+, 18 ESBL-/pACBL-/POR+, 4 ESBL+/pACBL+-/POR-, and 3 ESBL-/pACBL+/POR-. Antimicrobial susceptibilities to doripenem, ertapenem and imipenem were determined by broth microdilution (CLSI guidelines) with standard and 10-fold higher inocula. The inoculum effect was defined as an eight-fold or greater MIC increase on testing with the highest inoculum. Clinical categories were assigned applying the new CLSI interpretative criteria for carbapenems and Enterobacteriaceae (document M100-S20-U; June 2010).

Results: The MIC50 of doripenem, ertapenem and imipenem were 0.125, 0.5 and 0.5 mg/L respectively, the MIC90 were 1, 1.6 and 1 mg/L, respectively. Doripenem and Imipenem displayed an excellent activity against isolates POR-, irrespective of β-lactamase(s) produced, with 100% of susceptible strains whereas the percentage of susceptibility fell to 72% in the group of strains POR+. The percentage of susceptibility to ertapenem among POR+ isolates was 59% and fell to 17% in the group POR-.

Inoculum effect was observed for all three carbapenems, but almost always in isolates pACBL-, being more pronounced with doripenem, but with fewer changes in the clinical category than those observed in the cases of ertapenem and imipenem. Thereby, at standard inoculum the percentages of susceptibility to doripenem, ertapenem and imipenem were 90, 44 and 90%, respectively, whereas at 10-fold-higher inoculum were 82, 30 and 54%.

Conclusions: Doripenem and imipenem displayed an excellent activity against clinical isolates of Klebsiella pneumoniae producing ESBL, pACBL or both and expressing POR. Using the new CLSI interpretative criteria for carbapenems and Enterobacteriaceae, doripenem showed the lowest activity against these strains. Porin deficiency decreased the activity of all three carbapenems. Clinical categories of doripenem, in contrast to those of imipenem, were rarely affected by high inocula.

In vitro activity of the siderophore sulfactam BAL30072 against meropenem non-susceptible Acinetobacter baumannii

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Objectives: Multidrug-resistant Acinetobacter baumannii is a growing threat leaving few therapeutic options. Recently there has been a dramatic increase in carbapenem-resistance mediated mainly through the action of intrinsic and acquired OXA-type enzymes. Metallo-carbapenemases while rare in this species, are also detected and pose a significant potential threat. Efflux does not significantly affect carbapenems, however it plays a role in the intrinsic resistance to fluoroquinolones, tetracyclines, aminoglycosides and macrolides. BAL30072 is a novel siderophore sulfactam that enters the cells through a non-porin route and is not affected by efflux. The activity of BAL30072 was compared with anti-Acinetobacter reference drugs against defined A. baumannii isolates that had either an acquired OXA enzyme or its OXA-51-like enzyme.

Methods: Antimicrobial susceptibility testing was investigated by broth microdilution of 310 non-duplicate, meropenem non-susceptible A. baumannii isolates to BAL30072, amikacin ampicillin/sulbactam, aztreonam, cefepime, colistin, imipenem, levofloxacin, meropenem, rifampicin, tigecycline, and tobramycin.

Results: The MIC range, MIC90 and MIC50 values (µg/mL) of the 310 A. baumannii isolates are summarised in the table. BAL30072 showed greater activity than the β-lactam comparators, levofloxacin, amikacin,
tobramycin and rifampicin, MIC50 of BAL30072 was comparable to tigecycline. Elevated BAL30072 MICs were found, but there was no correlation with elevated MICs for the other antimicrobials.

**Conclusion:** BAL30072 is a promising new agent with good activity against carbapenem-resistant *A. baumannii*.

### Quinolones

**P1185** In vitro activity of delafloxacin against European isolates of *Staphylococcus aureus* from skin and soft tissue, respiratory, urine and blood specimens

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**Objectives:** Delafloxacin (DFX) is an investigational fluoroquinolone with excellent activity against a variety of Gram-positive bacteria, including quinolone-resistant methicillin-resistant *Staphylococcus aureus* (MRSA). We evaluated the in vitro activity of DFX against *S. aureus* strains isolated from wound, respiratory, urine, and blood specimens in Europe. Isolates represented inpatient, outpatient and ICU populations from 2006 to 2008 from eleven countries.

**Methods:** Minimum inhibitory concentration (MIC) values were determined by broth microdilution according to CLSI methodology. Comparator agents included levofloxacin (LVX), linezolid (LNZ), daptomycin (DAP), vancomycin (VAN), oxacillin (OXA), clindamycin (CLI), erythromycin (ERY), and tigecycline (TIG).

**Results:** Ninety-six percent of strains were found to be methicillin resistant by susceptibility testing using OXA. DFX MIC values (mcg/mL) are presented below by country for wound, respiratory, and blood isolates (N=681). By country MIC90 values for DFX ranged from 0.12 to 0.5 mcg/mL and MIC50 values ranged from ≤0.004 to 0.25 mcg/mL. Against all MRSA strains in Europe (n=676) MIC50/MIC90 values for DFX were 0.12/0.25 mcg/mL. Against all MRSA strains in Europe (N=676) MIC50/MIC90 were >32, 1/2, 1/1, 0.06/0.12 mcg/mL, >8, >8, 0.12/0.25, 16/16, 0.06/0.12 mcg/mL, respectively. LFX was at least 32-fold less active than DFX against all geographically grouped strains comparing MIC90s overall or by country.

**Conclusions:** DFX was more potent than LVX against MRSA, regardless of geographic region. The activity of DFX was largely consistent across regions, although isolates with slightly higher DFX MIC values were more frequently encountered in Germany, Belgium, and Central Europe.

| Country         | Number Strains(n) | MIC Range | MIC50 | MIC90 |
|-----------------|-------------------|-----------|-------|-------|
| Belgium         | 35                | ≤0.004-2  | 0.12  | 0.5   |
| Central Europe  | 23                | ≤0.004-2  | 0.25  | 0.5   |
| France          | 197               | ≤0.004-4  | 0.12  | 0.25  |
| Germany         | 95                | ≤0.004-1  | 0.25  | 0.5   |
| Italy           | 91                | ≤0.004-0.5| 0.12  | 0.25  |
| Spain           | 167               | ≤0.004-1  | 0.12  | 0.25  |
| Sweden          | 12                | ≤0.004-0.25| 0.12  | 0.25  |
| United Kingdom  | 121               | ≤0.004-0.5| 0.12  | 0.25  |

### P1186

**Antibacterial activity of the investigational fluoroquinolone finafloxacin under different pH conditions against *Staphylococcus aureus* small-colony variants**

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**Objectives:** Finafloxacin (FIN) is an investigational C-8-cyano-fluoroquinolone (FC) containing a unique chiral C7 substituent that exhibits broad-spectrum antibacterial activity. While all other marketed fluoroquinolones exhibit significantly reduced activity at slightly acidic pH (5.0–6.5), FIN’s activity is enhanced at these conditions typical for the environment of abscesses. *Staphylococcus aureus* represents a typical abscess-forming microorganism and its small colony variant (SCV) phenotype has been associated with chronic and relapsing infections. Here, the in vitro activity of FIN against methicillin-susceptible (MSSA) and methicillin-resistant (MRSA) *S. aureus* isolates displaying normal (NP) and SCV phenotypes was compared with those of ciprofloxacin (CIP), levofloxacin (LEV) and moxifloxacin (MOX) at neutral and low pH conditions.

**Methods:** In vitro activities of FIN, CIP, LEV and MOX were tested against 28 MSSA and 3 MRSA clinical strain pairs consisting of SCVs and their clonally identical *S. aureus* parental strains displaying the normal phenotype. Additionally, two *S. aureus* mutants displaying the SCV phenotype (hemin-auxotrophic hemB mutant and thymidine-auxotrophic thyA mutant) and their parental strains were included. Applying epilometer test, MIC50, MIC90 and MIC ranges were calculated at pH 5.8 and pH 7.2.

**Results:** Against MSSA, FIN demonstrated superior activity under acidic conditions for both phenotypes (MIC90 of NP:SCV, mcg/L: 0.125/0.094 (FIN); 1.5/2 (CIP); 0.75/0.5 (LEV) and 0.25/0.25 (MOX)). At neutral conditions, the activity against MSSA was as follows: MOX > FIN > LEV > CIP. In comparison to ciprofloxacin-susceptible NP isolates, CIP was less active against their corresponding SCVs. For other FQs, there was no marked difference in activity between NPs and SCVs observed. At pH 5.8, FIN showed highest activity also against the hemB and thyA mutants and their parental strains as well as against those NP-SCV pairs tested methicillin-resistant.

**Conclusion:** Abscesses still represent a therapeutic dilemma because available antimicrobial agents are often ineffective and their therapy regularly necessitates surgical intervention. Thus, in particular at acidic conditions, FIN appears to be a promising antibiotic agent for the treatment of persistent staphylococcal infections including those caused by SCVs.

**P1187** Comparative in vitro activity of sitafloxacin and other fluoroquinolones against bacteria isolated from patients with urinary tract infections and lower respiratory tract infections

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**Background:** Sitafloxacin (DU 6859a) is a broad-spectrum oral fluoroquinolone and it is very active against many bacteria including the strains resistant to other fluoroquinolones. The objective of the study was to determine in vitro activity of sitafloxacin and other antibiotics against common causative bacteria isolated from the patients with urinary tract infections and those with lower respiratory tract infections who attended five tertiary care hospitals in Thailand in 2010.

**Methods:** We studied 1,255 strains of *E. coli* (N=140), *K. pneumoniae* (N=196), *P. mirabilis* (N=100), *P. aeruginosa* (N=216), *A. baumannii* (N=198), Enterococcus spp (N=100), *S. pneumoniae* (N=100), *S. aureus* (N=105), *H. influenzae* (N=50), and *M. catarrhalis* (N=50) isolated from different Thai patients with urinary tract infection and those with lower respiratory tract infection in 2010. The minimum inhibitory concentrations (MICs) of sitafloxacin, levofloxacin, ciprofloxacin, moxifloxacin were determined by agar dilution method.

| Site of infection                          | Bacteria                  | MICs (mg/L) |
|--------------------------------------------|---------------------------|-------------|
| Urinary tract infection                    | *E. coli* (DU1188)        | ≤0.063      |
| Lower respiratory tract infection          | *K. pneumoniae* (DU1189)  | ≤0.063      |
|                                             | *P. mirabilis* (DU1190)   | ≤0.063      |
|                                             | *P. aeruginosa* (DU1191)  | ≤0.063      |
|                                             | *A. baumannii* (DU1192)   | ≤0.063      |
|                                             | *H. influenzae* (DU1193)  | ≤0.063      |
|                                             | *M. catarrhalis* (DU1194) | ≤0.063      |
|                                             | *S. pneumonia* (DU1195)   | ≤0.063      |
|                                             | Enterococcus spp (DU1196) | ≤0.063      |
|                                             | *S. pneumonia* (DU1197)   | ≤0.063      |
|                                             | *A. baumannii* (DU1198)   | ≤0.063      |
|                                             | *K. pneumoniae* (DU1199)  | ≤0.063      |
|                                             | *P. mirabilis* (DU1200)   | ≤0.063      |
|                                             | *P. aeruginosa* (DU1201)  | ≤0.063      |
|                                             | *H. influenzae* (DU1202)  | ≤0.063      |
|                                             | *M. catarrhalis* (DU1203) | ≤0.063      |
|                                             | *S. pneumonia* (DU1204)   | ≤0.063      |
|                                             | Enterococcus spp (DU1205) | ≤0.063      |

**Table:** MICs of sitafloxacin, levofloxacin, ciprofloxacin, moxifloxacin against all tested bacteria.
Results: The MIC50 and MIC90 of tested antibiotics and susceptibility rate of tested organisms are shown in Table.

Conclusion: Sitafloxacin is much more active than levofloxacin, meropenem against bacteria including multidrug-resistant strains isolated from Thai patients with urinary tract infections and lower respiratory tract infections.

Biofilm susceptibility of P. aeruginosa isolated from patients with otitis media to ciprofloxacin and N-acetylcysteine

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Objective: P. aeruginosa is a common cause of chronic otitis media in which biofilm formation favors bacterial resistance. N-Acetylcysteine (NAC) is a well known mucolytic agent which has limited in vitro antibacterial activity but synergy with Ciprodex (ciprofloxacin 0.3%/dexamethasone 0.1%). The combination may have promise for the treatment of chronic otitis media. Our objective was to demonstrate synergy between Ciprodex and or ciprofloxacin and NAC against biofilm forming P. aeruginosa strains isolated from otitis cases.

Methods: 15 clinical isolates of P. aeruginosa were tested using the bioFILM PA™ antimicrobial susceptibility assay (Innovotech, Edmonton, AB) to determine antimicrobial susceptibility of both planktonic and biofilm P. aeruginosa to Ciprodex and ciprofloxacin both alone and in combination with NAC. Isolates were grown on plastic pegs in a 96 well format to allow identical biofilms to form on all pegs. The pegs were then placed into 96 well microplates containing Ciprodex and ciprofloxacin both alone or in combination with NAC at various concentrations. Susceptibility of the planktonic population was measured by a microreader at 620 nm after overnight incubation. Viability of the sessile population was assessed after rinsing the pegs and placing them in recovery medium for 24 hrs.

Results: Of the 15 P. aeruginosa strains, one (7%) showed growth in all Ciprodex tested concentrations and two strains (13%) showed growth in two ciprofloxacin concentrations in the planktonic state. Higher resistance to Ciprodex and ciprofloxacin was observed in the sessile population where five (33%) strains showed growth in one or more ciprofloxacin or ciprofloxacin concentration. NAC, in a final concentration of 0.5 µg/mL, effectively inhibited both planktonic and sessile populations when added to any of the Ciprodex or ciprofloxacin concentrations. No growth was observed in any of the wells with added NAC.

Conclusion: Sessile P. aeruginosa were less susceptible than the planktonic populations to Ciprodex and ciprofloxacin when tested alone. The addition of NAC to Ciprodex or ciprofloxacin completely inhibited the growth of both planktonic and sessile populations. These results suggest there are differences between biofilm state and planktonic state in P. aeruginosa strains. Biofilm P. aeruginosa are more resistant to Ciprodex or ciprofloxacin. The combination of these agents with NAC may increase their clinical utility in the treatment of chronic otitis media.

H1N1

Bacterial co-infections in community-acquired pneumonia cases of 2009 pandemic influenza A/H1N1 in Spain

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Background: Bacterial co-infection in adult patients with pneumonia has been scarcely studied and their clinical significance remains unknown.

Objectives: To investigate the role and the incidence of bacterial co-infection in patients with influenza A (H1N1) pneumonia.

Results: The incidence of H1N1 was 19% at both hospitals. We studied 128 patients: 42 (32.8%) presented with bacterial co-infection. The most frequently isolated microorganism was Streptococcus pneumoniae (62%). The factors associated with bacterial co-infection were presence of COPD (OR 11.79, 95% CI 2.42 to 57.29, p = 0.002), C-reactive protein (+1 mg/dL increase, OR 1.04, 95% CI 1.00 to 1.07, p = 0.070), and platelets count (per mm3) (+10 units increase, OR 1.06, 95% CI 1.02 to 1.11, p = 0.009). Independent predictors of bacterial co-infection found in the multivariate analysis were presence of COPD (OR 9.66, 95% CI 1.93 to 48.31, p = 0.002) and platelets count (per mm3) (+10 units increase, OR 1.05, 95% CI 1.00 to 1.11, p = 0.041).

Twelve patients (9%) died, the mean age of deceased patients was 51 years. Factors associated with mortality were age ⩾65 years (OR 7.57, 95% CI 2.03 to 28.29, p = 0.003), prior influenza vaccination (OR 4.61, 95% CI 1.12 to 18.93, p = 0.034), serum creatinine (+1 mg/dL) (OR 1.84, 95% CI 1.04 to 3.28, p = 0.038), serum lactate dehydrogenase (+100U/L) (OR 1.11, 95% CI 1.00 to 1.25, p = 0.060), presence of chronic cardiovascular disease (OR 15.86, 95% CI 3.70 to 68.01, p < 0.001), PSI IV–V (OR 6.82, 95% CI 1.85 to 25.14, p = 0.004) and multilobar infiltration (OR 4.74, 95% CI 1.22 to 18.51, p = 0.025). Factors independently associated with mortality were age ⩾65 years (OR 5.75, 95% CI, 1.11 to 29.44, p = 0.037), presence of chronic cardiovascular disease (OR 8.60, 95% CI 1.63 to 45.33, p = 0.011) and multilobar infiltration (OR 5.56, 95% CI 1.12 to 27.68, p = 0.036).

Conclusions: Streptococcus pneumoniae is the most common pathogen. This study confirms that bacterial co-infection was associated with more severe clinical course among influenza virus (H1N1) infected patients, as shown by PSI, rate of ICU admission and MV.

Comparative analysis of positive and negative H1N1 hospitalised adult patients during the influenza A pandemic in Mar del Plata, Argentina

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Introduction: Since May 2009 and after the first cases of influenza A H1N1 detected in Mexico and California, the virus disseminated swiftly causing a strong impact in the health systems worldwide.

Materials and Methods: A descriptive, observational and multicenter study was performed including patients ⩾15 years with a severe acute respiratory infection (SARI) hospitalized in 5 centres of Mar del Plata, Argentina. Between May to August 2009 a prospective record was kept of all the patients hospitalized with a diagnosis of SARI. Patients were divided into 2 groups and a comparison was done between the confirmed and negative cases using the following definitions:

Confirmed A/H1N1 cases: positive PCR for H1N1 Influenza found in nasal pharyngeal swabs.

Negative Cases: negative PCR for H1N1 Influenza or positive for the seasonal influenza strain.

Results: 184 patients were hospitalized with a SARI during the study period as follows: 100 were confirmed, 84 were negative. Between confirmed patients the average age was 43, 52% were female and the
average time between the onset of symptoms and admission was 5 days. Among the patients defined as negative the mean age was 48, 47% were female and the average time from onset of symptoms to admission was 7 days. The main co-morbidities among the confirmed cases were smoking (37% and 32%); COPD (18% both groups); obesity (16% and 18%). The clinical presentation was similar in both groups; the majority reported fever, cough and dyspnoea. No differences were observed in the laboratory findings. PaO2 values were 68mmHg in confirmed cases vs. 73mmHg in negative cases. A bilateral interstitial pattern was observed in 71% of the confirmed cases and in 50% of the negative ones. 99% and 98% respectively of confirmed and negative cases received treatment with oseltamivir. Mechanical ventilation was required in both groups at a equivalent rate (13% and 14% respectively).

No significant differences were observed in mortality rates between the 2 groups (13% confirmed and 12% negative). The patients who died as a result of the H1N1 Influenza A infection were younger in average (46 vs. 53 years).

Conclusions: The H1N1 confirmed cases were younger and had a shorter clinical course. They presented a higher degree of hypoxemia and the predominant pulmonary radiological pattern observed was bilateral and interstitial. No differences were found between the groups in terms of comorbidities, clinical presentation, laboratory findings and mortality rates.

P1192 Prospective surveillance of 2009 pandemic influenza A/H1N1 at a university hospital: characteristics and factors associated with severe disease

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Objectives: Influenza A(H1N1) 2009 can lead to severe disease. The objective was to assess the characteristics and factors associated with severe disease in patients with laboratory-confirmed influenza A(H1N1) admitted to a University Hospital during the 2009–2010 influenza pandemic.

Methods: An analytic study based on a prospective surveillance system was conducted between September 2009 and January 2010 in the Edouard Herriot University Hospital (Lyon, France). All patients who had laboratory-confirmed influenza A (H1N1) were included. Cases were identified through daily monitoring in the Emergency department, medical consultation dedicated to immunocompromised patients and from virological laboratory results. We compared patients who had non-severe disease defined as patients not requiring hospitalisation, who had mildly severe disease defined as patient hospitalised in short stay units, and who had severe disease defined as patient requiring hospitalisation in Intensive Care Units (ICU). Patients with severe disease were compared 1) to patient with non severe disease, and 2) to patients with mildly severe disease using univariate logistic regression analysis.

Results: Totally, 69 patients were included. Those aged 15–44 years were most affected (n=36; 52%), 9 (13%) were older than 65 years; and 57 (83%) patients had at least one underlying medical condition. A total of 24 (35%) cases were non severe, 29 (42%) were mildly severe and 16 (23%) were severe. Among 45 hospitalised patients, 19 (42%) had complications and 5 (11%) died. The factors associated with severe influenza A(H1N1) were (Table 1): age (45–64 years), cardiovascular risk factors, heart disease, obesity (body mass index >30), renal disease, dyspnea and a long delay from onset to diagnostic.

Conclusion: An increase of vaccination coverage among these high risk patients could improve the management of a future influenza pandemic. In this way, the early detection of cases, as conferred by our prospective surveillance strategy, could be an important tool.

P1193 Immunogenicity and safety of two doses of AS03A-adjuvanted influenza A/H1N1 2009 vaccine in cancer patients on chemotherapy – VACANCE study

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Background: Influenza vaccination is recommended to cancer patients on chemotherapy but remains controversial because of vaccine efficacy concerns. During the 2009 influenza A H1N1v pandemic, French recommendations were to vaccinate immunocompromised patients with two doses of an adjuvanted H1N1v vaccine. The aim of this study was to evaluate the immunogenicity of the vaccination in cancer patients on chemotherapy.

Methods: VACANCE is a prospective, multicenter open-label study, evaluating the immunogenicity and safety of two doses of AS03A-adjuvanted H1N1v vaccine (Pandemrix®) administered on day 1 (D1) and D21 in cancer patients receiving cytotoxic and/or targeted therapies, allocated in four groups: cytotoxic drugs each 3 weeks (3W), each 2 weeks (2W), continuously (C), or targeted therapy alone (T). Serum Hemagglutination-Inhibition Antibody Titers (HI AT) were measured at D1, D21 and D42.

Results: 64 patients were included: 52% male, mean age 65±10, 74% metastatic 85% on cytotoxic drugs, 53% on targeted therapies. Treatments were 19% 3W, 56% 2W, 11% C and 14% T. At baseline, only 3 (5.2%) patients were seroprotected. Immunogenicity results after 1st and 2nd doses are presented in Table 1. Univariate logistic regression revealed positive associations of SC at D21 with T and C and negative with 3W and 2W (p=0.03). Adjusted for age, the associations remained significant (p=0.04). No association was found at D42.

Conclusions: A single dose of AS03A-adjuvanted A/H1N1 vaccine triggers a low immune response in cancer patients on chemotherapy and seroconversion seems to be dependent on treatment type and frequency. Two doses are needed in those patients, achieving satisfying immune response.

Table 1. Immunogenicity of the H1N1 adjuvanted vaccine after one and two doses

| Group | D1 | D21 | D42 |
|-------|----|-----|-----|
| Age (yrs) | 65±10 | 65±10 | 65±10 |
| M/F | 32/32 | 32/32 | 32/32 |
| HI AT | >1:40 | >1:40 | >1:40 |

1. % of patients with HI AT ≥1:40.
2. % of patients with D1 AT <1:10 and D21 or D42 AT ≥1:40, or a D1 AT ≥1:10 and at least a 4 fold increase in D21 or D42 AT.
3. geometric mean of the ratios of the reciprocal HI AT at D21 or D42 to HI AT at D1.

P1194 Knowledge, perception, attitude and behaviour regarding influenza immunisation in Lebanon

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Objectives: The objective of the study is to determine the differences in knowledge, perception, attitude and behaviour relating to influenza immunisation between healthcare workers (HCWs) and college students. The aim is to identify factors associated with vaccine use ultimately helping in the design of vaccination campaigns. This is, to our knowledge, among the few studies of its kind to be conducted following the H1N1 pandemic, and the first in Lebanon and the region.
Methods: A self-administered survey was distributed to registered nurses at the American University of Beirut Medical Centre, a 400 bed tertiary referral centre, as well as to non-medical students of the university’s main campus. Data were collected during the 2010 influenza vaccination campaign.

| Questions                                                                 | Students (n=99) | Health-care workers (n=100) | P-value |
|--------------------------------------------------------------------------|----------------|-----------------------------|---------|
| **Generalities**                                                         |                |                             |         |
| Vaccine-Received                                                         | Never          | 49 (50)                     | 41 (37.8) |
| 1 Time                                                                  | 43 (44.4)      | 36 (35.8)                   | 0.002   |
| Regularly/Yearly                                                         | 7 (7.1)        | 27 (25.5)                   |         |
| Vaccinated recommended by physician                                    | 48 (52.3)      | 32 (35.5)                   | 0.001   |
| You’ve got me vaccinated                                                 | 96 (97.1)      | 97 (97.0)                   |         |
| Your family                                                              | 5 (5.1)        |                             |         |
| Your partner                                                            | 3 (3.0)        |                             |         |
| All of the above                                                         | N/A            | N/A                         |         |
| Other                                                                   | 16 (16.2)      |                             |         |
| **Knowledge of (in, % correct answers)**                                |                |                             |         |
| Symptoms                                                                | 91 (91.9)      | 95 (95.0)                   | 0.716   |
| Spread                                                                  | 74 (74.7)      | 95 (95.9)                   | 0.005   |
| Transmission                                                            | 54 (54.3)      | 49 (49.0)                   | 0.448   |
| Influenza period                                                        | 43 (43.4)      | 41 (38.7)                   | 0.681   |
| Prevalence                                                              | 52 (52.5)      | 51 (46.8)                   | 0.072   |
| High-risk groups                                                        | 77 (78.6)      | 93 (87.7)                   | 0.079   |
| Effectiveness of vaccination                                             | 56 (56.5)      | 45 (42.4)                   | 0.050   |
| Treatment                                                               | 65 (66.3)      | 83 (78.9)                   | 0.030   |
| I believe the recommended to HCWs                                       | 99 (99.9)      |                             |         |
| No                                                                      | N/A            | N/A                         |         |
| Don’t know                                                              | 1 (1.0)        |                             |         |
| Wrong answers ST                                                        | 28 (28.3)      | 28 (24.9)                   | 0.542   |
| **Perception**                                                          |                |                             |         |
| Self-perceived knowledge of influenza                                   | Poor           | 25 (25.2)                   | 2 (1.9)  |
| Good                                                                   | 65 (65.6)      | 63 (58.9)                   |         |
| Very good                                                               | 10 (10.1)      | 29 (27.4)                   |         |
|                                         | (1)           | (12)                        | 0.000   |
| Source of health information                                             | Physician      | 33 (33.3)                   | 30 (29.2) |
| Family/Friends                                                          | 22 (22.2)      | 20 (20.0)                   |         |
| General Media                                                           | 37 (37.4)      | 33 (32.3)                   |         |
| Specialized Medical                                                     | 7 (7)          | 8 (7.8)                     |         |
| I think that influenza has a serious disease                            | True           | 76 (76.8)                   | 98 (92.6) |
| False                                                                   | 14 (14.1)      | 7 (6.8)                     |         |
| Don’t know                                                              | 9 (9.1)        | 1 (0.9)                     |         |
| What do you think of your likelihood of getting influenza                | Not likely      | 39 (40.4)                   | 16 (15.2) |
| Likely                                                                  | 54 (54.5)      | 60 (57.1)                   |         |
| Very likely                                                             | 5 (5.2)        | 27 (24.7)                   |         |
| **Vaccination effectiveness**                                           | Leaps than 50% | 13 (13.1)                   | 40 (37.7) |
| 50-90%                                                                  | 52 (52.5)      | 47 (44.6)                   |         |
| 90-100%                                                                 | 11 (11.1)      | 6 (5.7)                     |         |
|                                       | (23)          | (12)                       | 0.000   |
| Vaccination safety                                                       | Safe           | 4 (4.1)                     | 4 (3.8)  |
| Not safe                                                                | 58 (58.6)      | 72 (67.9)                   |         |
| Very safe                                                               | 15 (15.2)      | 25 (23.3)                   |         |
| Don’t know                                                              | 22 (22.2)      | 5 (4.7)                     |         |
| Benefit from the vaccine is more than risks                             | 90 (90.7)      | 79 (75.0)                   |         |

Results: The survey was fully completed by 99/100 students and 106/125 HCWs. We found that 7.1% of students and 25.5% of HCWs received vaccination on a yearly basis (p = 0.002). In addition, 28.3% of students and 24.5% of HCWs were deemed knowledgeable of influenza illness and its vaccination by making no more than one error in answering the knowledge questions. HCWs were significantly more concerned about side effects from the vaccine than college students. Table 1 shows the differences in the survey responses among students and HCW. Multivariable analysis revealed that knowledge was the only factor independently associated with vaccine receipt on a yearly basis among HCWs (OR=5.8, p = 0.016).

Conclusion: Our findings suggest that while HCWs have high self-perceived knowledge, they in fact demonstrate comparable knowledge to healthy college students. Also, although they recognize the potential seriousness of the disease, they show substantial scepticism about the effectiveness and safety of the vaccine. Their main motive for vaccination is self protection rather than the intended objective of protecting their patients. Compliance to yearly vaccination is strikingly low albeit comparable to reported numbers from European countries but lower than the USA. The only factor which significantly affected compliance to yearly vaccination among HCWs on multivariable analysis was knowledge. Vaccination campaigns should therefore be directed towards raising education, keeping in mind the importance of physician’s recommendations in the clinics, the effect of which was non negligible.

P1195 Individual determinants of seasonal and pandemic A/H1N1 influenza vaccination among healthcare workers
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Objectives: To compare the individual determinants of seasonal (SI) and pandemic (A/H1N1) (PAI) influenza vaccination among health-care workers (HCWs) during the 2009–2010 vaccination campaign.

Methods: A one-day questionnaire-based survey was conducted in 5 teaching hospitals of the Paris area between June, 25th and July, 8th, 2010. Anonymous participation was proposed to all HCWs on duty in emergency departments and wards on study days. Acceptance or refusal of SI and PAI vaccines were analyzed on the basis of socio-professional characteristics, history of SI vaccination, previous contacts with influenza cases, and 34 propositions elaborated by using the Health Belief Model (HBM) methodology. Vaccinated and non-vaccinated HCWs’ answers to HBM items were integrated in a Classification And Regression Tree (CART) algorithm.

Table 1. Factors associated with health-care workers’ vaccinations against seasonal influenza and pandemic A(H1N1) influenza: results of multivariate analysis

| Odd's Ratio | 95% Confidence Interval | P-value |
|-------------|-------------------------|---------|
| **Seasonal influenza (SI) vaccination** | | |
| History of SI vaccination during the 3 previous winters | | |
| Every year | 0.44 | [0.263 – 0.997] | >0.0001 |
| Twice | 0.71 | [0.410 – 1.11] | 0.014 |
| Professional category | | |
| Medical | 0.92 | [0.667 – 1.28] | 0.005 |
| **Pandemic (A/HIN1) influenza vaccination** | | |
| History of SI vaccination during the 3 previous winters | | |
| Every year | 0.73 | [0.47 – 1.14] | >0.0001 |
| Twice | 0.65 | [0.40 – 1.09] | 0.096 |
| Professional category | | |
| Medical | 0.95 | [0.6 – 1.5] | 0.723 |

Results: A total of 1977 HCWs participated in the study, including 1576 (80%) paramedical (PM) and 401 (20%) medical (M) HCWs. Vaccination rates of PM-HCWs and M-HCWs were 29% and 58% for SI, and 20% and 70% for PAI, respectively (P < 0.0001 for both comparisons). History of SI vaccination was strongly associated with both SI and PAI vaccines acceptance during the current campaign (Table 1). HBM analysis showed that, for PM-HCWs, self-protection and protection of the personnel are the leading determinants of vaccine acceptance. Willingness to protect the patient or contribute to limit the spread of influenza, and fear of side effects were not discriminatory for SI or PAI vaccines’ decision to accept or decline either SI or PAI vaccines. For M-HCWs, the leading determinants of
vaccination were willingness to be a model for other HCW for SI, and self-protection for PAI. Among HCWs vaccinated against PAI, 85% planned to get vaccinated on subsequent winters. Conversely, among HCWs vaccinated against PAI, 55% were reluctant or opposed to revaccination in case of a novel influenza pandemic.

**Conclusion:** SI and PAI vaccination rates were very low for PM-HCWs. Determinants of these 2 vaccinations differed between PM-HCWs and M-HCWs, suggesting a benefit of separate information campaigns to improve vaccination rates during subsequent SI epidemics or influenza pandemics. However, the association between refusal of SI vaccination during previous campaigns and refusal of SI and PAI vaccines during the 2009–2010 winter raises concerns about the efficacy of such educational programs, which may plead for the advent of mandatory vaccination.

**Objectives:** The effects of oseltamivir (Tm) have been documented among patients with mild influenza in the community. We sought to determine its effects on patients with 2009 H1N1 influenza requiring hospitalization, by comparing early vs. late initiation of Tm.

**Methods:** Retrospective cohort study, including all adult patients with 2009 influenza A(H1N1) documented by RT-PCR of respiratory samples at the national laboratory, hospitalized in 3 hospitals in Israel after 22 July 2009. During this period the national policy was to hospitalize only patients with suspected influenza complications or at risk. Data were collected through patient chart review by a single investigator. Early treatment was defined as start of Tm within 48hrs of symptom onset, documented prospectively by clinicians. The outcome assessed was influenza complications defined as: pneumonia, saturation <90%, ICU admission, need for hemodynamic support or in-hospital death. We assessed the effect of early Tm on the outcome adjusting for other significant variables through multivariable logistic regression. In sensitivity analyses we assessed the effects of Tm administered within or after 48 hrs of admission on outcomes post-admission (excluding outcomes present on admission).

**Results:** Out of 506 patients hospitalized with 2009 influenza A(H1N1), 449 patients treated with Tm were included. Early Tm was significantly associated with male sex, pregnancy/post-partum, high temperature, myalgia, lower lymphocyte count, lower LDH and specific hospital. Significantly more patients receiving Tm≤48hrs after symptom-onset experienced complications (150/260, 57.7% Tm≤48 hrs vs. 67/189, 35.4% Tm>48 hrs), p < 0.001. Similar results were observed when excluding outcomes present on admission (42/260, 16.2% vs. 15/189, 7.9%, respectively), p = 0.01. In the adjusted analysis, late Tm initiation remained significantly associated with complications, OR 2.25 (95%CI 1.45–3.51). Other risk factors independently associated with the outcome (p < 0.05) were dyspnea, SOFA score on admission and the specific hospital. Rhiinorrhea was protective. The model was highly predictive (ROC area 0.75, 95%CI 0.71–0.80). Initiation of Tm >48hrs after admission was significantly associated with complications whose onset was after admission, adjusted OR 4.09 (1.55–10.80), ROC area 0.77 (0.69–0.84).

**Conclusions:** Within the limitations of an observational study, early initiation of Tm was associated with fewer severe complications.

**Objective:** The benefit of early treatment with oseltamivir in hospitalised patients with documented 2009 influenza A/H1N1: retrospective study

**V Hiba* (Tel Aviv, IL)**

**Objectives:** A comparison of severity scores for identifying adult patients with severe 2009 pandemic influenza A/H1N1 pneumonia

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**Objective:** To assess the performance of severity scores for community-acquired pneumonia in adult patients with 2009 pandemic influenza A/H1N1.

**Methods:** From September 2009 to February 2010, all adult patients hospitalized with 2009 pandemic influenza A/H1N1 pneumonia at 17 Korean teaching hospitals were included. Clinical and laboratory findings were evaluated to the attack rate of HCP by the 2009 H1N1 influenza virus during the 2009 pandemic influenza season in Korea.

**Method:** This study was conducted during the 2009 H1N1 influenza season from September to December 2009 at nine university affiliated hospitals in Korea. Infection of HCP with H1N1 virus was confirmed by real-time or multiplex reverse transcriptase polymerase chain reaction (RT-PCR). The study subjects of HCP were classified into four groups based on job type: Group I (physicians), Group II (nurses and nurse/s aides), Group III (technicians, therapists, et al.), and Group IV (administrative workers and others employees not directly involved in patient care but having the potential of being exposed to infectious agents). HCP infected with H1N1 virus were asked to fill out a questionnaire, which included job type, method of diagnosis, facility type, history of contact with patients infected by H1N1 virus, vaccination status, place of isolation, and use of personal protective.

**Results:** A total of 328 HCP (31.4% male) was infected with H1N1 virus at the nine study centers. The highest attack rate was in physicians, followed by nurses and nurses’ aides. Transmission occurred primarily after contact with outpatients (36.6%), followed by contact with inpatients (28.9%). The attack rate of infection was highest in group I (2.87%, 95% confidence interval (CI) 2.29–3.46), followed by group II (2.80%, 95% CI 2.37–3.23), group III (2.13%, 95%CI 1.40–2.86), and group IV (1.00%, 95%CI 0.72–1.29). The mean interval between exposures to a patient with suspected H1N1 and onset of clinical symptoms was 3.27±2.51 days (group difference p = 0.303 by ANOVA). The mean interval between initial clinical symptoms and diagnosis was 2.46±3.71 days (p = 0.567). Most (90.7%) of the infected HCP never or intermittently used an N95 mask during contact with patients. Surgical masks were used always or usually used by 60.1% of the subjects. Peak incidence of the H1N1 infection among HCP preceded that among general population (Figure 1).

**Conclusion:** Among HCPs, physicians, nurses, and nurses’ aides were at greatest risk of H1N1 infection. HCP should be more vigilant and protect themselves with appropriate personal protective equipments during the influenza season.

**2009 H1N1 influenza infection in Korean healthcare personnel**

**C.S. Lee*, J. Yeom, J.H. Lee, I.G. Bae, W.S. Oh, C. Moon, K.H. Park, J.H. Lee, E.S. Kim, Y. Kwak, B.N. Kim (Jeonju, Seoul, Jinju, Chunchon, Busan, Gwangju, Iksan, Goyang, KR)**

**Objective:** Healthcare personnel (HCP) can acquire influenza and transmit it to patients and other hospital staff. The aim of this study was to evaluate the attack rate of HCP by the 2009 H1N1 influenza virus during the 2009 pandemic influenza season in Korea.

**Method:** This study was conducted during the 2009 H1N1 influenza season from September to December 2009 at nine university affiliated hospitals in Korea. Infection of HCP with H1N1 virus was confirmed by real-time or multiplex reverse transcriptase polymerase chain reaction (RT-PCR). The study subjects of HCP were classified into four groups based on job type: Group I (physicians), Group II (nurses and nurse/s aides), Group III (technicians, therapists, et al.), and Group IV (administrative workers and others employees not directly involved in patient care but having the potential of being exposed to infectious agents). HCP infected with H1N1 virus were asked to fill out a questionnaire, which included job type, method of diagnosis, facility type, history of contact with patients infected by H1N1 virus, vaccination status, place of isolation, and use of personal protective.

**Results:** A total of 328 HCP (31.4% male) was infected with H1N1 virus at the nine study centers. The highest attack rate was in physicians, followed by nurses and nurses’ aides. Transmission occurred primarily after contact with outpatients (36.6%), followed by contact with inpatients (28.9%). The attack rate of infection was highest in group I (2.87%, 95% confidence interval (CI) 2.29–3.46), followed by group II (2.80%, 95% CI 2.37–3.23), group III (2.13%, 95%CI 1.40–2.86), and group IV (1.00%, 95%CI 0.72–1.29). The mean interval between exposures to a patient with suspected H1N1 and onset of clinical symptoms was 3.27±2.51 days (group difference p = 0.303 by ANOVA). The mean interval between initial clinical symptoms and diagnosis was 2.46±3.71 days (p = 0.567). Most (90.7%) of the infected HCP never or intermittently used an N95 mask during contact with patients. Surgical masks were used always or usually used by 60.1% of the subjects. Peak incidence of the H1N1 infection among HCP preceded that among general population (Figure 1).

**Conclusion:** Among HCPs, physicians, nurses, and nurses’ aides were at greatest risk of H1N1 infection. HCP should be more vigilant and protect themselves with appropriate personal protective equipments during the influenza season.
at initial presentation were used to calculate severity scores using the pneumonia severity index (PSI), CURB, and CURB-65. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and the area under ROC curve (AUC) were compared for ICU admission or in-hospital death.

Results: A total of 260 patients were diagnosed with 2009 pandemic influenza A/H1N1 pneumonia, confirmed by real-time RT-PCR and chest radiography. Of these, 45 patients (17.3%) were admitted to ICU, and 27 (10.4%) died in hospitals. AUC (0.750 (95% confidence interval (CI), 0.672–0.829)) of PSI classes IV+V was higher for ICU admission or in-hospital death than those of CURB score of 2 or more (0.688 (95% CI, 0.599–0.777)) or CURB-65 score of 3 or more (0.566 (95% CI, 0.475–0.657)). The sensitivity, specificity, PPV, and NPV of PSI classes IV+V were 68.5% (95% CI, 54.3–80.1), 81.6% (95% CI, 75.4–86.5), 49.3% (95% CI, 37.7–61.0), and 90.8% (95% CI, 85.4–94.4), respectively. CURB-65: Ag+ (99.9% values). In samples with a high viral load (>26 IFs), the three cell lines

In conclusion, V.A.C. Silver® GranuFoam quickly reached and maintained levels of Ag+ in ranges that were bactericidal on the microorganism tested.

**Objective:** To evaluate the in vitro antimicrobial properties of a polyurethane foam used for treatment and prevention of wound infections with a novel release method of silver ions (Ag+).

**Methods:** V.A.C. GranuFoam Silver® (KCi Spain) was tested on Acinetobacter baumannii, Pseudomonas aeruginosa, Serratia marcescens, Klebsiella pneumoniae, Escherichia coli, Proteus mirabilis, Staphylococcus aureus, Enterococcus faecium, Streptococcus pyogenes and Corynebacterium minutissimum.

Time-kill study: Based on the E2149–10 method of the American Society for Testing and Materials International, 0.10g of silver foam were introduced in 25ml Phosphate Buffered Saline (PBS) isotonic medium (chlorine concentration 139 mM) with a minimum microbical concentration of 10–5 CFU/ml. Then, it was incubated with agitation at 36°C and 0.1ml aliquots were drawn and cultured for colony counting on Columbia agar.

Silver release study: The amount of silver ion released was determined in a PBS sample in contact with the foam at 0 min, 30 min, 1h, 3h, 6h, 12h and 24h by Inductively Coupled Plasma Mass technique.

**Results:** Ag+ release presented an exponential curve with a sharp increase and a plateau after 3 hours that was stable up to 24 hours with Ag+ levels of 0.22 to 0.24 mg/l. After 3h, there was a reduction over 99.9% of the Gram-negative initial inoculum, except for E. coli which reached 92.5%. Enterobacteria showed greater initial tolerance than non-fermentative Gram-negative rods, which had a very sharp decrease in viable cell after contact with Ag+: 99.9% in two hours, higher in the case of P. aeruginosa: 98.1% in the first hour. However, a very small subpopulation (<1 CFU/ml) of S. maltophilia was able to survive 8 hours. In the case of the Gram-positive, reduction over 99% was achieved within 2h for both S. pyogenes and C. minutissimum, 6 hour for S. aureus and 14h for E. faecium, for which a small subpopulation (<1 CFU/ml) was able to survive up to 24 hours. No decrease was observed in the controls.

**Conclusions:** Our data agree with previous studies that showed a higher susceptibility to Ag+ in Gram-negative versus Gram-positive. This could be explained by the thicker layer of peptidoglycan that should confer Gram-positive some protection against Ag+.

In conclusion, V.A.C. Silver® GranuFoam quickly reached and maintained levels of Ag+ in ranges that were bactericidal on the microorganism tested.

**Objective:** To prospectively evaluate three different commercial cell lines in the isolation of pandemic influenza A/H1N1 from respiratory samples

**Methods:** Over a 3 weeks period we studied the nasopharyngeal aspirates sent to the virology laboratory for the diagnosis of pandemic influenza. The samples were screened for IAp by RT-PCR (OneStep Real-Time; Applied Biosystems, USA). The comparative study was made with 72 positive samples by RT-PCR. Each sample was homogenized with 3 ml of PBS. For the shell vial culture 200 µl of the sample was inoculated into a MDCK, Vero and LLC-MK2 vials (Vircell, Granada, Spain). The vials were then centrifuged at 3500 rpm × 15 min. The vials were incubated for three days at 36°C and subsequently stained with an indirect immunofluorescence assay by a monoclonal antibody against the influenza A virus (clone IA-52) (Monofluokit Influenza, BioRad, France). Time-kill study: Based on the E2149–10 method of the American Society for Testing and Materials International, 0.10g of silver foam were introduced in 25ml Phosphate Buffered Saline (PBS) isotonic medium (chlorine concentration 139 mM) with a minimum microbical concentration of 10–5 CFU/ml. Then, it was incubated with agitation at 36°C and 0.1ml aliquots were drawn and cultured for colony counting on Columbia agar.

Silver release study: The amount of silver ion released was determined in a PBS sample in contact with the foam at 0 min, 30 min, 1h, 3h, 6h, 12h and 24h by Inductively Coupled Plasma Mass technique.

**Results:** Ag+ release presented an exponential curve with a sharp increase and a plateau after 3 hours that was stable up to 24 hours with Ag+ levels of 0.22 to 0.24 mg/l. After 3h, there was a reduction over 99.9% of the Gram-negative initial inoculum, except for E. coli which reached 92.5%. Enterobacteria showed greater initial tolerance than non-fermentative Gram-negative rods, which had a very sharp decrease in viable cell after contact with Ag+: 99.9% in two hours, higher in the case of P. aeruginosa: 98.1% in the first hour. However, a very small subpopulation (<1 CFU/ml) of S. maltophilia was able to survive 8 hours. In the case of the Gram-positive, reduction over 99% was achieved within 2h for both S. pyogenes and C. minutissimum, 6 hour for S. aureus and 14h for E. faecium, for which a small subpopulation (<1 CFU/ml) was able to survive up to 24 hours. No decrease was observed in the controls.

**Conclusions:** Our data agree with previous studies that showed a higher susceptibility to Ag+ in Gram-negative versus Gram-positive. This could be explained by the thicker layer of peptidoglycan that should confer Gram-positive some protection against Ag+.

In conclusion, V.A.C. Silver® GranuFoam quickly reached and maintained levels of Ag+ in ranges that were bactericidal on the microorganism tested.
different mixtures and concentrations of the used organic acids. As a novel combination, FA treatments as combinations with AA, LA, and PA, especially FA with LA, reduced bacterial loads greatly, up to 3 logs cfu/ml and eradicated inoculated bacteria. *E. coli* O157:H7 and *S. aureus* completely within 3–6 days. This reduction was higher than that incurred by other combinations. Significantly, higher log reductions by the used organic acids were obtained for *S. aureus* than for *E. coli* O157:H7.

**Conclusion:** It was concluded that the combination of LA and FA treatment was a highly promising, feasible, and economical method of decontamination of meat surface from both *E. coli* O157:H7 and *S. aureus* bacteria. Moreover, it is safe if compared with other approaches.

**P1202** Multi-resistant bacterial colonisation of reusable hospital tourniquets
A. Pinto*, T. Phan, G. Sala, E. Cheong, S. Stiarakas, T. Gottlieb (Sydney, AU)

**Objective:** To determine the prevalence of microbial colonisation of reusable venesection tourniquets in a Sydney teaching hospital.

**Methods:** One hundred reusable tourniquets were collected from critical care areas, general wards, and non-clinical areas. The study was performed over 11 weeks. Tourniquets were placed in enrichment media (BHI broth), incubated overnight and subcultured onto general and selective media. Isolates were divided into communal, “potentially significant” bacteria and multi-resistant organisms (MROs). MROs were defined as MRSA, VRE, MBL and ESBL producing enterobacteriaceae. Van B positive *Enterococcus faecium* isolates were typed using DiversiLab rep-PCR system.

**Results:** The overall bacterial colonisation rate was 78% (78/100). Seventeen grew commensal bacteria, viz. coagulase negative staphylococci and Bacillus species. Ten grew non multi-resistant Gram-positive organisms – methicillin sensitive *Staphylococcus aureus* (1) and *Enterococcus* species (9). Non multi-resistant Gram-negatives grew in 38 specimens, viz: *Pseudomonas* species (13) and coliforms (26). MROs were found on 25% of tourniquets, including three from MRO isolation rooms. An IMP-4 positive *Enterobacter cloacae* and an ESBL *E. cloacae* were isolated from a single specimen. Typing revealed five predominant VRE genotypes. MRSA and VRE were isolated together from nine tourniquets, and 24 tourniquets grew either one of these. Six of nine tourniquets collected from ICU grew at least one MRO. MROs were isolated consistently throughout the study period from a wide variety of hospital locations, including general wards, ICU, burns unit, operating theatre anaesthetic bay and the blood collection unit.

**Conclusion:** Reusable tourniquets are frequently colonised with MROs and may be a potential source of cross-transmission. They are also a surrogate marker for environmental colonisation and deficiencies in hospital cleaning. Using broth enrichment, 24% harboured either MRSA or VRE. Continued use of reusable tourniquets may not be justified in the current hospital setting.

**P1203** Influence of temperature and chemical decontamination on the prevalence of *Legionella pneumophila* in hot water
C. Colomba*, G. Senn, A. Bürgel, C. Rüf (Zurich, CH)

**Objectives:** To demonstrate the impact of water temperature and chemical decontamination on prevalence and concentration of *Legionella pneumophila* (L.p.) in hot water of high risk medical wards (oncology, radiation therapy, organ transplantation units and burn ICU).

**Methods:** Measurement of *L. pneumophila* concentration and temperature of hot water of patient care units of the University Hospital of Zurich monthly during the baseline period (A; 2008/2009), a preparatory period with reduced water temperature (B; February – June 2010), the phase prior to chemical decontamination (C; July – September 2010). Chemical decontamination with copper-silver and chlorine dioxide was started in separate hospital areas in October 2010 (period C). Concentrations of chemicals used were measured regularly.

**Results:** The proportion of positive peripheral sites as well as the concentration of L.p. increased significantly during period B (table), parallel to a significant reduction of the median temperature (p < 0.001). Serotype 1 was the predominant strain. After raising the temperature of the outlets to a median of 62 (period C), respectively 60°C during period D, the rate of positive samples as well as the concentration of L.p. dropped significantly (p < 0.001). Following the introduction of chemical decontamination in October 2010, only one sample was positive for L.p. This sample was found in a unit with copper-silver ionisation. The median residual concentration on peripheral outlets of chlorine dioxide (samples n = 29) was 0.02 mg/l. Between October and December 2010 we noted a significant decrease of mean (7.7 to 2.9 μg/l) and median (7.7 to 2.75 μg/l) concentration of silver (p < 0.001), but not of copper or chlorine dioxide. All measured concentrations were below the upper threshold concentration required for safety of potable water.

**Conclusion:** Our data show a strong correlation between low water temperature and the appearance of *L. pneumophila* as well as the positive effect of adequate water temperature (55°C) and the application of chemical decontamination on the prevalence and concentration of *L. pneumophila* in a hospital hot water system. Chemical decontamination enhances the effect of an increase in water temperature on the reduction of *Legionella* concentration. Further follow-up is needed to determine the long term effect of these measures.

| Time Period | 2008 | 2009 | 2010 |
|-------------|------|------|------|
|             | A    | B    | C    |
| Number of samples | 168  | 53   | 62   |
| Temperature min (°C) | 60   | 60   | 60   |
| Temperature max (°C) | 62   | 24   | 24   |
| Number of sites with water temperature >57°C (%) | 12 (7) | 10 (7) | 4 (7) |
| Positive L. pneumophila (%) | 32 (19) | 105 (66) | 11 (6) |
| CFU/L, median (min–max) | 8 × 10⁴–10⁵ | 2250 × 10⁴–10⁵ | 0–10⁴ | 0–10⁴ |
| CFU/L, average (SD) | 1.02 (1.91) | 6957 (6957) | 1075 (5647) | 47 |

**P1204** Impact of a single-room design on the spread of multidrug-resistant bacteria in an intensive care unit
T. Halaby*, R. de Wit, N. Al Naemi (Enschede, NL)

**Objectives:** Cross infection has been shown to occur more frequently in the intensive care unit (ICU) than in other wards of the hospital. This may be enhanced by several factors including heavy colonization of environment and ICU patients, invasive procedures such as mechanical ventilation and intravascular devices and crowding of both patients and health care workers (HCW). In such an ICU, high prevalence of multi-resistant (MDR) bacteria was observed despite extensive infection control measures, which led to the move of the ICU into a new single room designed facility. The aim of this study is to report the effect of this move on the prevalence of MDR-bacteria.

**Methods:** Study period is between January 2001 and November 2010. The ICU consisted of 18 beds, of which 5 in single ventilated rooms with ante-room and the remaining 13 in an open bay. Infection control measures included standard barrier nursing techniques, isolation of patients when indicated, education and reinforcement of hand hygiene among HCW, temporary closure of the unit for architectural modifications, and the introduction of selective decontamination of digestive tract (SDD). In April 2009 the whole ICU was converted to a new unit with the same number of beds but with single ventilated rooms with an ante-room, in the vicinity of the original unit. Nurse-patient ratio did not change. No changes were introduced in infection control protocols. Surveillance cultures have been obtained twice weekly from all admitted patients, before and after conversion. MDR-bacteria were defined decontamination national guidelines.

**Results:** Despite efforts, high prevalence of MRD bacteria continue to occur in the original facility. Only after transfer to the new unit could a sharp decrease in the prevalence be observed (Fig 1).

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1. **Authorship:** C. Colomba, G. Senn, A. Bürgel, C. Rüf (Zurich, CH)
2. **Table:** Time Period: 2008, 2009, 2010; Number of sites: A, B, C; Positive L. pneumophila: 32 (19), 105 (66), 11 (6); CFU/L, median (min–max): 8 × 10⁴–10⁵, 2250 × 10⁴–10⁵, 0–10⁴; CFU/L, average (SD): 1.02 (1.91), 6957 (6957), 1075 (5647).
3. **Figure:** Fig 1: Illustrates the impact of a single-room design on the spread of multidrug-resistant bacteria in an intensive care unit (ICU).
4. **Figure Legend:** No significant change in the prevalence of MDR bacteria observed after the move to the new single room designed facility.
5. **Conclusion:** The move to a single-room design significantly reduced the prevalence of MDR bacteria in the ICU.
Conclusions: Transformation of an ICU with an open bay to a single-room unit resulted in significant decline in the prevalence of MRD-bacteria. Major factors that may contribute to this effect are inherent in the design, and enable the block of contact routes of bacterial transmission between patients. These factors include decreased number and movements of persons near the patient, the feasibility of and adherence to adequate barrier patient care techniques before and after patient contact, and the possibility of adequate separation of medical waste.

Results: Baseline evaluation of 2,799 surfaces in 205 operating theatres in 12 highly diverse hospitals found that an average of 23.3% of recommended surfaces (hospital range 9–53%) had been disinfection cleaned according to policy. Only three types of objects in two hospitals were relatively well (>80%) cleaned prior to interventions. Of particular concern was the fact that only 25% of primary field lights and 15.5% of secondary field lights were cleaned. Following education and objective performance feedback to the Environmental Services staff along with multi-disciplinary collaborative efforts involving Infection preventionists and administrative champions, overall thoroughness of cleaning significantly improved to 70% (p < 0.0001) (Figure) with 61 types of objects in 10 hospital being cleaned >80% of the time.

Conclusion: The first multi-site objective evaluation of the thoroughness of operating theatre terminal cleaning demonstrated both unanticipated shortcomings in disinfection cleaning practice as well as the ability to improve such cleaning using educational interventions along with ongoing objective feedback to the environmental services staff in all participating hospitals.

Objective: Environmental bacteria, particularly coagulase negative staphylococci, are the most common pathogens associated with deep surgical site infections in all forms of implantation surgery. In light of this problem and in the context of our evolving understanding of the healthcare epidemiology of these organisms, optimization of operating theatre environmental hygiene is imperative. Given our previous studies demonstrating significant opportunities to improve hygienic cleaning practice in patient rooms, a multi-institutional process improvement project was implemented to evaluate and enhance compliance with widely accepted disinfection cleaning standards in the operating theatre.

Methods: An invisible fluorescent targeting method was used to covertly evaluate the cleaning of twelve standardized high-touch implantation operating theatre surfaces. Rooms were marked following terminal cleaning and re-evaluated after several implantation surgeries had taken place in the room and it had been temporarily cleaned on at least two occasions (after the last case of the day). Following process improvement interventions which incorporated specific performance results, the impact of the interventions was objectively evaluated.

Figure: The log-reduction (RF) of HPV against spores from three Clostridium difficile strains on two different surfaces. Horizontal bars represent the mean.

Results: Baseline evaluation of 2,799 surfaces in 205 operating theatres in 12 highly diverse hospitals found that an average of 23.3% of recommended surfaces (hospital range 9–53%) had been disinfection cleaned according to policy. Only three types of objects in two hospitals were relatively well (>80%) cleaned prior to interventions. Of particular concern was the fact that only 25% of primary field lights and 15.5% of secondary field lights were cleaned. Following education and objective performance feedback to the Environmental Services staff along with multi-disciplinary collaborative efforts involving Infection preventionists and administrative champions, overall thoroughness of cleaning significantly improved to 70% (p < 0.0001) (Figure) with 61 types of objects in 10 hospital being cleaned >80% of the time.

Conclusion: The first multi-site objective evaluation of the thoroughness of operating theatre terminal cleaning demonstrated both unanticipated shortcomings in disinfection cleaning practice as well as the ability to improve such cleaning using educational interventions along with ongoing objective feedback to the environmental services staff in all participating hospitals.

Objectives: Clostridium difficile spores present a particular challenge to effective decontamination. They are shed in high numbers by infected patients and they are resistant to desiccation and some disinfectants. We explored the in vitro activity of Hydrogen Peroxide Vapour (HPV) against several strains of C. difficile spores on common hospital materials.

Methods: The sporicidal capacity of HPV was tested using a spore-carrier test. The spore carriers used were small (2 cm²) pieces of vinyl polychloride (PVC) (representative of the room’s floor) or laminate (representative of the room’s furniture). Three strains of C. difficile were tested: a toxotype 0 reference strain (VPI 10463), a ribotype 027 historical strain (CD 196) and a current ribotype 027 epidemic strain (1067). For each assay, the 3 strains were tested simultaneously but separately on the 2 different spore carriers. Each assay was repeated 4 times. C. difficile spores were obtained by using the method described by Wullt et al. (Infect Control Hosp Epidemiol 2003; 24:765–768). Each carrier was experimentally contaminated with 100 microlitres of spore suspension to achieve an inoculum of approximately 10⁸ spores per carrier. After disinfection, test and control spore carriers were placed in 3 mL of neutralizing solution, vortex mixed and sonicated for 5 minutes. The spores from test and control spore carriers were counted by serial dilution on selective medium containing taurocholate 0.1%, with a threshold of sensitivity of 3 spores/spore carrier. The reduction in initial concentration was calculated as the difference between the number of spores on control carriers and the number on test carriers after HPV exposure.
Results: The concentration of spores on unexposed control carriers ranged from a mean of 4.7–6.9 log10 spores/carrier for the three strains. No C. difficile was cultured from carriers exposed to HPV (figure). Conclusion: HPV is effective for the inactivation of a range of C. difficile spores from common hospital materials in vitro. HPV should be considered for the eradication of C. difficile spores from the hospital environment.

P1207 Cross-transmission audit of environmental surfaces, clinical equipment and patient: who touches what?
S.J Smith, V. Young, S.J Dancer*, C. Robertson (Glasgow, UK)

Background: Most patients probably acquire pathogens from contaminated hands. We conducted an audit of hand-touch events that occur during patient care throughout the day.

Methods: Cross-transmission pathways were monitored during 40x30 minute covert observation periods (20 summer and 20 winter periods), beginning with entry of staff into one room on an acute ward. Hand-touch activities were recorded within the following categories: hand-hygiene on entry; hand contact with near-patient sites; hand contact with patient; hand contact with clinical equipment; hand-hygienic on exit; and hand contact with sites outside the room.

Results: There were 104 entries during the study periods: 77 clinical staff (59 nurses; 18 doctors), 21 cleaning and catering staff, one pharmacist and five relatives. Overall compliance with hand hygiene among clinical staff before and after entry was 25% (38/154), with markedly higher compliance during 20 summer (47%; 32/68) as opposed to 20 winter periods (7%; 6/86). Over half (58%; 45/77) of clinical staff touched the patient; nearly half (48%; 37/77) handled patient notes; 25% touched the bed (19/77); and 10% (8/77) touched the patient console. Most frequently handled equipment inside the room were IV drip (27%; 21/77) and BP stand (13%; 10/77); and computer (25%; 19/77), notes trolley (23%; 18/77) and telephone (22%; 17/77) outside the room.

Conclusions: Monitoring the sequence of hand-touch events during patient care has highlighted potential microbial transmission pathways. Understanding the most frequent interactions between hands and surfaces could target high risk sites for cleaning, since hand hygiene compliance remains poor during covert observation.

P1208 Absence of Clostridium difficile stool carriage in asymptomatic volunteers
M. Hell*, K. Sickau, G. Chmelizek, J.M. Kern, M. Maass, S. Huhulescu, F. Allerberger (Salzburg, Vienna, AT)

Objectives and Background: Clostridium difficile emerged as important nosocomial pathogen. It is considered a leading cause of hospital-acquired diarrhea and can cause a potentially fatal illness. Up to now there are only case-reports of symptomatic Health-Care-Workers (HCW) in the literature and only one report demonstrating a transmission of C. diff from patient to HCW on a molecular basis. Therefore we initiated a prospective study to evaluate the prevalence of asymptomatic C. difficile stool carriage among healthcare workers with direct patient contact at a single university hospital compared to non-healthcare workers to assess the risk for HCWs acquiring Clostridium difficile Infection (CDI).

Methods: The study population consisted of 113 healthy HCWs (without any previous history of diarrhea) of different clinical departments with a high incidence of CDI in inpatients who were considered to be at high risk for acquiring C. difficile. The 128 healthy controls were purchased from the administration department of a big Food Company and from frozen stool samples. Both groups were comparable in age- and sex-distribution. During the time period April – July 2010, in total 241 stool specimens were collected. Stool samples were tested within 24 hours for toxigenic culture of C diff using selective culture media (Cycloserine-cefoxitin agar plates (bioMérieux, Vienna, Austria)) and incubated for 48 hours at 35±2 °C under anaerobic conditions. 51% of stool samples (58/113) of the study population and all stool samples of the controls (n=128) were consecutively sent to the National Reference Laboratory for C. difficile at the Austrian Agency for Health and Food Safety (AGES) in Vienna for further confirmation through cultivation by broth enrichment technique.

Results: Both investigated study-groups (n-total= 241) were negative for Clostridium difficile by culture techniques (both direct plating and broth enrichment method).

Conclusion: We conclude therefore that healthy HCWs are probably not at risk for acquiring C diff spores from CDI-patients contacts and are themselves no risk for spreading C. diff spores in health-care facilities. Data about C diff carriage in the community (up to 3%) possibly reflects an overestimation.

P1209 Do silver-coated needleless intravascular catheter connectors reduce microbial contamination?
T.J. Karpanen*, A.L. Casey, P. Nightingale, M. Cook, T.S. Elliott (Birmingham, UK)

Objective: There have been several reports of elevated catheter-related bloodstream infection (CR-BSI) rates associated with the use of some needleless intravascular connectors. A connector with a unique silver coating has been developed. The objective of this trial was to compare the rate of microbial contamination associated with this antimicrobial connector to a control non-antimicrobial equivalent.

Methodology: Haemato-oncology patients who required a central venous catheter (CVC) were recruited into the study and were randomised to receive either the silver or control connector. Every 4 days the silver or control connectors were removed. To assess external contamination of the connectors, the silicone compression seals were imprinted onto an agar plate. The presence of internal microbial contamination was investigated by sampling in three stages to remove planktonic, loosely bound bacteria and bacterial biofilms from the connectors. All patients were monitored for symptoms of CR-BSI.

Results: No episodes of CR-BSI were observed during the study period. 36 of 119 (30.3%) silver connectors were externally contaminated compared with 41 of 117 (35.0%) controls (OR=0.8, 95%CI=0.47–1.39, P=0.49). 31 of 119 (26.1%) silver connectors were internally contaminated compared with 55 of 117 (47.0%) controls (OR=0.40, 95%CI=0.23–0.69, P=0.001). There was also a significantly lower level of internal microbial contamination in the silver connectors (P=0.001). Some connectors had blood residue in the internal interstitial space despite optimal flushing. However, this did not have any effect on the internal contamination rate.

Conclusion: Significantly fewer silver connectors were internally contaminated when compared to controls. This was probably related to the antimicrobial activity of the silver within the connectors. Such a difference was not seen on the external compression seal which is not coated with silver. Blood residue was observed in some connectors post-flushing but this was not associated with an increased internal microbial contamination rate. This highlights the advantage of using transparent connectors which allow internal inspection and facilitates device change if blood is detected. Although no episodes of CR-BSI were observed during the study period, the reduction in internal contamination associated with the silver connectors may lead to a reduced risk of such infection.

P1210 Disinfection of CVC catheters with ultraviolet-C light
T. Bogosic, T. Bjarnsholt, A. Nielsen, J. Bak* (Roskilde, Copenhagen, DK)

Objectives: To develop a method and a device based on UVC light emitting diodes (LED’s) that can be used to disinfect the intra-luminal part of catheters, as a mean to prevent biofilm to establish in central venous catheters (CVC’s). CVC’s are normally flushed with 0.9% saline solutions after they have been used for drawing blood or administering drug and nutrition before anti-coagulating agents are injected. Saline solutions are transparent for UVC light and the germicidal light can
then be transmitted from the Luer connector to the distal end of the catheter tube.

**Methods:** The device were tested on 20 cm tubes (inner diameter 4 mm) made of ethylene vinyl acetate (EVA) contaminated with 10^3 CFU/ml solutions of *E. coli*, *P. aeruginosa* and *S. aureus*. The bacteria in suspension were inoculated in the tubes for 3 hours prior to UVC disinfection. The device was connected to the Luer catheter hub and the UVC light was launched into the connector opening. The UVC fluence rates at the tube entrances were between 0.41 and 4.11 J/cm². After UVC treatment (2–20 min) the number of CFU’s was determined both in treated and control samples by plating aerobically at 37 °C for 24 hours. All tests were done in triplicate. Detection limit was less than 3 CFU/ml.

**Results:** We found for all three bacterial species that 2 min of UVC treatment were sufficient 100% disinfection of the tubes. The germicidal effect of the UVC device is reduced substantially when approaching the distal end of the tubes. This attenuation of the light is caused primarily by the absorption of UVC light in the wall of the tube material. We were able to measure the reduced fluence (dose) delivered to the distal end of the 20 cm tubes. During the short time of treatment (2 min) a dose (12 mJ/cm²) sufficient to kill all bacteria in the lumen were delivered to the distal end.

**Conclusion:** The UVC device presented here has several features that make it applicable in the clinic. The doses required for 100% kill of planktonic bacteria can be delivered within a few minutes. We envision the method to be applied as part of a preventative approach each time the catheter has been used to avoid biofilm to establish. Flushing with the UVC transparent saline solutions is part of the normal procedure to be applied as part of a preventative approach each time the catheter has been used to avoid biofilm to establish. Flushing with the UVC transparent saline solutions is part of the normal procedure to be applied as part of a preventative approach each time the catheter has been used to avoid biofilm to establish. Flushing with the UVC transparent saline solutions is part of the normal procedure to be applied as part of a preventative approach each time the catheter has been used to avoid biofilm to establish. Flushing with the UVC transparent saline solutions is part of the normal procedure to be applied as part of a preventative approach each time the catheter has been used to avoid biofilm to establish. Flushing with the UVC transparent saline solutions is part of the normal procedure to be applied as part of a preventative approach each time the catheter has been used to avoid biofilm to establish. Flushing with the UVC transparent saline solutions is part of the normal procedure to be applied as part of a preventative approach each time the catheter has been used to avoid biofilm to establish.

**Conclusions:**

1. The study shows for the first time a high rate of *Pneumocystis* colonisation among health-care workers of a respiratory endoscopy unit and the presence of DNA of the pathogen in air samples of the area.
2. These findings, besides the concordance of genotypes between HCW and air samples, support the possibility of nosocomial transmission of this infection, including sulfonamides-resistant strains.

(Support by ERA-NET PneumoPathoGenoMics and FIS-europeo 03/1743)

| Week 0 | Week 2 | Week 4 | Week 8 | Week 12 |
|--------|--------|--------|--------|---------|
| F1 (medical staff) | + (1) | + (2) | NA | + (1) |
| F2 (nurse) | + (2) | + (1) | - | - |
| F3 (resident physician) | + (1) | - | - | - |
| F4 (medical staff) | NA | - | - | - |
| F5 (resident physician) | NA | - | - | - |
| UNIT AREAS | - | NA | - | - |

NA, no available; +, positive; -, negative; *|*,Wildtype genotype.

**Nosocomial infection**

**P1213** Reduction of healthcare-associated infections after implementation of bundle programmes in a tertiary care teaching hospital in Saudi Arabia

W. Mazi*, D. Abdullah, G. Gasem, N. Helali, A. Senok (Taif, Riyadh, SA)

**Objectives:** The bundles program introduced in January 2010 at the King Abdul Aziz Specialist Hospital (KAASH), Taif, Saudi Arabia aimed to reduce the incidence of Central line-associated bloodstream infection (CLABSI), Catheter-associated urinary tract infection (CA-UTI), Ventilator-associated pneumonia (VAP) and cesarean section-healthcare associated infection (CSEC-HAI). We present data evaluating the effectiveness of this program in the intensive care unit (ICU) and surgical-gynecology unit (SGU).

**Methods:** This prospective study was carried out in the 20-bed medical and surgical ICU and the 30-bed SGU at KAASH. Laboratory-confirmed CLABSI, CA-UTI, VAP and CSEC-HAI in 2009 and 2010 (January-November) were identified using Centers for Disease Control and Prevention (CDC) criteria. To enable benchmarking with National Healthcare Safety Network (NHSN, USA), data collection and analysis was carried out in accordance with NHSN recommendations.

**Results:** In 2009 the incidence of CLABSI was 4.79/1000 central line days with a significant decline to 2.45/1000 central line days in 2010 representing a decline from 90th to 50th percentile when benchmarked with NHSN data. The incidence of CA-UTI was 3.73/1000 catheter days in 2009 dropping to 2.9/1000 catheter days in 2010. Increased device utilization ratio was observed for central lines and urinary catheters. The significant decline in VAP incidence from 5.79/1000 to 3.95/1000 ventilator days was not accompanied by increased utilization of ventilation ratio (Table). In the SGU, 2396 cesarean sections with 35 HAI events representing CSEC-HAI incidence of 1.46% per 100 cesarean section procedures was recorded for 2009. Post-bundles implementation HAI events dropped to 21 despite increased cesarean sections (n = 216) representing CSEC-HAI incidence of 0.80% per 100 cesarean section procedures. Relative to NHSN data, this represented a decline from the 75th to 25th percentile. Overall, the implementation of bundles was accompanied with 51% decline in Methicillin resistance *Staphylococcus aureus* (MRSA) HAI’s (2009: n = 49 vs. 2010: n = 24).
Conclusion: Reductions in HAIs including MRSA-HAI were observed in our ICU and SGU following the implementation of bundles. The trend of increased device utilization ratios for central lines and urinary catheters in ICU deserves further investigation as well as post-discharge surveillance for cesarean section surgery is needed.

| Year | BSI site | HAIs | Device-days | Patients | Rate to SSN (%) | Risk reduction to SSN (%) | Device utilization ratio | Risk reduction to device utilization (%) |
|------|----------|------|-------------|----------|----------------|--------------------------|------------------------|----------------------------------------|
| 2009 | CLA-ESI  | 14   | 2017        | 6225     | 4.79          | >50%                     | 0.46                   | 29%                                    |
|      | CLA-OTTI | 19   | 3502        | 6264     | 3.77          | 50%−70%                  | 0.81                   | 30%−70%                                |
|      | VAP      | 21   | 3940        | 6218     | 2.79          | 70%−90%                  | 0.55                   | 70%                                    |
| 2010 | CLA-ESI  | 13   | 4869        | 6357     | 3.45          | 50%−70%                  | 0.51                   | 29%−39%                                |
|      | CLA-OTTI | 17   | 5768        | 6357     | 2.84          | 50%−70%                  | 0.84                   | >50%                                   |
|      | VAP      | 15   | 4169        | 6375     | 3.92          | 50%−70%                  | 0.51                   | 29%−70%                                |

P1214 Intestinal carriage of colistin resistant Enterobacteriaceae during selective decontamination of the digestive tract

E.A. Oostdijk*, A.M. De Smet, M. J. Bonten (Utrecht, NL)

Objectives: Selective Digestive Tract Decontamination (SDD) was associated with improved patient outcome in Dutch ICUs. As part of SDD colistin (COL) – in topical form – is administered four times daily in the oropharynx and the gut throughout ICU stay. Although resistance to COL has been reported infrequently, the risks of prolonged use of SDD (including COL) on resistance development are unknown. As COL is increasingly important to treat infections with multi-resistant Gram-negative bacteria, we quantified the risk of COL resistance in intestinal tract. Where human gut is sterile at delivery, bacterial colonization occurs during the early postnatal days that presents a possible source of neonatal systemic infections. Extended-spectrum-β-lactamase (ESBL)-producing Enterobacteriaceae (ESBL-E) are susceptible to the intestinal tract. Although most Extended-Spectrum-β-Lactamases (ESBL)-Lactamases (ESBL-E) producing Enterobacteriaceae (ESBL-E) are susceptible to the SDD-regimen, the effect of SDD on eradication of ESBL-E from the intestinal tract is unknown. We quantified eradication rates of non-ESBL and ESBL-E in SDD-patients participating in a 13-center cluster randomized study (NEJM 2009;360:20).

Methods: All SDD-patients with culture results from >1 rectal sample (with the first sample obtained <2 days in ICU) were included. Rectal carriage with Enterobacteriaceae was determined at admission and twice weekly during ICU-stay. Extended-spectrum-β-lactamase (ESBL) producing Enterobacteriaceae were identified at an increasing prevalence in Neonatal Intensive Care Units (NICU). The aim of this surveillance study was to determine the incidence of intestinal colonization with ESBL producing Enterobacteriaceae in neonates and infants treated in NICU.

Results: 345 (21%) of 1613 patients meeting inclusion criteria were colonized with Enterobacteriaceae on ICU admission: 111 (32%; 6.9% of 1613) with ESBL-E. Median lengths of ICU-stay were 8 (range 2−134, IQR 9) and 10 days (range 2−79, IQR 10) for non-ESBL and ESBL colonized patients, respectively (p=0.13). Median duration of colonization was 4.5 days for non-ESBL patients and 5 days for ESBL patients (p=0.12, logrank test). The most frequent colonizers were E. coli (n=257; 31 ESBL-E (12%)), Enterobacter spp (n=43; 42 ESBL-E (98%)) and Klebsiella spp (n=37; 8 ESBL-E (22%)).

Conclusion: SDD was equally effective in eradicating ESBL and non-ESBL producing Enterobacteriaceae present at ICU admission from the intestinal tract.

P1215 Eradication of extended-spectrum β-lactamases during selective digestive tract decontamination

E.A. Oostdijk*, A.M. De Smet, M.J. Bonten on behalf of the Dutch SDD-SDD trialists group

Objectives: Selective Digestive Tract Decontamination (SDD) was beneficial for patient outcome in Dutch ICU patients. SDD consists of topical antibiotics containing tobramycin, colistin and amphotericin B to eradicate potential pathogens, such as Enterobacteriaceae, from the intestinal tract. Although most Extended-Spectrum-β-Lactamases (ESBL)-Lactamases (ESBL-E) producing Enterobacteriaceae (ESBL-E) are susceptible to the SDD-regimen, the effect of SDD on eradication of ESBL-E from the intestinal tract is unknown. We quantified eradication rates of non-ESBL and ESBL-E in SDD-patients participating in a 13-center cluster randomized study (NEJM 2009;360:20).

Methods: All SDD-patients with culture results from >1 rectal sample (with the first sample obtained <2 days in ICU) were included. Rectal carriage with Enterobacteriaceae was determined at admission and twice weekly during ICU-stay. ESBL-E was defined as resistance to either ceftazidime, cefotaxime, ceftriaxone or cefuroxime. If colonization with Enterobacteriaceae was present at admission, ESBL status and duration of colonization was determined. Kaplan-Meier analysis was performed to test for differences in colonization duration. Patients were censored at ICU-discharge.

Results: 345 (21%) of 1613 patients meeting inclusion criteria were colonized with Enterobacteriaceae on ICU admission: 111 (32%; 6.9% of 1613) with ESBL-E. Median lengths of ICU-stay were 8 (range 2−134, IQR 9) and 10 days (range 2−79, IQR 10) for non-ESBL and ESBL colonized patients, respectively (p=0.13). Median duration of colonization was 4.5 days for non-ESBL patients and 5 days for ESBL patients (p=0.12, logrank test). The most frequent colonizers were E. coli (n=257; 31 ESBL-E (12%)), Enterobacter spp (n=43; 42 ESBL-E (98%)) and Klebsiella spp (n=37; 8 ESBL-E (22%)).

Conclusion: SDD was equally effective in eradicating ESBL and non-ESBL producing Enterobacteriaceae present at ICU admission from the intestinal tract.

P1216 Screening of gastrointestinal carriage of ESBL-producing Enterobacteriaceae in neonates

É. Kenesei*, M. Szénessi, M. Szabó, M. Pataki (Budapest, HU)

Introduction: Whereas human gut is sterile at delivery, bacterial colonization occurs during the early postnatal days that presents a possible source of neonatal systemic infections. Extended-spectrum-β-lactamase (ESBL) producing Enterobacteriaceae are identified at an increasing prevalence in Neonatal Intensive Care Units (NICU). The goal of this surveillance study was to determine the incidence of intestinal colonization with ESBL producing Enterobacteriaceae in neonates and infants treated in NICU.

Materials and Methods: In 2008 and 2010, 2912 perianal samples were screened for the presence of ESBL producing bacteria. Samples were routinely taken from 1093 newborns and infants at the admission to the NICU and then once a week. Samples were inoculated onto ChromID ESBL and blood agar (BioMerieux), Combination discs, AmpC and ESBL Detection Set (Mast Diagnostics) were used to identify the producer present.

Results: 102 of 1093 newborns and infants were colonised with ESBL producing, and 14 newborns with multidrug resistant enterobacteria. ESBL producing enterobacterial colonisation was detected in 5, 18, 35 and 44 newborns and infants within the first 72 postnatal hour, by the end of first postnatal week, first and third postnatal months, respectively. Major species were Klebsiella pneumoniae, Enterobacter cloacae and Escherichia coli.

Conclusion: Our results show, that up to 10% of newborns and infants treated at NICU are colonised with ESBL producing species. These colonised organisms may serve as a potential source of sepsis and may be
Risk factors for acquiring extended-spectrum β-lactamase producing *Escherichia coli* infection from prior colonisation

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Objectives: Extended-spectrum β-lactamase-producing *Escherichia coli* (ESBLEC) is an increasing cause of hospital-acquired infection. Risk factors for ESBLE, faecal carriage and infection, have already been reported but risk factors for acquiring ESBLEC infection from prior documented colonization are not well established.

Material and Methods: A retrospective case control study was performed in the Necker-Enfants Malades hospital, a French tertiary-care hospital from March 2008 through May 2010. Cases and controls were defined as ESBLEC colonized (faecal carriage) patients who developed or not ESBLEC infection respectively. Controls were matched in a 2:1 ratio to case patients according to duration between colonization and infection. We recorded demographic characteristics, functional status, underlying diseases, the presence and duration of indwelling devices, any hospitalization, mechanical ventilation, surgery, endoscopic procedures, dialysis and antimicrobial use after colonization. Antibiotic exposure and duration were analyzed separately for each antibiotic. Risk factors for ESBLE infection were identified by univariate and multivariate analysis.

Results: Forty cases and 78 controls were included. The median time from colonization to infection was 12.5 days (range 0–522, mean 45 days). Thirty-two and 26% of patients were admitted in the nephrology and hematologic ward respectively. ESBLE infections predominantly included urinary tract infection (85%), bacteremia (7.5%) and lower respiratory tract infection (7.5%). Univariate analysis identified urinary catheterization (p = 0.001), use of antibiotic therapy (p = 0.002) and specifically β-lactam/β-lactamase inhibitor association (BLI) (p = 0.017), cephalosporins (p = 0.001), sulfamides (p = 0.025) and aminglycosides (p = 0.004) as risk factors of infection. Nevertheless, only 3 factors were significantly associated with infection in multivariate analysis: duration of urinary catheters (p = 0.01, OR=1.26, 95%CI 1.01–1.5), duration of cephalosporins (p = 0.03, OR=1.15, 95%CI 1.01–1.32) and duration of BLI treatments (p = 0.02, OR=1.15, 95%CI 1.01–1.31).

Conclusion: The identification of these risk factors will be helpful to identify ESBLEC colonized patients who will require broad spectrum antibiotic therapy in case of nosocomial infection. Restriction/limiting the use of specific antibiotics could be new targets for prevention of ESBLS spread.

Proportionate increase from 2004–2008 in *S. aureus* associated US hospitalization rates in children

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Objectives: To describe recent epidemiological changes in US *Staphylococcus aureus* hospitalization rates in children.

Methods: The study combined discharge data from the Nationwide Inpatient Sample (NIS), Healthcare Cost and Utilization Project, and 71,272 pediatric inpatient isolates from the TSN electronic surveillance database (Eurofins Medinet) for the years 2004–2008. *S. aureus* associated hospitalizations were identified using ICD-9 codes. Based on antibiotic susceptibility, isolates were classified as MRSA and CA-MRSA, stratified by age, sex, source of the isolate, and census region. Blood and bronchial isolates were categorized as invasive disease. Hospitalization rates, standard errors, proportions and rate ratios (RR) were obtained using appropriately weighted data that included the variability of the TSN isolate collection.

Results: While the overall *S. aureus* hospitalization rate increased by 7% from 2004 to 2008, among children less than 18 years of age the rate increased by 49% (p < 0.01), from 14.2 ± 1.7 to 21.2 ± 1.8 hospitalizations per 1,000 discharges. The hospitalization rate increased most among children in the 1–5 age group (86% rise), followed by those less than one year old (40%) and those aged 6 to 18 years (33%). There was no significant change in the rate of invasive disease, but there was a 74% (p < 0.001) rise in the rate of skin and soft tissue infection (SSTI) hospitalizations, from 8.6 ± 0.97 in 2004 to 14.9 ± 1.45 in 2008. SSTIs increased by a factor of 2.2 (p < 0.01) among the 1–5 age group, but only by a factor of 1.71 and 1.47 among those less than 1 and 6 to 18 years old respectively. MRSA hospitalizations in children increased by 64% (RR 1.6 P < 0.01), from 6.5 ± 0.78 to 10.7 ± 0.97 hospitalizations per 1,000 discharges respectively, but this increase was twice as high among children 1–5 years old (RR 2.2, p < 0.01) during the same period. CA-MRSA hospitalizations in children increased from 3.8 ± 0.45 in 2004 to 6.7 ± 0.62 in 2008, or 80% (RR 1.8, p < 0.001), with a RR of 2.47 for those between 1 and 5 years of age.

Conclusion: Between 2004 and 2008 US children experienced a nearly 50% increase in hospitalizations associated to *S. aureus*. The majority of this increase was due to SSTI hospitalizations. By 2008, 51% of all *S. aureus* hospitalizations in children were associated with MRSA, and 32% of them with CA-MRSA. The age group most affected by and participating this change was children aged 1 to 5 years old.
**P1220** Risk factors for and consequences of nosocomial infections caused by multidrug-resistant pathogens

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**Objectives:** To identify possible risk factors for nosocomial infections caused by multidrug-resistant pathogens and to assess the effect of these infections on treatment parameters and patient outcome.

**Methods:** Microbiologically confirmed nosocomial infections occurred at a tertiary care intensive care unit (ICU) between 2004 and 2009 were retrieved retrospectively and were classified according to the antibiotic sensitivity of the pathogen (according to CDC definitions). The following patient data were analysed: age, type of admission diagnosis, length of stay, length of antibiotic treatment, number of drains and/or indwelling catheters, patient severity (SAPS II score), patient outcome. Statistical analyses were performed with SPSS and included T- and Ï²- and Fischer exact test. P values less than 0.05 were considered as statistically significant.

**Results:** During the five years of assessment 4683 cases were treated on the ICU. The number of microbiologically confirmed nosocomial infections were 360 of which were caused by antibiotic sensitive (S group), 120 cases by multidrug resistant pathogen (R group). The distributions of admission diagnoses differed considerably between the two groups, multitrauma patients were 23.3% in the S, 39.2% in the R group. Age and SAPS II score of patients in the two groups did not differ considerably (S vs. R group: 57.4±18.7 vs. 51.2±21.6 years) and (39.2±1.4 vs. 38.6±10.5 points) while the number of catheters/ drains differed significantly 4.0±1.3 vs 4.9±1.6. The mean length of stay were significantly longer in the R group (S vs. R group: 9.5±6.3 vs. 16.3±22.5 days) and the length of the antibiotic treatment was two times longer in the R group (5.5±5.6 vs. 11.5±17.0 days). The mortality was similar in the two patient group (S vs. R group: 27 vs. 19 cases).

**Conclusion:** The appearance of multidrug resistant pathogens were irrespective of patient's age and patient's severity, while the central venous catheters diagnosis and the number of catheters/drains were identified as risk factors. Multidrug resistant pathogen caused nosocomial infections had lead to significantly longer antibiotic treatment course and length of stay but had not resulted in higher mortality.

**P1221** Antibiotic drug consumption and the emergence and spread of antimicrobial-resistant Klebsiella pneumoniae clones in an intensive care unit

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**Objectives:** Klebsiella pneumoniae has been isolated with an increasing frequency as reported by the Italian Nosocomial Infections Surveillance in Intensive Care Units (ICUs) (SPIN-UTI) project. Previous studies have suggested a causal relationship between antibiotic usage and antimicrobial resistance. Our study aimed to investigate the relationship between antimicrobial consumption and K. pneumoniae resistance in a Sicilian ICU.

**Methods:** The study design integrated the patient-based and the laboratory-based surveillance approaches (Agodi A., et al., in press J Hosp Infect 2010, doi:10.1016/j.jhin.2010.10.007). For each antimicrobial agent, resistance rates (RR) were calculated as the number of resistant isolates divided by the total number of the isolates tested. Monthly data on antibiotic use were obtained from the pharmacy. The amount of antimicrobial drugs was standardized by conversion to defined daily doses (DDD, WHO ATC/DDD Index 2008). Antimicrobial usage density (AD) was calculated as the DDD per 1000 patient days. Molecular typing of K. pneumoniae isolates was performed by macrorestriction analysis of the XbaI-digested genomic DNA.

**Results:** During a seven-month period, 80 patients were enrolled in the study and a total of 95 isolates were collected from 44 patients. PFGE analysis of K. pneumoniae isolates led to the identification of 4 clones associated to cross-transmission and 22 single patterns associated with sporadic strains. RR for K. pneumoniae belonging to epidemic clones were higher than those for isolates belonging to sporadic strains. The total AD was 13252. The three most used drug groups were tetracyclines (AD 4236.5), quinolones (AD 3066.2) and glycopeptides (AD 1298.5). The single most frequently prescribed antimicrobial agents were tigecycline (AD 4237), ciprofloxacin (AD 2002.5) and levofloxacin (AD 1003.7). Comparison with international reference AD percentile distributions revealed high AD values for most of the antimicrobial groups used. Thus, the high RR's observed in the presence of high ADs would suggest an overuse of these antimicrobials in the presence of a relatively high genodiversity.

**Conclusion:** Increased efforts are required towards controlling antibiotic use and raising awareness of the need for prudent use of antibiotics. Surveillance of antibiotic consumption and bacterial resistance is important to understand the relationship between antibiotic usage and the emergence of K. pneumoniae resistance.

**P1222** Is there a variation in microorganisms causing healthcare-associated infections in a tertiary care hospital? Analysis of five years data

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**Objective:** Surveillance of healthcare associated infections and to have a thorough knowledge of variation of antimicrobial resistance pattern of the isolates are crucial for planning antimicrobial therapy in hospitals. The aim of this study was to analyze the nosocomial infections surveillance data collected for five years and to detect the changes in antimicrobial susceptibility of the microorganisms.

**Methods:** Patient-based active nosocomial infections (NI) surveillance in intensive care units and laboratory-based NI surveillance in selected clinics has been performed in our hospital between January 2006 and November 2010. Hospital acquired infections were diagnosed according to CDC criteria. Conventional methods and automated systems were used for the identification and detection the antimicrobial susceptibility pattern of the microorganisms.

**Results:** In the year of 2006 and 2010, 810 and 852 microorganisms were isolated from the infected patients, respectively. The most frequently isolated microorganisms in 2006, were Staphylococcus spp (29.8%), Pseudomonas spp (14.6%), Acinetobacter spp (17.0%), Escherichia coli (12.6%) and Klebsiella spp (8.0%), whereas in 2010, Acinetobacter spp (30.3%) was the most frequently isolated microorganism and E. coli (7.4%) was the least isolated Gram-negative bacteria. There were a statistically significant increment in the isolation frequencies of Acinetobacter spp, Pseudomonas spp and Klebsiella spp (p < 0.05), whereas isolation rate of E.coli was decreased (p < 0.001) in 2010. The isolation rate of Staphylococcus spp was 10.3%, and the decrease in the isolation frequency was significant (p < 0.05). Methicillin-resistant S. aureus isolation frequencies in years 2006 and 2010 were 19.1% and 6.7%, respectively (p < 0.001).

Antimicrobial resistance rates of carbapenems were increased in Acinetobacter spp in 2010 when compared to year 2006 (p < 0.01), on the other hand there was an increase in antimicrobial susceptibility rates of these antibiotics among Pseudomonas spp (p < 0.05). Methicillin resistance rates in S. aureus were 94.5% and 79.2%, respectively.

**Conclusion:** Acinetobacter spp especially drug resistant ones have been isolated more frequently as a cause of NIs whereas isolation rates of MRSA decreased in recent years. Meticulous infection control are needed for the control of this resistant Gram-negative microorganism.

**P1223** Primary colonisation with coagulase-negative staphylococci in hospitalised newborns

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**Objectives:** Coagulase negative staphylococci (CNS) are the most often pathogens causing sepsis in very low birth weight preterm neonates and are also the most important inhabitant of our skin. Therefore the
Unexplored field of the dynamic of the primary colonization with CNS is an important field for the understanding of CNS infections in neonates.

**Methods:** We investigated the diversity of CNS on the skin of preterm neonates at the neonatal ICU as well as healthy newborns on the regular neonatal ward (NW) on day 3 to 5 after birth. For each newborn at least 50 to maximally 240 individual colonies from skin swabs of the ankle and the elbow were identified using MALDI-TOF identification and 50 to 192 isolates identified as CNS were phenotypically characterized for their capacity to form biofilm and the resistance phenotype against Oxacillin.

**Results:** We identified 4,549 bacteria of 40 neonates (23 ICU; 17 NW). On the ICU, CNS were predominately *S. epidermidis* (78.1%) and *S. haemolyticus* (18.4%) and only two other CNS species were identified (*S. hominis* and *S. warneri*). On the NW, the major species were *S. epidermidis* (75.8%) and *S. hominis* (19.7%), whereas *S. haemolyticus* was identified only in 1.5%. Additionally, 5 more CNS species (*S. capitis*, *S. cohnii*, *S. lugdunensis*, *S. pasteurella*, and *S. warneri*) were identified at the NW. A statistically significant (p < 0.005) higher prevalence of Oxacillin resistance was observed for ICU isolates (80.2%) compared to isolates from the NW (39.1%). The prevalence for the capacity to form a biofilm was very similar, with 72.1% biofilm forming isolates on the ICU and 69.3% biofilm-positive isolates identified at the NW.

**Conclusions:** Summarizing the epidemiological data it could be suggested that the residence in the environment of an ICU is a risk for primary colonization with Oxacillin resistant bacteria. The capacity to form adherent biofilms is not detrimental in the primary colonization of sterile skin in contrast to the observation on the skin of healthy adults (Rogers, AEM, 74:6155). A higher diversity of species in the skin flora of neonates at the NW could be a fact of a higher frequency with the flora of persons not associated with the health care system. Further studies should focus on the question if CNS identified as primary colonization are identical with the strains causing neonatal sepsis.

**Table P1225**

| Respiratory infection period (May 2008 to April 2009) | Respiratory infection period (May 2009 to April 2010) |
|-----------------------------------------------------|-----------------------------------------------------|
| Total No. in NVA (percentage in NVA)                 | Total No. in NVA (percentage in NVA)                 |
| *A. baumannii*                                      | *A. baumannii*                                      |
| 34 (18.9%)                                          | 35 (18.9%)                                          |
| *Acinetobacter* resistance S. *haemolyticus*         | *Acinetobacter* resistance S. *haemolyticus*         |
| 10 (7.9%)                                           | 13 (7.9%)                                           |
| *E. coli*                                           | *E. coli*                                           |
| 7 (5.4%)                                            | 9 (5.4%)                                            |
| *E. faecalis*                                       | *E. faecalis*                                       |
| 13 (9.8%)                                           | 15 (8.8%)                                           |
| *Escherichia coli*                                  | *Escherichia coli*                                  |
| 2 (1.6%)                                            | 1 (0.6%)                                            |
| Other Gram-negative bacilli                         | Other Gram-negative bacilli                         |
| 1 (0.7%)                                            | 1 (0.5%)                                            |
| Total                                               | Total                                               |
| 72 (40.6%)                                          | 72 (40.6%)                                          |

**Objective:** Acinetobacter species are important opportunistic pathogens responsible for nosocomial infections especially in intensive care settings. Management of infections caused by these strains is difficult, as the strains are often resistant to a wide range of antibiotics, including broad-spectrum β-lactams, aminoglycosides quinolones and carbapenems.

**Methods:** We here, retrospectively evaluated the risk factors and clinical outcomes of Acinetobacter infections in our pediatric wards including pediatric intensive care unit, hematological wards and newborn units between 2004–2009.

**Results:** According to our results, 137 children (84 boys and 53 girls) have been followed-up in our clinics with diagnosis of Acinetobacter infections. Median age was 95 days and median hospital stay was 40 days. 66.4% of these children had a previous history of hospitalization. 77.4% of these children had chronic underlying conditions, 8% had neutropenia, 83.2% have required intensive care unit stay (median 40 days), 52.6% had surgical interventions, 46.7% had urinary catheterization, 19.7% had central venous line, 78.1% have required mechanical ventilation and 78.8% have received total parenteral nutrition. Among these, 62 isolates obtained from tracheal aspirates (all cases are ventilatory associated pneumonia), 36 obtained from blood, 19 urine samples, 11 cerebrospinal fluid, 6 surgical site samples and 3 peritoneal fluid, 38.2% of these 137 children are died. Among these 137 isolates, 40.1% (n = 55) are imipenem-resistant isolates. Among 138 isolates 89.3% amikacin-resistant, 73% cefepime resistant, 86.9% ceftazidime resistant, 86.1% ciprofloxacin-resistant, 8% resistant to cefoperazone-sulbactam and 1 isolates resistant to colistin. The presence of imipenem resistance were not associated with age group, gender, presence of underlying conditions, presence of surgical interventions, requiring intensive care unit stay. Presence of imipenem resistance are 2.2 higher in children requiring mechanical ventilation (OR 2.2; 1.0–4.6;p < 0.05). Mortality rate also 2.7 fold higher in imipenem resistant group (OR 2.7;1.3–5.4; p < 0.05).

**Conclusion:** Carbapenem-resistant Acinetobacter infections still remains major challenge in pediatric age group and are common in children.
Nosocomial infection incidence and risk factors in elderly patients in intensive care units

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Objective: Gradually increased in patients over 65 year age accounts a specific population for nosocomial infections. Predisposition to infections in this age group is a result of impaired host defence such as diminished cellular immunity. Despite the importance of this fact there is not much data about the incidence and risk factors of nosocomial infections in elderly. The purpose of this study is to investigate the risk factors, the factors effecting mortality and incidence of nosocomial infections in elderly patients in the medical ICUs of our hospital.

Methods: The patients treated over 48 hours at four ICU’s between January and December 2008 were enrolled in the study. Data were collected by active prospective surveillance. Elderly and adult patients were compared according to the factors effecting the development of nosocomial infection, causative agents, and mortality rates. Moreover, elderly patients with/without nosocomial infection were compared for the risk factors.

Results: A total of 433 patients were included in the study. 288 of the patients were over 65, and 205 of them were 18–65 age. The incidence of nosocomial infection was found 51% in the elderly group and 48.8% in the adult group. There was no difference in the incidence of nosocomial infection between the elderly and adult groups. The presence of diabetes, COPD and chronic diseases were statistically higher in the elderly patients with nosocomial infection. Pneumonia was the most frequent nosocomial infection in both groups, and mechanical ventilator utilisation was found to be high in both groups. Mortality rate and duration of hospitalization were found significantly higher in patients with nosocomial infection than those of without nosocomial infection. Increased age, lengthened duration of hospitalization, deterioration of conscious, existence of underlying chronic diseases, the presence nosocomial infection and malignancy as well as the use of MV and CVC are found to be independent risk factors for mortality.

Conclusion: Nosocomial infection incidence among the elderly patients in ICU’s was high but not statistically different than the adult patients in our study. However, the mortality was significantly higher in elderly group. We found that use of invasive techniques were related with increase in the incidence of nosocomial infections. Limited application of invasive procedures will make a contribution to decrease these infections.

Device-associated infection surveillance in intensive care units of Duzce University Hospital, 2008–2009, Turkey

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Objectives: Device-associated infections (DAIs), such as catheter-associated urinary tract infections (CAUTIs), central line-associated blood stream infections (CABSIIs), and ventilator-associated pneumonia (VAP) pose the greatest threat to patient safety in intensive care units (ICUs). The purpose of this study was to evaluate the device-associated infections in Intensive Care Units of Duzce University Hospital, in Turkey.

Methods: A prospective surveillance was performed to determine DAIs rates during 2008–2009. Hospital-acquired infections were identified according to the definitions of Centers for Disease Control and Prevention. DAIs rates were calculated as the number of infections per 1000 device-days.

Results: The hospital-acquired infection incidence was 31.4 per 1000 patient days in 2008, and 28.3 per 1000 patient days in 2009. Ventilator-associated pneumonia rate was 17.4 and 21.6, CAUTIs rate were 6.3 and 4.8, and CABSIIs rate were 8.6 and 6.9 in 2008 and 2009, respectively. There was not any statistically significant difference between DAIs rates in 2008 and 2009. Pseudomonas spp., Staphylococcus aureus, Escherichia coli, and Acinetobacter spp. were the most common causative agents.

Conclusion: In this study, high incidence rates of device-associated infections were found. Ventilator-associated pneumonia, and high ratios of Pseudomonas infections were particularly present in our ICUs. To reduce infection rates and improve quality of care in ICUs in developing countries such as Turkey, comprehensive infection control programs are required.

Increase of patients co-colonised or coinfected with methicillin-resistant Staphylococcus aureus, vancomycin-resistant Enterococcus faecium or extended spectrum β-lactamase-producing Enterobacteriaceae

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Objectives: We aimed to determine the incidence of patients with co-occurrence of MRSA, VRE faecium or ESBL producing enterobacteriaceae in four German tertiary care hospitals and to describe the change...
in the incidence of patients with co-occurrence of those pathogens over the last three years (2007–2009).

**Methods:** Co-colonization or co-infection was defined as patients with positive cultures for at least two of the following resistant pathogens: MRSA, VRE faecium or different species of ESBL-producing Enterobacteriaceae within one calendar year.

**Results:** A total of 896,822 patients were analyzed. 10,066 patients harboured MRSA, VRE faecium and/or ESBL producing enterobacteriaceae and 542 patients co-harboured those resistant pathogens. In 2009, 7.6% of MRSA patients, 13.7% of the VRE faecium patients and even 16.1% of the ESBL producing enterobacteriaceae patients were co-colonized or co-infected. The total number of co-infected or co-colonized patients increased from 157 to 219 over the last three years and accounted for 6% of all patients with MRSA, VRE faecium or ESBL producing enterobacteriaceae in 2009. The incidence of patients with co-infection or co-colonization increased steadily from 5 (2007) to 7 per 10,000 patients (2009).

Patients harbouring ESBL producing enterobacteriaceae or VRE faecium had a higher risk to be co-colonized or co-infected than what was to be extrapolated from their overall incidence. This might be linked to their gastro-intestinal reservoir and impracticality to decolonize the gut of resistant patients. Therefore, three points are paramount: prevention of transmission, limitation of antibiotic use to curtail the selection and persistence of predominant clones and, strategies to influence carriage specifically in the intestinal tract being an important reservoir of antibiotic resistance genes.

**Conclusion:** The burden of patients with co-colonization or co-infection with MRSA, VRE faecium or ESBL producing enterobacteriaceae increased from 2007 to 2009 by 40%. This was especially the case in patients carrying ESBL-producing enterobacteriaceae. Clinicians can expect to face increasingly patients with co-occurrence of resistant pathogens. Therefore, three points are paramount: prevention of transmission, limitation of antibiotic use to curtail the selection and persistence of predominant clones and, strategies to influence carriage especially in the gastrointestinal tract being an important reservoir of antibiotic resistance genes.

**P1230 Frequency of colonisation by multiresistant organisms among patients hospitalised in a giraditic ward: a one-year prospective observational study**

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**Objectives:** To determine the prevalence, incidence and risk factors of asymptomatic carriage of extended-spectrum β-lactamase producing Enterobacteriaceae (ESBL) methicillin-resistant *Staphylococcus aureus* (MRSA), and vancomycin-resistant enterococcus (VRE) in elderly subjects admitted to hospital in a geriatric ward.

**Methods:** During one year, nasal, oropharyngeal, groin, axilla and rectal swabs were prospectively collected upon admission and at discharge for microbiological culture on selective chromogenic agar and broth enrichment. Identification and susceptibility testing of the target pathogens was performed according to conventional laboratory methods. Genotypic characterisation of resistance determinants was performed by multiplex PCR assays.

**Results:** Out of 445 admitted patients between 12.2009 and 12.2010, 350 were included in the study. The estimated prevalence upon admission of ESBL, MRSA and VRE carriage was respectively 13% (Confidence Interval 95% (95% CI): 10–17%), 7% (95% CI: 5–11%) and 0.6% (95% CI: 0.1–2.0%). *Escherichia coli* (*E. coli*) was the most frequently isolated microorganism among ESBL+ isolates (89%). Only 1% of the patients were co-colonized by ESBL and MRSA on admission. The incidence density of ESBL and MRSA carriage was respectively of 2.25 and 0.86 new cases for 1000 hospitalization-days. No cases of VRE acquisition were found. Using a logistic regression model, the following risk factors for ESBL colonization on admission were: multiple contacts with hospital within the previous year (Odds Ratio (OR): 3.2; 95% CI: 1.5–6.9; P-value: 0.003), chronic bladder catheter use (OR: 3.4; 95% CI: 1.3–9.1; P-value: 0.01) and a high level of dependency measured by the 24 items Katz scale (OR: 1.1; 95% CI: 1.0–1.2; P-value: 0.01). For MRSA, the two following risk factors were obtained in the model: previous known MRSA colonization (OR: 26.8; C95%: 8.0–90.0; P-value <0.001) and a high level of dependency measured by the 6 items Katz scale score (OR: 0.7; 95% CI: 0.5–1.0; P-value: 0.08).

**Conclusion:** This study shows a high prevalence of asymptomatic colonization by ESBL producing *E. coli* on admission in an acute geriatric ward. This rate is almost twice higher than the prevalence of MRSA carriage. VRE colonization remains low in our setting. A low functional status is a common risk factor for both ESBL or MRSA colonization and highlights the need to reinforce infection control procedures.

**P1231 ICU – always the holobed of resistance?**

R. Reynolds*, K. Maher, R. Hope on behalf of BSAC Working Party on Resistance Surveillance

**Objective:** Intensive care (ICU) is often considered a hotspot for development and dissemination of antibiotic resistance. We compared non-susceptibility (NS) in hospital-acquired infections (patients in hospital ≥48 hours) between ICU and other wards, and between blood and respiratory infections (RTI) monitored in the BSAC Resistance Surveillance Project.

**Methods:** Laboratories in the UK and Ireland provided isolates from blood (28 centres) and RTI (22 centres) in 2008–2009. Isolates were tested by the BSAC agar dilution MIC method at two central laboratories and categorised by BSAC/EUCAST breakpoints. Excluding patients in hospital <48 hours or missing data for speciality left 2622 isolates for analysis.

**Results:** In *S. aureus*, MRSA was much less prevalent in ICU than other wards (30 vs. 55%) among RTI but not blood (35 vs. 29%), with corresponding differences in NS to ciprofloxacin (CIP; 40 vs. 60%), erythromycin (38 vs. 54%) and penicillin (PEN, 83 vs. 95%). Among MSSA, blood isolates had less CIP-NS in ICU (0% vs. 13%) and appeared to have less PEN-NS (77 vs. 84%), but teicoplanin NS was more common in ICU (7 vs. 0.3%); RTI MSSA similarly appeared to have lower NS in ICU for CIP (14 vs. 21%) and PEN (75 vs. 89%). Among MRSA, RTI isolates showed more NS in ICU for gentamicin (18 vs. 1%) and mupirocin (32 vs. 4%; perhaps also in blood, 18 vs. 5%). For *E. coli*, CIP-NS was substantially lower in ICU for RTI (18 vs. 29%) and appeared so also in blood (7 vs. 21%), although there was no difference in ESBL prevalence. Other NS all appeared similar or lower in ICU than other settings for RTI *E. coli*, with a more mixed picture for blood. *Klebsiella* too tended to have less CIP-NS in ICU (11 vs. 18%, RTI; 6 vs. 14%, blood) and no clear examples of higher NS in ICU. *Enterobacter* had a general but unclear tendency to more NS in ICU. For *Pseudomonas*, in both RTI and blood, NS appeared more prevalent in ICU for all five agents tested, the difference for imipenem being most striking (28 vs. 10%, RTI 22 vs. 5%, blood).

**Conclusion:** For hospital-acquired infections, non-susceptibility was not universally more prevalent in ICU than other settings. In some cases (e.g. mupirocin in RTI *S. aureus*; ciprofloxacin in RTI *E. coli* and blood MSSA), non-susceptibility was significantly less prevalent in ICU than elsewhere. Relationship of non-susceptibility to ICU varied with site of infection as well as organism and antibiotic, and was not clearly explained by patient age.

| Organism Group | non-ICU | ICU | non-ICU | ICU |
|----------------|--------|-----|--------|-----|
| **RTI**        | N      | (%) | N      | (%) |
| *S. aureus*    | 337    | 93  | 404    | 48  |
| *E. coli*      | 150    | 120 | 237    | 30  |
| *Klebsiella*   | 106    | 115 | 188    | 32  |
| *Enterobacter* | 72     | 82  | 190    | 37  |
| *Pseudomonas*  | 132    | 101 | 179    | 51  |
| **Blood**      | N      | (%) | N      | (%) |
| *S. aureus*    | 337    | 93  | 404    | 48  |
| *E. coli*      | 150    | 120 | 237    | 30  |
| *Klebsiella*   | 106    | 115 | 188    | 32  |
| *Enterobacter* | 72     | 82  | 190    | 37  |
| *Pseudomonas*  | 132    | 101 | 179    | 51  |
Molecular typing by pulsed-field gel electrophoresis of an outbreak of multidrug-carbapenem-resistant Acinetobacter baumannii bacteremia in a haematology department

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Objective: To characterize multidrug-resistant (MDR)-A. baumannii strains involved in an outbreak within a 41-bed haematology department (HD) of an university hospital.

Methods: An increase in the number of cases of A. baumannii bacteremia was observed among patients of HD. The outbreak lasted for 12 days, seven patients involved and all of them died due to A. baumannii sepsis. Outbreak investigation procedures was followed according to the CDC recommendations on outbreak investigation. Case control study was designed in order to find any association between MDR-A. baumannii bacteremia and some patients’ characteristics and variables. Molecular typing by PFGE of the digested genomic DNA was performed according to WHO protocols with modifications. In this method, each isolate was digested with Apal macrorestriction enzyme separately. PFGE patterns were analyzed both visually and with computer assisted analysis with Gene Directory software (Syngene, Cambridge, UK). A similarity index was determined by using Dice coefficient, unweighted pair-group method average (UPGMA) method with 1% band tolerance. Isolates displaying more than three DNA fragment differences and a similarity of <80% following dendrogram analysis were considered to represent unrelated PFGE types; isolates with between 1−3 fragment differences and a similarity of >80% were considered to represent PFGE subtypes, while isolates showing identical DNA banding patterns were considered to be clonal.

Results: Three of six MDR-A. baumannii strains from outbreak cases tested gave an identical banding pattern and were considered to be clonal, which was designated as clone A. These three isolates also gave similar antibiotic susceptibility patterns. Remaining three isolates were unrelated to clone A, two isolates in this group were in clone B, and one remaining isolate was in clone B1. The isolates from other departments and environmental isolates tested gave DNA fingerprint patterns distinct from outbreak isolates tested.

In case control study a statistically non-significant association between outbreak isolates and environment isolates gave DNA fingerprint patterns distinct unrelated to clone A, two isolates in this group were in clone B, and one remaining isolate was in clone B1. The isolates from other departments and environmental isolates tested gave DNA fingerprint patterns distinct from outbreak isolates tested.

Conclusion: The MDR-A. baumannii outbreak occurred in hematology department was caused by the spread of two epidemic clones, which was probably selected because of its resistance to major antimicrobial agents.

Outbreaks

P1233 Molecular typing by pulsed-field gel electrophoresis of an outbreak of multidrug-carbapenem-resistant Acinetobacter baumannii bacteremia in a haematology department

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Introduction and aim of the study: A. baumannii (A) is a Gram negative, non fermenting coccobacillus, widespread in the environment, that can survive on inanimate surfaces for up to 6–8 weeks. The clinical relevance of A. is linked to its involvement in nosocomial infections in critically ill patients and especially its inherent multidrug resistance (MDR) makes therapy very complex and influences the outcome. In the period from February to November 2009 A. was isolated from 20 patients hospitalized in the S. Gerardo Hospital of Monza, Italy. 18/20 strains (90%) were isolated from patients in ICU; 13 developed clinical infections while 7 cases were considered colonizations.

Material and Methods: Bacterial identification and antibiotic sensitivity were assessed using Vitek system (bioMérieux) and confirmed with MIC by Etest. All MDR strains were resistant to aminoglycosides, carbapenems, quinolones, and cephalosporins and sensitive to Colistin. All the isolates responsible for colonization and infection were genetically related to each other as shown by molecular analysis with rep-PCR. A space-time analysis of the epidemic spreading was carried out.

Results: 14/20 patients (70%) had lung as primary location:12 with clinically manifest infection (60%) and 2 (10%) were considered colonizations. Multiple sites infections occurred in 35% of the cases. Pulmonary infections were treated with Colistin 2−3 MU × 3 IV + Ampicillin/Subl IV 4 gr × 4 + endobronchial Colistin 1 MU × 3 by aerosol. In two cases was added Tigecycline 50 mg × 2 IV. The crude mortality rate overall was 45% (9/20), but in 8 cases mortality was attributed to other causes not A.-correlated as shown by negative cultures for A. to document the effectiveness of antibiotic therapy instituted. In one case mortality was related to infection: the patient did not start antibiotic therapy as the identification of cultured A. and communication to the department had taken place just in 24 hours from the exitus. Therefore, antibiotic therapy was effective, as documented by microbiological monitoring, in 100% of 12 cases treated.

Conclusion: The attributable mortality by A. Infections observed in our series is 7,6% (1/13), better than the 10−43% reported in literature in patients in ICU of which our population represents the vast majority. The combination of systemic therapy and endobronchial Colistin was effective against lung infections and there have been no major side effects.

P1234 Outbreak of methicillin-resistant Staphylococcus aureus in a neonatal intensive care unit

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Nosocomial outbreaks caused by Methicillin-resistant Staphylococcus aureus (MRSA) are a major health problem in neonates. Outbreaks of MRSA colonization and infection in neonatal intensive care units (NICUs) can lead to significant morbidity and mortality.

Objectives: To assess the presence of an epidemic strain of MRSA in a NICU using genotyping and epidemiological methods, and the probable relationship between neonates and healthcare workers (HCWs).

Methods: During a period of two months (July-August 2010) surveillance cultures were obtained from all neonates (150) admitted and all HCWs (157). A total of 79 MRSA strains were collected from 14 (8,9) NICUs and 65 (43,3%) neonates.

First of all, molecular typing of 52 MRSA isolates was performed by repetitive extragenic palindromic PCR (rep-PCR) using Diversilab® system (BioMérieux). Secondly, protein A-encoding gene (spa) of 18 MRSA strains was investigated, to monitoring the clonal distribution of MRSA strains.

Results: Cluster analysis of rep-PCR fingerprints indicated an heterogeneous population of MRSA isolates, especially one major cluster (similarity >95%) and another distinct group composed only by one case, isolated in a healthcare worker.

Spa-typing was performed only on 18 MRSA strains from both clusters. Overall, the largest individual group of spa types (17/18) belonged to the EMRSA-15 complex, with one spa type, 032, predominating (16/17) and one spa type t515 that is recently considered to belong to this clonal group EMRSA-15.

Only one of the 18 MRSA isolates showed the spa type t127 (the healthcare workers clustered alone) in agreement with the cluster analysis of rep-PCR.

Conclusion: Automated rep-PCR assays on the DiversiLab system, used for MRSA, proved as a rapid and reliable method for molecular analysis of nosocomial outbreaks. In addition, use of spa-typing can give more information about the current epidemiological distribution of clones.
Outbreaks

P1235 Epidemiological characterisation of epidemic and non-epidemic clones of Acinetobacter baumannii: is surveillance of carriage in intensive care units useful? M. Barchitta*, G. Valent, A. Quattrrocchi, M.A. Romeo, L. Giaquinta, C. Sntango, G. Castiglione, A. Agodi (Catania, IT)

Objectives: The emerging role of Acinetobacter baumannii as well as an increasing trend in time of specific resistance patterns has been reported in Italian intensive care units (ICUs) (Agodi et al., 2010). Active surveillance for A. baumannii acquisition was implemented simultaneously in multiple ICUs in order to define the genetic relationships of isolates, both for local and for global epidemiological purposes, using Pulsed-Field Gel Electrophoresis (PFGE) and Multilocus Sequence Typing (MLST) analyses.

Methods: Patterns of A. baumannii acquisition in ICU, during the period of the study, were carriage, colonization and infection. Identification of A. baumannii was confirmed by Amplified rDNA Restriction Analysis (ARDRA), genotyping by PFGE of the Apal-digested genomic DNA, and MLST analysis using the Institute Pasteur's MLST scheme (Diancourt et al., 2010).

Results: During the six months period, 182 patients were enrolled in the patient-based surveillance of 3067 patient-days and a total of 149 isolates, all identified as A. baumannii by ARDRA, were collected from 84 patients. Carriage was associated to 3.3% of isolates; colonization to 25.8%, colonization/infection to 47.5%, and infection to the remaining 23.4% of isolates, using international definitions. A total of 12 unrelated pulse-types were identified. Particularly, two major clones were identified involving respectively 42.7% and 34.3% of isolates, showing inter-hospital spread and intra-ICU spread. All A. baumannii isolates were multidrug resistant. Most isolates exhibited susceptibility only to colistin and tigecycline. For each ICU a specific epidemic curve was plotted and outlier analysis suggested a carrier patient as the source of the outbreak at least for one ICU. MLST will further provide characterization of epidemic and non-epidemic strains as well as carriage or colonization/ infection associated clones.

Conclusions: Our study underlines the importance of early detection of A. baumannii epidemic clones in the ICU setting, and suggests careful efficacy evaluation of screening on admission for all patients at high risk. Therefore, multicentre studies are needed to address the increasing import of resistant A. baumannii into the ICU and to minimize the risk of transmission. Furthermore, global epidemiological scenarios are required in order to define the efficacy of infection control strategies in ICUs.

P1236 Nosocomial pseudo-outbreak of XDR Pseudomonas aeruginosa urinary tract infections due to analytical contamination in the laboratory M. Hallin*, A. Deplano, S. Roi., V. Boyart, R. De Ryck, C. Nonhoff, O. Denis (Brussels, BE)

Background: By the end of may 2010, an increase in the number of urinocultures positive for extremely drug-resistant (XDR) P. aeruginosa (defined as resistant to all but one antipseudomonal antibiotics, except colistin) was observed in our 750-bed university hospital and led to an infection control alert. No epidemiologic link between the patients and no increase in the frequency of XDR P. aeruginosa in non-urine samples was observed. Therefore, a pseudo-outbreak due to analytical contamination in the laboratory was rapidly suspected.

Methods: A retrospective search of cases (defined as patients with an urinoculture positive for P. aeruginosa non-susceptible to ceftazidime, imipenem, meropenem and ciprofloxacin) was initiated. Sampling of the automated urinary analysers used in the laboratory was performed. Antibiotypes of clinical isolates as well as the isolate recovered from one of the analysers were performed by disk diffusion. Genotypes were determined by PFGE and the presence of VIM and IMP genes was searched by PCR.

Results: From February to July 2010, 15 patients, admitted to 11 different departments, four outpatient and one staff member were included. The mixing device of the cytometric analyser used for enumeration of urinary particles (Sysmex UF1000i) was proved to be heavily contaminated. Isolates recovered from 12 patients belonged to the same antibio- and PFGE type as the isolate recovered from the analyser, all were VIM-positive. Four isolates harboured unrelated PFGE types and 4 isolates were unavailable. Extensive disinfection with a broad spectrum disinfectant and replacement of the entire tubing was necessary to achieve complete negativity of culture samples taken from the analyser. A weekly disinfection procedure followed by sterility control of the analyser was implemented.

Conclusion: A pseudo-outbreak caused by a VIM positive XDR P. aeruginosa clone was proved to be due to the contamination of the cytometric analyser for urinary sediment. Users of such analysers should be aware that contamination can occur and should perform regular disinfection and sterility control procedures of their analyser.

P1237 High-throughput molecular screening of methicillin-resistant Staphylococcus aureus during a major nosocomial outbreak caused by various genotypes L. Roorda*, W. Hendriks, A. van der Zee, J. Ossewaaarde, J. Buitenwerf (Rotterdam, NL)

Objective: A major outbreak of methicillin-resistant Staphylococcus aureus (MRSA) was detected in one of the two locations C and Z of our hospital. Using genotyping 4 epidemic MRSA clones were identified in hospital C that spread to location Z of our hospital and to other health care related institutions in the region. To efficiently handle the huge numbers of MRSA screening cultures, a molecular approach for screening was designed and implemented.

Method: A fully automated high-throughput detection system of MRSA was installed. Screening was carried out by means of chromosome-SCCmec PCR.

Results: The major epidemic clones of MRSA in the outbreak were eradicated after implementation of a rapid high throughput molecular screening system, reducing the time needed to deliver the results, and reducing the time of containment measures as prescribed by the Dutch search and destroy policy.

Conclusion: Rapid molecular screening contributed to fast eradication of epidemic MRSA clones.

P1238 Outbreak of Pseudomonas (Flavimonas) oryzihabitans bacteremia in a neonatal intensive care unit H. Prifti*, D. Dkomomidou, O. Pappa, K. Tryfonompoulos, K. Vatzeli, K. Karaiskos, E. Kottis, A. Vatopoulos, K. Zanetos (Athens, GR)

Objective: To report an outbreak of nosocomial bacteremia caused by P oryzihibans in a NICU of a tertiary care hospital.

Patients and Methods: Six premature neonates nursed at the NICU developed bacteremia at the same day. The mean age, gestational age and birth weight were 13 days, 30 weeks and 1362 g respectively. All neonates had intravenous catheter (2 of them had also umbilical) and received total parenteral nutrition. Blood samples were inoculated in PedsPlus bottles and incubated in BACTEC 9050 system. The API 20NE and MicroScan were used for bacterial identification. Susceptibility testing was performed by disk diffusion technique and MICs were determined by MicroScan and Etest. Specimens for culture were taken from the environment of NICU and milk kitchen, from IV solutions, as well as from milk preparations in order to identify the source of infection. The clonal relationship of isolates was investigated by PFGE.
Results: All blood isolates from the six neonates were identified as *P. oryzihabtans* (a yellow-pigmented, Gram-negative bacillus), and had an identical susceptibility pattern. All were susceptible to piperacillin, piperacillin/tazobactam, ticarcillin/clavulanate, carbapenems, amnoglycosides, ciprofloxacin, levofloxacin, and tetracycline and resistant to aztreonam and trimethoprim-sulfamethoxazole. After removal of IV catheter and appropriate antibiotic treatment (imipenem combined with gentamicin) five neonates recovered, and one developed necrotic enteritis. Culture results showed contamination of a sponge from milk kitchen which yielded *P. oryzihabtans* with a biotype and a susceptibility profile identical to those of blood isolates. All other specimens taken from parenteral IV fluids and milk were negative. All blood isolates showed genetically indistinguishable PFGE patterns; this finding combined with the concurrent onset of bacteremia led to the suspicion of IV fluid contamination, despite the causative agent was not isolated from the IV fluid preparations. Infection control measures during preparation and handling of parenteral solutions resulted in elimination of outbreak.

Conclusions: (a) Although *P. oryzihabtans* has been rarely reported as a cause of bacteremia should be considered as a nosocomial pathogen in neonates with IV fluid administration. (b) Infections are mild and respond well to treatment. (c) A strict aseptic technique in the preparation of parenteral fluids may reduce the risk of bacteremia in newborns.

**MALDI-TOF mass spectrometry proteome profiling for identification and typing of multidrug-resistant *Klebsiella pneumoniae* strains in a paediatric nosocomial outbreak**

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Objectives: Rapid and reliable description of genetic relatedness of clinical isolates of *Klebsiella pneumoniae* is crucial for nosocomial outbreak investigations. We report an outbreak of severe sepsis in 3 patients hospitalized at the Onco-Haematology Unit of the Paediatric Hospital “Bambino Gesù” of Rome, Italy. The patients, who underwent allogenic haematopoietic stem cell transplant, were infected by extended-spectrum β-lactamase (ESBL) and carbapenemase (KPC)-producing *K. pneumoniae* strains. Proteomic phenotypes from MALDI-TOF MS were employed as analytical and typing expression profiling of *K. pneumoniae*. These strains were clustered by using a hierarchical analysis based on (Unweighted Pair Group Method with Arithmetic Mean) UPGMA algorithm and Pearson’s coefficient to investigate correlation similarity.

Methods: Twelve clinical isolates of *K. pneumoniae* (4 ESBL-KPC-producing and 8 antibiotic-sensitive controls) were investigated. Bacterial identifications (ID) and initial antibiotic susceptibility testing was performed by using the VITEK II instrument (BioMérieux, Marcy l’Etroite, France) and drug resistance was confirmed by Etest (AB Biodisk, Solna, Sweden). ID was confirmed by MALDI-TOF-MS assay. Spectra profiling from the 12 isolates were converted into virtual gel-like format and UPGMA analysis was performed to investigate strain typing correlation. The typing analysis based on repetitive-sequence PCR (rep-PCR) (DiversiLab, BioMérieux) was performed to confirm the results.

Results: Concordant results for *K. pneumoniae* strain IDs were achieved with VITEK II and MS methods. The MS typing analysis of ESBL and KPC-producing isolates proved that three ESBL-KPC isolates had a similarity of 92% (close-related clustering), one ESBL-KPC belonged to a different clade (similarity around 60%), while the eight controls showed a very low similarity value. Rep-PCR corroborated the MS-based clustering.

Conclusion: In immunocompromised patients the evolution of *K. pneumoniae* colonisation of the upper respiratory tract into episodes of sepsis is a frequent clinical course. Furthermore, the antibiotic prophylaxis of patients subjected to allogenic haematopoietic stem cell transplant represents a strong risk factor for selecting multidrug-resistant (MDR) *Klebsiella* strains. Therefore, the MS IDs and typing might provide a new fast and accurate tool for the implementation of epidemiological controls aiming to impede the spread of nosocomial pathogen outbreaks.

**Consecutive outbreaks of *Serratia marcescens* infections in a neonatal ICU are due to the repeated introduction of new clones**

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Objective: The epidemiological investigation of three consecutive outbreaks of *S. marcescens* bloodstream infections in a NICU of a tertiary hospital in Athens over a 3-year period.

Material and Methods: The outbreaks have occurred from December 2007 to August 2008 (outbreak 1) involving 5 neonates, from September 2009 to February 2010 (outbreak 2) involving 10 neonates and from May 2010 to September 2010 (outbreak 3) involving 5 neonates. During all three outbreaks extensive screening of patients, health care workers and inanimate environment was conducted. Typing performed by Pulsed-field gel electrophoresis (PFGE) of Spe-1 digested bacterial DNA.

Results: A total of 58 clinical (20 from neonatal infections and 38 from patients’ colonization) and 9 environmental *S. marcescens* isolates were recovered. PFGE allocated the 58 clinical isolates into 9 PFGE types (>85% similarity), however 4 types were found to prevail during the three outbreaks. In outbreak 1 all clinical *S. marcescens* isolates and one isolate recovered from a sink drain were genetically indistinguishable belonging to PFGE type I. In outbreak 2 two PFGE types prevailed, type III in the first part of this outbreak and type VII in the last part of the outbreak. In outbreak 3 a new PFGE type VIII was recovered from the vast majority of patients and also from the sink drain in the milk kitchen. Moreover, 5 other PFGE types were recovered from 5 cases (IV, V and VI during outbreak 2 and IX and X during outbreak 3). The rest of the environmental *S. marcescens* isolates were genetically distinct belonging to PFGE type II.

Conclusion: Molecular typing elucidate that the three consecutive outbreaks in the NICU during a 35-months period had been caused by genetically distinct *S. marcescens* clones, a fact consistent with the repeated introdution and spread of new clones in the NICU. The fact that no *S. marcescens* PFGE type was found to persist during the whole period under investigation, indicates good decontamination and disinfection policies in the ICU. However the entrance and explosive spread of new clones in the ICU underlines possible gaps in infection control practices. Thorough review of these practices resulted in the containment of the epidemic.

**Super spreader and nosocomial outbreaks**

L. Danzmann, P. Gastmeier, R.P. Vonberg* (Hannover, Berlin, DE)

Objectives: The role of infected and/or colonized health care workers (HCWs) as a potential source of a nosocomial outbreak is yet unclear. The present study summarizes data from such outbreaks in order to elucidate areas of special interest and to clarify potential reasons for transmission.

Methods: We conducted a systematic review of the medical literature based on PubMed, the Outbreak Database (www.outbreak-database.com), and on a hand search of so retrieved articles. We extracted data on the setting, type of infection, number of patients, duration of the epidemic, and infection control measures.

Results: A total of 45 outbreaks and 1,449 patients got included thereof 51 fatal cases. The main route of transmission was direct or indirect contact (53% of all outbreaks). Duration of the outbreaks ranged from 1 to 287 weeks (mean: 28 weeks; median: 10.5 weeks). The number of affected patients per outbreaks ranged from 1 to 75 (mean: 9.5; median: 7). Surgical (40%) and neonatological departments (24%) were most often involved in outbreaks primarily caused by staff. Transmission took frequently place in surgical theaters (40%) and on peripheral wards (38%). The primary types of infection were surgical site infections (27%) and hepatitis B (22%). The corresponding causative
main agents were *Staphylococcus aureus* (32%), hepatitis B virus (14%), and *Streptococcus pyogenes* (12%). Physicians (59 thereof 30 surgeons) and nurses (56) were the predominant staff spreading the pathogen to patients. Nine per cent of them were aware of their carrier status. When checking for the compliance to hand hygiene (HH) of the person causing the outbreak, we found that HH was considered “adequate” in 14% of the outbreaks but “poor” in 10.5%. The infection control measures most often implemented in order to terminate the outbreak included screening of personnel (90%) and patients (77%), education of staff and disinfection/sterilization procedures (53% each), application of antimicrobial substances (47%), improvement in HH (46%), and use of protective clothing (39%).

**Conclusion:** Nosocomial outbreaks caused by staff are an important problem in the field of epidemiology and infection control. In the next step we will check for characteristics of HCWs that may predispose for an increased pathogen spread to patients.

**P1242 A retrospective review of hospital MRSA outbreaks: does UK15 cause increased colonisation of healthcare workers?** J. Hart*, R. Lee, K. Christiansen, J.O. Robinson (Perth, AU)

**Objectives:** In Western Australia (WA) the epidemiology of healthcare-associated methicillin resistant *Staphylococcus aureus* (MRSA) strains has evolved over the last decade, with ST239-MRSA-III (AUS2/3) decreasing in incidence and a concomitant rise in ST22-MRSA-IV (UK15). A “Search and destroy” policy that includes screening and isolating all patients exposed during an outbreak and screening and decolonising Healthcare workers (HCW) has successfully controlled nosocomial spread. As result, MRSA is not endemic in WA teaching hospitals but outbreaks do occur. Assessing factors that contribute to HCW colonisation will allow better management of outbreaks. It has been anecdotally noted that the increasing prevalence of UK15 appears to be associated with increased HCW colonisation during outbreaks; therefore we aimed at comparing AUS2/3 and UK15 colonisation rates of patients and HCWs during outbreaks.

**Methods:** A retrospective review was performed of MRSA outbreaks occurring between January 2000 and December 2009 at Royal Perth Hospital. Records were examined and data extracted including demographic information on colonised patients and HCWs and the number of patients and HCWs colonised with the outbreak strain of MRSA.

**Results:** Six AUS2/3 outbreaks (median duration 49 days [range 20–392]) were compared with 10 UK15 outbreaks (median duration 52 days [range 22–128], p = 0.69). The number of patients screened during the outbreaks were similar between AUS2/3 and UK15 (median 124 [range 39–338] and 106 [range 39–338], p = 0.59). Likewise, the number of HCWs screened were similar between AUS2/3 and UK15 outbreaks (median 159 [range 120–288] and 125 [range 72–330], p = 0.30). The number of patients colonised were comparable between AUS2/3 and UK15 outbreaks (median 25 [range 5–26] and 7 [3–20], p = 0.07) but the number of HCWs colonised was significantly lower in AUS2/3 compared to UK15 outbreaks (median 0.81 [range 0.56–2.17] and 3.66 [0–5.56]) respectively, p = 0.013.

**Conclusion:** HCWs are more likely to become colonised during outbreaks of UK15 compared with outbreaks of AUS2/3. Given that UK15 has largely replaced AUS2/3 as the dominant outbreak strain in hospitals in Western Australia, and is the dominant strain in many countries, this highlights the importance of introducing early screening and decolonising of HCWs to outbreak strategies for swift control.

**P1243 A systematic review of nosocomial outbreaks caused by multidrug-resistant Gram-negative bacteria** E. Zhuchenko, K. Graf*, R.P. Vonberg (Hannover, DE)

**Objectives:** Multi drug resistant Gram negative bacteria (MRGN) represent an increasing problem in many health care setting. Until now there are only few data on sufficient infection control measures that should be applied in the case of an outbreak.

**Methods:** We conducted a systematic review of the medical literature based on PubMed, the Outbreak Database (www.outbreak-database.com), and on a hand search of so retrieved articles. Pathogens of interest were *Acinetobacter* spp. (ACI), *Pseudomonas* spp. (PAE) and ESBL-producing Enterobacteriaceae (ESBL). We extracted data on the setting, type of infection, number of patients, duration of the epidemic, and infection control measures.

**Results:** A total of 59 ACI outbreaks, 109 PAE outbreaks, and 57 ESBL outbreaks were included.

| Type of Infection | ACI Outbreaks | PAE Outbreaks | ESBL Outbreaks |
|-------------------|--------------|--------------|---------------|
| Blood stream infection | 34 (57%) | 64 (59%) | 42 (74%) |
| Respiratory tract infection | 37 (62%) | 60 (55%) | 32 (56%) |
| Urinary tract infection | 17 (28%) | 42 (39%) | 38 (67%) |
| Wound infection | 18 (30%) | 44 (40%) | 15 (26%) |
| Menigitis | 8 (13%) | 6 (6%) | 11 (19%) |

| Age Group (Years) | ACI | PAE | ESBL |
|-------------------|-----|-----|------|
| < 10 | 10 (17%) | 18 (17%) | 18 (32%) |
| 11-17 | 10 (17%) | 16 (15%) | 3 (5%) |
| 18-50 | 20 (34%) | 32 (29%) | 6 (10%) |
| >50 | 14 (23%) | 36 (33%) | 10 (18%) |
| Not mentioned | 29 (49%) | 53 (49%) | 29 (49%) |

**Route of Transmission**

| Route of Transmission | ACI | PAE | ESBL |
|-----------------------|-----|-----|------|
| Contact | 16 (27%) | 73 (67%) | 28 (49%) |
| Unknown | 43 (73%) | 36 (33%) | 29 (51%) |

**Infection Control Measures**

| Measure | ACI | PAE | ESBL |
|---------|-----|-----|------|
| Disinfection/sterilization | 29 (49%) | 39 (36%) | 25 (44%) |
| Environmental screening | 34 (58%) | 47 (43%) | 21 (37%) |
| Hand hygiene | 31 (53%) | 28 (26%) | 24 (42%) |
| Isolation precautions | 19 (32%) | 15 (14%) | 19 (33%) |
| Closure of the ward | 9 (15%) | 6 (6%) | 8 (14%) |
| Screening of patients | 19 (32%) | 19 (17%) | 22 (39%) |
| Screening of personnel | 15 (25%) | 20 (18%) | 8 (14%) |
| Immediate feed back | 24 (41%) | 39 (36%) | 23 (40%) |
| Extensive education of staff | 4 (7%) | 4 (4%) | 7 (12%) |
| Protective clothing | 15 (25%) | 9 (8%) | 17 (30%) |
| Patient-to-patient ratio | 1 (2%) | 5 (5%) | 8 (14%) |
| Medical devices | 6 (10%) | 25 (23%) | 6 (11%) |
| Vaccination | 0 (0%) | 0 (0%) | 0 (0%) |
| Not mentioned | 22 (37%) | 52 (48%) | 27 (47%) |

**Source of Pathogen**

| Pathogen | ACI | PAE | ESBL |
|----------|-----|-----|------|
| Index patient | 5 (8%) | 3 (3%) | 11 (19%) |
| Environment | 2 (3%) | 10 (9%) | 1 (2%) |
| Medical devices | 7 (12%) | 33 (30%) | 7 (12%) |
| Multiple dose vials | 3 (5%) | 11 (10%) | 1 (2%) |
| Staff | 0 (0%) | 5 (5%) | 2 (3%) |
| Other | 2 (3%) | 7 (6%) | 5 (9%) |
| Unknown | 46 (88%) | 40 (37%) | 30 (53%) |

**Results:** A total of 59 ACI outbreaks, 109 PAE outbreaks, and 57 ESBL outbreaks were included.

The median duration of the outbreak was 183 days (ACI), 92 days (PAE), and 210 days (ESBL) respectively. The average number of affected patients in the three groups was 25 (ACI outbreaks); thereof infected: 19 patients; thereof died: 4 patients), 23 (PAE; 16; 3),
MRSA – colonisation and screening

[Introduction: The emergence of Community-acquired Methicillin-resistant Staphylococcus aureus (CA-MRSA) in the 1990s posed new challenges for preventive and therapeutic approaches of staphylococcal infections. Studies that assess prevalence and characteristics of CA-MRSA in high risk populations can improve our knowledge of its epidemiology.]

Objective: To identify prevalence, risk factors and molecular characteristics of nasopharyngeal colonization with CA-MRSA among men in a correctional facility in the municipality of Avaré, Southeastern Brazil.

Methods: In a cross-sectional survey, nasopharyngeal swabs were obtained from 302 men from Avaré Correctional Facility in the municipality of Avaré, Southeastern Brazil.

Results: The general prevalence of S. aureus colonization was 16.5%, while MRSA was found in 5.0% of study subjects. Disk diffusion tests failed to identify most strains that harboured mecA gene. PVL-related genes were not found in any of the study isolates. Colonization with S. aureus was independently associated with “sex with men” (OR=4.37, 95% CI=1.50–12.74) and the presence of pulmonary (OR=12.16, 95% CI=1.99–74.41) or skin (OR=2.44, 95% CI=1.24–4.81) diseases. Risk factors for MRSA colonization were: “sex with men” (OR=6.07, 95% CI=1.99–19.74) or skin diseases (OR=2.44, 95% CI=1.24–4.81) and pulmonary (OR=12.16, 95% CI=1.99–74.41) and skin diseases. The general prevalence of MRSA colonization was 16.5%, while MRSA was found in 5.0% of study subjects. Disk diffusion tests failed to identify most strains that harboured mecA gene. PVL-related genes were not found in any of the study isolates. Colonization with S. aureus was independently associated with “sex with men” (OR=4.37, 95% CI=1.50–12.74) and the presence of pulmonary (OR=12.16, 95% CI=1.99–74.41) or skin (OR=2.44, 95% CI=1.24–4.81) diseases. Risk factors for MRSA colonization were: “sex with men” (OR=6.07, 95% CI=1.99–19.74) or skin (OR=2.44, 95% CI=1.24–4.81) and pulmonary (OR=12.16, 95% CI=1.99–74.41) and skin (OR=2.44, 95% CI=1.24–4.81) diseases.

Conclusion: The prevalence of MRSA colonization was high. While MRSA colonization was related to sex with men (mostly inmates from the correctional facility), M SSA was associated with (mostly heterosexual) intimal visits with partners that came from the community. Even though there was an association between S. aureus carriage and skin diseases, no PVL-producing isolates were found.

Risk factors for methicillin-resistant Staphylococcus aureus colonization on admission to rehabilitation medicine

A. Pan*, F. Antonioli, R. Lazzari, A. Patroni, S. Garilli, F. Bernieri, P. Mangoni, C. Meinecke (Cremona, Esine, Brescia, IT)

Objective: M SSA may colonize a discrete proportion of patients transferred to rehabilitation medicine (RM). Defining risk factors associated with M SSA carriage upon admission could possibly permit to better allocate resources for microbiologic screening and to identify patients at higher risk, for preventive isolation.

Methods: All patients admitted to a rehabilitation ward of the hospital of Cremona, where M SSA nasal screening is routinely performed 48 hours within admission, were investigated for risk factors associated with M SSA carriage. The clinical chart was analyzed to identify information regarding: reason of admission, previous diseases, recent surgery, recent antibiotic therapy, general conditions (Barthel, FIM, CIRS scales). Univariate and multivariate analysis were performed. A score to identify patients at higher risk of M SSA carriage was defined.

Results: We evaluated 301 patients, 13 (4.3%) previously unknown M SSA positive upon admission. Univariate analysis ha identified 12 risk factors associated with an increased risk of M SSA positivity:
Prevalence and basal characteristics of persistent and intermittent colonization of methicillin-resistant Staphylococcus aureus among residents living in long-term care facilities

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Objective: To determine the prevalence and basal characteristics associated with persistent and intermittent methicillin-resistant Staphylococcus aureus (MRSA) colonization among subjects living in long-term care facilities (LTCFs) in southern Spain.

Methods: From April 2009 to November 2009, all subjects living in six LTCFs of our area were initially included in a prospective longitudinal study. Subjects were visited by the investigators at baseline and one year later. Only the residents with two visits were the final population of our study. In each visit, clinical data and cultures of nasal were included. Subjects were screened using nasal swabs and these were cultured in a chronogenic media. Individuals were classified as non-carriers (both samples were negative), persistent MRSA nasal colonization (both samples were positive) and intermittent MRSA nasal colonization (at least one sample was positive). The following data were obtained in residents to identify risk factors for persistent MRSA carriers: age, sex, time of living in residence, comorbidities (Charlson index), previous antibiotic treatment, prior MRSA isolation, presence of decubitus ulcers, functional status (Barthel index), use of invasive devices and invasive procedures (surgery, endoscopies) in the last year. Data were analyzed using SPSS version 14.

Results: A total of 326 subjects were evaluated, of which 258 (79%) were classified as no colonized. Among 68 residents with positive cultures for MRSA, 54 (79%) were intermittent carriers and 14 (21%) were persistent carriers. The annual incidence of MRSA acquisition was 10.4%. The only significant baseline characteristic related to persistent MRSA colonization was an invasive procedure in the last year (4 [28%] vs. 3 [5%], respectively, p=0.035).

Conclusions: A single sample of subjects living in LTCFs could misclassify the SARM colonization status. It is necessary a major knowledge about the dynamic of MRSA colonization in residents of LTCFs. There is a high annual incidence of SARM in these institutions. An invasive procedure, including surgery in the last year, was the only factor associated with persistent MRSA carrier.

Nasal carriage of methicillin-resistant staphylococci among sick and healthy horses in Portugal

N. Couto*, P. Tilley, J. Simões, J. Sales-Luís, C. Pumba (Lisbon, PT)

Objectives: To investigate the frequency of methicillin-resistant staphylococci-MRSA carriage in a random sample of equines entering the Faculty of Veterinary Medicine Teaching Hospital, Lisbon, Portugal.

Methods: From March 2008 to October 2010, nasal swabs were obtained after admission to the hospital from 71 horses (38 from horses with Recurrent Airway Obstruction – RAO; 13 from horses with Equine Gastrointestinal Ulcer Syndrome – EGUS and 20 from healthy horses that were admitted to either elective surgery (castration) or as part of a control group for a RAO study). The swabs were enriched in 3 ml of Muller-Hinton broth containing 65 g/l NaCl. Five-hundred ul were inoculated into 3 ml Tryptone Soy Broth with 3.5 mg/l cefoxitin and 75 mg/l aztreonam. Ten ul were plated onto a chromogenic agar selective for MRSA including Staphylococcus aureus, MRSA-IA – bioMérieux. Antimicrobial susceptibility testing was performed using the microbroth dilution method (Sensititre CMV1AMAF and BOPO6F microplates, Trek Diagnostic Systems). MRSA isolates were typed with BBL™ Crystal™ system (BD Diagnostic System, USA) and identified by PCR for the mecA gene. MRSA were subjected to spa typing, SCCmec typing, and ST398 PCR.

Results: From a total of 71 equines 15 MRSA were isolated (13 were methicillin-resistant coagulase-negative staphylococci-MRCoNS and 2 were MRSA). In the RAO group 5 animals carried 5 MRCoNS isolates. Equines with EGUS carried 1 MRCoNS and 1 MRSA (spa type t062, SCCmecII, ST5). From healthy horses 7 MRCoNS and 1 MRSA (spa type t011, SCCmecIV, ST398) were isolated. ST398 MRSA isolate was co-resistant to tetracycline, gentamicin and spectinomycin. The ST5 MRSA isolate was co-resistant to erythromycin and clindamycin. Among MRCoNS, 1 Staphylococcus sciuri was co-resistant to gentamicin, spectinomycin, clindamycin and fluoroquinolones.

Conclusions: Our study detected for the first time MRSA isolates among equines in Portugal including MRSA carriage of an associated animal clone (ST398) and the second human main clone in Portugal (ST5). In horses it is known that MRSA can cause varying types of infection in both colonized and non-colonized individuals. Resistance to β-lactams, namely penicillin G and associated resistance among these isolates may compromise future antimicrobial chemotherapy in equines. The question of potential interspecies and zoonotic transmission exists and MRSA carriage among equines is a relevant animal and human health issue.

Methicillin resistance, virulence factors and genetic lineages of Staphylococcus aureus isolates from nasal samples of healthy sheep in Tunisia

H. Gharsa*, K. Ben Slama, C. Lozano, E. Gómez-Sanz, N. Khibi, A. Jouini, M. Zarazaga, A. Boudabous, C. Torres (Tunis, TN; Logroño, ES)

Objective: To study the carriage rate, resistance mechanisms, virulence traits and genetic lineages of nasal S. aureus of healthy sheep in Tunisia.

Methods: Nasal swabs of 163 healthy sheep were obtained in two farms (n = 59 animals) and in one big abattoir that receives sheep from farms of all Tunisia (104 animals, sampled in 6 different days during a period of two months). Samples were inoculated into Baird Parker and ORSAB plates for S. aureus and methicillin-resistant S. aureus (MRSA) recovery, respectively. Isolates were identified by biochemical methods and mec-nuc-gene PCR. Antibiotic susceptibility profile was determined by disk diffusion. The presence of 32 resistance genes, 18 staphylococcal enterotoxin genes and lukF/lukS (encoding Panton-Valentine leucocidin, PVL), lukE/lukD, LukM, hla, hlb, hld, hlg, hlv, eta, ets and tst were studied by PCR. S. aureus isolates were typed: spa, agr, SCCmec, MLST and Small-PFGE.

Results: 73 of 163 samples contained S. aureus (44.8%) and one isolate per sample was studied. Five of 73 isolates were MRSA (3% of tested animals) and the remaining 68 isolates were methicillin-susceptible
Effective screening for methicillin-resistant Staphylococcus aureus on the basis of hospital-specific risk factors

A. Bühling*, R. Tech, K. Mydlak, P. Thorausch, F. Schwab, K. Schenke

Objectives: The aim of this study was to determine the MRSA-prevalence and the risk factors for MRSA-carriage of patients admitted to a hospital and therefore to find the most effective search policy as an additional part of anti-MRSA-measures.

Methods: A 9 month MRSA-admission screening was performed in a 230-bed hospital. Risk factors to be colonised with MRSA at admission were determined using a questionnaire based on recommendations of the Robert Koch-Institute (RKI), the German institution for disease control and prevention. In addition to these factors we included the age in the analysis.

Results: From all 7906 admitted patients 6296 (79.6%) provided nasal swabs. 5244 patients of them (83.3%) had a fully filled questionnaire and were included in the further analysis. 47 MRSA-carrier-cases were detected (prevalence of 0.9 MRSA/100 admissions). Independent risk factors were previous MRSA-carriage, need for long-term care, receipt of antibiotics during the previous six months and age. We found 761 patients with RKI-risk factors (14.5%) and 2653 patients older than 67 years (50.6%). Only 24 (51%) of all MRSA-colonised patients performed official RKI-risk factors including 11 (23.4%) patients with previous carriage. Remarkably, 36 (76.6%) of all MRSA-carriers aged >67 years.

Conclusion: The results have shown that for this particular hospital age >67 years and previous MRSA-carriage are effective and sensitive indicators for a MRSA colonisation at admission. These independent and easy to establish risk factors were included in screening policy. To find a search policy for MRSA-carriage on admission for an individual hospital it can be recommended to perform a short time screening of all patients with parallel analysis of risk factors.

Study of Staphylococcus aureus nasal carriage in diabetic patients and non-diabetic controls: preliminary results

C. Loupa*, A. Karaitianou-Velonaki, C. Chiotis, G. Kouppari, D. Voyatzoglou, M. Lelekis (Athens, GR)

Objectives: Staphylococcus aureus nasal carriage is an important predisposing factor for subsequent infection. Aim of the present study was to estimate the prevalence of S. aureus nasal carriage in diabetic patients and to compare it with that of non-diabetic controls. We report our preliminary results.

Methods: 101 consecutive diabetic persons (36 men, 65 women, age 65.2±11.9 years) of Diabetes Outpatient Clinic and 105 non-diabetic controls of Internal Medicine Outpatient Clinic (30 men, 75 women, age 67.9±11.3 years) of the same hospital were enrolled in this study. People with recent antimicrobial treatment were excluded. Cotton-wool swabs and transportation media were used for nasal cultures. We used SPSS 11.5 for the statistical analysis.

Results: In 27/101 patients (26.7%), cultures were positive for S. aureus (3 MRSA/24 MSSA isolates). 2 of these patients had a recent history of both hospitalization and antibiotic treatment, 4 of antibiotic use only and 4 of hospitalization only. Out of the 14/27 positive patients in whom culture could be repeated, 10 proved persistent carriers, while in the rest 4 the second culture was negative (2 of these were treated with antibiotics in the meantime). Regarding non-diabetic controls, 11/105 (10.5%) were colonized (4 MRSA/7 MSSA isolates). 3/11 reported antibiotic use and nobody had a recent history of hospitalization. None of the 11 positive controls had a second nasal culture performed. A statistically significant difference in nasal carriage was found between patients and controls (p=0.003, x2, SPSS 11.5).

Conclusions: According to our preliminary results, prevalence of S. aureus nasal carriage in diabetic patients was high (26.7%, more than 1 out of 4 patients), and more than 26% higher than in non-diabetic people. It is worth noting that a significant proportion of positive cases were persistent carriers and that among isolates there were methicillin-resistant strains (MRSA). If our preliminary results are confirmed in the long term and due to the clinical significance of S. aureus carriage, diabetic patients might have to be checked for S. aureus nasal carriage during routine visits and carriers have to be treated.

Staphylococcus aureus colonisation and recurrent furunculosis in young Greek adults: the role of CA-MRSA

V. Sakka, P. Nikon*, L. Galani, A. Fragou, K. Karaiskos, T. Panagia, S. Kanellaki, G. Petrakos, H. Giamarellou (Athens, GR)

Background: The precise role of nasal carriage of Staphylococcus aureus in recurrent furunculosis remains unclear.

Objectives: To seek correlations between epidemiological characteristics, S. aureus carriage, and clinical manifestations in patients with community-acquired furuncles.

Methods: Since 2006, we studied clinical data and bacteriological samples (cultures from lesions and nasal, axillary and inguinal carriage) prospectively collected from patients presenting with chronic furunculosis in an Outpatient Infectious Disease Department.

Results: 100 patients, 15–70 years of age (mean: 34.5±11.6 years), 40% men with a history of “heavy” disease (mean of 1.4 preceding years and ≥3 episodes per year), Diabetes mellitus or impaired glucose tolerance was recorded in 13/75 (17.3%) patients. Other classical predisposing factors e.g. chronic renal disease, immunoglobulin deficiency, athletes, recruits and iv drug abusers were not identified. 43/83 (51.8%) reported a roommate with same degree of skin infections and 5/80 (6.2%) an indoor pet. 81 (81.8%) had active furuncles at the initial visit. Thirty six patients had no S. aureus carriage at any site, where as 50% had nasal, 20.6% axillary and 41% inguinal carriage. S. aureus was confirmed in 74 patients, either from a clinical sample or from carriage. 75.7% were methicillin (cefoxitin) resistant, whereas the percentage of resistance for other antibiotics was: erythromycin 14.9%, clindamycin 12.2%, rifampicin 1.3% and trimethoprim-sulfamethoxazole 5.4%. No strain was resistant to quinolones, minocyclin, and mupirocin. All patients were treated with chlorhexidine gluconate for hair and body bathing for a month, whereas 66% (mostly the nasal carriers) also received intranasal mupirocin tid for two weeks. 73% of the patients received an antibiotic regimen for 2 weeks, consisted of a combination of minocyclin 100 mg bid plus rifampicin 600–300 mg (54%), cotrimoxazole 960 mg bid plus rifampicin 600–300 mg (9%), clindamycin alone or plus rifampicin...
External quality assessment scheme for MRSA screening

E. Fagan*, S. Seaton, C. Walton (London, UK)

Objectives: To evaluate the results from clinical diagnostic microbiology laboratories taking part in the United Kingdom National External Quality Assessment Service (UK NEQAS) for Microbiology MRSA screening scheme between 2009 and 2011.

Methods: Quality assessment of MRSA screening was performed on twenty simulated nasal swab specimens. Specimens sent included 13 positive and seven negative for MRSA.

Results: Analysis of the results showed that with the exception of one specimen (specimen 9281) performance by culture was good with over 93% of participants reporting correctly on the detection of MRSA. Specimen 9281 contained a methicillin resistant S. aureus and a coagulase negative staphylococcus; only 84.6% of participants reported correctly. The overall false positive rate for the MRSA negative specimens was 1.4% (20/1463). Whilst the overall false negative rate for the MRSA positive specimens was 2.5% (72/2907). One specimen contained a ciprofloxacin susceptible community MRSA which 12 laboratories incorrectly reported as negative for MRSA. The most commonly used culture method is Oxoid Brilliance MRSA chromogenic agar plates. Overall performance by molecular methods was good with 92.4% (789/854) of participants reporting correctly on the detection of MRSA. The most commonly used molecular method is Cepheid Xpert MRSA. During the 21 months the scheme has been running, the number of laboratories reporting molecular screening results has risen from 22 to 67, which was an increase of 200%

Conclusion: The UK NEQAS for Microbiology MRSA screening scheme has been operating for 21 months and provides participants with the opportunity to assess the quality of culture and molecular screening techniques they use to detect MRSA. The results from participating laboratories demonstrate a good performance. The scheme has highlighted problems with identifying ciprofloxacin susceptible community MRSA and also that some molecular techniques give false positive results when confronted with a mecA knockout gene.

Estimating the effectiveness of isolation and decolonisation measures in reducing MRSA transmission in hospital general wards: a model-based analysis

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Objectives: Methods used to control the spread of hospital pathogens such as methicillin-resistant Staphylococcus aureus (MRSA) include both non-specific interventions (e.g. hand hygiene) and targeted interventions (such as screening combined with patient isolation and/or decolonisation treatment). Such targeted interventions, while widely used, are controversial due to the lack of robust evidence of their effectiveness at reducing transmission. In this study we aimed to estimate the effectiveness of isolation and decolonisation measures in reducing transmission of MRSA.

Methods: We used prospectively collected MRSA surveillance data from over 14,000 admissions across ten hospital general wards at a London teaching hospital between January 2006 and April 2007. MRSA carriage was assessed with admission and discharge culture swabs; known MRSA patients were given decolonisation treatment with chlorhexidine and mupirocin and were isolated using side rooms where possible, and patient cohorting and contact precautions otherwise. Data were analysed in a Bayesian framework using a Markov Chain Monte Carlo (MCMC) algorithm to infer the unobserved colonisation times from the screening data. We estimated the overall effect of isolation and decolonisation measures by comparing transmission from isolated and unisolated individuals separately, and calculating the relative risk of acquisition.

Results: We estimated that the combined effect of isolation and decolonisation reduced transmission by 64%: relative risk of acquisition 0.36 (95% CI: 0.21, 0.63). Unisolated patient days were found to be a determining factor in transmission with background (e.g. environmental) transmission minimal. Moreover, we estimated that 54% (95% CI: 46%, 60%) of all colonised patient days were spent without any isolation precautions, mainly due to undetected carriage.

Conclusions: This is the first strong evidence that isolation measures combined with decolonisation treatment are associated with a reduction in MRSA transmission in general hospital wards. While these findings provide support for active methods of MRSA control, further research is needed to determine the relative importance of isolation and decolonisation in preventing transmission, and their cost effectiveness.

Surveillance of topical antibacterial usage in primary care in Wales

M. Heginbotham* (Cardiff, UK)

Objectives: Antimicrobial stewardship programs tend to focus on the appropriate use of systemic antimicrobials. Little data exists regarding the extent of topical antibacterial use, which also has the potential to select resistant organisms. The objective of this study was to determine the extent of topical antibacterial use across Wales.

Methods: Topical antibacterial dispensing was obtained from the Prescribing Services Unit (PSU) of Health Solutions Wales. Primary care dispensing data comprised prescriptions submitted to PSU by dispensing contractors for re-imbursement purposes.

Results: In 2009, the total number of antibacterial items (oral, parenteral or topical) dispensed in Wales was 3,016,112, of which 618,519 (20.5%) were topical preparations. Topical antibacterials accounted for 48% of topical antibacterial dispensing across Wales in 2009. Fusidic acid (FUS) was the most commonly dispensed topical skin preparation, with an average dispensing of 54.3 items/1000 ppa (See Figure 1).

Ear preparations were the second most commonly dispensed items, accounting for 31% of topical dispensing across Wales. Chloramphenicol (CHL) and fusidic acid were the two most commonly dispensed eye preparations, with an average of 43.5 items/1000 ppa and 15.8 items/1000 ppa respectively. 

Conclusions: This is the first strong evidence that isolation measures combined with decolonisation treatment are associated with a reduction in MRSA transmission in general hospital wards. While these findings provide support for active methods of MRSA control, further research is needed to determine the relative importance of isolation and decolonisation in preventing transmission, and their cost effectiveness.

Measuring and influencing community antibiotic use

P1255

Surveillance of topical antibacterial usage in primary care in Wales

M. Heginbotham* (Cardiff, UK)

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Figure 1. Topical Antibacterial Agents
More awareness of antibiotic risks and benefits are needed amongst Polish general public

B. Mazinska*, W Hryniewicz (Warsaw, PL)

Objectives: Widespread and inappropriate antibiotic use leading to antibiotic resistance directs national and international antibiotic resistance control strategies to educate not only of health-care professionals but also of the general public in order to promote prudent antibiotic use. The aim of the study was to assess the level of knowledge of the Polish general public regarding antibiotics and whether actions undertaken during educational campaigns resulted in an increased knowledge of this subject.

Methods: The survey was conducted in Poland between 2009 and 2010, in four waves before and after the European Antibiotic Awareness Day campaign. The survey was based on 14 self-designed questions. The questionnaire was divided into four sections: previous antibiotic exposure, patient’s expectation of antibiotic prescription, sources of information, effectiveness of campaign on prudent antibiotic use. The survey was carried out by Millward Brown SMG/KRC on a representative sample of 1000 people, using Computer Assisted Telephone Interviews (CATI). Statistical analysis was performed by x² test.

Results: Overall, the percentage of people using antibiotics was high. In the fourth wave, 41% of adults had used antibiotics in the last 12 months and 63% in the last 24 months. The majority (91%) had used antibiotics prescribed by physicians, mostly general practitioners. Respondents considered that antibiotics were warranted for common colds (28%), sore throats (27%), the flu (14%) and coughs (18%). The results of the fourth wave show that the majority of interviewees expected antibiotics to be prescribed for pneumonia (85%), bronchitis (77%) or urinary tract infections (59%). More than half of the respondents (63%) believed that antibiotics kill viruses. The percentage of people who had encountered any information on the prudent use of antibiotics within the last year was significantly higher in comparison to the third wave of the survey. The most reliable sources of information on antibiotics were physicians (92%), hospital staff (77%) and pharmacists (60%). Almost half (47%) of the respondents who had encountered information/initiatives within the last year declared a change in their attitude towards antibiotic use.

Conclusions: Inappropriate antibiotic use is highly prevalent in Poland. New positive trends sustained by public campaigns have emerged. Additional didactic and systematic education regarding appropriate antibiotic use is needed.

The impact of antibiotic education sessions in primary care in the Republic of Ireland – a case study

M. Murphy*, S. Byrne, C.B. Bradley (Cork, IE)

Objectives: In 2008, antibiotic use in primary care in the Republic of Ireland (ROI) declined for the first time. The reduction coincided with European led educational campaigns on prudent antibiotic use for the public and general practitioners (GPs). The observed rate of antibiotic use in primary care in Ireland for 2009 was 8.5% lower than the expected rate as projected by the Health Protection Surveillance Centre in ROI. Interventions in primary care such as antibiotic workshops have shown to reduce and rationalise antibiotic prescribing. The objectives of this study were to observe if antibiotic consumption was reduced where educational meetings took place in ROI.

Methods: All GP continuing medical education (CME) groups in ROI were invited to participate. GPs recorded data on all antibiotics prescribed by them in 100 consecutive patients’ consultations and the conditions being treated. Educational sessions through CME groups took place to distribute prescribing feedback and discuss the findings. Two Local Health Offices (LHOs) in nearby locations (A and B) were chosen to compare the impact of the study. LHO A did not have an antibiotic CME session for GPs. LHO B had the CME session in January 2009. Data on systemic antibiotic items were obtained from the General Medical Services (GMS) database before and after the antibiotic CME sessions took place. The GMS database contains data from patients receiving free medical services. Antibiotic prescribing rates were calculated using the total number of patients who received a prescription in the time period.

Results: There were 17 GP tutors (43.20%) who ran 65 meetings nationally. In LHO B, CME sessions on antibiotic use took place in January 2009. In December 2008, the rate of antibiotic prescriptions was 244 per 1000 patients. There was an immediate reduction in antibiotic use seen in February 2009 and March 2009 (176, 178/1,000 patients). The reduction was also sustained 12 months later (December 2009: 162 prescriptions/1,000 patients). There was a smaller reduction seen in antibiotic use in LHO A (December 2008: 259/1,000 patients, December 2009: 224/1,000 patients).

Conclusion: These results demonstrate that the CME sessions on prudent antibiotic use played a role in the reductions seen in antibiotic use in ROI since 2008. This further supports the benefit of educational initiatives for both the public and health professionals.

A national study of antimicrobial stewardship structures and antimicrobial use in Irish long-term care facilities

M.P. Cotter*, S. Donlon, F. Roche, H. Byrne, F. Fitzpatrick (Dublin, Cork, IE)

Objectives: Essential components of the prevention and control of healthcare associated infection (HCAI) and antimicrobial resistance (AMR) in long term care facilities (LTCF) are staff education, the availability of specialist antimicrobial stewardship advice, and AMR surveillance. Presently, there is limited information available on this in Irish LTCFs.

Methods: A point prevalence study was conducted in June 2010 as part of a European study of HCAI in European LTCFs (HALT). Sixty-nine Irish LTCFs completed a questionnaire detailing information on LTCF antimicrobial use, antibiotic stewardship practices and protocols. Data was collected by trained local senior clinical staff (nursing or medical) and/or infection prevention and control nurses. Information was collected on; HCAI risk factors, signs and symptoms of infection and antimicrobial use from eligible residents. The aim of this study was to evaluate medical care, infection control and antibiotic stewardship practices and protocols in Irish LTCFs.

Results: A total of 4,170 residents in 69 LTCF were surveyed; 1664 (40%) males and 2506 (60%) females. Antimicrobial stewardship activities included: availability of local antimicrobial guidelines (28%), antimicrobial consumption surveillance (16%), AMR surveillance (12%) and prescribers education (7%). The most common HCAIs were urinary tract (n = 62, 39.7%), respiratory (n = 44, 28.2%) and skin infections (n = 31, 19.9%). Of the 426 (10.2%) residents on antibiotics, 25 (6%) were prescribed two or more antibiotic types. Antibiotics were prescribed for treatment (57.8%, n = 262) and prophylaxis (40.2%, n = 182) of infection. The most common indications for therapy included respiratory tract (35.1%), urinary tract (32.1%) and skin infections (21.8%). Prophylactic antibiotics were predominantly prescribed for prevention of urinary tract infection (35.8% of total prescriptions) with trimethoprim most frequently prescribed. Seventeen (10.7%) residents on UTI prophylaxis had a urinary catheter in situ.

Conclusion: This study provides an important baseline on antimicrobial stewardship activities and antimicrobial consumption in Irish LTCFs.
to inform future HCAI/AMR preventative strategies. The frequency of prophylactic antimicrobial prescribing underlines the importance of antimicrobial stewardship in this setting and highlights the need for national antimicrobial stewardship guidelines specifically for LTCF.

**P1250** Variation in outpatient antibiotic prescribing

J. Zweigner*, F. Schwab, E. Meyer, S. Ebert, P. Gastmeier (Berlin, DE)

**Objectives:** The overuse and inappropriate prescription of antibiotics drove bacterial resistance. In Germany ~85% of all antibiotics are prescribed in ambulatory care but studies about antibiotic prescribing practice in this setting are rare. Thus, the aim of this study was to analyse in detail antibiotic prescribing in the outpatient setting.

**Methods:** We analysed data from the largest regional public-sector health insurer AOK in the German federal state Brandenburg in respect to diagnosis and antibiotic prescription in ambulatory care in the 1st quarter 2009. The AOK provides statutory health insurance to more than 1 million out of ~3 million inhabitants of Brandenburg. We included data from physicians of all specialisations who treated at least 50 AOK outpatients per quarter with one or more diagnosis and/or prescription.

**Results:** A total of 1,983 physicians prescribed 74,529 times antibiotics for systemic therapy to 685,379 patients. In median, every 10th patients received an antibiotic. The four top prescribed antibiotics were ciprofloxacin (n = 8283) followed by roxithromycin (n = 6471), azithromycin (n = 6118) and cefuroxime (n = 5415). The leading infections made acute upper respiratory tract infections and acute bronchitis (n = 34,175) followed by urinary tract infections (n = 7,909), acute tonsillitis (n = 5,453) and viral infections (n = 5,145). Paediatricians and general practitioner (GP) saw the most patients with infection followed by urologists and ear, nose and throat (ENT)-specialists. Paediatricians prescribed in median only 0.4 time an antibiotic per encoded infection. GPs prescribed an antibiotic 1.1 times, urologists 1.4 times and ENT-specialists even 3.1 times per infection. The range of the number of prescription (10th-90th percentile) varied substantially by medical specialist from 0.7 within the paediatricians to 1.6 within the GPs. Variation was 8.4 within the ENT doctors and peaked at 8.5 within the urologists.

**Conclusion:** Upper respiratory tract and urinary infections were the most common infections in the outpatient setting and chinolones, macrolides and cephalosporines the most frequently prescribed antibiotics. Paediatricians were more conservative to prescribe antibiotics in relation to the numbers and kind of infections. The amount of antibiotics prescribed by urologists and ENT-specialists varied largely (up to 8.5 times). Analysis and feedback of outpatient use data are the basis for improving outpatient antibiotic prescribing.

**P1260** How to improve parental knowledge and awareness about antibiotics? A unique Swedish Strama educational programme in child health centres

B. Jönsson*, L. Eriksen, M. Ertell (Halmstad, SE)

**Objective:** The use of antibiotics in Sweden increased during 2004–2007, particularly in children age 0–4. The overuse of antibiotics and increasing bacterial resistance is of major concern. The Swedish governmental initiative on patient safety includes appropriate antibiotic use. This highlights the needs for educational outreach. We have developed and implemented a parental educational programme. The aim was to educate parents about common childhood infections, appropriate use of antibiotics and resistance. The target audience is parents of young children in parental groups at Swedish Child Health Centres (CHC). All children age 0–6 are enrolled in the CHC, which enables a continuing dialogue. Parental education is an established part of the Swedish CHC programme.

**Method:** The educational programme “Children, infections and antibiotics” is based on national consensus and guidelines. It consists of a PowerPoint presentation of 20 slides and a pamphlet, teaching parents about common childhood infections, antibiotics and bacterial resistance. Included are interactive case studies. In addition, there is a manual with facts and pedagogical advice to assist the CHC-nurse as the group leader. It was introduced to 51 CHC in the county of Halland in 2009. Each CHC received an USB. The material is also available on the website of Strama Halland: www.regionhalland.se/strama. The programme was evaluated to assess the implementation and usefulness by a survey to the CHC in 2010.

**Result:** A total of 87% of CHC in Halland had used the programme. The CHC-nurses found the programme accurate and easy to use, a satisfaction rate of 99%. Nurses reported that parents gained important information, awareness and confidence in handling common childhood infections. They also experienced an interactive and dynamic discussion within the parental group.

**Conclusion:** A communication strategy on antibiotic resistance requires a multidimensional approach. “Children, infections and antibiotics” targets both parents and health care providers. We have shown it is a relevant, pedagogical and interactive tool to increase knowledge and awareness about appropriate antibiotic use. The programme has been distributed and implemented by CHC in other Swedish counties. The material is easily updated and can be used in various ways on an international level.

**P1261** Improved compliance to antibiotic guidelines in long-term care facilities in Region Västra Götaland, Sweden

P. Ulleryd*, L. Karlsson, A. Arvidsson, R. Eklund, M. Eriksson, L. Osebeck, M. Schewenius, T. Wältberg, S. Öberg (Region Västra Götaland, Borås, Skövde, Uddevalla, Gothenburg, SE)

**Objectives:** The overuse and inappropriate prescription of antibiotics drive bacterial resistance. In Germany ~85% of all antibiotics are prescribed in ambulatory care but studies about antibiotic prescribing practice in this setting are rare. Thus, the aim of this study was to analyse in detail antibiotic prescribing in the outpatient setting.

**Methods:** We analysed data from the largest regional public-sector health insurer AOK in the German federal state Brandenburg in respect to diagnosis and antibiotic prescription in ambulatory care in the 1st quarter 2009. The AOK provides statutory health insurance to more than 1 million out of ~3 million inhabitants of Brandenburg. We included data from physicians of all specialisations who treated at least 50 AOK outpatients per quarter with one or more diagnosis and/or prescription.

**Results:** A total of 1,983 physicians prescribed 74,529 times antibiotics for systemic therapy to 685,379 patients. In median, every 10th patients received an antibiotic. The four top prescribed antibiotics were ciprofloxacin (n = 8283) followed by roxithromycin (n = 6471), azithromycin (n = 6118) and cefuroxime (n = 5415). The leading infections made acute upper respiratory tract infections and acute bronchitis (n = 34,175) followed by urinary tract infections (n = 7,909), acute tonsillitis (n = 5,453) and viral infections (n = 5,145). Paediatricians and general practitioner (GP) saw the most patients with infection followed by urologists and ear, nose and throat (ENT)-specialists. Paediatricians prescribed in median only 0.4 time an antibiotic per encoded infection. GPs prescribed an antibiotic 1.1 times, urologists 1.4 times and ENT-specialists even 3.1 times per infection. The range of the number of prescription (10th-90th percentile) varied substantially by medical specialist from 0.7 within the paediatricians to 1.6 within the GPs. Variation was 8.4 within the ENT doctors and peaked at 8.5 within the urologists.

**Conclusion:** Upper respiratory tract and urinary infections were the most common infections in the outpatient setting and chinolones, macrolides and cephalosporines the most frequently prescribed antibiotics. Paediatricians were more conservative to prescribe antibiotics in relation to the numbers and kind of infections. The amount of antibiotics prescribed by urologists and ENT-specialists varied largely (up to 8.5 times). Analysis and feedback of outpatient use data are the basis for improving outpatient antibiotic prescribing.

**Method:** The educational programme “Children, infections and antibiotics” is based on national consensus and guidelines. It consists of a PowerPoint presentation of 20 slides and a pamphlet, teaching parents about common childhood infections, antibiotics and bacterial resistance. Included are interactive case studies. In addition, there is a manual with facts and pedagogical advice to assist the CHC-nurse as the group leader. It was introduced to 51 CHC in the county of Halland in 2009. Each CHC received an USB. The material is also available on the website of Strama Halland: www.regionhalland.se/strama. The programme was evaluated to assess the implementation and usefulness by a survey to the CHC in 2010.

**Result:** A total of 87% of CHC in Halland had used the programme. The CHC-nurses found the programme accurate and easy to use, a satisfaction rate of 99%. Nurses reported that parents gained important information, awareness and confidence in handling common childhood infections. They also experienced an interactive and dynamic discussion within the parental group.

**Conclusion:** A communication strategy on antibiotic resistance requires a multidimensional approach. “Children, infections and antibiotics” targets both parents and health care providers. We have shown it is a relevant, pedagogical and interactive tool to increase knowledge and awareness about appropriate antibiotic use. The programme has been distributed and implemented by CHC in other Swedish counties. The material is easily updated and can be used in various ways on an international level.

**Children, infections and antibiotics**

An interactive parental educational programme

**Strama**
Hygiene risk factors such as having a wound or an indwelling urinary catheter were found in 48% and 15% of the patients on antibiotics, in comparison with 7% and 8% of the patients without antibiotic treatment. Most of the wounds were located on the lower limb.

**Conclusion:** We found a decrease in total antibiotic use in long-term municipality care as well as an improved compliance to regional antibiotic guidelines in Region Västra Götaland, particularly regarding urinary tract infections. They who were admitted in short-term care and they with hygiene risk factors such as wounds and indwelling urinary catheters were more often committed to antibiotic treatment.

Skin and soft tissue infections are now the major indication for antibiotic treatment, and the predominance of patients with wounds being treated signal a too liberal prescribing policy for these patients. Educational packages concerning wound care are launched.

**P1262 European Surveillance of Antimicrobial Consumption (ESAC): disease-specific quality indicators for outpatient antibiotic prescribing**

N. Adriaenssens, S. Coenen*, S. Tonkin-Crine, T. Verheij, P. Little, H. Goossens and the ESAC Ambulatory Care Subproject

**Objectives:** To develop a set of evidence-based disease-specific outpatient antibiotic prescribing quality indicators in Europe.

**Methods:** Within the ESAC Ambulatory Care Subproject 2 meetings were convened in 2008 and 2009 to produce a list of proposed evidence-based disease-specific outpatient antibiotic prescribing quality indicators, building on previous and similar development of drug-specific quality indicators, and in close collaboration with CHAMP (www.champ-antibiotics.org) and HAPPY AUDIT (www.happyaudit.org). 62 experts from 33 countries were asked to complete 2 scoring rounds of the proposed indicators on 7 dimensions, i.e. their relevance to 1 reducing antimicrobial resistance, 2 patient health benefit, 3 cost-effectiveness, 4 policy makers, 5 individual prescribers, 6 their evidence base, and 7 their range of acceptable use, using a scale ranging from 1 (= completely disagree) to 9 (= completely agree). According to the UCLA-RAND appropriateness method, proposed indicators were judged relevant if the median score was not within the 1–6 interval and if there was consensus, i.e. the number of scores within the 1–3 interval was less than one third of the panel.

| No. | Title | Label |
|-----|-------|-------|
| 1a. | The percentage of patients aged between 18 and 25 years with acute bronchitis/bronchiolitis (ICPC-2: R47) prescribed antibiotic(s) for systemic use (ATC: J01) | [R78, R01, %] |
| 1b. | ≥ 6a. receiving the recommended antibiotic(s) (ATC: J01C or J01D) | [R78, RECOM, %] |
| 1c. | ≥ 6a. receiving quinolones (ATC: J01D) | [R01, J01D, %] |
| 2a. | The percentage of patients aged 18 years and above with acute upper respiratory infection (ICPC-2: R47) prescribed antibiotic(s) for systemic use (ATC: J01) | [R78, R01, %] |
| 2b. | ≥ 6a. receiving the recommended antibiotic(s) (ATC: J01C) | [R78, RECOM, %] |
| 2c. | ≥ 6a. receiving quinolones (ATC: J01D) | [R01, J01D, %] |
| 3a. | The percentage of female patients aged between 15 years and below with cystitis/other urinary infection (ICPC-2: R131) prescribed antibiotic(s) for systemic use (ATC: J01) | [R78, %] |
| 3b. | ≥ 3a. receiving the recommended antibiotic(s) (ATC: J01E or J01X or J01X) | [R78, RECOM, %] |
| 3c. | ≥ 3a. receiving quinolones (ATC: J01D) | [R01, J01D, %] |
| 4a. | The percentage of patients aged 18 years and above with acute tonsillitis (ICPC-2: R47) prescribed antibiotic(s) for systemic use (ATC: J01) | [R78, R01, %] |
| 4b. | ≥ 6a. receiving the recommended antibiotic(s) (ATC: J01C) | [R78, RECOM, %] |
| 4c. | ≥ 6a. receiving quinolones (ATC: J01D) | [R01, J01D, %] |
| 5a. | The percentage of patients aged between 18 and 25 years with acute/even chronic sinusitis (ICPC-2: R47) prescribed antibiotic(s) for systemic use (ATC: J01) | [R78, R01, %] |
| 5b. | ≥ 5a. receiving the recommended antibiotic(s) (ATC: J01C or J01D) | [R78, RECOM, %] |
| 5c. | ≥ 5a. receiving quinolones (ATC: J01D) | [R01, J01D, %] |
| 6a. | The percentage of patients aged between 18 years and above with acute/chronic otitis media/mastoiditis (ICPC-2: R43) prescribed antibiotic(s) for systemic use (ATC: J01) | [R78, R01, %] |
| 6b. | ≥ 6a. receiving the recommended antibiotic(s) (ATC: J01D or J01X) | [R78, RECOM, %] |
| 6c. | ≥ 6a. receiving quinolones (ATC: J01D) | [R01, J01D, %] |
| 7a. | The percentage of patients aged between 18 years and above with pneumonia (ICPC-2: R47) prescribed antibiotic(s) for systemic use (ATC: J01) | [R78, R01, %] |
| 7b. | ≥ 7a. receiving the recommended antibiotic(s) (ATC: J01C) | [R78, RECOM, %] |
| 7c. | ≥ 7a. receiving quinolones (ATC: J01D) | [R01, J01D, %] |

**Results:** For each of the 6 mean indications for antibiotic prescribing (acute otitis media, acute upper respiratory infection, acute/chronic sinusitis, acute tonsillitis, acute bronchitis/bronchiolitis, cystitis/other urinary infection) and for pneumonia (labelled by ICPC codes H71, R74, R75, R76, R78, U71 and R81, respectively), 3 quality indicators were proposed, i.e. a. the percentage of patients with age and/or gender limitation prescribed an antibiotic; b. the percentage of patients with age and/or gender limitation prescribed an antibiotic, receiving the recommended antibiotic; c. the percentage of patients with age and/or gender limitation prescribed an antibiotic, receiving quinolones (see Table). This set was scored by 40 experts from 25 countries. Already after the first scoring round, all indicators were rated as relevant on all 7 dimensions, except 3a. was scored 6 on cost-effectiveness.

**Conclusion:** All 21 (7x3) proposed disease-specific evidence based quality indicators for outpatient antibiotic prescribing have face validity and are potentially applicable. In line with the main objectives of antimicrobial use surveillance at the European level, this set could be used to better describe antibiotic use and assess the quality of antibiotic prescribing patterns in ambulatory care.
**Impact of the H1N1 pandemic on antibiotic consumption in the adult population in Emilia-Romagna, Italy**

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**Objectives:** Antibiotic use in Italy increases significantly over the winter period, and the influenza epidemic is a major cause of an increase in the inappropriate use of antibiotics. The H1N1 pandemic has been an relatively mild infection in the elderly, while it has been more aggressive in the younger class groups. We analysed the antibiotic use in the community among adults (≥15 years), in the period 2007–2009, in Emilia-Romagna, Italy, aiming to identify differences in antibiotic prescription during the H1N1 pandemic period.

**Methods:** Data regarding antibiotic use were obtained from the regional public health system data-bases. Antibiotic consumption was evaluated using prescriptions, i.e. the number of prescribed antibiotic boxes of a certain drug reported in the same recipe. Prescriptions were calculate as per 1000 inhabitant-years and 1000 inhabitant-months. Prescriptions were calculated for the different age groups among the population aged ≥15 years: 15–19 years, 20–59, 60–79, and ≥80.

**Results:** Yearly prescription rate was 778 prescriptions/1000 inhabitant-years in 2007, 795 in 2008 and 797 in 2009. The analysis on a monthly base showed that antibiotic prescriptions peaked in January, with rates ranging between 71 prescriptions/1000 inhabitant-months in the younger age groups and 130 in the elderly. The lowest prescription rates were observed in August, ranging between 36 and 74/1000 inhabitant-months. Antibiotic prescription rate increased by 41% in the group of patients aged 15–19 years, in October 2009 and by 90% in November 2009, during the peak of H1N1 pandemic, as compared with the same months during 2007 and 2008 (see figure). A milder increase (37%) was also observed in the 20–59 years age group between November 2008 and 2009, only. Antibiotic use returned to baseline levels in December 2009. No other difference in antibiotic consumption was observed for any month in any other age group.

**Conclusion:** The H1N1 pandemic did not cause a significant increase in the overall antibiotic consumption on a yearly base in the overall population. Nonetheless, a striking increase in the group aged 15–19 years and, to a lesser extent 20–59, was observed. It is possible to assume that a discrete proportion of antibiotics prescribed during the H1N1 peak were used inappropriately in patients with flu-like symptoms. Campaigns aiming to a better definition of antibiotic indications, particularly during the cold season, are needed.

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**Identifying determinants of antibiotic use in Europe**

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**Objectives:** To identify determinants contributing to the most observed differences in outpatient antibiotic use between European countries and over time.

**Methods:** Data on outpatient antibiotic use (DDDs and packages) were collected for 35 European countries from 1999 to 2007 through the European Surveillance of Antimicrobial Consumption (ESAC) network. For these country-years, through databases (eg, EUROSTAT) and surveys, 180 variables were collected in the following categories: Agriculture (7 variables), Culture (26), Demography (21), Disease burden (35) Education (6), health care (73) and socioeconomics (12). Multiple imputation generalized estimation equations with a backward model selection procedure were applied to select the best fitting model for the data using outpatient antibiotic use as dependent variable.

**Results:** The following variables were found to be significant in the overall model: (1) % attaining upper secondary education, (2) population density, (3) death rate due to chronic liver disease, (4) existence of restrictions on pharmaceutical companies to pay physicians for attending conferences, (5) death rate due to respiratory disease, (6) existence of financial incentives for patients to register with one GP, (7) household out of pocket payment on health as a % of total health expenditure, (8) density of GP practices, (9) Corruption Index score, (10) number of antibiotics available, (11) male life expectancy, (12) extent to which most people are trusted, (13) death rate due to ischaemic heart disease, (14) extent to which people respect authority, (15) private health expenditure as a % of health expenditure. In analyses focused on more developed countries (human development index >0.9), variables (2), (4), (6), (7), (12), (15) above were still significant, whereas the others above were not. Instead, the following variables were also significant: whether or not official guidelines for antibiotic prescribing are available for GPs and pulmonologists, death rate due to AIDS and production of turkey. Note that some significant determinants may act as a surrogate for combinations of others. In different subgroups of antibiotics similar results were found.

**Conclusion:** Our analysis reveals that there are diverse significant determinants and that these vary according to the scope of analysis. At each level of analysis, some significant determinants are inherent to culture and populations, but others could be changed through governance.

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**Antibiotic prescribing for adults with acute cough/LRTI: congruence with guidelines**

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**Objective:** European guidelines for treating acute cough/lower respiratory tract infection (LRTI) aim to reduce non-evidence based variation in prescribing, and better target and increase the use of first line antibiotics. However, application in primary care is unknown. We explored congruence of both antibiotic prescribing and antibiotic choice with European Respiratory Society-European Society Clinical Microbiology and Infectious Diseases (ERS-ESCMID) guidelines for managing LRTI.

**Methods:** Analysis of prospective observational data from patients presenting to primary care with acute cough/LRTI. Clinicians recorded symptoms on presentation, and their examination and management. Patients were followed up with self-complete diaries.

**Results:** 1776 (52.7%) patients were prescribed antibiotics. Given patients’ clinical presentation, clinicians could have justified an antibiotic prescription for 1915 (71.2%) patients according to the ERS-ESCMID guideline. 761 (42.8%) of those who were prescribed antibiotics received...
a first choice antibiotic (i.e. tetracycline or amoxicillin). Ciprofloxacin was prescribed for 37 (2.1%) and cephalosporins for 117 (6.6%).

**Conclusion:** A lack of specificity in definitions in the ERS-ESCMID guidelines could have enabled clinicians to justify a higher rate of antibiotic prescription. More studies are needed to produce specific clinical definitions and indications for treatment. First choice antibiotics were prescribed to the minority of patients who received an antibiotic prescription.

**P1267** Severity assessment for lower respiratory tract infections: potential use and validity of CRB-65 in primary care

N.A. Francis*, J. Cals, C. Butler, K. Hood, T. Verheij, P. Little, H. Goossens, S. Coenen on behalf of the GRACE Project Group

**Objectives:** The CRB-65 (Confusion, Respiratory rate, Blood pressure, and age ≥65 years) is a useful rule for predicting pneumonia outcome in hospitals, and has been recommended for use in the community. We aim explore potential use in primary care by describing the extent to which components of the CRB-65 rule are routinely assessed in adults presenting with lower respiratory tract infection (LRTI), and to assess the validity of CRB-65 for predicting poor prognosis in these patients in this prospective observational cohort in general practices in 14 research networks in 13 European countries.

**Methods:** Clinicians recorded antibiotic treatment and clinical features for 3,402 adults presenting with LRTI. Patients recorded daily symptoms for up to 28 days. Multi-level regression models determined the association between an elevated CRB-65 score and prolonged moderately severe symptoms, hospitalisation, and time to recovery, controlling for antibiotic prescribing. Sensitivity analyses used zero imputation.

**Results:** Respiratory rate and blood pressure were recorded in 22.7% and 31.9% of patients respectively. 2,690 patients completed symptom diaries. A CRB-65 could be calculated for 334 (12.4%) of these. A score of one or more was not significantly associated with prolonged moderately severe symptoms, hospitalisation, and time to recovery but not associated with prolonged moderately severe symptoms. Larger studies are required to determine the role of CRB-65 in predicting mortality and/or risk of hospitalisation in this setting.

**P1268** Antimicrobial prescribing for urinary tract infections in European nursing homes

K. Latour*, E. Broex, A. Muller, N. Drapier, V. Vankerkhoosten, R. Stroobants, H. Goossens, B. Jans on behalf of the European Surveillance of Antimicrobial Consumption (ESAC) Nursing Home subproject group

**Objectives:** To explore antimicrobial (AM) prescribing for urinary tract infections (UTI) in European nursing homes (NH).

**Methods:** In November 2009 the European Surveillance of Antimicrobial Consumption NH subproject organised a second point prevalence survey (PPS) in 1435 residents in 21 European countries (including 2 UK administrations). Out of 1435 residents receiving AMs, 702 residents received one or more AM for an indication related to the urinary tract (81% female; median age=85 year). In total 714 molecules were registered (crude mean prevalence (CMP) of AM use for UTIs=2.4%; range by country: 0–10.0%) of which 48% served as uroprophylaxis (CMP=1.5%; range by country: 0–5.0%). Empirical treatments counted for 32% of all UTI therapies (CMP=0.9%; range by country: 0–5.3%) and documented treatments for 20% (CMP=0.5%; range by country: 0–2.0%).

The most frequently prescribed molecules for uroprophylaxis (n=343) were nitrofurantoin (29.7%), trimethoprim (19.8%) and nitrofurantoin (16.0%). For empirical treatment (n=229) UTI methenamine (14.4%), ciprofloxacin (13.5%) and nitrofurantoin (12.7%) were most commonly used, while for documented treatment (n=142) the main molecules were nitrofurantoin (24.7%), ciprofloxacin (16.9%), amoxicillin & enzyme inhibitor (7.8%) and pivmecillinam (7.8%). Isolated microorganisms (MO; optional question) were reported in 58.2% of the cases where a culture sample was taken prior to AM therapy (n=304). In total 195 MO were reported. Escherichia coli (n=98 of which 6 resistant (R) to 3rd generation cefalosporines), Proteus mirabilis (n=13; 1 R to 3rd generation cefalosporines), Pseudomonas aeruginosa (n=11; 1 R to carbapenem) and Klebsiella pneumoniae (n=10; 1 R to 3rd generation cefalosporines) were most frequently documented.

**Conclusion:** The prevalence of uroprophylaxis is high in European NHs. Further in-depth research is needed as AM therapy for asymptomatic bacteriuria has not been shown to be of benefit in elderly living in NHs and can even be harmful, for instance in light of the development of AM resistance.

**P1269** Repeated point-prevalence surveys on antimicrobial prescriptions in Finnish nursing homes, 2009–10

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**Objectives:** Finnish nursing homes (NH) participated in 3 European Surveillance of Antimicrobial Consumption (ESAC) Point Prevalence Surveys (PPS) in April and November 2009 and May 2010 which were conducted in order to assess the antimicrobial (AB) consumption in European NHs. We analyzed Finnish ESAC PPS data and compared the results with those previously published by the ESAC project group.

**Methods:** All residents present in NH for >24 hours and receiving systemic ABs on the day of the survey were included. Data on ABs and their indications were collected from residents’ charts: prophylaxis or treatment and the type of infection.

**Results:** In 2009 8 NHs and in 2010 9 NHs participated in the survey. In total, there were 5791 eligible residents (range by survey, 1706–2320); 737 (12.7%; range, 9.7–17.2%) of them received at least one AB. The most common indication was prophylaxis (487/737, 66.0%; range 56.8–73.4%), mainly for urinary tract infection (UTI) (460/737, 62.4%). Of the residents, 250 (4.3%, range, 3.5–5.2%) were on AB treatment.

**Conclusion:** AB consumption in Finnish NHs was high. Most ABs were used for UTI prophylaxis. Especially the use of methenamine was very common, even though it reduced by half during the study period. If methenamine consumption were excluded, the Finnish AB prevalence would be in line with the ESAC results from spring 2009 (median 5.4%). Differences in AB consumption between countries may also be related to differences in NH patient population and patients’ underlying conditions.
Antimicrobial consumption and stewardship in nursing homes in European regions

R. Jans*, K. Latour, E. Broeckx, A. Muller, N. Druyf, V. Vankerkhoven, R. Stroobants, H. Goossens on behalf of the European Surveillance of Antimicrobial Consumption (ESAC) Nursing Home subproject group

Methods: In April 2009 the ESAC point prevalence survey (PPS) on antimicrobial (AM) use in European nursing homes (NH) was held. Institutional determinants for AM use and current AM stewardship resources were collected through a NH questionnaire. For each resident with an AM on the PPS day, a questionnaire was completed for measuring AM use in the NH.

Results: A total of 304 NHs from 20 EU countries participated: 15 in Eastern Europe (E-EU), 86 in Northern EU (N-EU), 43 in Southern EU (S-EU) and 157 in Western EU (W-EU). In all participating settings, 5.4% (median) of the residents used an AM on the PPS day (range by NH: 0–30%). The prevalence of AM use was significantly higher in N-EU NHs (10.4%, range: 0–28.3%) compared to S-EU (4%, range: 0–30%; p < 0.001), E-EU (5%, range: 0–20.7%, p = 0.002) and W-EU (4.9%, range: 0–20%; p = 0.001). This variation could not be explained by differences in care load (incontinence, disorientation, impaired mobility) nor by the prevalence of wounds in the total NH populations. In N-EU NHs, urinary catheters were more frequently used (5.3%) compared to NHs from the other EU regions (2.5%, p = 0.003). Medical care was only provided by the general practitioner (GP) in 66.2% of all EU NHs (N-EU: 60.3%, other regions: 68.5%). In 87.2% of all N-EU NHs only working with GPs, no medical coordinator was present while in the other regions medical coordination was absent in only 10.5% of the NHs.

In N-EU NHs, compared to NHs from other regions, some AM stewardship resources were significantly less available such as written guidelines for prudent AM use (N-EU: 24.6% versus other regions: 59.2%, p < 0.001), a therapeutic formulary (4.3% versus 60.2%, p < 0.001), annual data on AM consumption (8.7% versus 26.2%, p = 0.003), a restrictive list of AM to be prescribed (6.6% versus 29%, p = 0.004) and the use of a motivation form for prescription outside the formulary (0% versus 9.9%, p = 0.007). N-EU NHs were also more encouraged to take microbiological samples for guidance of AM prescriptions (92.8% versus 53.4%, p = 0.004) and the use of a motivation form for prescription outside the formulary (0% versus 9.9%, p = 0.007). N-EU NHs were also more encouraged to take microbiological samples for guidance of AM prescriptions (92.8% versus 53.4%, p = 0.004) and the use of a motivation form for prescription outside the formulary (0% versus 9.9%, p = 0.007). This variation could not be explained by differences in care load (incontinence, disorientation, impaired mobility) nor by the prevalence of wounds in the total NH populations. In N-EU NHs, urinary catheters were more frequently used (5.3%) compared to NHs from the other EU regions (2.5%, p = 0.003). Medical care was only provided by the general practitioner (GP) in 66.2% of all EU NHs (N-EU: 60.3%, other regions: 68.5%). In 87.2% of all N-EU NHs only working with GPs, no medical coordinator was present while in the other regions medical coordination was absent in only 10.5% of the NHs.

In N-EU NHs, compared to NHs from other regions, some AM stewardship resources were significantly less available such as written guidelines for prudent AM use (N-EU: 24.6% versus other regions: 59.2%, p < 0.001), a therapeutic formulary (4.3% versus 60.2%, p < 0.001), annual data on AM consumption (8.7% versus 26.2%, p = 0.003), a restrictive list of AM to be prescribed (6.6% versus 29%, p = 0.004) and the use of a motivation form for prescription outside the formulary (0% versus 9.9%, p = 0.007). N-EU NHs were also more encouraged to take microbiological samples for guidance of AM prescriptions (92.8% versus 53.4%, p < 0.001). In general, the prevalence of AM use was significantly higher (6.6%) in NHs promoting sampling prior to prescribing, compared to those who didn’t (4.6%, p = 0.0001).

Conclusions: In EU NHs a lack of medical coordination might contribute to a more important device use and less AM stewardship. Promoting microbiological sampling could produce adverse effects when positive cultures are treated without considering the clinical status of the residents.

European Surveillance of Antimicrobial Consumption (ESAC): outpatient antibiotic use in children and teenagers in Europe

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Objectives: To provide a detailed description of outpatient systemic antibiotic use among children and teenagers in Europe, and to assess differences between two outcome measures in the context of the first European Antibiotic Awareness Day (EAAD) focusing on this target group.

Methods: Since 2004, the ESAC Ambulatory Care subproject collects outpatient antibiotic consumption data by age using the Anatomical Therapeutic Chemical (ATC) Defined Daily Doses (DDD) methodology. We analysed 2007 and 2008 data on outpatient use of antibacterials for systemic use (ATC J01) for children and teenagers up to age 20 in countries where use could be expressed in both DDD (WHO version 2010) and packages per 1000 inhabitants per day (DID and PID, respectively).

Results: In 2008, outpatient antibiotic use up to the age of 20 (10) represented 15% (6%) of total antibiotic use in DID compared to 22% (14%) of total antibiotic use in PID. In DID, use varied with a factor 2.6 between highest (18.7 DID in Luxembourg) and lowest (7.2 DID in Norway) use. In PID, use among children varied with factor 3.3 between highest (3.0 PID in Luxembourg) and lowest (0.9 PID in Norway) use. Between 2007 and 2008 outpatient antibiotic use in Belgium, Denmark, Luxembourg and Norway decreased both in DID and PID when assessed by age from the age of 0 until the age of 18 years (see figure). Use in DID (age 0–20) decreased with 5% and in PID with 8% as compared to 2007. Total outpatient antibiotic use (all ages) decreased in PID, not in DID.

In children, mainly penicillins (J01C) and macrolides (J01FA) are commonly used, and to a lesser extent second-generation cephalosporins (J01DC), sulfonamides and trimethoprim (J01EE). Cephalosporin use is negligible in Norway. Denmark only uses penicillins and macrolides in children.

Conclusion: Quantities and classes of antibiotic substances used by children vary substantially between European countries. The proportion of antibiotic use in children on the total antibiotic use is highly dependent on the outcome measure. To evaluate the effect of interventions targeting antibiotic use in children, e.g. the EAAD, antibiotic use data expressed in DID should be linked to the patient’s age, and if information on age is missing, be complemented with used data expressed in PID. Applying this methodology, the 2008 EAAD appears to have been a success.

Comparative effects of the extended and the immediate release formulations of ciprofloxacin on normal human intestinal microflora

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Objectives: Ciprofloxacin is a well-known fluoroquinolone, active in vitro against many Gram-negative and Gram-positive bacteria. The purpose of the present study was to evaluate the ecological effects of an orally administered extended release ciprofloxacin formulation in comparison with an immediate release ciprofloxacin formulation on the normal human intestinal microflora.

Methods: Thirty-six healthy female subjects (18–45 years of age) were included in the study. The extended release formulation ciprofloxacin Unimux® 500 mg (Rottapharm Madaus SpA, Monza, Italy) was taken by 18 volunteers for 3 days and the immediate release formulation ciprofloxacin Ciproxin® 250 mg (Bayer HealthCare AG, Leverkusen, Germany) was taken by 18 volunteers bid for 3 days. Faeces was collected for determination of ciprofloxacin concentrations and analysis of faecal microflora. The faecal specimens were cultured on non-selective and selective media. Different colony types were counted, isolated in pure culture, and identified to genus/species level by biochemical and molecular tests. All new colonizing bacteria were tested for susceptibility to ciprofloxacin. The faecal concentrations of ciprofloxacin were determined by a microbiological method.
Results: The volunteers receiving the extended release formulation had a mean concentration of ciprofloxacin 453 mg/kg at the end of treatment. In the immediate release formulation group, the mean concentration of ciprofloxacin at the end of treatment was 392 mg/kg. No ciprofloxacin was detected in faeces before and after treatment. In volunteers receiving the extended release formulation, the numbers of E. coli were significantly suppressed while the enterococci decreased moderately. No significant effects were observed on the other Enterobacteriaceae and B. fragilis. In the volunteers receiving the immediate release formulation, the numbers of E. coli decreased significantly at the end of treatment while the numbers of other Enterobacteriaceae, enterococci and B. fragilis were moderately suppressed. In the extended release group, one volunteer became colonized with resistant E. coli strains (MIC >1mg/l) and nine volunteers with resistant enterococci. In the immediate release group, six volunteers were colonized with resistant enterococci.

Conclusion: No major differences were observed between the two studied formulations on the normal human intestinal microflora.

**P1273** Effect of telavancin on the normal human intestinal microflora

M. Rashid, A. Weintraub, C.E. Nord* (Stockholm, SE)

Objectives: Telavancin is a new glycopeptide being developed for the treatment of complicated skin and skin structure infections and pneumonia caused by Gram-positive bacteria. Investigating the impact of antibiotics on the normal microflora is important since alteration of the balance may facilitate colonization by new potentially pathogenic strains or enable microorganisms in the normal flora to develop resistance. The purpose of the present study was to investigate the effect of administration of telavancin on the intestinal microflora of healthy subjects.

Methods: Thirteen healthy subjects (6 males and 7 females) 18–30 years of age received telavancin 10mg/kg over a 60-minute period by intravenous infusion q 24h during 7 days. Faeces was collected for determination of telavancin concentrations and analysis of faecal microflora. Faecal specimens were cultured on non-selective and selective media. Different colony types were counted, isolated in pure culture, and identified to genus level by biochemical and molecular tests. All new colonizing bacteria were tested for susceptibility to telavancin. The faecal concentrations of telavancin were determined by a microbiological method.

Results: No measurable faecal concentrations were found on days 1, 2, 5, 7, 9, 14 and 21. The numbers of staphylococci, enterococci, lactobacilli and bifidobacteria were within the normal variations (1 log cfu/g faeces). Mean numbers of clostridia decreased by approximately 1.5 log cfu/g faeces from Day 1 to Day 7 with recovery of baseline counts on Day 21. No Clostridium difficile strains or toxins were detected. No significant effects (>2 log cfu/g faeces) in the number of Escherichia coli, Enterobacteriaceae species and Bacteroides fragilis group were observed during or after the administration of telavancin. The number of Candida albicans in the intestinal microflora was not changed within the observation period. No new colonizing aerobic and anaerobic Gram-positive bacteria resistant to telavancin (MIC >2mg/l) were found.

Conclusion: Telavancin has a favourable ecological impact on the human intestinal microflora.

Antibiotic use, stewardship and resistance in the hospital: optimising outcomes

**P1274** Results of Irish National point-prevalence studies 2009 and 2010

D. Lambert*, M. Philbin, A. Oza on behalf of the IAPG

Objectives: A one-day point prevalence study of antimicrobial prescriptions is a methodology used to gather data on the amount and type of antimicrobials used.

The purpose of this study was to collate and analyse the results of nationwide antimicrobial point prevalence studies conducted in Ireland in 2009 and 2010. These studies were conducted by antimicrobial pharmacists in the majority of participating hospitals.

Methodology: A point prevalence study of antimicrobial prescribing was carried out between June & July 2009 by 23 Irish hospitals as part of the 2009 ESAC PPS. The methodology used was that of the 2009 ESAC project.

Data collected included: age & sex of patient, details of systemic antimicrobial therapy, diagnoses and indication, compliance with local guidelines & documentation of reason for therapy. The HPSC analysed the data of the Irish participants and participants were also asked to complete a questionnaire to gather feedback and suggestions for future studies.

Following on from this work, a national PPS was designed, organised and carried out in September/October 2010. The data collection form used in 2010 included additional fields such as demographic data on all inpatients (not just those on antimicrobial therapy) and information on IV/PO switch suitability.

Results: In 2009 5824 patients’ records were examined, of which 2000 received systemic antimicrobial therapy. The median prevalence was 34.3% (range 21.4–55.3%) compared with the European median of 29%. The average number of antimicrobial drugs per patient was 1.58, the maximum was 6.

In 2010 28 Irish hospitals participated (6414 patients). Of these a median of 36.5% were prescribed antimicrobial therapy. Despite this increase, the average number of antimicrobial drugs per patient was 1.51 (median 1.43), a slight reduction.

Conclusion: This 2009 study allowed comparison between Irish hospitals and their European counterparts, and the HPSC analysis facilitated inter-hospital comparison within Ireland.

Positive feedback following participation in the 2009 study prompted organisation of a national PPS to be conducted again in 2010 with a slightly modified methodology.

The results show usage in Irish hospitals to be marginally above the European average in the 2009 study.

Comparing 2009 and 2010 results a slight increase in the percentage of all inpatients prescribed antimicrobials was seen.

**P1275** The association between “guideline-adherent” empirical treatment of community-acquired pneumonia and mortality in the Netherlands

S.M. Huijs*, M.J. Bonten on behalf of the CAP-therapies investigators

Objectives: The Dutch guidelines for the empirical antibiotic treatment of community-acquired pneumonia (CAP) are based on either severity of disease classification by the Pneumonia Severity Index (PSI) or CURB65-score (categorizing patients in ‘mild’, ‘moderate severe’ or ‘severe’ CAP) or a ‘pragmatic classification’ based on the level of care that is needed (general ward or ICU). Empirical treatment is based on these different severity classifications and physicians may choose which classification they prefer. We determined the association between guideline adherence for empirical antibiotic treatment for each of these classifications and in-hospital mortality for hospitalized patients with CAP in the Netherlands.

Methods: In a prospective, observational study in 23 Dutch hospitals, patients (≥18 years) admitted to hospital with a clinically suspected CAP were included between January 2008 and March 2009. Patients with missing information on antibiotic use were excluded. Choices of empirical antibiotic treatment were not dictated by protocol. Changes in antibiotic treatment after admission were not recorded.

The Dutch guidelines advise treatment with amoxicillin or doxycyclin for patients with ‘mild’ CAP, β-lactam, macrolide or quinolone monotherapy for patients with ‘moderate-severe’ CAP depending on the Legionella antigen test and moxifloxacin monotherapy or a combination of penicillin, ciprofloxacin, macrolide or a cephalosporin for patients with ‘severe’ CAP. Antibiotic treatment with a narrower spectrum was
Adherence to antibiotic guidelines for empirical treatment
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to Dutch guidelines and in-hospital mortality.

When adjusted for disease severity, there were no statistically significant associations between 'undertreatment' according to Dutch guidelines and in-hospital mortality.

|                | OR    | 95% CI       | p-value |
|----------------|-------|--------------|---------|
| PSI            | 0.78  | (0.37–1.62)  | 0.28    |
| CURB           | 0.78  | (0.37–1.62)  | 0.28    |
| Pragmatic      | 0.78  | (0.37–1.62)  | 0.28    |

Conclusions:
- The pragmatic classification has the highest percentage of guideline adherence.
- The pragmatic classification is an effective tool to prevent antibiotic resistance and increase savings in addition to providing direct and indirect costs.

P1276 Adherence to antibiotic guidelines for empirical treatment of pneumonia in the Netherlands
S.M. Hauts*, M.J. Bonten on behalf of the CAP-diagnosticians investigators

Objectives: The Dutch guidelines for the empirical antibiotic treatment of CAP are based on either severity of disease classification by the Pneumonia Severity Index (PSI) or CURB-65-score (categorizing patients in ‘mild’, ‘moderate severe’ or ‘severe’ CAP) or a ‘pragmatic classification’ based on the level of care that is needed (general ward or ICU). Empirical treatment is based on these different severity classifications and physicians may choose which classification they prefer. We compared guideline adherence for empirical antibiotic therapy, according to the 3 classification schemes, in hospitalized patients with CAP in the Netherlands.

Methods: In a prospective, observational study in 23 Dutch hospitals, patients (>18 years) admitted to hospital with a clinically suspected CAP were included between January 2008 and March 2009. Patients with missing information on antibiotic use were excluded. Choices of empirical antibiotic treatment were not dictated by protocol. Changes in antibiotic treatment after admission were not recorded. Antibiotic treatment with a narrower spectrum was defined as 'undertreatment', and treatment with broader antibiotics as 'overtreatment'. Logistic regression was used to calculate odds ratios for 'undertreatment' versus 'correct' or 'overtreatment' with adjustment for disease severity based on the PSI score.

Results: 1036 hospitalized patients with confirmed CAP were included, of whom 69 (6.7%) died in hospital. According to the classification scheme 88, 143 and 31 patients received 'undertreatment' for the PSI, CURB and pragmatic schemes, respectively. The crude OR for in-hospital mortality for 'undertreatment' was 3.75 (2.04–6.90), 2.58 (1.47–4.53) and 2.14 (0.73–6.31) for PSI, CURB or pragmatic severity classifications, respectively (table). The adjusted OR were 0.78 (0.37–1.62), 1.06 (0.57–1.98) and 1.90 (0.59–6.06), respectively.

Conclusion: When adjusted for disease severity, there were no statistically significant associations between 'undertreatment' according to Dutch guidelines and in-hospital mortality.

P1278 Antimicrobial consumption in VINCat hospitals: stratified data by hospital size
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Objectives: VINCat is a nosocomial infection surveillance program in Catalonia (7.5 million population), Spain. In 2009, a total of 46 acute care hospitals supplied data on antimicrobial use. Information about antimicrobial use stratified by hospital size is scarce. The aim of the study is to assess the evolution of the antimicrobial consumption in acute care hospitals stratified by hospital size.

Methods: A specific training in data management on antimicrobial consumption was given to all participant hospitals before the implementation of the program. During the first trimester from 2008 to 2010, the VINCat program requested annual data, from 2007 to 2009, on antimicrobial use from participant hospitals. Hospitals were stratified in 3 groups: I (more than 500 beds), II (200–500 beds) and III (less than 200 beds). Defined daily doses of 2009 were applied for calculations. Defined daily doses per 100 occupied bed-days (DDD/100 OBDs) were used to calculate the average consumption rate among all hospitals. Antifungal consumption was excluded from the study.

Results: Number of hospitals Group I: 7; Group II: 13; Group III: 19 (2007), 25 (2008); 26 (2009). Mean DDD/100 OBDs Group I: 85.00
Junior and senior residents’ knowledge and perceptions about antimicrobial resistance

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Objective: To evaluate the knowledge and perceptions about antimicrobial resistance (AMR) of junior and senior medical residents in 5 Spanish hospitals.

Methods: An online survey was conducted between Sept 15th and Nov 15th, 2010. Both junior (postgraduate year [PGY] PGY1) and senior (PGY4 and PGY5) residents of 5 Spanish academic medical centres were targeted. The survey explored the perception of doctor residents about the relevance and scope of the problem of AMR in hospitals. Knowledge of residents regarding AMR was explored through two questions about the rate of quinolone-resistant E. coli (QREC) and the rate of MRSA in each medical center. Residents were also asked to evaluate the relevance of several factors potentially linked to AMR as suggested by Pulcini et al. (Clinical Microbiology and Infection. 2010).

Result: 279 responses were received during the study period, corresponding to 33.05% of all targeted residents. Response rate was higher among junior doctors (40.71% vs 25.47%). Respondents belonged mainly to medical specialties (43.4%) while 17.6% and 10.8% of the responses arose from surgical and critical care residents. Residents of all hospitals, specialties and seniority mostly considered that AMR was a significant problem. Regarding the extent of the problem, respondents considered AMR to be a problem at national level (94.3%), at their institution (91.3%) and for their daily practice (83.8%). The proportion of residents that correctly identified the rate QREC and MRSA in their institution (91.3%) and for their daily practice. A majority of the residents considered AMR to be a problem at national level (94.3%), at their institution (91.3%) and for their daily practice (83.8%). The proportion of residents that correctly identified the rate QREC and MRSA in their centres was 40.8% and 25.3%, respectively. There were differences among hospitals in the proportion of residents identifying the rate of resistance but no differences were found when different specialities or seniority was considered. Residents’ perceptions about the relevance of several factors on the magnitude of the problem of AMR in hospitals are described in table 1.

Conclusion: The vast majority of residents find AMR to be a significant problem but, to some extent, they perceive that it affects others more (country > hospital > own daily practice). A majority of the residents cannot accurately identify local rates of resistance involving two major pathogens, especially MRSA. Spanish residents believe that the main factor driving AMR in hospitals is an excessive number of antimicrobial prescriptions. Senior resident doctors perceive that the duration of therapy is more relevant for AMR than junior resident doctors do.

![Table 1. Perception of the importance of several factors linked to antibiotic resistance](image-url)
Consequences of failure of initial antibiotic therapy in complicated skin and skin structure infections in US hospitals, 2000–2009
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Objective: Initial antibiotic therapy in patients hospitalised for the treatment of complicated skin and skin structure infections (cSSSI) is typically empiric, as causative pathogens often are unknown at the time. While initial treatment is usually successful, many patients fail to respond to such therapy. The clinical and economic consequences of failure of initial antibiotic therapy in patients with cSSSI have not been well described. The objective of this study was to examine this issue.

Methods: Using a US multi-hospital database, we identified all patients hospitalised for cSSSI (acute infection [eg, abscess, cellulitis], chronic/ulcerative infection [eg, decubitus ulcer], surgical site infection) between 1/1/2000 and 6/30/2009 who received parenteral antibiotic therapy for >24 hours (except in event of death) beginning >24 hours of hospital admission. Initial therapy was defined as all antibiotics received within the first 24 hours. Focusing attention on the 40 most frequently used regimens, we defined initial antibiotic failure as: (1) receipt >24 hours of an antibiotic not used in the first 24 hours, excluding agents of similar/narrower spectrum and those begun at hospital discharge; or (2) drainage, debridement, or amputation after 72 hours. We stratified patients according to whether they experienced initial antibiotic failure, and then compared hospital length of stay, total billed charges, and mortality between the two groups. Student’s t-tests were used to assess the statistical significance of differences in continuous measures; for mortality, a χ²-test was used.

Results: We identified 22,382 patients admitted to hospital for the treatment of cSSSI, of whom 17,543 (78%) received one of the 40 most frequently used regimens and met all other inclusion criteria. A total of 3403 patients (19.4%) experienced failure of initial antibiotic therapy. Patients who experienced failure of initial antibiotic therapy averaged 5.1 additional days in hospital (mean [SD], 9.2 [8.0] days vs 4.1 [3.1] days for those not experiencing failure) and $14,304 in additional hospital charges ($25,626 [$43,052] vs $11,322 [$21,632]). Case fatality was more than 7-fold higher in patients with failed initial therapy (1.5% vs 0.2%, respectively) (p < 0.01 for all comparisons).

Conclusion: Patients hospitalised for cSSSI who experience failure of initial antibiotic therapy have significantly worse clinical outcomes, longer lengths of stay, and higher costs of care.

Dynamic relationship between fluoroquinolone use and emergence of E. coli ESBL producers
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Introduction and Objectives: During the past decade, Escherichia coli producing Extended Spectrum β-lactamases (EC-ESBL) has become a new important threat in our sanitary setting. At the same time, classical fluoroquinolones were replaced by new ones. We aimed in this study to investigate the possible relationship between an increase in the use of newer fluoroquinolones and the emergence of the above cited bacteria through a time-series analysis as a routinely activity in ViReSSST (www.viresst.org).

Methods: From January 2001 to May 2010 we studied all inpatients and outpatients with a non duplicate positive culture for E. coli. We calculated the monthly percentage of EC-ESBL and estimated the use of fluoroquinolones. Classicals −CF − (ciprofloxacin, norfloxacin and ofloxacin) and newer − NF − (levofloxacin and moxifloxacin) were separated. We measured the use as monthly Defined Daily Dose (DDD) per 1000 inpatients-days (DDD/1000pat-days) and monthly DDD/1000 inhabitants-days, respectively for hospital setting and community. We identified and calculated a Transfer Function Model to found a model for a non contemporaneous relationship between time-series of resistance and antibiotic use.

Results: During the past decade, the monthly percentage of EC-ESBL, an unusual phenomenon in our laboratory before the study period, rises until 14%. The use of CF decreases from 3 to around 1.5 DDD/1000 inhabitants-days and from 100 to 70 DDD/1000pat-days, while NF use rises from 0.5 to 2 DDD/1000inhab/days and from 0 to 90 DDD/1000pat-days. We observed that 62% of the variability of EC-ESBL was explained by the lagged use of NF: 2 months before in hospital setting and 6–12
months before in community. No relationship was observed with use of classical fluoroquinolones.

**Conclusion:** We observed a strong, dynamic and retarded relationship between the use of newer fluoroquinolones and the emergence of ESBL, but not so with the classical ones. Reducing the use of these drugs could help to control this threatening bacteria. We think that ViResiST approach would potentially improves the knowledge of the relationship between antibiotic use and resistance due to its ability to handle vast amounts of data.

**P1284**  
Antibiotic prophylaxis in surgical procedures in the Latium region, Italy  
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**Objective:** Antibiotic prophylaxis (ABP) is one of the main prevention measure against Surgical Site infections (SSI) and the adherence to guidelines (GL) is considered an important indicator. ABP usage in 25 hospital centers in the Latium Region (Central Italy) participating on a voluntary basis, was studied.

**Methods:** In 2008 (Apr-Jun), the Regional Center for Health Associated Infections of the Latium coordinated a SSI surveillance based on the HELICS project in which 10 different surgical procedures were investigated: appendicectomy, caesarian section, colon-surgery, cholecystectomy and herniectomy (in laparoscopy or not), arthro- and hip-prosthesis, cardiovascular surgery, laminectomy and mastectomy. Information on ABP was collected, and its appropriateness was considered according to Italian GL adapted from the Scottish Intercollegiate Guidelines Network (SIGN), and to Surgical Care Improvement Project (SCIP) indicators: time at starting, drug used, and duration (SCIP1, SCIP2 and SCIP3, respectively), for each indicators a “best” and “worst” scenario were defined, considering all cases with missing information adherent or non adherent to GL, respectively.

**Results:** A total of 2835 surgical interventions (73% in males; 25% in emergency; 10% of all analogue procedures performed in the Region in the same period) was studied. ABP was administered in 2664 (94%) cases: 2346/2468 (95%) pts for whom it was to be provided according to GL, and in 318/367 (86.6%) even if not indicated. Considering only the 2346 procedure where ABP was indicated and administered, SCIP1 was in agreement with GL for 1172 pts (50%), and undetermined in 15.4% cases; SCIP2 for 84.5% pts, while in the remaining cases second line drugs (66.4% 3rd class cephalosporin, 32.2% penicillin-β-lactamase) were used; SCIP3 was consistent with GL in 48% of cases, and undetermined in 7% cases. Considering the “best” scenario, all SCIP indicators were followed in 37% of cases; two of the three in 18% cases, only one in 38% cases, and in 7.4% cases all indicators were not followed, in the “worst” these proportions were respectively 22.7%, 23.1%, 45.6% and 8.6%.

**Conclusions:** The appropriateness of ABP use appears to be low, mostly because of prolonged duration and of large use of second line antibiotics. This suggests the need for further efforts and educational initiatives aimed to reduce the improper use of antibiotics in the surgical setting.

**P1285**  
Assessing stewardship programmes as a potential determinant of antibiotic management in European intensive care units: a survey  
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**Objective:** Inappropriate antibiotic use is associated with poor patients outcome, especially in ICUs. Understanding the current antibiotic practice is extremely important to build effective strategies for improving antibiotics use. A survey was designed to investigate the self-assessed rate of compliance with national or international guidelines on antibiotic usage and diagnostic tests. In addition, as a potential determinant of antibiotic management, the local implementation of antibiotic stewardship programmes was assessed.

**Methods:** A 32-point structured questionnaire was elaborated and sent to 118 ICUs throughout Europe. ICUs were selected according to the prevalence of methillin-resistant Staphylococcus aureus (MRSA) bacteremia (low, moderate and high MRSA rate).

**Results:** Completed questionnaires were provided by 35 ICUs (response rate, 30%). Geographical distribution among Europe was fairly good. In the majority of cases, ICUs were closed (29, 83%), mixed (18, 51%) and belonged to medium-large size University hospitals (29, 83%). Ninety one percent of ICUs declared to follow local guidelines for empirical antibiotic therapy (see Table). The survey documented a high rate of blood cultures performance (94%) before starting antibiotics, while cultures from other site was less frequently described (urine and BAL, 54% and 31%, respectively). Concerning timing, antibiotics were not started within 4 hours in 4 centers (11%). Infection markers, mainly serum procalcitonin to start (71%) and to monitor response to antibiotics (83%), were routinely used in 66% of centers. Interestingly, high percentage of ICUs declared to regularly apply de-escalation (94%) and daily reassessment of therapy (77%). Antibiotic stewardship programmes were not present in 34% of ICUs. High percentages of centers did not have limitation system for specific drugs (54%), antibiotic order form (52%), antibiotic formulary (43%) and automatic stop order (69%). Only 17% of centers used computer decision support system to help antibiotic choice.

**Conclusions:** Although the appropriateness of antibiotic use is of paramount importance in ICU patients, our survey shows that antibiotic stewardship is far to be standardized in such setting. Interventions to improve antibiotic use in ICUs need to be urgently designed on a European level.

**P1286**  
Comparison of inhaled and intravenous colistin combined with tigecycline in treatment of multidrug-resistant Acinetobacter baumannii pneumonia  
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**Objectives:** Increasing antimicrobial resistance in Acinetobacter baumannii lead physicians to search for alternative therapeutic options. The aim of this study was to investigate the effectiveness of inhaled and intravenous (i.v.) colistin combined with tigecycline in multidrug-resistant (MDR) A. baumannii pneumonia.

**Methods:** Medical records of the patients receiving i.v. or inhaled colistin combined with tigecycline for at least 72 h for MDR A. baumannii pneumonia were reviewed retrospectively from 1 October 2009 to 30
April 2010. Daily dosage of inhaled colistimethate sodium was 2 million IU divided into 2 doses which was used via ventilator in patients under mechanical ventilation and via nebulized oxygen flow in spontaneously breathing patients. Daily dosage of i.v. colistin was 3 million IU divided into 3 doses. Follow up cultures were evaluated at 7th and 14th days of treatment. The primary outcome measure was 30 day in hospital mortality, and secondary outcome was microbiological eradication.

**Results:** 43 patients with MDR *A. baumannii* pneumonia were included in the study. Of these patients, 22 (51.2%) were female and the mean age for all patients was 64.4±13.6 (range, 22−91) years. Ventilator associated pneumonia was present in 39 (91%) patients. APACHE II scores were as follows: <10 (n = 2, 4.7%), 10−20 (n = 14, 32.6%), >20 (n = 27, 62.8%). Among 43 patients, 42 (97.7%) had a co-morbidity. All patients were followed up in intensive care units. Duration of ICU stay until pneumonia was 7.5±7 days. In addition to tigecycline, i.v. colistin was used in 20 (46.5%) and inhaled colistin in 23 (53.5%) patients. Nephrotoxicity was not observed in both groups, however in one patient bronchospasm was developed after administration of inhaled colistin. Microbiological eradication rate was higher in inhaled colistin group than i.v. group both at day 7th (30% vs 0%, p = 0.027) and 14th (53% vs 16%, p = 0.050). In 10 patients microbiological and clinical cure were obtained, in 4 patients only clinical cure were obtained. In one patient colistin resistance was developed at the 14th day of inhalation treatment. All cause 30 day in hospital mortality was 67.4%. There was no significant difference between i.v. and inhaler groups for mortality (70% vs 65.2%, p = 0.738). In hospital mortality was 67.4%. There was no significant difference in mortality and or oral switch (0% vs 21%, P = 0.052, N = 66).

**Conclusion:** Both inhaler and i.v. colistin combined with tigecycline could be used in treatment of MDR *A. baumannii* pneumonia. Inhaler colistin combined with tigecycline provided better microbiological eradication in this study.

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**Table 1. Characteristics of the 66 cases of infective endocarditis**

| Characteristic | N | % |
|---------------|---|---|
| Community-acquired | 55 | 83 |
| Endocarditis type | | |
| Native valve | 40 | 59 |
| Prosthetic valve, early infection (<1 year after surgery) | 2 | 3 |
| Prosthetic valve, late infection >1 year after surgery | 14 | 21 |
| Previous valve, early infection | 16 | 24 |
| Previous valve, late infection | 0 | 0 |
| Other | 3 | 4 |
| Microbiological etiology | | |
| Staphylococci | 42 | 65 |
| Streptococci | 25 | 38 |
| Enterococci | 7 | 10 |
| Other | 14 | 24 |
| Negative culture findings (all due to previous antibiotic therapy) | 4 | 6 |

**Complications**

| Cardiac failure | 22 | 35 |
| Shaken | 10 | 15 |
| Endocarditis, mycotic | 19 | 29 |
| Intravascular access | 11 | 17 |
| Surgical therapy | 38 | 42 |
| Outcome | | |
| In-hospital mortality | 10 | 15 |
| Refuse | 1 | 2 |
| Medium durations of follow-up, in days | 50 | |

*pSome infections may be polybacterial.* **The same patient could have two complications.**

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**P1287 Audit of antibiotic therapies in 66 cases of endocarditis**

E. Demonchy, E. Cua, P. Roger, P. Dellamonica, C. Pulcint* (Nice, FR)

**Objectives:** We wanted to assess the quality of antibiotic therapies prescribed for infective endocarditis on our ward. Such audits have rarely been reported in the literature.

**Methods:** We conducted a retrospective audit of all adult cases of endocarditis hospitalised over a 3-year period in the Infectious Diseases department of Nice University Hospital, France. The quality of antibiotic therapies was assessed using the 2004 European Society of Cardiology guidelines as a reference, since no national guidelines were available in France. The antibiotic therapy was considered as appropriate only if all the following items were in accordance with the guidelines: molecule, dose, route and interval of administration, duration of antibiotic treatment.

**Results:** Sixty-six patients were included, aged 63 yo on average. The main characteristics of the cases of endocarditis are presented in Table 1. One-hundred and fifty-two antibiotics were prescribed, representing 7 single therapies, 36 combinations of two antibiotics and 23 combinations of three antibiotics. The molecules that were the most frequently prescribed were: gentamicin (n = 49), amoxicillin (n = 29), rifampin (n = 25), vancomycin (n = 17) and oxacillin (n = 14). The antibiotic therapies were in accordance with the guidelines in 14% of the cases. The most frequent causes of inappropriate therapies were: gentamicin prescribed as a single daily dose in 55% of the cases, too long durations of gentamicin treatments in staphylococcal endocarditis in 28% of the cases and unnecessary prescriptions of rifampin in 72% of the cases. An intravenous-oral switch of antibiotic therapies was done in 29% of the patients (n = 19), 18±9 days after starting therapy on average, for staphylococcal (n = 12) or streptococcal endocarditis (n = 7); these endocarditis were mainly left-sided (n = 12) and complicated in 79% of the cases. There was no significant association between mortality and inappropriate antibiotic therapies (14% vs 22%, P = 0.62, N = 66) or between mortality and oral switch (0% vs 21%, P = 0.052, N = 66).

**Conclusion:** Infective endocarditis was rarely treated using an antibiotic regimen in accordance with the 2004 European guidelines, but misuse did not have a negative impact on mortality. Intravenous-oral switch of antibiotic therapies was common practice, even in complicated left-sided endocarditis, and was associated with a favourable outcome in all cases.
Results from a non-interventional study: daptomycin as empiric treatment of Gram-positive infections

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Objectives: Inadequate empiric treatment (ET) of *S. aureus* infections is frequent and is associated with poor outcome. MRSA coverage is recommended for ET in regions with high prevalence or for severely ill patients (pts). Vancomycin is however inferior to penicillin to treat MSSA infections, a therapeutic dilemma. The objective of this analysis is to evaluate if treating physicians’ prediction of MRSA or MSSA infection is accurate and also the role of daptomycin (DAP) in the ET of Gram positive infections.

Methods: Answers to standardized questions concerning suspected pathogens in pts receiving DAP as ET were analyzed and compared to culture results received after treatment initiation in the European Cubicin® Outcomes Registry and Experience, a retrospective, non-interventional, multicenter study describing characteristics and outcomes of pts treated with DAP. Among all 3621 pts enrolled from 2006 to 2010 only those empirically treated were included. Additionally, outcomes (cured and improved = success, failure, non-evaluable) were assessed at the end of DAP therapy by the investigators.

Results: A total of 2041 pts were treated empirically. Information on suspected pathogens was provided in 1841 cases (62% MRSA, 17% MSSA and 21% other pathogens. Table 1 shows common discrepancies between the final culture results and the suspected pathogens. Overall, ET with DAP was given to 746 pts (37%) with cSSTI, 330 pts (16%) with bacteraemia, 317 pts (16%) with uSSTI, 158 pts (8%) with foreign body/prosthetic, 134 pts (7%) with endocarditis and 105 pts (5%) with osteomyelitis. Most frequently used DAP doses in ET were 6mg/kg and 4mg/kg with no clear influence of the suspected pathogen on dose choice. Doses higher than 6mg/kg as ET were used in 13% and 10% of MSSA and MRSA cases, respectively. Clinical success of ET with DAP in pts with confirmed culture results were: 82% for MSSA, 85% of pts for MRSA, 78% for coag-neg staphylococci, 72% for *E. faecalis* and 81% for *E. faecium*. Success rate for pts without identified pathogen was 77%.

Conclusion: This study confirms that in routine practice clinical suspicion of MRSA or MSSA infections are frequently not confirmed by laboratory results which commonly leads to inappropriate therapy (Ammerlaan et al. 2009). The study also expands the results of DAP pivotal trials, which support DAP as effective empiric treatment of G+ infections, regardless of the presence of MSSA or MRSA.

Table 1 – Culture results in patients with suspected infections caused by MRSA, MSSA and others

| Confirmed pathogens by culture | MRSA N (%) | MSSA N (%) | cong. neg. Staph | E. faecium N (%) | E. faecalis N (%) | Others* N (%) | Culture negative |
|-------------------------------|------------|------------|-----------------|----------------|----------------|-------------|-----------------|
| Suspected MRSA (n=1134)       | 147 (13%)  | 103 (9%)   | 124 (11%)       | 11 (1%)        | 33 (3%)        | 403 (35%)   | 313 (28%)       |
| Suspected MSSA (n=312)        | 9 (3%)     | 30 (10%)   | 32 (10%)        | 1 (0.3%)       | 3 (1%)         | 146 (47%)   | 91 (25%)        |
| Suspected “other” (n=395)     | 0 (0%)     | 12 (3%)    | 83 (21%)        | 24 (6%)        | 20 (5%)        | 155 (40%)   | 101 (26%)       |

*Other pathogens include Streptococci and other G+ pathogens.

Clinical response and nephrotoxicity according to the trough serum vancomycin concentration among patients with methicillin-resistant *Staphylococcus aureus* bacteraemia

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Background: Vancomycin is an important antibiotic for the treatment of MRSA infection, but has narrow therapeutic window. We conducted the study to assess the clinical response and nephrotoxicity according to the trough serum vancomycin concentration among patients with methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia.

Methods: A retrospective cohort study was conducted among patients who received vancomycin for MRSA bacteremia during the period from 1 April 2002 and 31 March 2010 at Korea University Ansan Hospital. Patients were included in this study if they (1) were ⩾15 years old, (2) received vancomycin for more than 48 hours, (3) had ⩾1 vancomycin trough level collected within 3−7 days from the initiation of vancomycin therapy, and (4) had serum creatinine level collected within 7−14 days from the initiation of vancomycin therapy. Patients were excluded if they (1) had a diagnosis of infective endocarditis, (2) received hemodialysis or peritoneal dialysis during the vancomycin therapy, or (3) received vancomycin before the first MRSA bacteremia episode. Clinical response was assessed by the eradication of MRSA bacteremia within 7−14 days from the initiation of vancomycin therapy or the time required for the resolution of fever. Nephrotoxicity was defined as an increase in serum creatinine level of 0.5 mg/dL or ⩾50% from baseline. Patients was classified according to the trough serum vancomycin level; <15 mg/L, 15−20 mg/L and >20 mg/L.

Results: During the study period, 200 patients had MRSA bacteremia and 73 patients were included in this study according to the study protocol. Among 73 patients, 37 (50.7%) had trough serum vancomycin concentration of <15 mg/L, 19 (26.0%) had trough concentration of 15−20 mg/L, and 17 (23.3%) had trough concentration of >20 mg/L. MRSA eradication rate was not different between the three groups:
Superinfection during the treatment of nosocomial infection with tigecycline

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Introduction: Tigecycline is a antibiotic active against Gram positive, Gram negative included multiresistant drugs agents. Proteae genera present low sensible and Pseudomonas aeruginosa is inherently resistant. In large FIII, clinical trials the superinfection rate of tigecycline is 6.7% but are not specified focus or aetiology. We observed that superinfection rates of P aeruginosa during the treatment with tigecycline is higher that the previously described (EJC MID 2010 Jul; 29(7): 867–71).

Objective: The aim of this study was to evaluate the superinfection rates and colonization including by P aeruginosa, during tigecycline treatment, along the time.

Methods: We performed an observational study retrospective (November 1st, 2007 – October 31th, 2008) and prospectively (November 1st, 2008 – October 31th 2010) of all adult patients, who received at least 72 hours tigecycline as the loading dose, followed by 50 mg every 12h. The duration of treatments was a decision of the physicians in charge of the patients and was related to the clinical conditions. In each patient, we analyzed all cultures made until hospital discharge or death. Patients without follow up cultures during or after tigecycline treatment and those with prior or concomitant isolation of P aeruginosa at the beginning of the treatment were excluded.

Results: We included 130 patients, 51 (39%) retrospectively and 79 (61%) prospectively. Median of days of treatment with tigecycline was 13 days (9; 20.5) (p25; p75). In the first period the time the superinfection rate 12/51 (23.5%), seven of them (55%) due to P aeruginosa and the five resting were Enterobacter cloacae, Morganella morgani, Proteus mirabilis, Providencia stuartii and Enterococcus faecalis. In the second period the superinfection rate was 18/79 (22.7%), nine of them (95%) due to P. aeruginosa and five due to Proteus mirabilis, three due to Morganella morgani, and one due to Enterococcus faecalis.

Conclusion: The superinfection rate during treatment with tigecycline may be higher than previously reported, and consistent along the time. Considering the potential risk of infection with P aeruginosa and other resistant bacteria, a tight surveillance in the follow up of patients treated with tigecycline must be performed in order to disregard or confirm this potential risk.

Clinical and economic impact of rapid microbiological information of the samples more frequently processed in the Department of Clinical Microbiology

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Objectives: Up to 50% of the antibiotic prescriptions in hospitals are considered inappropriate. Antibiotics can represent up to 30% of hospital pharmacy expenses. The aim of this study was to measure the clinical and economic impact of rapid microbiological information of the samples more frequently processed in the Department of Clinical Microbiology.

Methods: 574 hospitalized patients with a bacterial infection confirmed by culture were assigned to a control group (n = 284; results information in the following day of their obtaining) or an intervention group (n = 290; results information in the same day of their obtaining). Viték® 2 system (bioMérieux) was used for identification and susceptibility testing in both groups. Outcome parameters were length of hospital stay (conventional hospitalization unit, intensive care unit) and mortality rates (global, attributable to infection). Treatment recommendations were made by an infectious diseases physician. Direct fixed costs, direct variable costs and indirect costs during hospitalization were considered. Costs related to tests, Laboratory, Microbiology, Pharmacy, hospital stay and “other costs” (“blood bank, anesthesia, clinical assistance, operating room”) were calculated.

Results: The samples more frequently processed were wound and abscess cultures, blood cultures and genitourinary tract samples cultures. Baseline characteristics of the patient groups in each study period based on the McCabe-Jackson criteria (severity of underlying illness) and on the Charlson score (comorbidity index) were similar. Faster reporting of identification and antimicrobial susceptibility results (p < 0.001) led to a significant reduction in the length of hospitalization for those patients in which wound and abscess samples (p = 0.014) and genitourinary tract samples (p = 0.039) were analyzed. Mortality rates did not differ significantly between the two groups. Global costs in the intervention group were lower than in the control group for those patients in which wound and abscess samples (p = 0.003) and genitourinary tract samples (p = 0.038) were analyzed.

Conclusions: Rapid microbiological information was associated with a significant reduction in length of hospital stay and in global costs for those patients in which wound and abscess samples and genitourinary tract samples were analyzed. However, identification and antimicrobial susceptibility results of blood culture isolates did not lead to significant clinical and financial benefits.

Forgotten antibiotics: an inventory in Europe, US, Canada and Australia

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Objectives: In view of the alarming spread of bacterial resistance in the absence of new antibiotics, this study aimed at collecting structural, reliable information on the availability of potentially useful but sometimes old antibiotics.

Methods: A survey on the availability of antibiotics was performed among hospital pharmacists, microbiologists or infectious diseases specialists in Europe, the US, Canada and Australia. Systemic antibacterial drugs (ATC code J01) were selected from the textbook “Kucers: The use of antibiotics” by an international expert panel. The experts assessed (potential) activity on resistant microorganisms and/or narrow spectrum “niche” characteristics for these antibiotics, handling defined criteria.

Results: The experts identified 33 valuable antibiotics, 13 because of their potential activity on resistant Gram negative bacteria and 8 on the
basis of their narrow spectrum. Availability data were obtained for the US, Canada, Australia and 27 European countries (Figure 1). The number of antibiotics available and the way they were available (marketed or via a special system) differed considerably from one country to another. Twenty out of the 33 selected antibiotics were available in less than 15 countries. Economic motives are the major cause for (discontinuation of) marketing of these antibiotics.

**Conclusion:** Many potentially useful antibiotics, usually low cost and marketed as generics, are not available in all countries. This is a worrisome situation, taking into account the current worldwide bacterial resistance crisis. Measures to improve the availability of these antibiotics are needed on a global scale.

**P1294 Getting acquainted with aztreonam: a six-month audit of use in a tertiary referral hospital**

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**Objectives:** An outbreak of Clostridium difficile infection occurred in this hospital during summer 2008. A fluoroquinolone-ban was introduced as one of the outbreak control measures. The non-availability of fluoroquinolones led the clinical microbiology team to use alternative agents, including aztreonam. In Ireland, aztreonam accounted for just 0.1% of total antimicrobial use in 2008 (0.08 defined daily doses/100 bed days used). An audit was carried out to determine the appropriateness of aztreonam prescription for patients treated between January and June 2009 and to correlate its use with microbiology results, patient characteristics and outcomes.

**Methods:** Following the fluoroquinolone ban, aztreonam was prescribed on the advice of the clinical microbiology team and a record kept of patient details; indication for aztreonam prescription; use as monotherapy or in combination with other therapy; microbiology results and patient outcomes.

**Results:** From January to June 2009, aztreonam was administered to 77 patients (40 male). Mean patient age was 64 years (range 14–89 years); 26 patients were admitted to the intensive care unit. Aztreonam was prescribed for 41 medical and 36 surgical patients for the treatment of:
- Pneumonia (20 patients)
- Wound/soft tissue infection (15 patients)
- Urinary tract infection (15 patients)
- Intra-abdominal infection (12 patients)
- Bacteraemia (10 patients)
- Infection of uncertain source (5 patients)

For 23 patients, aztreonam was used as monotherapy and for the remaining 54, it was given in combination with other agents). Mean duration of therapy was 8 days (range 1 to 42 days). Positive microbiology results were recorded for 59 patients. Ninety-six percent of Gram-negative organisms isolated were susceptible to aztreonam, 44.8% exhibited reduced susceptibility or resistance to piperacillin/tazobactam and 14% were resistant to fluoroquinolones. Extended spectrum β-lactamase producing Gram-negative organisms were detected on two occasions necessitating discontinuation of aztreonam. One patient developed *C. difficile* infection whilst on combination therapy of aztreonam and clindamycin. Sixty-two patients recovered from infection, 12 died (7 admitted to ICU) and for 3 patients, the outcome was unknown. The average length of stay was 51 days.

**Conclusion:** Aztreonam is a safe and useful alternative to fluoroquinolones for the treatment of a variety of infections due to Gram-negative organisms.

**P1295 Drug content, powder characterisation and particulate matter contamination in imipenem/cilastatin powder for injections from Asia region**

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**Introduction:** The report of clinical inferiority of generic imipenem/cilastatin (IC) injection, compared to the original one, was recently reported in Thailand. Due to extremely hygroscopic property of cilastatin sodium and highly agglomerated powder of imipenem monohydrate, the manufacturing process of these products is usually not simple. Most of generic IC products in Asia region are provided by the same manufacturing sources.

**Objectives:** Samples of IC powder for injection were collected from China, India, Japan, Taiwan, and Thailand for drug content analysis, powder characterization and investigation of particulate matter contamination.

**Methods:** This was a double blinding study. Powder characterization of IC was investigated using scanning electron microscope (SEM). Two laboratory methods were applied to investigate the contamination of particulate matter after reconstitution, i.e. microscopic and electrical sensing zone methods. The contents of imipenem and cilastatin were measured simultaneously using newly developed HPLC technique.

**Results:** There were eight IC powders for injections, collected during June 2009 to December 2010. Most of IC products were manufactured by dry mixing of crystalline imipenem and sodium bicarbonate powder. The original imipenem powder was prepared differently by spray drying process resulting in minimal degree of agglomeration and more complete reconstitution. The particles burden found under SEM were undissolved drug/excipient particles as well as materials from the containers and stoppers. The numbers of particles in all samples with sizes ≥25 and ≥10 microns were within the acceptable range according to pharmacopoeia standard, with significantly lower numbers of particles found in the original samples. The contents of imipenem and cilastatin in the original samples were also higher than those of the others. The difference in drug contents between generic and original samples was up to 14% for imipenem and 17% for cilastatin, respectively. The particle burden trapped on the membranes under SEM also reflected the heavy load of contamination in some of generic brands which also had the low contents of the active drugs.

**Conclusion:** The commercially available products of IC in Asia region were different in the contents of active drugs and the contamination of particulate matter. These differences possibly lead to inferior clinical safety and efficacy of generic IC, as reported earlier.

**P1296 Multiple-dose pharmacokinetics of anidulafungin during continuous veno-venous haemofiltration**

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**Objective:** Clinical studies support a role of anidulafungin as a first-line treatment of invasive candidiasis in critically ill patients and postulate no need of dose adjustments in mild to severe renal failure. Although intensive care patients with organ failure are in particular at risk of invasive fungal infections, no pharmacokinetic (PK) data on anidulafungin in continuous renal replacement therapy is available. Therefore we wanted to study the PK of anidulafungin in critically ill patients with venovenous haemofiltration (CVVHF).

**Methods:** Ten critically ill patients with CVVHF due to acute renal failure were included. Anidulafungin was infused on 3 consecutive days starting with a loading dose of 200mg on day 1, followed by doses of 100mg on days 2 and 3, respectively. During the 72h study phase of CVVHF, blood and ultrafiltrate samples were collected at corresponding times and anidulafungin concentrations were determined by high-pressure liquid chromatography (HPLC).

**Results:** Peak plasma concentrations were reached 3h after the start of infusion and were 8.5±3.6µg/ml at the pre-filter port. The mean arterial area under the curve (AUC) of the study population was 109.9 ± 49.82, the total clearance was 1.08±0.41 L/h, the volume of distribution 41.97 ± 22.64L, and the elimination half-life 28.78 ± 10.40h. Trough levels were above previously published minimal inhibitory concentrations (MIC90) of anidulafungin for relevant pathogens. Anidulafungin was not filtrated, but CVVHF resulted in a substance loss reflected by a difference between venous and arterial AUC of about 20%, due to adherence to synthetic surfaces.

**Conclusion:** Pharmacokinetics of anidulafungin in CVVHF resembled findings in healthy adults and adults with fungal infections. Therefore we recommend a loading dose of 200mg intravenous anidulafungin on the first treatment day and 100mg on consecutive days in anuric patients during CVVHF.
A 3-year survey of antifungal therapy results in febrile neutropenic patients with haematological malignancies: an analysis of 335 febrile episodes

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Objectives: One of the reasons for the continuing high mortality rates due to invasive fungal infection is the delay in administering appropriate therapy in febrile neutropenic patients with hematological malignancy. Empirical antifungal therapy is the standard form of care for neutropenic patients with hematologic malignancies who remain febrile despite broad-spectrum antibacterial treatment. However, targeted and pre-emptive antifungal therapy is a more effective strategy in the treatment of fungal infections.

Our aim in this study was to evaluate antifungal treatment in febrile neutropenic patients with hematological malignancies in our university hospital.

Methods: 335 febrile neutropenic episodes in 170 patients with hematological malignancies monitored between October 2005 and October 2008 at an university hospital in Turkey were included in the study, which evaluated the antifungal treatments administered.

Results: The average time for the onset of antifungal treatment for all antifungals was 7.0 ± 4.6 days. Classical amphotericin B (CAMB) (6.8%), liposomal amphotericin B (LAMB) (9.6%), caspofungin (CASP) (38.2%) and voriconazole (VOR) (17.6%) were used in antifungal treatment. Mean time to bring fever under control with antifungal therapy modification was 4.4 ± 5.1 days. Duration of antifungal therapy was 15.2 ± 0.9 days during hospitalization. Nephrotoxicity, hepatotoxicity, allergic reaction, electrolyte imbalance, polyneuropathy and hallucination were determined as antifungal treatment-associated side effects. Side effects were most frequently observed in patients using CAMB, at a level of 44%. The most common CAMB-associated side effects were nephrotoxicity and electrolyte imbalance, while polynuropathy was a significant side effect in patients using VOR. Analysis of antifungal treatments in terms of mortality determined no significant findings. Analysis of antifungal treatments is shown in the table.

Conclusion: Despite new antifungal treatments, invasive fungal infection mortality is still high. Definition of fungal infections and treatment being directed toward a likely agent/cause or target will enable spending on antifungal treatment to be used effectively, as well as enhancing treatment success. Bearing in mind the toxic side effects of other chemotherapeutic drugs used by patients with malignities, there is a need for antifungal agents with greater target efficacy in the treatment of fungal infections and with less toxicity.

Early antibiotic use during pregnancy changes the vaginal microflora near time of birth

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Objectives: Antibiotics are widely used through pregnancy, mainly to treat symptoms of urinary tract infections. Symptoms of UTI are very common among pregnant women, however only a small percentage do have bacteriuria within the group complaining about typical symptoms of UTI. Antibiotics are also administered during birth either prophylactic or because of symptoms. It is known, that antibiotic treatment can eliminate pathogenic bacteria causing disease, but it does also influence the normal colonisation. The aim of this study was to analyse the effect of antibiotic use during pregnancy on the vaginal colonisation near the time of birth.

Methods: The novel Copenhagen Prospective Study on Asthma in Childhood (COPSAC2010) prospective unselected birth cohort is an ongoing study of 700 pregnant women and their children. Vaginal samples from the pregnant women are characterised by culture at gestational week 36 and detailed information on antibiotic use during pregnancy is collected prospectively from participants and double checked in national registers.

Results: To date 485 vaginal samples have been cultured. 144 of the 485 women included in the study had received antibiotics during their pregnancy in a total of 235 treatments. Antibiotic treatment at any point during pregnancy was associated with a decrease in number of different species colonising the vagina. Antibiotic treatment resulted in detection of fewer species in the vaginal samples compared to those from women that did not receive antibiotic treatment.

Conclusion: A change of bacterial diversity was found in the vaginal microflora of the pregnant women, who received antibiotics during pregnancy. This change is not only observed in the weeks immediately after antibiotic administration as expected, but remains throughout pregnancy even though treatment was given as early as the first trimester. Thus, vaginal colonisation seems to be affected more definitely than expected, even at early treatments.
Nanosensors for antibiotic discovery and therapeutic drug monitoring in real time

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Today the emergence of new infections and the re-emergence of old enemies, including methillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci (VRE) is a major global healthcare problem. The frequent use of antimicrobial agents in developing countries where they are readily available without prescription and failure to complete treatment courses have escalated development of resistance to antibiotics. Faced with shortages of new antibiotics in the drug pipeline, prudent use of current antibiotics is necessary to curb the incorrect usage of antibiotics which has contributed to the fuelling of anti-microbial drug resistance. Alternative approach is to focus on precise dosing by employing novel technologies to optimise drug therapy for better disease management.

Here we report the development of a new point-of-care (POC) nanosensor, which in combination with a new model, can be used to probe in real time, the concentrations of active “free drug” circulating in serum as well as directly quantify the drug-target interactions at the receptor site. Using model bacterial cell wall peptides terminating in Lysine D-Alanine D-Alanine (DAla) on the cantilever surface and solution drug-serum interactions were simultaneously quantified in near-physiological conditions. Thereby, the effect of serum binding on the antibiotic activity is determined, allowing precise tuning by adjusting the antibiotic doses to optimize antimicrobial activity and monitor drug resistance. These findings provide a new paradigm for POC applications in personalised medicine and drug discovery process.

Evidence-based recommendations for antibiotic usage in the intensive care unit: a systematic review

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Objective: Inappropriate antibiotic use is associated with increased patients morbidity and mortality. Building and implementing strategies to reduce the inappropriate use of antibiotics, especially in ICU patients, is of crucial importance. A systematic review was performed to identify evidence-based-recommendations and tested tools to drive antibiotic use in ICU setting.

Methods: We searched MEDLINE and COCHRANE databases up to July 2010. No restriction of language was applied. National and international guidelines (GLs), randomised (RCTs) and non randomised controlled trials (CCTs), systematic reviews (SRs) and controlled before–after studies (CBAs) were included.

Results: Overall 937 potentially relevant studies identified, 11 (4 international GLs, 1 Spanish GL, 3 RCTs, and 3 SRs) were included. All GLs were published between 2006 and 2008. All the following recommendations were classified as “strong” (see Table): performing cultures (≥2 blood cultures and 1 from each vascular access) before starting antibiotic; starting antibiotic as soon as possible (within 1 hour for septic shock); using empirical antibiotic therapy covering likely pathogens with good penetration into presumed sources; establishing site of infection within 6 hours from presentation; evaluating patients for a focus of infection amenable to source control measures; discontinuing therapy if diagnostic tests are negative; removing vascular catheter if potential infected; reassessing therapy daily and performing chest X ray, if patient is ventilated. Surprisingly, the level of evidence those recommendations relied on was very low or very low (9/12) or moderate (3/12). All RCTs and SRs focused on the diagnostic power and the efficacy of serum susceptibility test results.

Conclusions: Evidence-based recommendations for appropriate antibiotic use in ICU patients rely on a very low quality evidence. Public health officers should be aware, when designing intervention strategies, of the level of the evidence provided by the scientific literature for antibiotic usage in the ICU population.

Results from the 2010 antimicrobial susceptibility testing external quality assessment exercise organised for EARS-Net participants

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Objectives: The United Kingdom National External Quality Assessment Service for Microbiology (NEQAS) provides external quality assessment for antimicrobial susceptibility testing to the EARS-Net (formerly EARS) participants. In 2010 the annual EQA exercise was the first distribution in collaboration with European Centre for Disease Prevention and Control (ECDC) but the ninth in succession to EARS-Net laboratories.

Methods: An analysis was carried out on the performance of participants in the quality assessment exercise. Participation was invited from 827 laboratories in 30 countries and results were returned by 769 laboratories. The organisms distributed were one each of Klebsiella pneumoniae, Escherichia coli, Streptococcus pneumoniae, Enterococcus faecium, Pseudomonas aeruginosa and Staphylococcus aureus. Participants’ results for identification and susceptibility testing were assessed.

Results: The level of performance with these quality assessment specimens was generally high; with concordance of the intended results over 95% for most organism-antibiotic combinations. For the K. pneumoniae, (plasmid mediated AmpC) participant reporting of susceptibility to cephaplatin was more variable (cefotaxime susceptible (S) 20.1%, intermediate (I) 18.5%, resistant (R) 64.1%; cephradine S 16.1%, I 24.6%, R 59.3%; cefazidime S 2.8%, I 22.9%, R 74.3%); the piperacillin-tazobactam discrepancy rate was also higher. The. coli was borderline in susceptibility to amikacin (MIC 8–16mg/L) and results were variable (51.4% S, 31.7% I, 16.9% R). The E. faecium had vanA-mediated low-level teicoplanin resistance (MIC 8mg/L) and results were more variable among those using CLSI guidelines than those following EUCAST guidelines. The P. aeruginosa was borderline in susceptibility to piperacillin-tazobactam and again more variability in reporting was seen among CLSI users. There were no significant problems with the S. pneumoniae (penicillin S, ciprofloxacin R, erythromycin R) or the S. aureus (ST 239, multi-resistant).

Conclusion: EQA is a valuable tool in the quality assurance of antimicrobial susceptibility testing and indicates the validity of comparing collated data between laboratories in resistance surveillance studies. In this exercise concordance between participating laboratories was high except where there was borderline susceptibility or where different breakpoints in the guidelines were used, resulting in discrepancies in susceptibility test results.
Methods: Prospective unit and laboratory based surveillance in German ICUs from 2001–2010. The data were calculated on proportions of non-duplicate resistant isolates (RP), resistance densities (RD; i.e. the number of resistant isolates of a species per 1000 patient days) of a total 55 ICUs and antimicrobial usage density (AD) expressed as daily defined doses (DDD) and normalised per 1000 patient-days (pd) of a total of 75 ICUs. Linear trends were calculated using linear regression analysis with monthly data and χ²-trend test for resistance rates with yearly data.

Results: Total mean antibiotic use (without sulbactam) stayed stable over time. AD was 1180 in 2001 and 1238 in 2010. Carbapenem use more than doubled to an AD of 176 in 2010. Significant increases were also calculated for quinolones (AD of 169 in 2010 which equals 14% of antibiotic use) and 3rd and 4th generation cephalosporin (3 and 4GC) use (AD of 121 in 2010) and macrolides (AD 105 in 2010). The most prominent decrease faced aminoglycoside use (AD was 86 in 2001 and 28 in 2010).

RPs were as follows in 2001 and 2010: MRSA 26 and 23%, VRE faecium 2.3 and 5.1%, 3GC resistant E. coli 1.2, 11.9% and 3GC resistant K. pneumoniae 3.8 and 18.2%. The burden of resistance or the RPs did not change for MRSA but increased significantly for VRE and 3GC resistant E. coli and K. pneumoniae. RD of MRSA was 4.2 in 2010, of VRE faecium 0.26 and of 3GC resistant E. coli 1.64 and K. pneumoniae 1.07 resistant pathogens/1000 pd.

Conclusion: Total antibiotic use did not change over time with 1.2 DDD per patient per ICU day. However, there was a significant increase in the use of broad spectrum antibiotics like carbapenems. The burden of MRSA did not change in contrast to an increase in the burden of 3GCR E. coli and K. pneumoniae in German ICUs. In the face of resistant pathogens we prefer to describe and compare the burden of resistance and the magnitude of the public health problem by resistance densities besides resistance proportions which are essential for empirical antibiotic therapy decisions.

P1302 ISIS-AR and ISISweb: a new surveillance system for antimicrobial resistance surveillance
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Objectives: With the increasing antimicrobial resistance rates, surveil lance is an essential tool to monitor trends, rapidly detect and actively react to new resistance elevations, evaluate policies and compare laboratory results for quality improvement. For this purpose, the Dutch Infectious Diseases Surveillance Information System on Antibiotic Resistance (ISIS-AR) and the interactive database ISISweb were developed. This paper gives a summary of the achievements and possibilities of this new surveillance system.

Methods: From 2008 onwards, ISIS-AR has connected 27 medical microbiology laboratories to a central database, where antimicrobial susceptibility test (AST) results and epidemiological data from 40 routinely cultured bacterial species are uploaded monthly. Data undergo thorough quality control and automated feedback reports including an overview of EUCAST exceptional resistance phenotypes are send to the laboratories for confirmation. After confirmation, data from ISIS-AR are freely accessible via a query interface at www.ISIS-web.nl, a joint venture of the Dutch Society of Medical Microbiology (NVMM) and the National Institute for Public Health and the Environment (RIVM).

Results: Professionals and policymakers can view the national resistance trends via ISISweb and data can be used in clinical patient care. For example the prevalence of ESBL in E. coli blood isolates increased from 2.3% in 2008 (n = 1943) to 3.6% in 2010 (n = 996, until June, p < 0.05) and the prevalence of MRSA remained stable around 1%. The system has also proven successful in the detection of several cases of carbapenemase producing Enterobacteriaceae (CPE) in the Netherlands. As a spin-off, national ESBL and CPE guidelines have been developed for laboratory detection and infection control purposes. On ISISweb, data can also be viewed and compared by institution type, hospital department and age groups (ao). For example, in 2009 the proportion of ciprofloxacin resistance from E. coli urine isolates collected in hospitals (11.1%, n = 10,834), outpatient departments (14.9%, n = 10,587) and GPs (8.3% n = 34,660) differed substantially. Furthermore, participating laboratories can compare their results to those of their peers on a closed domain.

Conclusions: In the 3 years existence, ISIS-AR has proven very successful, which was largely due to the joined efforts of RIVM, NVMM and all participating laboratories. This national framework can be extended to include other pathogens.

P1303 Antimicrobial resistance surveillance for hospital- and community-acquired strains from southwestern Romania
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Objectives: It is a preliminary study, part of the PNDCHI 42121/2008 national research project, aiming the screening of bacterial strains isolated from intensive care units (ICUs), surgical departments and also from the community (Timis county, Romania), with the selection and preservation of multidrug resistant (MDR) strains for future molecular studies, scheduled in the next stages of the project.

Methods: The participant laboratories (Emergency Clinical County Hospital Laboratory – P1, Institute of Cardiovascular Diseases Laboratory – P2 and S.C. Bioclinica S.A. Laboratory – P3), transferred the MDR selected strains (collected from bronchial aspirates, blood samples, urines, surgical wound secretions, etc), for confirmation, to the University Microbiology Department’s Laboratory. These strains have been reidentified and phenotyped, with the help of automatic VITEK 2 compact system, using VITEK 2 GP/GN identification cards and AST cards for antimicrobial sensitivity tests. We have also performed Hodge tests for extended spectrum β-lactamase (ESBL) producing carbapenem resistant enterobacteria.

Results: From 2437 samples, collected in 2010, we isolated 842 MDR strains (607 from hospitals and 235 from the community). The percentage of Methicillin-resistant S. aureus strains (MRSA) varied from 35.44% (P1) to 8% (P2) in hospital environment, being 23.13% in the community (P3). Regarding hospital acquired ESBL producing enterobacteria, their prevalence was situated between 37.32% (P1) and 16.67% (P2), with 11.97% for the community (P3). The area of MDR percentage in the case of carbapenem-resistant non-fermentative strains, varied from 43.94% (P1) to 11.11% (P2) in hospitals, respectively, 14.70% in the community. All the Hodge tests were negative. Also, as a part of the project, both ICUs have estimated an average number of antimicrobial treatment days/MDR infectious episode, which varied from 9.11 days (P1) to 29.57 days (P2), with an average hospitalisation cost of 3657.35 €/patient (P1) and 3780.87 €/patient (P2).

Conclusions: Bacterial MDR is more frequently described not only in Romanian hospitals but in our community as well, representing an alarming phenomenon through the drastically restriction of therapeutic options. However, in comparison with 2009 (the first year of our research project), we assist to a slow decreasing of MDR prevalence in both studied hospitals with aproximately the same prevalence in community.

P1304 Japan Nosocomial Surveillance System (JANIS), a national surveillance system of antimicrobial resistance and nosocomial infections in Japan
S. Suzuki*, K. Yamane, A. Tsutsui, T. Yamagishi, Y. Arakawa (Tokyo, JP)

Objectives: A surveillance system for antimicrobial resistance is crucial to provide information for implementing control measures to limit the spread of antimicrobial resistant bacteria.

Methods: Japan Nosocomial Infections Surveillance (JANIS) was launched as a program of Ministry of Health Welfare and Labour of Japan in 2000 followed by system renewal in 2007 to provide practical and useful information for infection control practices in hospitals. It includes hospital with more than 200 beds with voluntary participation. It consists of five different divisions and Antimicrobial Resistant Bacterial
Infections (ARBI) division was assigned to monitor all patients with infections due to five major types of drug-resistant bacteria, while Clinical Laboratory (CL) division describes prevalence of major bacterial species among clinical isolates including antimicrobial susceptibility data.

**Results:** Up to January 2011, there are 504 participating hospitals in ARBI division and 681 in CL division, which covers about 1/4 to 1/3 of target hospitals in Japan. Data submission rate of those participating hospitals are around 90% and detailed feedback information for participating hospitals and the summary of JANIS data which is open to the public are both available thorough website.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is still the leading cause of nosocomial infections in Japanese hospitals. In 2009, among 17065 cases reported to JANIS ARBI division, 15093 (88%) were caused by MRSA and its incidence was 5.3 brasile/1000 admissions. On the other hand, infection caused by Vancomycin-resistant enterococci is still rare as only five cases were reported in 2009. Most remarkable change notified by CL division during the last decade is the increasing antimicrobial resistance in *Escherichia coli*. In 2001, only 0.6% of *E. coli* isolates were resistant to cefotaxime but it reached up to 10% in 2009. Fluoroquinolone resistance also increased dramatically as 9% of *E. coli* isolates were resistant to cefotaxime in 2001 but rose up to 17% in 2009. Resistance in *Klebsiella pneumonia* increased gradually but not as significant as *E. coli*. Unlike European countries and United States, multildrug-resistant *Acinetobacter* spp. isolates are quite rare and its prevalence is less than 0.5% among *Acinetobacter* spp. isolates.

**Conclusion:** JANIS has been established as a national surveillance system and provides useful epidemiological information of antimicrobial-resistant bacteria in Japan.

### P1305 National antimicrobial resistance alerts – an early warning and quality assurance system

**A.T. Eastaway**, J. Wilson, P. Webb, C. Wuff (Glasgow, UK)

**Objectives:** To develop and test an alert system at national level for rare organism/antimicrobial resistance (AMR) combinations ensuring early identification, confirmation and reporting of emerging problems to enhance the national AMR surveillance programme.

**Methods:** Laboratories in Scotland use CLSI sensitivity testing and report results via the Electronic Communication of Surveillance Scotland (ECOSS) from the Laboratory Information System directly to Health Protection Scotland (HPS) in line with ECOSS reporting protocols. Specific rare organisms/antibiotic resistance patterns were defined. A weekly automated search was run against ECOSS records which flagged organisms matching these combinations. An alert was automatically generated for the AMR surveillance team at HPS. Two alerts were piloted, penicillin resistance in *β*-haemolytic streptococci and carbapenem resistance in *E. coli* and *Klebsiella* species. The AMR team followed up all alerts. Where results were confirmed the laboratory was requested to forward to the appropriate reference laboratory for further investigations. If not confirmed the ECOSS record was altered accordingly.

**Results:** The system has been tested over 9 months. To date there have been seven reports of *β*-haemolytic streptococci resistant to penicillin. None were confirmed on follow up. The HPS database in ECOSS was amended accordingly. Nine reports of Meropenem resistance in *Klebsiella pneumoniae* have been received. Three of these were confirmed. Two were identified as possessing a CTX-M and permeability defect and one was confirmed as a KPC producing strain. Early awareness at a National level allowed a prompt alert to be sent to all infection control teams and Microbiologists of possible circulating strains.

**Conclusion:** A national surveillance programme can detect uncommon resistance mechanisms in key organisms using an automated alert system. This allows timeous follow up ensuring appropriate action by the referring laboratory where resistance is confirmed, early warnings to other laboratories and as a quality assurance measure to ensure the national dataset is as robust as possible.

### P1306 Real-time local epidemiological data through the internet as a management tool for infections in the community

**G.S. Simonsen**, T. Chomutare, L. Ilebrekke, P.A. Bakkeoll, A. Spaceian, J.G. Bellika (Tromsø, NO)

**Objectives:** To provide real-time epidemiological data on infectious diseases through the internet as a management tool for general practitioners and infection control personnel in the community. Increase the knowledge of pre-test probability of various infectious agents in order to optimize diagnostic strategies and treatment.

**Methods:** The Snow system extracts, classifies and summarizes data from microbiology production environments. The summarized data are transferred to a server on the internet every night and communicated to the users using web based spatiotemporal visualization. Simple heuristics is used to detect and provide alerts of unusual disease events in the patient populations.

**Results:** The Snow system is an open source software tool for extraction, classification, summary and spatiotemporal visualization of epidemiological data. Privacy issues are handled by a two stepped approach to production of spatiotemporal disease data as preparation for visualization. The rule based approach to transformation and classification of the data makes it possible to mask variations in coding of microbiology analysis and result codes. It also enables input from a variety of sources, such as labs, GP practices and casualty clinics. The Snow system currently covers two microbiology laboratories and the three northernmost counties of Norway with a population of approximately 470,000.

**Conclusion:** The Snow system provides real-time epidemiological data on infectious diseases through the internet as a management tool for general practitioners and infection control personnel in the community. The web based spatiotemporal visualization tool enables increased knowledge of the pre-test probability of various infectious agents and optimization of diagnostic strategies and treatment.

### P1307 Social media and infectious disease surveillance: tweets indicate norovirus outbreak at a university

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**Background:** It is not clear whether or not information from social media can enhance already established indicator-based surveillance of known infectious diseases. We examined a recent community outbreak of Norovirus at a university in Lower Saxony, Germany to determine if news articles and information posted to the websites Facebook, Twitter and blogs was faster than reporting through established indicator-based surveillance. This study was part of the Medical Ecosystem (M-Eco)
project, an EU FP7 funded project, which aims to develop a new platform for information collection about health events.

**Methods:** We compared information from each day of the outbreak from local and state authorities to information from Internet sources. We obtained news articles from an existing RSS feed established to collect news on health and infectious disease in Lower Saxony from local online news websites. We entered outbreak-related search terms into Topsy, a search engine to analyse Twitter content, and Google to search for information from blogs or other Internet forums.

**Results:** An initial report from the university canteen to the local public health department occurred a day after first signs of infection, and a timely press report was formulated on August 12, 2010. Activity in all information sources followed this inquiry closely (Peak 1), and there was an increase of coverage from social media after official pathogenic test results from infected individuals were reported on Friday August 13, 2010 (Peak 2). The majority of news media, however, occurred after the weekend, on Monday August 16, which in turn also spawned more social media coverage that could be linked to the content in newspaper reports (Peak 3). A final peak occurred in social media a day later (Peak 4) (Fig. 1).

**Conclusion:** The first notification of this case was made by the canteen to the local health department, a report not required by established laws for indicator-based surveillance of Norovirus in Germany, and news and social media followed. Furthermore, social media was also used for information exchange about the event, creating more information and more noise, and potentially amplifying possible signals for monitoring purposes. These cases show that individual infections might be caught by noise, and potentially amplifying possible signals for monitoring purposes. These cases show that individual infections might be caught when scanning social media and that investigating social media may lead to potential outbreak indications.

**Results:** The ECDC pilot Point Prevalence Survey of healthcare-associated infections and antimicrobial use: feasibility analysis

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**Objectives:** The ECDC pilot Point Prevalence Survey of healthcare-associated infections (HAI) and antimicrobial use (AU) in European acute care hospitals was coordinated by ECDC and outsourced to a consortium led by the University of Antwerp in collaboration with the French Institute for Public Health Surveillance and the Belgian Scientific Institute of Public Health. The objectives of the survey were to test and finalize a European protocol allowing to describe and estimate the prevalence of HAI and AU in hospitals. Two protocols, a patient-based (PB) with risk factors collected for each patient and a unit-based (UB) with aggregated denominator data at ward level, were tested. National questionnaires were sent to collect figures on hospitals and programmes/campaigns related to infection control (IC). A feasibility survey was conducted to assess and compare the burden of each protocol.

**Methods:** A questionnaire was sent to the 23 participating countries and to the 66 hospitals. Following items were surveyed: (i) at the national level: list of hospitals according to a proposed scheme based on hospital specialties, workload for training and for data collection; (ii) at the hospital level: workload for data collection and entry, number of health-care workers (HCW) involved in the survey.

**Results:** The information needed to classify the hospitals according to the protocol was not available at the national level in some countries, requiring a protocol adjustment. At the hospital level, the average time to fulfill the survey was 5 working days (wd; 1wd equals 8 hours) and 4 wd per 100 patients for the PB and UB protocols, respectively. The difference was mainly due to the data collection time. On average, 6 HCWs were involved in the survey (max: 21) classifiable in 4 different categories (max: 9). The most frequently involved HCWs were IC nurses and doctors. In 20 hospitals, ward staff were also involved in the survey. In 11 countries, national coordinating staff participated in the data collection or data entry processes.

**Conclusions:** The ECDC-PPS protocol was well implemented by national and hospital staff and no major feasibility problems were encountered. The patient protocol was slightly more labour intensive than the unit-based version, but brought much more valuable information and therefore has been chosen as the preferred protocol. The final adjustments to the ECDC-PPS protocol have been done in view of its implementation in all EU Member States in 2011–2012.

**Methods:** KONIS Steering Committee reviewed surveillance data from January through March 2008 and from July through August 2010. All KONIS hospitals were divided into six groups according to the healthcare-associated infection rates. Each one hospital was randomly selected from six groups. The trained members of KONIS Steering Committee visited each hospital and they reviewed medical records. The samples included patients with HAIs reported to KONIS and randomly selected patients without reports of these infections.

**Results:** We reviewed 195 (11% of total HAIs) reported cases and age and sex matched 427 patients with no reported infection randomly selected from 96 ICUs of 56 hospitals in 2008 and 101 ICUs of 57 hospitals in 2010. Sensitivities of UTI, BSI, and pneumonia were 95%, 89%, and 67%, respectively. And specificities of UTI, BSI, and pneumonia were 98%, 99%, and 99%, respectively. The overall positive predictive value for the hospital reports was 93%, and the overall negative predictive value was 95%. Using KONIS WRAP with standardized manual, the accuracy of KONIS was similar to that of other countries such as National Nosocomial Infections Surveillance System (NNIS) in United States and Krankenhaus Infektions Surveillance System (KISS) in Germany.

**Conclusions:** The findings of this study suggest that there was under-reporting of healthcare-associated pneumonia to KONIS. Continued validation and training will be needed in Korea to improve completeness of reported healthcare-associated infection data and especially to assure that healthcare-associated pneumonia data are valid.
The ECDC Pilot Point-Prevalence Survey of healthcare-associated infections and antimicrobial use: healthcare-associated infection results

C. Suetsens*, A. Muller, B. Coignard, J. Griskeviciene, S. Hopkins, K. West, M. Goossens, B. Catry, S. Vierenberg, V. Vankerkhoven, H. Goossens, P. Zarb on behalf of the ECDC-pPPS Study Group

Objectives: The ECDC pilot Point Prevalence Survey of healthcare-associated infections (HAIs) and antimicrobial use (AU) in European acute care hospitals (ECDC-pPPS) was coordinated by ECDC and outsourced to a consortium led by the University of Antwerp (UA) in collaboration with the French Institute for Public Health Surveillance (InVS) and the Belgian Scientific Institute of Public Health (WIV-ISP). The objectives of the ECDC-pPPS were to test and finalize a European protocol allowing to: describe and estimate the prevalence of healthcare-associated infections (HAI) and antimicrobial use (AU) in participating hospitals; stratify estimates by patients’ characteristics and invasive procedures and; provide a standardized tool for hospitals within Europe to identify targets for quality improvement.

Methods: All patients on ward by 8:00am and not discharged at the time of survey were included. Each ward was surveyed in one day. Two protocols – a patient-based with risk factors collected for each patient and a unit-based with aggregated denominator data at ward level – were tested.

Results: The overall prevalence of patients with HAI (n = 1408/19888 patients) was 7.1%. Of 1531 HAI, pneumonia represented 22%, surgical site infections 19%, urinary tract infections 17%, bloodstream infections 12% and gastro-intestinal infections 7%. The median length of stay before onset of HAI acquired during the current hospitalisation (n = 1378) was 12 days. Of 437 (24%) HAI present at admission, 58% was associated to a previous stay in the same hospital. Isolated microorganisms are given in the table. Carbapenem resistance was reported in 3.2% of enterobacteriaceae, 23.4% of P. aeruginosa and 20.4% of Acinetobacter spp. The percentage MRSA was 34.2%, and resistance to glycopeptides in Enterococcus spp (VRE) was reported in 5.4%.

In patient-based logistic regression analysis (n = 14329), HAI was independently associated with length of hospital stay (until infection date if HAI), number of invasive devices, surgery since admission, McCabe score, and specialty. Based on this model, expected HAI prevalence at the hospital level correlated better with observed prevalence than the rate of HAI, number of invasive devices, surgery since admission, McCabe score, and specialty. Based on this model, expected HAI prevalence at the hospital level correlated better with observed prevalence than the expected prevalence based on specialty only (R-squared = 0.69 vs 0.46).

Conclusions: The HAI prevalence in this pilot with 66 European hospitals was similar to the prevalence of 7.1% found earlier from a European PPS review [1]. The patient-based protocol was shown to be superior for risk adjustment than the unit-based protocol.

Reference(s)
[1] ECDC Annual Epidemiological Report 2008.
than in the hospitals with 700–899 beds and more than 900 beds, the rates of urinary catheter-associated UTIs were higher in the hospitals with 400–699 beds than in 700–899 beds. The rate of central line-associated BSI was 2.15 (0.409–0.411). During the past 4 years, there was no significant difference in the rate of urinary catheter-associated UTIs and central line-associated BSIs. On the other hand, the rate of ventilator-associated PNEUs was lower in July 2009-June 2010 than in July 2007-June 2008 and July 2006-December 2006. The most distinctive finding in the antimicrobial susceptibilities of major pathogens was that the incidence of carbapenem-resistant *Acinetobacter baumannii* increased from 43.6% to 82.5% during the study period.

**Conclusion:** It appears that the KONIS influences the reduction in the rate of device-associated infections, especially ventilator-associated PNEUs. Carbapenem-resistant *A. baumannii* was identified as an emerging Gram-negative pathogen of nosocomial infections in Korea. Therefore, ongoing targeted surveillance and infection control strategies are needed to control device-associated infections and the major antimicrobial-resistant pathogens.

### [P1314] Assessment of European practices in infection control. First results from PROHIBIT

M. Martin*, A. Conrad, W. Zingg, S. Hansen, P. Gastmeier, D. Pittet, M. Dettenkofer and the PROHIBIT consortium

**Objectives:** The European Commission (FP-7) funded project “Prevention of Hospital Infections by Intervention and Training” (PROHIBIT www.prohibit.unige.ch) was established in 2010. PROHIBIT aims to analyse existing guidelines and practices to prevent healthcare associated infections (HAI) in Europe, to identify factors that influence compliance with best practices and to test the effectiveness of interventions of known efficacy.

**Methods:** In August 2010, the European Centre for Disease Prevention and Control (ECDC) HAI surveillance National Contact Points (NCP) and HAI experts in 34 countries (27 EU member states [where UK counts as 4 countries: England, Northern Ireland, Scotland, Wales], Croatia, Iceland, Norway and Switzerland) were invited to complete an online questionnaire about (i) existing national guidelines for prevention of surgical site infection (SSI), ventilator-associated pneumonia (VAP), urinary tract infection (UTI), catheter-associated blood stream infection (CA-BSI), *C. difficile* associated infection (CDI), as well as on (ii) national HAI surveillance systems and (iii) public reporting policies.

**Results:** 28 of 34 NCPs (82%) completed the questionnaire. (i) Guidelines: 2 countries reported to have no guidelines, 2 to have 1, 3 to have 2, 2 to have 3 and 6 countries to have 4 guidelines. 13 countries reported to have guidelines on all 5 topics. Scientific level of supporting evidence and strength of recommendation are rated in 46% (12/26) of the guidelines. (ii) Surveillance: A nationwide HAI-surveillance-system for public hospitals is established in 86% (24/28) of the countries. Participation is compulsory in 12 of 24 (50%) countries. Surveillance for CA-BSI, SSI, VAP, UTI and CDI is implemented in 22, 21, 18, 15 and 15 countries, respectively. 9 countries monitor all 5 topics. (iii) Public reporting: 7 countries have established public reporting of data on HAI from individual hospitals.

**Conclusions:** National HAI surveillance systems are well established in Europe, especially for SSI and CA-BSI, whereas evidence-based guidelines for prevention of HAI are still to be developed. Countries with public reporting do so after HAI-surveillance is established and declared mandatory. Thus, mandatory surveillance may be considered a first step towards public reporting. Although reasons for this must be further elucidated, we speculate that once data on HAI are retrieved systematically, political pressure may be exerted to make the data public.
MRSA carriage and to check infection control measures in inpatients in the rural district Hannover.

**Method:** A point-prevalence study was conducted in 17 hospitals from October 1, 2009 through March 31, 2010. Inpatients were screened by cultures from nose, throat and broken skin and patient data was recorded. MRSA isolates were tested for PVL and were analysed by staphylococcal protein A (spa) typing.

**Results:** MRSA was isolated from 118 (3.9%) of 3013 consenting inpatients. The MRSA prevalence ranged from 0.3% to 12.5% in the respective hospital. The spa typing revealed 26 different types. With 67% was the spa type t032 or so called “Barminer” strain predominant followed by t003 (3%), t011 (3%), t020 (3%) and t002 (2%). The PVL gene was present in two (1.7%) MRSA strains. Factors were found to be significantly associated with MRSA carriage at univariate analysis were age older than 71 years, prolonged hospital stay (23d vs. 17d), history of hospitalization (RR, 2.2; CI95, 1.5–3.15) or surgery (RR, 1.5; CI95, 1.01–2.01) or antibiotic therapy (RR, 2.0; CI95, 1.42–2.91), current antibiotic therapy (RR, 1.4; CI95, 1.01–2.06), dialysis patient (RR, 2.3; CI95, 1.13–4.87), and presence of open skin lesions (RR, 2.0; CI95, 1.35–2.90). 42% of all identified MRSA carriers were detected on internal medicine wards and 30% on surgery wards. 92 (78%) were newly identified MRSA carriers.

**Conclusion:** The results of our study revealed 78% of all MRSA-positive inpatients had been missed. Screening for MRSA on admission is useful to identify the imported cases and should be performed on wards harboured patients at risk, e.g. internal medicine and surgery. On the basis of our results, the respective hospital of the rural district Hannover can optimize its comprehensive infection control strategies against MRSA.

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**P1316 Guidelines for infection control staff: goals and reality in Germany**

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**Objectives:** In 2009 the German infection control commission (KRINKO) at the Robert Koch Institute released guidelines regarding needs of infection control (IC) personnel in hospitals based on risk assessment criteria. Our aim was to check the guideline requirements with reality in German hospitals.

**Methods:** 70 hospitals visited in 2010 by an external IC physician were assessed for IC staff working in the hospital, IC training for non-IC staff, and annual meetings of the IC team with the heads of departments. The numbers were compared to the numbers recommended by the KRINKO guidelines, and percentages of adherence to the guidelines were calculated.

**Results:** Hospitals were divided into three groups: I: small (<150 beds), II: medium-sized (151–400 beds) and III: large (>400 beds). In group I (n = 18), two hospitals (11%) employed the recommended number of infection control practitioners (ICPs), four (22%) had 75% and 12 (67%) less than half. IC link nurses were present in four hospitals, IC link physicians for every specialty in 11 (61%) and one for the whole hospital in five (27%). Continuous IC training for non-IC staff was available in 17 institutions (94%), and regular meetings of the IC team with the heads of department were held in all hospitals.

In group II (n = 36) two hospitals (5%) provided the recommended number of ICPs, six hospitals (17%) had 75% and 18 (50%) half. IC link nurses were available in nine hospitals (25%), IC link physicians in 35 (97%), 21 of which had one per specialty. All held regular meetings and in 34 hospitals (94%) IC training for non-IC staff was offered regularly.

In Group III (n = 16) 11 hospitals (69%) employed less than half the number of ICPs recommended, four (25%) employed half and one hospital 75%. All hospitals had IC link physicians, 11 (69%) one per specialty. Seven hospitals (44%) had IC link nurses as well. IC training for non-IC staff was offered in 15 hospitals (94%) and all hospitals held regular IC team meetings.

**Conclusion:** No hospital in the sample fulfilled all recommendations regarding IC personnel requirements and supporting IC link staff. In most hospitals, the gap between recommended and actual staff was large, often more than 50%. Thus infection control in hospitals has to be managed by non-specialized staff – often as an additional duty. However, there is a shortage of available IC personnel in Germany, so that increased efforts will be necessary to ensure training of sufficient numbers of skilled IC staff.

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**P1317 Preliminary evaluation of an automated detection tool for healthcare-associated infections, based on screening natural language medical reports**

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**Objectives:** Surveillance of healthcare associated infections (HAI) is an important activity in the context of control and prevention. Due to the limited resources allocated to this activity, developing tools using hospital information systems for monitoring HAI is required. An ongoing collaborative project ALADIN is aimed at developing an automated detection tool of HAI based on natural language processing of medical documents. The objective of this presentation was to evaluate the performance of this tool in its current development.

**Methods:** This project is conducted in three steps.

1. First step: development of rules associating expressions, concepts extracted from multiple medical terminologies and semantic relations for the detection of HAI. The rules were defined by an interdisciplinary work between Infection Control Practitioners (ICPs), semantic interoperability experts and linguists. For this step, it is planned to analyze 1200 medical documents, which will be gathered from four French University hospitals and will focus on surgical activity (digestive, neurosurgery and orthopaedics) and intensive care units.

2. Second step: development of the detection tool (Xerox Incremental Parser) based on semantic analysis of natural language.

3. Third step: evaluation of the performance of the detection tool in terms of sensitivity and specificity (Evaluation of the linguistic rules is outside the scope of this presentation). For this step, new medical reports will be analyzed (n=800). The gold standard will be the manual analysis of these medical reports by two independent ICPs.

| W | True | False | TP | FP | Sensitivity (%) | Specificity (%) | P1317 | P1318 |
|---|---|---|---|---|---|---|---|---|
| Intensive Care Unit | 25 | 10 | 12 | 6 | 0 | 62.5 | 97.5 | 100.0 | 100.0 |
| Digestive surgery | 21 | 14 | 4 | 2 | 1 | 67.5 | 90.0 | 100.0 | 100.0 |
| Neuro-surgery | 20 | 14 | 30 | 4 | 0 | 90.1 | 96.2 | 100.0 | 100.0 |

*Number of medical documents

**Results:** From the 2000 medical documents planned for the project, 635 (31.8%) were already available for this preliminary evaluation.
Surgical site infections after cardiac surgery in Oslo, Norway

T. Tollefsen*, B. Andersen, Ø. Vangen (Oslo, NO)

Objectives: To gain insight into the incidence of postoperative infections after ACB during a follow-up of 30 days. Surgical Site Infection (SSI) is one of the most common infections in hospitals and is a serious complication of cardiac surgery. In 2005, incidence registering of coronary artery bypass graft surgeries (ACB) was introduced at the Department of Thoracic- and Cardiac surgery, Oslo University Hospital – Ullevål (OUS), according to the National Nosocomial Infections Surveillance system (NOIS-1). After postoperative stay of only 3 days (mean), the patient is discharged to another hospital for further postoperative care.

Methods: A questionnaire was sent to all operated patients. The infection status was registered after surgery according to CDC criteria. Patients were asked to contact primary health care after 30 days for evaluation of the surgical sites. Any SSI was verified by a doctor and evaluated by the head surgeon and infection control personnel.

Results: Included were 1257 ACB-operated patients during the period 2005–2009, who responded to the questionnaire (ca 80%). SSI was detected in sternal-, leg- or drain wounds within 30 days after operation in 14.3% of the cases. The highest incidence of infection was registered in 2006, and there seemed to be some seasonal variation concerning SSI. Leg wound infection predominated (7.3%), followed by superficial sternal wound infection (4.7%) and drain wound infection (1.3%). A few patients had deep sternal wound infections (1.1%). Staphylococcus aureus was found in 23.2% of the cases with SSI and was the most common isolated pathogen. Methicillin resistant S aureus (MRSA) was detected in a leg wound infection of one patient (0.6%).

Conclusion: SSI is mainly detected after discharge from the hospital. Infections predominate in the incision site on leg after vein graft harvesting, and S aureus is the main pathogenic bacteria. Deep sternal wound infections occur relatively seldom. Incidence registering gives an important feedback to the surgeons – to evaluate and improve infection control in cardiac surgery.

Procedures for isolation and management of patients with highly infectious diseases in emergency departments: EuroNHID (European Network for Highly Infectious Diseases) data from 40 centres in 13 European countries

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In Health-care settings, isolation of patients with Highly Infectious Diseases (HIDs, e.g. Viral Hemorrhagic Fevers and SARS) is fundamental for the prevention of the spreading of these infections. In particular in the Emergency Departments (EDs), (i) the presence of infectious patients and many susceptible individuals in the same space; (ii) the lack of isolation because of mis-diagnosis or unavailability of adequate areas; (iii) the frequent and close contacts among patients and HCWs often not protected by PPE, all these are factors increasing the risk of spreading of transmissible diseases. For HIDs, which may cause large nosocomial outbreaks, the prompt and effective application of respiratory hygiene/cough etiquette measures, adequate procedures, availability of isolation areas, and well-trained personnel, are the most important measures for reducing the risk of transmission in EDs.

The EuroNHID (European Network for Highly Infectious Diseases) project collected data about the management of patients with proven or suspected HIDs from 48 isolation facilities in 16 European countries. Among these, 40 isolation facilities in 13 countries refer that a ED is operating in the same centre. We present data, collected with standardized checklists, about the management of HIDs patients in these EDs.

Among 40 surveyed EDs, 13 report to have a waiting area large enough to allow the right distancing among patients, while 19 among remaining refer to have preparedness plans to enlarge waiting areas if necessary. Isolation rooms are available in 33 EDs (82.5%): these rooms have an anteroom in 18, a dedicated entrance in 15, are equipped with negative pressure in 17, and with HEPA filtration of exhausting air in 12. In 6 EDs only (15%) isolation rooms have all these characteristics. Triage personnel specifically trained for the recognition of a suspected HIDs is available in 23 EDs; management protocols for these patients, including indications for diagnosis and infection control, are available in 34 EDs. Surveyed EDs are in the same centre as an isolation facility. These EDs are the most likely to manage patients with HIDs in their countries. Despite that, their preparedness level is partially adequate: isolation rooms are available, but these rooms are mostly not appropriate. Protocols are present in most EDs, the half only among HCWs has a specific training for the recognition and management of these patients. Targeted interventions including training are needed.

Does infection control regulation lead to less nosocomial infections? A comparison of federal states in Germany with infection control regulations and without

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Objectives: To investigate whether federal states in Germany with infection control regulations have less nosocomial infections than federal states without such regulations.

In summer 2010, two hygiene scandals in German hospitals were widely reported in the media and resulted in a call for tougher laws. The legislative framework in Germany is as follows: The German Preventing and Combating of Infectious Diseases in Humans Act (IfSG) took effect in all federal states already in 2001. However, 5 out the 16 federal states have additional federal regulations for infection control in hospitals. These federal regulations clarify responsibilities, explain in detail existing law, constitute structural quality parameters (e.g. the number of infection control staff) and set rules and authorities (e.g. to grant inspection of surveillance data). Policy and public opinion considered these federal regulations necessary for the solution of – perceived – increasing infection control and resistance problems in hospitals.

Methods: We analyzed data of 437 intensive care units (ICUs) taking part in the German hospital surveillance system for nosocomial infections (KISS) from 2007 to 2008. Nosocomial infections are reported according to the CDC definitions and in accordance with the National Nosocomial Infection Surveillance system (NNIS)/National Healthcare Safety Network (NHSN) method. Nosocomial infections included blood stream infections, pneumonia, bronchitis and urinary tract infections and multidrug resistant pathogens.

Results: The incidence density of all nosocomial infections did not differ between federal states with or without federal regulations (see table). Neither were less nosocomial infections observed with multidrug
resistant pathogens (methicillin resistant S. aureus, vancomycin resistant enterococci or extended spectrum β-lactamase producing bacteria) in federal states with regulations.

Conclusion: The call for more or tougher laws was a reflex after hygiene scandals in Germany. However, we could not substantiate the simple assumption that more regulations are per se associated with less nosocomial infections or less multidrug resistant pathogens in nosocomial infections.

**P1321** Outbreak investigation because of neonatal listeriosis cases in a Swiss maternity unit

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Objectives: Since 2008, the Federal Office of Public Health in Switzerland has reported no single case of neonatal listeriosis. For this reason, outbreak investigation was immediately started after occurrence of severe invasive infection with detection of L. monocytogenes in two term born neonates within one week in a Swiss maternity. The aim was to identify potential sources of this foodborne pathogen and consecutively being able to prevent further illness.

Methods: Confronted with a foodborne disease outbreak, guidelines for investigation and control as recommended were applied.

Results: Listeria monocytogenes serotype 4 was cultured in the cerebrospinal fluid of case 1 as well as 4 days later in the blood of case 2. Both neonates had pleocytosis and responded to antibiotic treatment with high dose amoxicillin. Further investigation of listeria isolates by pulsed field gel electrophoresis typing in the national listeria reference centre identified both as type 7, a type not detected in the 20 other human isolates that has been tested before. The mother of case 1 showed symptoms of a viral illness 2 weeks before giving birth. Listeria infection was suspected because of a 4-fold rise in specific antibody titters. The mother of case 2 stayed seronegative even 4 weeks post partum. Neither direct nor indirect contacts (separated rooms, different health care workers), nor common used subjects or food exposure could be identified except breast milk of both mothers that has been stored during one night together in one fridge. After implementation of clearly spatial separation in clean-contaminated and mother-nurses areas within the milk kitchen and individually packaged bottles and related accessoirs no further cases of listeriosis infections occurred.

Conclusion: Although transmission by contaminated hands of health care workers is considered the most frequent reason for nosocomial infection, this route appears implausible in this outbreak. In fact, breast milk was identified as the most likely source of nosocomial infection. As a consequence, system changes and structural changes concerning breast milk administration were implemented. As breast milk administration errors are not infrequent events, breast milk should be considered in foodborne outbreak investigations and strict prevention guidelines should be implemented and controlled in all maternities.

**P1322** Epidemiology of Serratia marcescens colonisations and infections in a neonatal intensive care unit

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Objectives: Serratia marcescens is a well-known causative agent of nosocomial infections in newborns with severe morbidity and mortality. The aim of the present work is to elucidate the molecular epidemiology of S. marcescens colonizations/infections among neonates in a 30-bed, university-affiliated, level III-IV Neonatal Intensive Care Unit (NICU) at a tertiary pediatric hospital in Athens. The incentive for this study came from the 4 cases of bacteremia that occurred in the NICU between July and October 2009.

Methods: All cases of S. marcescens colonization and/or infection in the NICU between June 2009 and November 2010 were retrospectively identified from the laboratory archives and the respective medical records. Routine surveillance of the patients’ bacterial flora included cultures of oropharyngeal and rectal swabs upon admission and weekly thereafter. Culture of samples and identification of organisms were made by standard methods. Susceptibility to antibiotics was performed by disk diffusion technique according to the CLSI criteria. Genetic relationships among the isolates were assessed by the Pulsed-Field Gel Electrophoresis (PFGE) after digestion of genomic DNA with XbaI.

Results: During the study period, 607 neonates were admitted in the NICU. Eight neonates (1.31%) born in public or private maternity hospitals in the greater Athens region, parts of southern Greece and many of the islands, were found colonized with S. marcescens upon admission (referred cases), whereas ten neonates (1.64%) were colonized during their NICU stay (NICU-acquired cases). All isolates showed identical susceptibility pattern (wild type). Bacteremia occurred in 4 premature neonates with gestational age <34 wks (27, 29, 32, and 34) and birth weight <2300 g (880, 1000, 1350, and 2300). Of those neonates, the most premature died. Three of the bacteremia cases were characterized as NICU-acquired. Among NICU-acquired cases of colonization 3 occurred in July 2009, displaying identical PFGE type I, 3 in October 2009 (two of them displaying identical PFGE type III and 4 between December 2009 and March 2010 displaying identical PFGE type V1). Genetic similarity among referred cases was not found.

Conclusion: Molecular typing is consistent with possible introduction and limited spread of various PFGE types in the NICU. Aggressive infection-control measures in maternity units and NICUs are necessary to prevent the spread of S. marcescens.

**P1323** Infection control in daily practice: improvement in prevention of catheter-related bloodstream infections with audit tools

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Objective: Catheter Related bloodstream infections (CRBSIs) are considered adverse events but almost all of them are preventable. These infections in hematologic patients can be overwhelming and potentially lethal. Adherence in infection control policies and establishment of safe practices in the daily care of venous catheters is imperative. Recording of determinants such as the date of catheterization, the date of dressing changes, the date of administration sets and stopcocks changes and the use of the appropriate dressing are crucial in order to avoid these serious events.

Methods: We conducted a 6-month prospective cohort study in 3 hematologic wards in a 990-bed tertiary hospital. This study used two interventions to improve the compliance in recording: an educational intervention and audit tools (ATs) in daily care practices. During the first month we implemented a survey with the use of an audit tool to identify the care practices and compare them with the standards of care. The first AT was performed to evaluate the baseline characteristics of daily care practices. After this the educational process was done. The second (3rd month) and the third (6th month) ATs were performed to evaluate the improvement of standards of care after education.

Results: The baseline compliance in recording was very low. It was 3.75% in the first ward, 14% in the second and 20% in the third ward. The compliance in the second audit tool increased and was 7% in the first ward, 17.5% in the second and 85% in the third. The compliance in the third audit tool was 56% in the first ward, 51% in the second and 91% in the third ward.

Conclusion: The audit tool seems to be effective in infection control of CRBSIs. The presence of Infection Control nurses in clinical wards substantially improves compliance. The results of our study were striking and suggest that the continuation of the intervention is mandatory.
Impact of an intervention based on contact precautions in hospital-acquired infections in a neonatal unit

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Objectives: To evaluate the impact of an educative intervention based on contact isolation precautions and colonization detection in a neonatal intensive care unit.

Materials and Methods: After a period of observation of 5 months, an educative intervention about contact isolation precautions based on skin samples to detect bacterial colonization was done in a reference neonatal unit. Proportion of hospital-acquired infections (HAI) during the two periods (before and after the intervention) was evaluated and an unconditional logistic regression model was used to adjust confusing variables.

Results: 450 newborns were included in the study, HAI were diagnosed in 78 patients, 45 (18.2%) in the period before and 33 after the educative intervention (16.2%). HAI were detected in 17 of 25 patients colonized (66.7%). 15 of 44 patients without colonization (34%) and just one case in 134 patients without any skin sample (0.75%; p < 0.01). The logistic regression model showed an increased risk of infection in the group without intervention (O.R. 2.99; CI 95%: 1.39–6.46). Other factors associated with HAI were the use of parenteral nutrition (OR 4.12; CI 95% 1.4–12.3), the presence of congenital cardiopathy (OR 3.1; CI 95% 1.4–6.6) and a longer length of stay in the unit (1.1 per hospital day).

Discussion: An educative intervention about appropriate isolation precautions based on surveillance skin cultures to evaluate bacterial colonization was associated with a lower rate of nosocomial infection in the intervention group in a NICU.

Perioperative antibiotic prophylaxis: results of a second audit in 7 major hospitals in North Italy

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Objectives: Perioperative antibiotic prophylaxis (PAP) is a cornerstone of good surgical care and a key-point in surgical site infections (SSI) prevention. A multicentric protocol providing PAP management evidence-based recommendations was introduced in 2009 in 7 major hospitals in North Italy (986 beds each on average). Data regarding local PAP management before protocol implementation (T0) had been obtained through a cross-sectional study in 2007. The aim of the present study was to evaluate the impact on PAP management after the implementation of the new protocol (T1).

Methods: 2285 clinical charts randomly collected from surgical activities from June to December 2009 were analyzed. Three indicators were evaluated: appropriateness (PAP correctly performed + PAP correctly omitted), adherence to the following criteria a) choice of drug b) dosage c) time of administration (full adherence: 3 criteria fulfilled; partial adherence: at least 1 criterion fulfilled; noncompliance: no one criterion fulfilled) and PAP duration.

Results: PAP was performed in 70% of the procedures. The appropriateness recorded was of 83% (1457 PAP correctly performed + 445 PAP correctly omitted). In 7% of cases PAP was administered without indication. Full and partial adherence were achieved in 49% (785/1610) and 43% (690/1610), respectively. Drug choice was correct in 63%, dosage in 96%, timing in 74%. PAP duration was correct in 55%; remarkably in 64% of PAP with an incorrect duration, supplement doses exceeding 48 hours after the intervention date were given. Full adherence and correct PAP duration were fulfilled simultaneously in 33% (532/1610); adding these cases to the correctly omitted PAP a correct protocol application rate of 43% was calculated. No statistically-significant differences between T1 and T0 data were detected.

Conclusion: Our study found a good appropriateness but a scant adherence rate. Whereas adherence to a single criterion is acceptable, the different criteria are rarely fulfilled concomitantly, likely due to organization and sanitary-staff interplay difficulties. It appears critical the unjustified protracted administration of PAP, potentially leading to antibiotic-resistance selection and costs expansions. T0 and T1 results are comparable, suggesting a recallicitance in changing clinical and organization behaviors; use of incentive patterns and targeted hospital policies could be useful tools to obtain improvement in this type of infection control.

A one-stop shop real-time national decision support system for infectious disease control in England

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Objectives: Prior to 2010, various disparate systems have been in use in England for the control of infectious disease resulting in significant challenges for consistent and timely reporting, surveillance and response. With the rise of emerging diseases, there is an urgent need for:

1. a single national decision support system to strengthen frontline infectious disease control;
2. a seamless integration with the laboratories;
3. a real-time overarching view of all infectious disease control at local, regional and national levels.

Methods: The work began with the development of a dynamic risk assessment model for Infectious disease embedded in a pilot Decision Support System (DSS) conceived via various regional and national Delphi workshops. Challenges encountered included standards, changing notification legislation, clinical governance, articulation of national workflows, collection of national reference data sets and integration with other software suites including the laboratories.

Results: The research has resulted in the design, development and implementation of the following web-based tools:

1. HPZone: a DSS for the management of Enquiries, Cases and Outbreaks at 26 Local Units readily providing consistent data. Basic coincidence and threshold alerts are built in so appropriate information is automatically brought to the attention of those who need to know.
2. HPZone-Cosurv interface: A module which automatically harvests laboratory data from encrypted emails and incorporates them into HPZone with tight rules for data insertion.
3. Dashboard: a module providing aggregate Case and Outbreak data collected and presented in different views including geographic, temporal and disease specific. Consideration has been given to key types of data that are informative to those charged with surveillance and incident management at local, regional and national levels.

Conclusions: The three tools offer immediate benefits in providing key User defined data, enhanced visibility of real-time critical data for prompt response, and a ready opportunity for surveillance and epidemiological research regionally and nationally. The involvement of frontline users has improved tools’ ownership amongst all users and has paved the way for more work on data sharing regionally and nationally in time for the Olympic games in 2012.
### [P1328] Transporting patients with highly infectious diseases: a survey in 14 EU member states

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**Objectives:** Highly Infectious Diseases (HIDs) are defined as transmissible from person to person, causing life threatening illness and presenting a serious hazard to the public. A harmonised response to natural or man-made HIDs plays a vital role in the management of such emergencies. In most European Union (EU) member states (MS), specialised hospitals are responsible for the management of such cases. Ground vehicle transportation capacities for HID patients are often collocated with such facilities but lack a common design. To date, no data on technical specifications and operational procedures of ground vehicles for HID transport are available in the EU.

**Methods:** Until 2010, the European Network for HIDs conducted a cross-sectional analysis of 46 hospitals in 14 MS responsible for the management of HID patients. The availability of ground vehicle capacities and management procedures regarding HID patient transport were assessed. Categories evaluated included (i) legal issues; (ii) technical and infrastructure issues; (iii) management procedures; and (iv) promotion and monitoring of procedures.

**Results:** Only half of all centres evaluated have guidelines for the transportation of HID patients (n=23/46), neither national nor local guidelines are in place in the majority of MS assessed (n=10/14). In contrary, the majority of centres evaluated do have access to ground vehicles (n=24/42 centres; 8 MS). In MS with specific regulations adherence broadly differs: If specifically designed vehicles are recommended, 90% of centres (10/11) do adhere to such regulations; adherence to recommendations without technical specifications is found in only 5/14 centres evaluated. Exclusive pathways for the admission of patients and transport within the facility exist in the majority of centres (31/43 and 29/24, respectively). Protocols for the disinfection of ambulances and equipment exist in 33 and 29 centres, respectively; adherence to correct practice while transporting patients is monitored in 29/42 centres.

**Conclusion:** Within the EU, quantity and technical specification of ground vehicles broadly differs. Although all centres evaluated are responsible for the management of HIDs only 50% provide specific or reserved ground vehicles. As rapid and short-distance relocation are most desirable for HID patients, regulations for domestic and cross-border transportation to the closest HLIU should be harmonised throughout the EU.

### [P1329] Implementing peripheral vascular catheter care bundle in a tertiary care hospital: no room for complacency?

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**Background:** Peripheral vascular catheter (PVC)-related infections are an important cause of device related blood stream infection and are potentially preventable. The 2009 Irish guidelines on the prevention of intravascular catheter-related infection recommend introduction of “care bundles” as an important component of an intravascular catheter related infection prevention programme. The PVC care bundle was introduced in Beaumont Hospital, a 750-bed tertiary referral hospital in Dublin in April 2010. We assessed compliance with bundle components and reviewed its impact on the incidence of PVC-related *Staphylococcus aureus* blood stream infections (SABSI).

**Methods:** Compliance with the PVC care bundle was audited by the Infection Prevention and Control Team in June and November 2010 using the national audit tool. Four components of the PVC care bundle namely, clinical requirement for a PVC, presence of extravasation or inflammation, status of PVC dressings and duration of PVC <72 hours were assessed. In addition, documentation in the daily PVC documentation sheet was assessed. As an outcome measure, the incidence of PVC-related *Staphylococcus aureus* bacteraemia (SAB) was measured.

**Results:** Overall compliance with the PVC care bundle was 66% and 74% in June and November 2010, respectively. Compliance varied with ward speciality – In November, compliance was 74% in the medical wards, 91% in the surgical wards, 74% in the neurosurgical and ENT wards, 100% in the critical care units and 35% in the renal/transplant wards. Since April 2010, there have been 10 cases of PVC-related SABSI, five of which were in surgical patients.

**Conclusion:** Overall compliance with the PVC care bundle has improved since its hospital wide implementation. There has been a slight decrease in the number of PVC-related SABSI since PVC bundle implementation when compared to 2009. However, the number of PVC-related SAB remains high with half in surgical patients despite these wards having the highest hospital ward compliance. We plan to re-audit these wards to investigate the reasons for this further. We believe that it is therefore essential to measure outcome in addition to process measures (such as care bundles) in order to fully truly reduce PVC-related infection.

### [P1330] Multidrug-resistant organisms: concurrent burden and implication on isolation

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**Objective:** In 2010, at our hospital, 45% of *Staphylococcus aureus* were methicillin-resistant (MRSA), 25% *Escherichia coli* (EC) and 39% *Klebsiella pneumoniae* (KP) carried extended-spectrum β-lactamase (ESBL), and 14% *Pseudomonas aeruginosa* (CRPA) and 83% of *Acinetobacter baumannii* (CRAB) were resistant to carbapenems. In planning isolation and contact precautions for multidrug-resistant organisms (MDRO), we wish to know the concurrent prevalence of different MDRO’s in our patients.

**Methods:** We studied the first admission and the first isolate for patients with MDRO’s in 2009 from computerised surveillance of all clinical cultures since 1 January 2006. We studied the concurrent prevalence of other MDRO’s for each MDRO, time to first positive MDRO culture from admission, and prevalence and duration of prior MDRO within our records.

**Results:** In 2009, there were 860 MRSA, 340 CRAB, 455 ESBL EC, 419 ESBL KP and 111 CRPA fulfilling our study criteria.
Development of an epidemiological tool to assess the spread of organisms in rehabilitation centres

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Introduction: MOSAR-WP5 is a study directed at reducing the spread of antibiotic resistant organisms (MDRO) in rehabilitation centers. Since isolation of patients is contradictory to many principals of rehabilitation, we aimed to focus on altering patients activities which are likely to spread organisms. To estimate the potential of various activities to contribute to the spread of MDRO, we developed and validated epidemiological tools.

Methods: In a 3-day multidisciplinary workshop of MOSAR-WP5 participants, we described the risk of spread related to activities as the actual risk per unit of activity multiplied by the frequency of the activity. Patients’ activities taking place during rehabilitation were analyzed, and hypothetical risk of transmission defined. By consensus, universal parameters related to the activities believed to be important to the risk of spread were agreed upon and graded. Validation of the parameters was performed using experimental design of contamination. These parameters were included in the “risk observation score” (ROS) measuring the potential risk with a unit of activity. This tool was validated using environmental sampling with tryptic soy agar and measuring the potential risk with a unit of activity. This tool was found to be reproducible.

Results: The “risk assessment tool” included 5 parameters, 3 of which were believed to determine potential spread: body area in contact, duration of contact, and whether body secretions were in contact; and 2 parameters which reduce the potential of spread: protective measures (such as gowns or gloves) and cleaning.

Preliminary microbiological validation supported the ROS scoring system. The risk assessment score, was used in 5 rehabilitation centers. In each center at least 30 types of activities (or objects) were examined, and scored for 30 encounters. Frequency (load) of each activity or object were samples for a total of 5 hours. The combination of the ROS and the frequency of use allowed each center to define the “risk assessment score”, and identify high risk activities.

Conclusions: The tools developed and methodology used can help direct infection control measures to combat the spread of MDRO.

Outbreak of Burkholderia cenocepacia bacteremia in an intensive care unit: an epidemiologic and molecular study

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Objectives: Burkholderia cenocepacia complex is a widespread Gram-negative environmental bacillus associated with nosocomial infection. We present epidemiology, diagnosis and surveillance of a 10-month outbreak of nosocomial Burkholderia cenocepacia bacteremia involving twenty-one critically ill patients in a 9-bed multidisciplinary Intensive Care Unit (ICU).

Methods: Medical records of patients infected were reviewed and the following data were collected: Age, sex, type of admission (medical or surgical) and APACHE II score, day of bacteremia identification following ICU admission, result of antimicrobial sensitivity tests, response to therapy, length of ICU stay (LOS) and outcome. PCR amplification of B. cepacia complex recA gene and sequence were used for molecular identification of Burkholderia sp. isolates. Molecular typing of Burkholderia cenocepacia isolates was performed by Pulsed Field Gel Electrophoresis (PFGE).

Results: Thirty episodes of B. cenocepacia bacteremia (3 catheter-related infections) were diagnosed in 21 patients (15 males), mean age 66±15 years. APACHE II score on admission was 17.8±8.7, 8 cases being medical and 13 surgical admissions. Median time for a positive blood culture was 9 days after admission. The pathogen grew in blood cultures after 3.3±1 days of incubation and was susceptible in meropenem, piperacillin/tazobactam, ciprofloxacin and trimethoprim/sulphamethoxazole. Surveillance involved environmental and patients-personnel cultures: chlorhexidine and povidone-iodine disinfectants, heparin solutions, ventilator tubing condensate, water taps, patients’ skin, personnel hands, blood gas analysers, intravenous lines, saline and dextrose solutions and bronchial secretions suction equipment. All samples were negative for B. cepacia. PFGE molecular typing showed that all isolates were undistinguishable. Complete elimination of the outbreak was achieved only after ICU’s disinfection. All patients responded to the antimicrobial therapy and had median LOS of 24 days. Among our study population, 6 patients died in septic shock and multorgan failure due to other multiresistant Gram negative pathogens.

Conclusions: Although B. cenocepacia outbreaks are quite frequent, true bacteremias are rare and in our cohort did not affect ICU mortality. Environmental and epidemiological investigations are necessary to identify the source of infection. Infection control measures are essential for hospital outbreaks eradication.

Infection control and MRSA in nursing homes – the perspective of nursing home staff

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Objectives: In 2008 a cluster randomised controlled trial (cRCT) involving an infection control education and training intervention in nursing homes in Northern Ireland found no significant difference in the prevalence of Mecillin resistant Staphylococcus aureus (MRSA) in homes which received the intervention compared to homes where usual practice continued. Furthermore, at the end of the study only 37.5% of intervention homes were deemed compliant when audited against infection control standards. The purpose of this qualitative study was to explore the reasons for these results and to determine the feasibility of carrying out a MRSA decolonisation programme in the nursing home environment.

Methods: Following ethical approval, staff who were trained in the intervention nursing homes during the cRCT were invited to participate. Six one-to-one interviews with nursing home managers and 6 focus groups each consisting of 4–7 participants i.e. nurses, care assistants and infection control link workers were conducted between November 2009 and February 2010. Transcripts were reviewed and coded into major themes using framework analysis.
Results: Participants identified that some audit standards could not be met due to financial reasons. Gloves, aprons and hand-gels were readily available; however, replacing worn furniture and equipment was more difficult. It was reported that care assistants were poorly paid, which may explain the high levels of staff turnover and the difficulties in ensuring that all staff are adequately trained in infection control. The majority of participants stated that they would like nursing homes to be MRSA free and favoured the concept of decolonisation. However, there were concerns about carrying out the decolonisation process in frail, ill and confused residents, about the risk of recolonisation particularly with frequent hospital transfers, and about the extra workload associated with decolonisation. It was reported that doctors were not in favour of prescribing for the decolonisation of MRSA in nursing home residents. Conclusions: Owners of nursing homes need to review their budgets to ensure that there are adequate resources to enable infection control standards to be met. The feasibility of carrying out widespread MRSA decolonisation in nursing homes may be questionable. Therefore, the focus must continue to be on good infection control practices.

Development of a preparedness plan to control the spread of carbapenemase-producing Gram-negatives in a tertiary hospital serving a major international airport

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Objectives: To develop a preparedness plan to prevent the spread of carbapenem producing Gram negatives (CPGNs) focusing on detection, screening and containment in the setting of a tertiary hospital providing specialist care for areas such as Burns, Transplant, Cystic Fibrosis and serving a major international airport.

Methods: Phase 1: Detection. An algorithm for detection of CPGNs was prepared involving automated sensitivity testing, MIC confirmation, a Modified Hodge Test and reference laboratory referral for confirmation by PCR on any suspicious isolate. Data on all CPGNs was collected prospectively over a 10 month period, January-October 2010. Phase 2: Screening. A risk assessment and screening policy was incorporated into the current A+E Management of Sepsis protocol. “At risk” was defined as any patient hospitalized abroad, or in a UK hospital with a known outbreak of CPGN, in the last 6 months. Where a CPGN was isolated from a ward patient, screening of all patients within the shared bay was carried out. Phase 3: Infection Control. An algorithm for isolation and management of high risk or confirmed cases was rolled out via Infection Prevention. Early liaison with microbiology was encouraged. A patient information leaflet was designed for any patient undergoing screening. Educational presentations were given to laboratory, infection control, ICU and A+E staff.

Results: 13 CPGN isolates have been detected with a variety of resistance mechanisms. All high risk patients are screened in A+E and isolated until confirmation of a negative result. Priority is given over MRSA or C. difficile positive patients. High risk or confirmed patients receive a ward visit by the Infection Prevention Team. Strict Infection Control precautions are initiated including side room isolation with en-suite facilities until discharge. Single use items are recommended wherever possible; sphygmanomanometer, stethoscope, floor cloths, medicine pots, host slings and slide sheets. On discharge a terminal clean with 1,000ppm Chlor-clean is carried out and environmental screening swabs collected.

Conclusion: The Preparedness Plan has raised awareness of the carbapenem resistance threat. The laboratory has changed practices to increase confidence in detection, including isolates with borderline MICs. Screening has enabled early patient isolation. Infection Control measures have been strengthened. To date only one confirmed case has led to cross contamination within the hospital.

From IPSE to TRICE: evolution in Europe of the state of the art of infection control

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Introduction: Training Infection Control in Europe (TRICE) was an ECDC funded project aimed to update the information regarding Infection Control/Hospital Hygiene (IC/HH) training in European countries. This paper presents key results related to the evolution in Europe of infection control resources.

Methods: The project, built on the approach and developed a questionnaire based on Work Package 1 (2006) EU funded project “Improving Patient Safety in Europe” (IPSE). IPSE questions considered still relevant were accepted while new areas of interest were added. National representatives for the project (NR) were identified by ECDC’s national competent bodies for training on ECDC’s request and contacted to participate in the project between April and August 2010. Participants in the 2006 IPSE survey were asked to compare situations between 2006 and 2010. The response rate was 100% amongst the 33 nominated NCPICTs.

Results: Most Countries (81.8% (27/33)) reported the presence of a definition of the Infection Control Team within national programmes or regulations. Six more countries reported it compared to 2006. 87.9% (29/33) of the respondents had recommendations for managing IC/HH at a national level. The professionals in charge of IC/HH were mostly associated (78.8%; [26/33]) with microbiological or infectious diseases departments followed by global or medical management and management of quality and safety (each 42.4% [14/33]). The usual background for IC/HH Nurses was a “graduated/certified nurse” (84.4%) followed by Intensive Care Unit and operating theatre (each 18.2% [6/33]). Definitions of a professional profile had increased between 2006 and 2010 (IC/HHDoctors from 58.1% (18/31) to 84.8% (28/33), IC/HHNurses from 71.0% (22/31) to 81.8% (27/33)). The presence at national or professional levels of a national curriculum or programme for IC/HH training showed a significant increase between 2006 and 2010: doctors from 32.6% (10/31) to 57.6% (19/33) and for nurses from 54.8% (17/31) to 63.6% (21/33) respectively.

Conclusions: These data show that Europe is moving towards a higher priority for HAI prevention and control and it is apparent that it is feasible to define and attain new targets in the application of EU Council recommendations provided that all stakeholders (governmental institutions, universities, health care providers, managers, professionals, patients and their advocates) are engaged and support and sustain new initiatives specifically for professional training.

Respiratory isolation in HIV patients with suspected pulmonary tuberculosis: comparison of clinical predictive models

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Objectives: The latest estimates of tuberculosis global burden show that there are 9.27 million new cases of TB in 2007 including 1.37 million cases among HIV positive people. Current recommendations to control TB spread call for immediate isolation of any patient suspected to have infectious TB. We tested 4 predictive models currently available for early identification of patients at risk for pulmonary TB (Wisnivesky, Rakoczy, Tattevin and Reid model) in a HIV population in order to identify the best performing score.

Methods: 119 HIV infected patients admitted to an urban hospital in Milan from January 2008 to September 2010 with suspicion of pulmonary TB were retrospectively studied. Cases were those in whom
Evaluation of control measurement procedures in the prevention of carbapenemase-2 (KPC-2)-producing Klebsiella pneumoniae nosocomial transmission

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Objective: Antibiotic-resistant pathogens constitute an important and growing threat to public health. The aim of the present study was to evaluate the effectiveness of the implementation of control measures in the prevention of carbapenemase-2 (KPC-2)-producing Klebsiella pneumoniae transmission in a university hospital in Italy.

Methods: All isolated of Klebsiella species with reduced susceptibility to carbapenems and positives by boronic acid and Hodge test were prospectively collected from December 2009 through December 2010. One isolate per patient was submitted for further study. MICs of meropenem, colistin, gentamicin, tigecycline and cotrimoxazole were evaluated by E-test and were classified according to Clinical and Laboratory Standards Institute (CLSI) breakpoint. The isolates were evaluated using rep-PCR (Diversilab bioMérieux) for clonal diffusion. Contact-isolation precautions for patients colonized or infected with KPC-2-producing K. pneumoniae were implemented. All patients with KPC-2-producing K. pneumoniae isolates, if available, were transferred to Infectious Diseases ward. Other measures were as follow: improvements in hand hygiene, an educational program for all hospital department by a protocol, introduction of screening tests of active souveilance.

Results: From December 2009 through December 2010, 59 patients were infected and 2 colonized by KPC-2-producing K. pneumoniae. Rep-PCR analysis showed a cluster of 57 strains. A single case patient, infected at a long-term facility, introduced the strain into our hospital, in Geriatric ward, in December. 15 patients were hospitalized in the ICU when the KPC-producing isolate was detected, 35 in medical ward and 9 in surgical ward.

Before the introduction of control program 9–11 patients/month were infected by KPC-2-producing K. pneumoniae. After the application of the control program a reduction of KPC-2-producing isolates was observed: in October 10 patients, in November 3 patients, in December 1 patient.

Conclusion: This is one of the largest outbreak of Carbapenemase-2 (KPC-2)-producing K. pneumoniae reported in Europe. The application of strict combined control measures was effective in the reduction of cross-transmission and should be immediately adopted in order to control the spread of KPC-2-producing K. pneumoniae.

Evaluation of control measurement procedures in the prevention of carbapenemase-2 (KPC-2)-producing Klebsiella pneumoniae nosocomial transmission

| WEBBER 2009 | RAKOCZY 2009 | TATTEVIN 1999 | REDD 1997 |
|-------------|-------------|--------------|----------|
| Sensitivity (Current Study) | 0.69 | 0.94 | 0.60 | 0.50 |
| Specificity (Current Study) | 0.29 | 0.65 | 0.71 | 0.36 |
| Sensitivity (Previous Studies) | 0.94 | 0.97 | 1.00 | 0.96 |
| Specificity (Previous Studies) | 0.30 | 0.62 | 0.49 | 0.54 |
| PPV (Current Study) | 0.16 | 0.33 | 0.26 | 0.12 |
| NPV (Current Study) | 0.05 | 0.95 | 0.97 | 0.60 |
| Diagnostic accuracy (Current Study) | 0.38 | 0.69 | 0.78 | 0.38 |
| Diagnostic accuracy (Previous Study) | 0.07 | 2.69 | 3.07 | 0.78 |
| Specifity (Current Study) | 0.06 | 0.69 | 0.15 | 1.36 |
| Sensitivity (Current Study) | 0.29 | 69.89 | 28.47 | 0.56 |

Bonferroni corrected p-value < 0.01) counts. However, these samples were collected 3 days before the cut-off. The incidence of nosocomial MRSA and Acinetobacter baumannii on a burns unit.

Methods: Our 10-bed burns was closed for maintenance in July and August 2008 and we took the opportunity to decontaminate the entire unit using a HPV decontamination service (Bioquell) and implement a bundle of infection control interventions when the unit was reopened in September 2008, including: regular HPV decontamination of the rooms following discharge of patients colonized/infected by multidrug-resistant bacteria, pre-emptive cohort isolation of newly admitted patients before proven culture negative, cohorting of colonised patients, installation of two air disinfection systems in the corridors of the unit (Plasmair) and improvement of material storage. Surfaces were sampled for bacteria and fungi and air samples were collected for fungi to monitor the effectiveness of the HPV. The incidence of nosocomial MRSA and A. baumannii was compared from January 2007-June 2008 (pre-closure) with September 2008-December 2009 (post-closure). Statistical comparisons were performed using Fisher’s Exact Test for categorical variables and the Mann-Whitney U test for continuous variables.

Results: HPV decontamination of the entire unit reduced significantly bacterial surface counts (from mean 4.0 to 0.7 cfu/100 cm², p < 0.02) and fungal surface (from 3.5 to 0 cfu/100 cm²) and air (from 3.7 to 0.5 cfu/m³ air, p < 0.01) counts. However, these samples were collected 3 days before the cut-off.
days after HPV decontamination was performed so we cannot rule out the possibility of post-process contamination. Regular HPV decontamination of patients’ rooms also reduced significantly bacterial surface counts (from mean 2.9 to 0.1 cfu/100cm²) and fungal surface (from 1.0 to 0 cfu/100cm²) and air (from 5.5 to 0 cfu/m³, air = 0.01) counts. The incidence of nosocomial MRSA fell by 90% from 7.6 to 0.8 cases/1000 patient days (p<0.001) and the incidence of nosocomial A. baumannii fell by 89% from 7.3 to 0.8 cases per 1000 patient days (p<0.001) (figure).

**Conclusion:** A HPV decontamination service is effective for the removal of environmental contamination on a unit or room scale. A bundle of infection control interventions resulted in a significant reduction in the incidence of nosocomial MRSA and A. baumannii. However, we were not able to determine the relative contribution of each of the interventions to the reduction but it is likely that all were important.

**Effect of multimodal interventions and healthcare-worker influenza vaccination on reducing all-cause nosocomial pneumonia in haematopoietic stem cell transplant recipients**

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Nosocomial respiratory virus infections (RVI) cause significant clinical disease and poor outcomes in haematopoietic stem cell transplant (HSCT) recipients, particularly if acquired during the transplant admission.

**Objectives:** To improve compliance with recommended methods to prevent nosocomial RVI in HSCT recipients and evaluate the effect on all-cause nosocomial pneumonia.

**Methods:** Multimodal interventions (influenza vaccination, hand washing, ward redevelopment, entry restriction, screening patients for RVI, staff, patient and family education) were instituted in yearly increments from January 2004. Time to first episode of nosocomial pneumonia was recorded for each HSCT patient admitted to the haematology ward, Westmead Hospital, Australia, January 2003 to September 2007.

**Results:** Vaccination of haematology staff against influenza increased from 59 to 67% during 2004–2007. Compliance was five-fold higher in haematology than intensive care staff (OR 5.3, 95% CI 3.1–9.0, P<0.0001).

Nosocomial pneumonia was diagnosed in 182/810 (22.5%) admissions. Patients admitted for HSCT were most at risk (48.9% and 4.6% in those with prior HSCT, OR 3.42, 95% CI 2.96–3.96, P<0.0001). While more episodes occurred in admissions for allogeneic than autologous HSCT (25.5% and 16.8% respectively, OR 1.69, 95% CI 1.15–2.49, P=0.007), this difference disappeared after adjustment for length of stay (P=0.8). RVI(s) were identified in 13.4% of all nosocomial pneumonia episodes. After adjustment for other variables, nosocomial pneumonia was associated with admission for HSCT (HR 4.23, 95% CI 2.72–6.58), female sex (HR 1.49, 95% CI 1.10–2.01), and age >46 years (HR 1.59, 95% CI 1.17–2.16). In patients receiving an allogeneic HSCT, year of admission, use of stem cells from an unrelated donor (HR 2.03, 95% CI 1.37–3.01, P<0.0001) and age >46 years (HR 2.15, 95% CI 1.46–3.16, P<0.0001) were associated with nosocomial pneumonia on multivariable analysis. The adjusted odds of developing nosocomial pneumonia in patients admitted in 2005, 2006 and 2007 were 49–55% less than in those admitted in 2003 (P<0.03).

**Conclusion:** Over five years, the incidence of nosocomial pneumonia in patients receiving allogeneic HSCT at a single centre was halved by the introduction of multimodal infection control measures. A multicentre study with randomisation of control and intervention sites is required to confirm these results.

**Epidemiology of antimicrobial resistance**

**Emergence of novel blaKPC-12 and blaKPC-13 variants in carbapenem non-susceptible Enterobacter cloacae: a first report of blakPC gene in Thailand**

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**Objectives:** To survey for carbapenem susceptibility and carbapenem-resistant determinants among Enterobacteriaceae clinical isolates at a university hospital in Thailand, and characterise blakPC-carrying isolates reported for the first time in this country.

**Methods:** All Enterobacteriaceae clinical isolates collected at Siriraj Hospital, a 2300-bed tertiary care in Bangkok (Thailand), during 2009–2010 were included. Isolates non-susceptible to a carbapenem (ertapenem, imipenem, meropenem or doripenem) as confirmed by MIC determination using E-test® method were tested for carbapenemase production by modified Hodge test (MHT) and were subject to genotypic characterisation (PCR sequencing) of various bla genes including bla(CTX-M, TEM, SHV, VEB, OXA, AmpC, KPC, IMP, VIM and NDM). Isolates harbouring blakPC were investigated for their cloinality by pulsed-field gel electrophoresis (PFGE).

**Results:** A total of 172 out of 12,741 Enterobacteriaceae isolates (1.3%) were non-susceptible to at least a carbapenem. Only 22 isolates (12.8%) yielded a positive MHT and 45 isolates (26.2%) were resistant to all four testing carbapenems. Two multidrug-resistant isolates of Enterobacter cloacae, both from urine specimens, demonstrated positive PCR for blakPC, but were negative for MHT. The first isolate also carried bla(TEM-1 and CTX-M-15), and the latter isolate also carried bla(TEM-1, CTX-M-3, VEB-1 and OXA-10). MICs of ertapenem, imipenem, meropenem and doripenem, respectively, for the first isolate were 6, 1.5, 0.5 and 0.8, and for the latter isolate were >32, 3, 4 and 4 mg/L. blakPC sequences of the first isolate was most closely related to blakPC-3 (99.8%) with A272G and C513T mutations, and was named blakPC-12. blakPC sequence of the latter isolate, namely blakPC-13, was most closely related to blakPC-12 (99.7%) with T297C and C487T mutations, and A844G mutations, and carried at least six nucleotide differences from other previously identified blakPC genes. Both isolates were not clonally related based on PFGE result.

**Conclusions:** This study identified the first and novel blakPC genes in Thailand. blakPC-12 was less resistant and more related to known blakPC genes compared to blakPC-13. Monitoring of the emergence and spread of carbapenem non-susceptible Enterobacteriaceae is critical for infection control. Further characterisation of both novel blakPC genes and other carbapenem non-susceptible isolates are warranted for better understanding of their resistance mechanisms.

**Plasmid-carried blakPC-2 genes in Enterobacteriaceae in Brazil**

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**Objectives:** Globally spread KPC carbapenemases have been sporadically detected in Brazil and other South American countries. We analyzed 52 carbapenem resistant K. pneumoniae (Kp) (clinical and fecal isolates) from an university hospital in Brazil (Hospital das Clinicas of Medical School – University of Sao Paulo) during 2007 to 2009.

**Methods:** Clonal relatedness was established by PFGE and MLST. Plasmid characterization (line group, RFLP analysis) and identification of the blakPC genetic environment (Tn4001-PCR mapping) was assessed. Results were compared with previously available information about KPC in Brazil.

**Results:** Kp isolates were grouped in 8 PFGE types and 3 sequence types: KpA-ST258 (n=50; 6 subtypes), KpA6-ST11 (n=1) and KpE-ST48 (n=1). All isolates were resistant to fluoroquinolones, susceptible to polymixin B and tigecycline and variably resistant to amikacin,
KPC-producing Klebsiella pneumoniae from Rome, Italy
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Nosocomial infections sustained by carbapenem-resistant Klebsiella pneumoniae (KP) are a global health problem. Active surveillance has been maintained during the last three years at the Policlinico Umberto I Hospital, demonstrating that the increasing carbapenem resistance in KP was mostly associated to the production of Extended-Spectrum β-lactamases (ESBLs) in specific successful clones, showing the alterations of the major OmpK35, OmpK36 nonspecific porins. However, the KP clone ST258 producing KPC was recently introduced in this hospital and the first strain was fully characterized.

Material and Methods: Identification and antimicrobial susceptibility were performed by Vitek 2 with the AST-N089 cards and disk tests with meropenem and boronic acid and/or EDTA. Strains were analysed by PFGE, MLST, OmpK-35/36 and ESBL bla gene PCR and sequencing. Plasmid analysis was done by PBRT and whole plasmid sequencing by the 454GS-FLX (Roche).

Results: The first KPC-3 positive strain was isolated on June 2010 from an abdominal drainage, belonged to the epidemic clone ST258 and was resistant to all β-lactam-β-lactamase inhibitor combinations, trimethoprim/sulfamethoxazole, fluoroquinolones, tobramycin and amikacin.

Porin analysis demonstrated porin alteration because of the loss of OmpK35 by a termination of translation, and a novel OmpK36 variant showing two additional amino acids, located within the internal loop 3, one of the highly conserved domains of the protein.

The KPC-3 plasmid was fully sequenced revealing high homology with plasmid pKpQIL identified in Israel (NC_014016), including the transfer locus, the replicon (classified as FlpF), the variable region conferring multi-drug resistance and the Tn4401 transposon, carrying the blaKPC-3. This plasmid also showed a second repA3 and a partitioning system of probable phage origin.

Conclusions: The high and fast spread of blakPC-3 gene could be explained by the clonal expansion of the ST258 clone, but also by its location on the FlKl plasmid. The FlKl family seems very common in KP of different origin (Villa et al. 2010) but these plasmids were undetected and ignored since negative for resistance gene markers. As result of genomic and plasmid high-throughput DNA sequencing, Flk plasmids appear as potential virulence plasmids.

In the epidemic clone ST258, a plasmid of this family acquired the Tn4401 transposon carrying KPC-3 and the clone and the plasmid successfully spread worldwide.

[1344] Dissemination of DHA-1 and CMY-2 β-lactamases among Klebsiella spp. and E. coli from Portuguese clinical settings

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Objectives: Acquired AmpC β-lactamases (qAmpC) have increasingly been recognized as an emerging problem worldwide. We investigate the occurrence and diversity of genes coding for qAmpC enzymes among Enterobacteriaceae from Portuguese clinical settings over a 6 year period (2002–08).

Methods: We analysed 100 presumptive qAmpC producers (34 E. coli, 52 K. pneumoniae, 13 K. oxytoca, and 1 P. mirabilis) recovered from 3 Portuguese hospitals and 2 ambulatory laboratories (North and Centre, 2002–08). Antimicrobial susceptibility testing was performed by standard methods (CLSI). Production of qAmpC and ESBL enzymes was investigated by the PBA (phenylboronic acid) and the DDST tests, respectively. Identification of genes coding for qAmpC (blaCMY/FOX/LACT/MIR/DHA/MOR/ACC) or ESBL (blaTEM/SHV/CTX-M) was performed by PCR and sequencing.

Clonal relatedness was established by XbaI-PFGE. E. coli phylogenetic groups and clonal complexes were identified by PCR and MLST, respectively. Transferability of blaAmpC was tested by conjugation.

Results: qAmpC expression was observed in 59% (59/100) of the isolates and the enzymes were identified as DHA-1 and CMY-2. DHA-1 was the most common variant (80%; 47/59), being found in K. pneumoniae (n=41; 12 PFGE-types), K. oxytoca (n=4; 2 PFGE-types) and E. coli (n=2; 2 PFGE-types; B1-ST1196 and B2-fumC24), throughout the studied period. CMY-2 was only detected among E. coli (5%, 3/59; 3 B1 PFGE-types; 1 ST101 complex and 1 B1-fumC65) from one hospital. An epidemic K. pneumoniae clone was identified in different hospitals. DHA-1 and CMY-2 producers were mainly recovered from urine (n=19, 38%) or respiratory samples (n=13, 26%). ESBL co-production (SHV-5, -12, -64, -90, CTX-M-32) was observed in 85% (40/47; 15 PFGE-types; all species) of DHA-1-producing isolates.

Co-resistance to non-β-lactams was frequently observed, mainly to kanamycin (98%), tobramycin (96%), sulfonamides (96%), streptomycin (84%), ciprofloxacin (82%), and trimethoprim (80%). blaAmpC were not transferred by conjugation.

Conclusions: Our study demonstrates the spread of qAmpC (DHA-1, CMY-2) enzymes in Portuguese clinical settings at least since 2003 within different Enterobacteriaceae species and clones which also produced ESBL. A shift in ESBL-epidemiology in Portugal might be anticipated by the dissemination of qAmpC enzymes among particular ESBL-producing species.

[1344] Emergence of VIM-1 carbapenemase-producing Enterobacter cloacae isolates in the Tyrol, Austria
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Objectives: The rapid emergence and dissemination of carbapenem producing Enterobacter sp and other Enterobacteriaceae poses a considerable threat to clinical patient care and public health. In this study, Enterobacter isolates demonstrating decreased susceptibility to carbapenems detected at the Division of Hygiene and Medical Microbiology, Innsbruck Medical University, were evaluated for the production of metallo-β-lactamases, serine-β-lactamases and OXA carbapenemases using polymerase chain reaction (PCR) tests. Additionally, the isolates were epidemiologically typed to determine the genetic relatedness.

Methods: From January 2006 to December 2010, isolates of Enterobacter sp. with reduced susceptibility to carbapenems were collected from the University hospital Innsbruck, district hospitals and general practitioners in the Tyrol. All isolates were tested for bla(VIM-1), bla(NDM-1), bla(IMP), bla(KPC) and bla(OXA-48, 23, 24, 58) using a multiplex PCR with published primers. Two typing methods – pulsed field gel electrophoresis (PFGE) and repetitive-sequence-based PCR (rep-PCR) – were performed to analyse the clonality of the isolates.
Results and Conclusion: In total, 34 isolates (29 Enterobacter cloacae and 5 Enterobacter aerogenes) were collected during the study period. From 2006 to 2009, between 2 and 7 isolates were found per year. In 2010, a significant increase of carbapenem-resistant strains was observed (n = 12). In 25 isolates of Enterobacter cloacae, the blaVIM-1 gene was detected. Typing of Enterobacter cloacae by PFGE and rep-PCR revealed five patterns each. Seventeen isolates showed an identical genotype with both methods. The five Enterobacter aerogenes isolates were clearly separated from Enterobacter cloacae and yielded different patterns each. These findings demonstrate the emergence of VIM-1-producing Enterobacter cloacae in our geographical area (the Tyrol, Western Austria). The clonal relationship confirms the risk of spread of these organisms and their possible persistence over the time.

Objective: Enterobacter cloacae can be harmless commensals or causative agents of diarrhoea or extraintestinal infections among animals and humans. The recent rapid increase in the occurrence of extended-spectrum β-lactamases (ESBL)-producing E. coli that are often resistant to multiple antimicrobial agents in both animals and humans poses a serious therapeutic challenge. In order to understand the diversity and distribution of ESBL-producing E. coli among different host species and different countries and to gain insight into their transmission, we have screened ESBL-producing E. coli isolates for their virulence (VIR) and antimicrobial resistance (AMR) genes.

Method: Approximately 800 E. coli from farm animals (cattle, broilers, turkeys), animal food products and humans and from different countries (UK, Netherlands and Germany) shown to produce ESBLs were analysed for their carriage of 120 VIR and 153 AMR genes using miniaturised microarrays (IdentiBac). Data were analysed by clustering algorithms, k-mean partition, and multivariate analysis of variance (MNOVA) whilst diversities were assessed by Simpson’s diversity index (D).

Results: The majority of isolates were similar to those from the same host species and different from those from other host species in terms of their carriage of the VIR and AMR genes (p < 0.001), but other isolates from different countries and host species were indistinguishable (Fig. 1). Human isolates from the Netherlands (122 isolates, D= 0.83 and 0.72) and Germany (14 isolates, D= 0.83 and 0.68) were considerably more diverse than those from the UK (196 isolates, D= 0.62 and 0.46) based on the clustering analysis and k-mean partitioning results respectively.

Conclusion: Although some strain types appeared to be mainly associated with certain host species or country, others were found in multiple host species or countries suggesting cross-species and cross-country transmissions. The different levels of diversity observed among human isolates from different countries indicate that the human population are receptive to different types of ESBL-producing E. coli strains. This demonstrates an ongoing requirement to monitor and control both person-to-person as well as cross-species transmission of ESBL-producing E. coli. We thanks FSA for financial support and contributions from Federal Institute for Risk Assessment and Institute of Farm Animal Genetics, FLI, Germany, Health Protection Agency UK and the Reference Laboratory on Antimicrobial Resistance in Animals, Netherlands.

Objective: A genetically distinct strain of E. coli sequence type (ST) 131 harbouring CTX-M-15, as well as producing other extended-spectrum β-lactamases (ESBL) and plasmid-encoded AmpC β-lactamases (p-AmpC), has emerged worldwide. In our area third-generation cephalosporins-susceptible isolates belonging to this clone have been also detected. The annual trend of these bacteria is not known and longitudinal observations are lacking. The aim of this study was to assess the prevalence of ST131 isolates in a prospective systematic collection of E. coli isolates recovered from 2005 to 2010.

Methods: Between 2005 and 2009, the first 25 consecutive E. coli isolates were collected each year during January-February period and in 2010 all isolates during the same period. One isolate per patient was selected. The clone was screened by PCR with specific primers for O25b serogroup, allele 3 of the pabB gene and a multiplex PCR assay for B2 phylogroup. The genetic relatedness among E. coli isolates was determined by XbaI pulsed-field gel electrophoresis (PFGE). Susceptibility pattern was obtained with MicroScan panel. Screening of ESBL was carried out by using double-disc method on Mueller Hinton agar and p-AmpC on 200 mg/l cloxacillin Mueller Hinton agar. ESBL-type was determined by PCR assay using specific primer sets for ESBL groups.

Results: A total of 506 E. coli isolates were analyzed during the study period (125 from 2005–09 and 381 from 2010) and 157 O25b/pabB3/B2 positive (13.2%) cases were found. The annual trend of ST131 E. coli isolates was 3.7% in 2005, 14.8% in 2006, 8.3% in 2007, 12% in 2008, 11.5% in 2009 and 14.5% in 2010. Three (5.5%) O25b/pabB3/B2 positive isolates were CTX-M-1 producers and were recovered in 2010 and the rest were non-ESBL-p-AmpC producers. O25b/pabB3/B2 positive isolates showed 75% similarity by PFGE. Antibiotic resistance analysis revealed that ST131 isolates were significantly more resistant to gentamicin, tobramycin, and quinolones.

Conclusion: Our findings suggest that O25b/ST131 emerged in our area before 2005 and a steady increase was observed, becoming a prevalent clone in the last three years. Isolates harbouring CTX-M-1 group enzymes were detected only in 2010.

Objective: The main objective was to detect and characterize ND1-positive Enterobacteriaceae in Bangladesh.

Methods: A total of 1789 Escherichia coli and 90 Shigella from diarrhoeal patients and healthy controls in 2009–2010 and 1816 clinical samples isolated in October 2010 were analysed for imipenem resistance. Gram-negative strains were isolated from 403/1816 samples. 100 ESBL-producing E. coli isolated in 2008–2009 were also analysed. Imipenem susceptibility was determined and the presence of ND1 gene was
assessed by PCR. NDM-1 positive isolates were characterized. Plasmid profiles were analyzed and presence of transmissible R-plasmid was determined by conjugation and hybridization. All NDM-1 positive patients were contacted and interviewed using a short standard questionnaire.

Results: All E. coli and Shigella isolates from diarrheal patients and healthy controls were susceptible to imipenem. Among 403 Gram-negative clinical isolates, 55/403 (13.6%) were resistant to imipenem and 14/403 (3.5%) were NDM-1 positive. These 14 isolates were cultured from 12 patients with the following clinical diagnoses: UTI (n = 5), RTI (n = 4), wound infections (n = 1), unknown (n = 2). Of these 12 patients, 10 could be contacted. All 10 patients had received antibiotics during the last 3 months, 5 were treated with carbapenem.

All patients reported hospitalization in last 3 months. The mean age of patients was 55 years and the male to female ratio was 1:2 (N=12). Of the 10 patients, outcome of treatment was not clear for 3, condition improved for 3 and 2 patients died. Among 100 (N=12). Of the 10 patients, outcome of treatment was not clear for 3, condition improved for 3 and 2 patients died.

All patients reported hospitalization in last 3 months. The mean age of patients was 55 years and the male to female ratio was 1:2 (N=12). Of the 10 patients, outcome of treatment was not clear for 3, condition improved for 3 and 2 patients died. Therefore, epidemiological data regarding this topic in Switzerland are lacking.

Methods: Cloacal swabs from poultry were incubated at 37°C in MacConkey broth with 5 mg/L of ceftazidime overnight. Then, 50 μL were plated on selective chromID ESBL (bioMérieux) and re-incubated. Resistance colonies were identified at species level using MALDI-TOF mass spectrometry and antibiotic susceptibility profiles were determined using Vitek 2. PCR and DNA sequencing were performed to characterize the main blaESBL and blamppm genes. Rep-PCR was used to study the clonality of Escherichia coli strains. Sequence typing analysis was carried out using the RFLP method. Antimicrobial susceptibility was determined by disk diffusion and MICs were determined by Etest. The b-lactamase content of non-susceptible strains was examined by isoelectric focusing and the blagenes were identified by PCR and sequencing. Thirty-two representative, non-susceptible strains were genotyped by PFGE after digestion of genomic DNA with XbaI.

Results: One hundred fifty-eight strains (62%) exhibited resistance or decreased susceptibility to 3rd generation cephalosporins. Carbapenemase production was confirmed in 83.5% of them and in particular, 103 strains produced KPC, 22 VIM, and seven strains both carbapenemase types. All blaKPC amplicons were identical to blaKPC-2. Twenty-three of the blaVIM amplicons were identical to blaVIM-1 and the remaining six to blaVIM-19. Imipenem and meropenem MICs for the above strains ranged from 4–32 μg/mL and 2–32 μg/mL, respectively. The remaining strains (16.5%) produced ESBLs (SHV-12, CTX-M-15, VEB) and/or the acquired cephalosporinase CMY-4. The majority of the carbapenemase-producing strains were confirmed to co-produce SHV-12 (69.7%), CTX-M-15 (3%) and/or CMY-4 (7.6%) enzymes. PFGE analysis of 26 strains revealed three major pulsotypes (A–C) and the remaining six strains exhibited unique patterns. KPC-2–SHV-12-producers belonged to type A and VIM-1-producers were clustered in type B whereas type C was associated to strains producing CMY-4 b-lactamase.

Conclusion: Carbapenemase-producing strains are prevalent in Greek hospitals. KPC-2-producers, despite their recent emergence (2007), constitute the majority of multidrug resistant K. pneumoniae, whereas VIM-producing strains have declined in frequency. In general, KPC production was accompanied by the presence of multiple other b-lactamases, which may contribute to the increased resistance levels in all newer b-lactams.

**[P1348] Surveillance of b-lactamase production in a recent sample of Klebsiella pneumoniae isolated in Greek hospitals**

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Objectives: Several reports have clearly indicated that the spread of extended-spectrum b-lactamases (ESBLs), mainly of CTX-M-type, is responsible for increasing prevalence of cephalosporin-resistant (CR) Enterobacteriaceae in poultry. This resistance phenotype could also be due to the production of plasmid-mediated AmpC (pAmpCs) enzymes. However, only a few cases of pAmpC-producing Enterobacteriaceae have been reported in poultry. Furthermore, epidemiological data regarding this topic in Switzerland are lacking.

Methods: Cloacal swabs from poultry were incubated at 37°C in MacConkey broth with 5 mg/L of ceftazidime overnight. Then, 50 μL were plated on selective chromID ESBL (bioMérieux) and re-incubated. Resistance colonies were identified at species level using MALDI-TOF mass spectrometry and antibiotic susceptibility profiles were determined using Vitek 2. PCR and DNA sequencing were performed to characterize the main blaESBL and blamppm genes. Rep-PCR was used to study the clonality of Escherichia coli strains. Sequence typing analysis was carried out using the Institute Pasteur MLST methodology. Antimicrobial susceptibility and plasmid profile analyses were also performed.

Results: Fifty swabs collected during October–December 2010 from several slaughter houses located in 10 different Swiss cantons were processed. Fourteen (28%) swabs contained at least one CR E. coli. More than a half of the isolates were blaCMY-2-positive and were fully susceptible to aminoglycosides, quinolones, and tetracycline. These strains shared similar rep-PCR profiles and belonged to ST48 or ST37. The remaining E. coli isolates were usually blaCTX-M-1-like-positive and showed different rep-PCR and ST patterns. These strains were also susceptible to aminoglycosides but displayed resistance to quinolones, tetracycline and trimethoprim/sulfamethoxazole (approximately 80%, 100% and 20%, respectively). The CMY-2-producing E. coli strains were found in different slaughter located in several cantons.

Conclusion: This is the first epidemiological analysis regarding the spread of CR Enterobacteriaceae in poultry in Switzerland. Our findings showed that the diffusion of CTX-M-type ESBLs is likely due to the spread of common multidrug-resistant plasmids in different clones, whereas the high prevalence of clonally-related CMY-2-producing E. coli seems to be associated to a common source.

**[P1350] High frequency of the ST131-O25b pandemic Escherichia coli clone among uropathogenic isolates from companion animals and humans in Portugal**

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Objectives: To evaluate the frequency of the Escherichia coli sequence type 131 (ST131) serotype O25 variant b worldwide pandemic clone among companion animal and human urinary tract infection (UTI) strains in Portugal and its association with fluoroquinolone-resistance and ESBL production.

Methods: The veterinary E. coli isolates (n = 119, 87 from dogs and 32 from cats) were collected from 2004 until 2009 at the Veterinary Teaching Hospital of the Faculty of Veterinary Medicine and at veterinary private practices mainly in the Lisbon area. Human strains (n = 138) were isolated in hospitals and a community Diagnostic Laboratory in

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Extended-spectrum cephalosporin resistance and Persistence of ciprofloxacin- and ceftriaxone-resistant

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We report the results of molecular analyses of human cephalosporin-resistant salmonellae. ESBL- and AmpC-producing human strains were isolated from transconjugants. The majority of isolates (n=123) showed the typical pattern of the ESBL enzyme SHV-12 and CTX-M-1 and CMY-type enzymes were found frequently. Here (n=68), Enteritidis (n=9), Infantis (n=8), Bareilly (n=6), Kentucky (n=5), Goldcoast (n=4), Virchow (n=3) and others (n=33) from the strain collection of the German National Reference Centre. β-lactamase genes were identified by PCR and sequencing. Conjugation experiments, plasmid analyses and PFGE-typing were performed.

Results: The National Reference Centre for Salmonella and other Enterics in Germany determined serovars, phage types and antimicrobial susceptibilities of 21,167 human Salmonella isolates from 2005–2010. Resistance to 3rd gen. cephalosporins ceftazidime or cefotaxime was demonstrated concomitantly in 2002. Despite a significant decline of S. Choleraesuis infection, clinical isolates of CIPr/CROr S. Choleraesuis persist in Taiwan. Furthermore, ceftriaxone resistance increased significantly among nontyphoid Salmonella isolates in 2009. Molecular investigation was conducted to explore the resistance mechanisms.

Conclusion: The first Salmonella enterica serotype Choleraesuis that demonstrated concomitant resistance to ciprofloxacin and ceftriaxone (CIP/CRO) was identified in 2002. Despite a significant decline of S. Choleraesuis infection, clinical isolates of CIP/CROs S. Choleraesuis persist in Taiwan. Furthermore, ceftriaxone resistance increased significantly among nontyphoid Salmonella isolates in 2009. Molecular investigation was conducted to explore the resistance mechanisms.

Methods: Genetic relatedness of the isolates was investigated by pulsed-field gel electrophoresis (PFGE). Quinolone resistance was examined by PCR/sequencing of the plasmid resistance-determining regions of gyrA, gyrB, parC, and parE genes. Ceftriaxone resistance was investigated by PCR/sequencing of SHV, TEM, CTX-M, and AmpC genes. Plasmid profiling and DNA-DNA hybridisation were performed to identify the resistance plasmids. Self-transferability and replication types of the resistance plasmids were determined by published methods. Conjugative resistance plasmids were further characterised by a recently described plasmid multilocus sequence typing (pMLST) method.

Results: Ten cases of CIP/CROI S. Choleraesuis infection were identified during 2003–2009; only 7 blood isolates were available for study. One 2008 isolate demonstrated a different PFGE pattern from the others. Quinolone resistance was associated with amino acid changes at codon 83 (serine to phenylalanine) and codon 87 (aspartic acid to asparagine) of gyrA and codon 80 (serine to isoleucine) of parC. Ceftriaxone resistance was due to the presence of a plasmid-mediated CMY-2 gene located on the transposon Tn6092. The Tn6092-containing plasmids were non-conjugative in early CIP/CROs S. Choleraesuis isolates. In the two isolates collected after 2007, the resistance plasmid became self-transferable due to the recombination with a conjugative Inc1 plasmid. The Tn6092-harboring conjugative Inc1 plasmid was also found in all the 6 ceftriaxone-resistant isolates of Salmonella enterica serotypes Typhimurium, Agona, and Enteriditis recovered in 2010. Although 6 new sequence types were revealed among the 8 Inc1 plasmids identified, they formed a separate group from the two clonal complexes, CC-2 and CC-12, commonly found among the Inc1 plasmids in Europe and North America.

Conclusion: Spread of the Inc1 resistance plasmid among nontyphoid Salmonella represents a threat to the public health and should be closely monitored.

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Conclusion: Spread of the Inc1 resistance plasmid among nontyphoid Salmonella represents a threat to the public health and should be closely monitored.
Results: These isolates were positive for PMQR-genes. The qnrB19 gene was present in nine strains isolated in different years in different German regions: eight S. Hadar (six isolated from turkey meat, one from poultry and once from ND meat), and one S. Uganda (ND meat). All S. Hadar showed an identical XbaI-PFGE pattern, and all except one carried three small plasmids (<4 kb) suggesting the clonal spread of a qnrB19-positive strain. The qnrS1 gene was found in five S. Saintpaul (four of them from turkey meat, one of them from ND minced meat, different years and different regions). These isolates showed two similar PFGE-patterns (differing in only band in one isolate), and three different plasmid profiles, all of them carrying a small plasmid of about <10 kb. Finally, the qnrA gene was present in a S. Typhimurium isolate from turkey, and qnrB2 in a S. Uganda isolate from ND meat. Plasmid location of these genes was confirmed. No qnrC, qnrD or qepA genes were detected among the isolates. Conclusions: Our results show that various determinants conferring PMQR are present in Salmonella isolates originating from turkey/turkey meat. This kind of food can contribute to the further spread of these determinants, and this fact needs to be further investigated.

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**Multidrug-resistant Salmonella clones in slaughtered swine**

E. Gomes Nexes*, P. Antunes, A. Tavares, P. Themudo, J.M. Correia da Costa, L. Peixe (Porto, Vairão, Lisbon, PT)

**Objectives:** Particular multidrug-resistant (MDR) Salmonella clones mainly attributed to pork products have been increasingly involved in human infections, as Salmonella S.4,5,12:i:-, Typhimurium DT104 or S. Rissen. Occurrence and characterization of these clones at slaughterhouse were scarce. In this study we characterize clonal relationships, antibiotic resistance profiles and associated genetic determinants of isolates obtained from slaughtered swine and meat handlers in Portugal.

**Methods:** Fifty Salmonella isolates, from swine (ileoceacal lymph nodes, carcass surface and meat) and meat handlers collected in 8 abattoirs (July 2007-August 2008) were included: 26 S.Typhimurium, 10 S.4,5,12:i:-, 10 S.Derby, 4 S.Rissen, 3 S.Mbandaka, 3 S.London, 2 S.Give, 1 S.Enterriditis, and 1 S. Sandiego. Antimicrobial susceptibility was determined by the disc diffusion method according to CLSI standards. PCR and sequencing were used to identify antimicrobial resistance genes and class 1 integrons. Genetic relatedness was analyzed by PFGE.

**Results:** Resistance was found in 75% of Salmonella isolates, being 68% MDR, dispersed in the different abattoirs and samples. The most common resistance phenotypes were to tetracycline (Tet) (65%, tet(A), tet(B) tetG), sulfamethoxazole (Sul) (63%, sul1, sul2, sul3), streptomycin (Sm) (62%, aadA), ampicillin (A) (57%, blaPSE-type, blaTEM-type) and chloramphenicol (C) (15%, floR, cmlA). Thirty-seven percent were positive for class 1 integrons, with a variable region between 400–2000 bp. The isolates studied (9 serotypes) corresponded to 17 PFGE types. S. Typhimurium (12 PFGE types) was observed in swine samples and in meat handlers of 6 and 2 abattoirs, respectively. Mostly of them revealed features of the main human clones spread in Portugal (DT104 and sul3). S.4,5,12:i:- recovered from swine in 3 abattoirs clustered with some S. Typhimurium isolates in 2 PFGE types and were mostly associated to ASuT type (blaTEM, strA-strB, sul2 and tetB), Isolates belonging to the emerging S. Rissen (1 PFGE type), recovered in swine and meat handlers of 3 and 2 abattoirs, respectively, differed in the resistance profile (Tet; AS; Tet; ASuT; Tet; A) being Tet resistance associated to Tet(A), A to blaTEM-1, Sul to sul1 and/or sul3.

**Conclusions:** Swine and abattoir environment seems to contribute to the load of MDR Salmonella isolates and to the emergence of monophasic variant S.4,5,12:i:- in humans.
ciprofloxacin susceptibility and the presence of blaCTX-M are described in Table1. According to the serotypes, almost all of the K1 serotype isolated harbored five virulence genes. In K2 isolates, the prevalence of only rmpA and aerobactin was high. In K54 isolates, all of the five isolates had wcaG but the prevalence of other virulence genes were low (20–40%). For K57 isolates, all harbored rmpA and aerobactin, but none harbored other virulence genes. There was no difference in distribution of virulence genes (except rmpA) or serotypes according to the isolates.

Conclusions: The prevalence of virulence determinants was significantly higher in ciprofloxacin-susceptible isolates and it was different according to the serotypes. The K1 and K54 serotype was more frequent in ciprofloxacin-susceptible isolates and blaCTX-M-positive isolates, respectively.

Table 1. Prevalence of degree virulence genes and serotypes according to the susceptibility and presence of blaCTX-M.

| Virulence determinants | Serotypes | CPD (%) | P Value | AcT/C-TIg-p | AcT/C-TIb-p | P Value |
|-----------------------|-----------|---------|---------|-------------|-------------|---------|
| rmpA                   | K1        | 0.59    | 0.002   | 7.35        | 25.122      | 0.070   |
| aerobactin             | K2        | 0.93    | 0.003   | 5.35        | 34.122      | 0.120   |
| fimbria                | K54       | 0.75    | 0.111   | 4.95        | 25.122      | 0.025   |
| wcaG                   | K57       | 0.73    | 0.003   | 8.35        | 46.122      | 0.587   |
| idE                    | K1        | 0.19    | 0.001   | 11.35       | 34.122      | 0.280   |
| K1                     | 0.75      | 0.003   | 3.35    | 19.121      | 0.429    |
| K2                     | 0.75      | 0.003   | 3.35    | 19.121      | 0.429    |
| K54                   | 0.75      | 0.003   | 4.35    | 11.121      | 0.000    |

Conclusions: The major human and avian E. coli ST clones associated with FQ-resistance and MDR phenotype were identified. Although our results do not support the hypothesis of the possible avian origin of the major ST131 clone affecting humans, the sharing of the ST23 and ST10 clones across strains from human and avian sources poses a zoonotic risk.

[PI358] Spread of plasmid-mediated quinolone resistance and pathogenic islands in Escherichia coli isolated from water

N Mendonça*, J Ramalho, P Vieira, A Tavares, P Pereira, G Da Silva (Coimbra, Aveiro, Leiria, PT)

Objectives: We report the search for plasmid-mediated quinolone resistance (PMQR) genes and virulence factors encoded in pathogenic islands (PAIs) among Escherichia coli strains isolated from waters of different origins.

Methods: Between May 2009 and February 2010, 37 isolates were recovered from drinking, recreational, waste, surface and ground waters. The antimicrobial susceptibilities were determined by the disk diffusion method by using CLSI guidelines. PMQR (qnr, aac(6’)-Ib-variant and qepA) determinants and PAIs markers were screened by PCR. Determination of the E. coli phylogenetic group was also performed by PCR.

Results: Most strains were isolated from surface waters (49%), and during the Summer 2009 (49%). 19% of the strains were resistant to nalidixic acid, 11% to ciprofloxacin and 5% to gentamicin. Molecular methods detected one type of PMQR: qnrA was detected among 16% of strains, all of which susceptible to quinolones. Pathogenicity islands (PAIs) IV356 was detected among surface (8%), drinking (5%) and ground waters (3%). While PAI IJCF/T073 was detected among drinking water (3%). According to the phylogenetic groups, we detected 41% and 27% of strains belonging to the less-virulent groups A and B1, while 30% and 3% were from groups D and B2, respectively. Our results showed 45% of strains from group D with the PAI IV356, a phylogenetic group also considered virulent. The PAI IV356 was also found in an isolate belonging to group B1 carrying a qnrA gene. All the qnrA genes were detected in group B1 strains collected in waters of different origin, geographical location and season.

Conclusions: The results showed the presence of potential pathogenic strains encoding resistance determinants and/or pathogenic islands among surface, ground and drinking waters. These strains can represent an increased risk for public health, as they were isolated from samples collected from waters with potential contact with humans and animals. Thus, it's of crucial importance a periodic monitoring of the water quality, not only for the presence of microorganisms, but also for screening antibiotic resistance and virulence factors.

[PI359] Occurrence of plasmid-mediated quinolone resistance among bacteria isolated in animals in Portugal

E. Ferreira, L. Clemente, D. Jones-Dias, A.P Francisco, V. Managiero, P Themudo, T. Albuquerque, A. Amado, M. Cañita* (Lisbon, PT)

Background: Plasmid-mediated quinolone resistance (PMQR) is increasingly identified worldwide in Enterobacteriaceae. The aim of this study was to evaluate the extension of PMQR in isolates from animals in Portugal.

Methods: We screened 186 Enterobacteriaceae isolates for the presence of PMQR determinants, identified at National Laboratory of Veterinary Research (2008–2009). A total of 92 Salmonella isolates were isolated from broilers, layers and pigs, and 94 Escherichia coli were from farm animals, birds and mammals. Susceptibility testing of all isolates was performed by disk diffusion method, and MICs against nalidixic acid, ciprofloxacin, gatifloxacin, levofloxacin, ofloxacin, enrofloxacin, morfloxacin and norfloxacin were determined by E-test for PMQR-positive isolates. PCR and nucleotide sequencing, by using specific primers, were used to screen for the presence of PMQR-encoding genes. The genetic context of PMQR genes was evaluated by using different molecular methods.
Results: We identified 5 qnrC-positive isolates: 2 Salmonella enteritidis collected in 2 layer chicken and in 3 E. coli from 2 broilers and one pig; 3 qnrS1 genes were detected in E. coli isolates from a broiler (co-expressing a qnr-C gene), a dog and a turtle-dove. The aac(6′)-Ib-cr gene was detected in an E. coli isolated from a mammalian. Seven PMQR-positive isolates showed diminished susceptibility to at least one quinolone, and one was detected in the range of susceptibility against the seven (fluoro)quinolones tested. Three E. coli and one S enteritidis were PMQR- and TEM-1 and/or CTX-M-15-producing isolates. An E. coli with qnrS, qnrS1, and blaTEM-1 genes and an E. coli with qnrC gene were positive for genes coding to class 1 integrons.

Conclusions: This survey showed that PMQR determinants are present in animals from different environments in Portugal, including food-producing animals, with a high frequency (3%) of QnrC-producing isolates. Susceptibility results demonstrate the difficulty to predict the PMQR mechanisms by phenotypic methods. Overall, the study suggests that PMQR genes are undergoing a dissemination process, which needs surveillance.

Aminoglycoside resistance in Escherichia coli in western Norway
P. C. Lindemann*, K. Risberg, H. Myhuganam (Bergen, NO)

Objective: There had been a steady increase in gentamicin resistance from 1.2% in 2000 to 4% in 2009, in E. coli isolated from blood cultures in Norway. Aminoglycoside modifying enzymes (AME) are important in resistance development and are acquired by horizontal transfer of the respective genes located on plasmids. In E. coli the most prevalent AMEs are AAC(6′)-Ib and AAC(3)-II. Our objective was to find the prevalence of the genes encoding these AMEs among Norwegian E. coli isolates that were resistant to aminoglycosides (AGs) and to use their resistance profiles for different AGs to consider their value in predicting the responsible AME.

Methods: A total of 108 clinical isolates of E. coli from Western Norway were included, consisting of 42 from blood culture isolates that had reduced susceptibility to gentamicin/netilmicin, and 65 isolates from other sites that were non-susceptible to gentamicin/ tobramycin, and also produced extended-spectrum β-lactamases (ESBL). Minimum inhibitory concentrations (MIC) were determined by E-test/MIC test strips for gentamicin, netilmicin, tobramycin, kanamycin, amikacin and streptomycin. The isolates were screened by PCR for aac(6′)-Ib-b and aac(3)-Ia/a. aac(6′)-Ib was sequenced to identify its variant aac(6′)-Ib-cr, which confers resistance also to fluorquinolones.

Results: The prevalence of aac(3)-Ia/a or -Ic was 79.6% (86/108) with no differences seen in the two groups. Resistance to gentamicin was seen in all isolates that had aac(3)-II, however 1–2 isolates were susceptible to netilmicin/tobramycin. aac(6′)-Ib was found in 35.2% of the isolates (38/108), of which 37 were identified as aac(6′)-Ib-cr, with a higher prevalence in ESBL isolates. The substrate specificity was as expected for kanamycin, netilmicyn, tobramycin, but amikacin resistance was not found, although the MICs were marginally elevated in 9 of the 38 isolates. Isolates with aac(3)-II had higher MIC values for netilmicin than isolates with aac(6′)-Ib, and aac(6′)-Ib was associated with higher MICs for tobramycin. The prevalence (91.7%) and the degree of qunolone resistance could not be attributed solely to the presence of aac(6′)-Ib-cr. Conclusion: aac(3)-II and aac(6′)-Ib-cr possibly contributed to the recent increase in gentamicin resistance. The resistance profile, MIC values for netilmicin and tobramycin and association with ESBL might be useful in predicting the AME.

Carbenapenemase-producing Acinetobacter spp. in Kyoto University Hospital, Japan: the occurrence of metallo-β-lactamases
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Objectives: In recent years, carbenapenemase-producing Acinetobacter spp. has increased substantially. The carbenapenem-hydrolyzing β-lactamases in Acinetobacter spp. are either metallo-β-lactamases (MBLs) or oxacillinases (carbenapenem-hydrolyzing class D β-lactamases [CHDLs]). Although CHDLs were reported as more major carbenapenemase than MBLs, blaIMP-2 detection in Acinetobacter spp. was previously reported in Japan. The aim of this study was to evaluate the occurrence of CHDL- and MBL-encoding genes among Acinetobacter spp. isolates recovered from Kyoto University Hospital, Japan.

Methods: During 2004–2009, 435 Acinetobacter spp. were recovered and 28 (6.4%) isolates were carbenapenem-non-susceptible. Only the first isolation was included in this study. 16 of 28 carbenapenem-non-susceptible Acinetobacter spp. isolates were preserved in our hospital. Carbenapenem-non-susceptibility, MIC >4mcg/ml for imipenem or meropenem was determined by the broth microdilution method according to CLSI M100-S20 guideline. These isolates were all screened for CHDL-encoding genes (OXA-23, OXA-24, OXA-51, OXA-58) and MBL-encoding genes (IMPL2/VIM1, SPM1, SIM1, GIM1), and confirmed by sequencing. Clonality of A. calcoaceticus/ A. baumannii complex was analyzed by MLST.

Results: 16 carbenapenem-non-susceptible Acinetobacter spp. included 8 A. baumannii, 2 A. lwoffii, and 4 other Acinetobacter spp. CHDL- or MBL-encoding genes were detected in 14 (87.5%) isolates. All 8 of carbenapenem-non-susceptible A. baumannii sp. 3 harboured MBL-encoding gene (1 IMP-1 and 7 IMP-2), and 1 isolate...
Evolution of Spread of imipenem-resistant Acinetobacter baumannii

A. baumannii has been identified as a multidrug-resistant (MDR) pathogen increasingly affecting severely ill patients. The aim of the study was to analyze the genetic relatedness and the evolution of clonal lineages in A. baumannii isolates recovered from Greek hospitalized patients during a long-term period.

Objectives: Acinetobacter baumannii is a multidrug-resistant (MDR) pathogen increasingly affecting severely ill patients. The aim of the study was to analyze the genetic relatedness and the evolution of clonal lineages in A. baumannii isolates recovered from Greek hospitalized patients during a long-term period.

Methods: The study included 97 A. baumannii isolates randomly selected among those recovered during 1994–2008 in seven institutions, located in four different Greek regions. Carbapenem MICs were determined by Etest and broth microdilution. PCR and sequencing analysis were used for testing the presence of class D OXA-type carbapenemase genes (blaOXA-23, blaOXA-24 and blaOXA-51). The isolates were typed by PFGE, the triokinase ST protocol developed by the Pasteur Institute followed by eBURST analysis of MLST types.

Results: In 2000, 2001, and 2002, the predominant blaOXA-type among the 272 isolates studied. Genes encoding MBLs were not identified. Though blaAmpC was found in all the isolates, its association with ISAba1 was not found among some isolates. Eight isolates harbored the disrupted carO gene or lacked this kind of gene. In 36 representative A. baumannii isolates, except that isolates from Hangzhou did not belong to European clone I; or II, European clone II, was identified for the other isolates. Ten STs, including 3 novel types, were identified and clustered into clonal complex 92 (CC92) and two distinct singleton.

Conclusion: Our observations suggest that imipenem-resistant A. baumannii of European clone II, which carried acquired ISAba1 preceding blaOXA-23-like gene and belonged to CC92, could spread widely in western China.

P1365 Evolution of Acinetobacter baumannii clonal lineages in Greek hospitals over the last 15 years

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Objectives: Acinetobacter baumannii is an emerging and widespread nosocomial pathogen, often difficult to treat. The aim of the present study was to evaluate the distribution of resistance genes and clonal relationship in the imipenem-resistant Acinetobacter baumannii isolates from ten hospitals in western China, and compare the epidemiologic data with those of the isolates from two hospitals in Hangzhou and Beijing.

Methods: The genes encoding OXA carbapenemases, metallo-β-lactamases (MBLs), AmpC cephalosporinase, and Carbapenem Resistance-Associated Outer Membrane Protein (CarO) were screened by PCR and sequencing. PCR mapping were performed to determine whether ISAba1 elements preceded OXA carbapenemases and AmpC cephalosporinase respectively. International clonal lineages were identified by sequence-type multiplex PCR and sequencing recA gene. Multilocus sequence typing (MLST) was performed to determine the sequence types (STs), and then eBURST algorithm was applied to assign clonal complexes (CCs).

Results: In this study, acquired ISAba1 preceding blaOXA-23-like was the predominant blaOXA-type among the 272 isolates studied. Genes encoding MBLs were not identified. Though blaAmpC was found in all the isolates, its association with ISAba1 was not found among some isolates. Eight isolates harbored the disrupted carO gene or lacked this kind of gene. In 36 representative A. baumannii isolates, except that 2 isolates from Hangzhou did not belong to European clone I; or II, European clone II, was identified for the other isolates. Ten STs, including 3 novel types, were identified and clustered into clonal complex 92 (CC92) and two distinct singleton.

Conclusion: Our observations suggest that imipenem-resistant A. baumannii of European clone II, which carried acquired ISAba1 preceding blaOXA-23-like gene and belonged to CC92, could spread widely in western China.

P1366 Retrospective analysis of carbapenem-resistant Acinetobacter baumannii strains in a tertiary hospital

M. Pirs*, T. Cerar, R. Kofol, M. Mueller Premru, V. Krican Hergovsk, J. Ambrozic Augustin, K. Sieme (Ljubljana, SI)

Objective: Carbapenem-resistant Acinetobacter baumannii (CR-AB) isolates have been isolated with increased frequency in our tertiary hospital, mostly in the surgical ICUs. The aim of the present study was to analyze antimiobacterial susceptibility of A. baumannii and to detect the presence of carbapenemase genes using molecular methods.

Methods: Laboratory records of patients with A. baumannii isolates were analyzed from 2005 to 2010. CR-AB was isolated in 109 patients and 14 non-repetitive isolates of CR-AB were analyzed. Characterization of blaKPC, blaVIM, blaIMP was performed using Hyplex KPC/MBL kit (Amplex BioSystem GmbH, Germany), detection of blaNDM-1 was performed using PCR as described previously. Class D carbapenemase detection was performed using Hyplex CarbOXA kit (Amplex BioSystem GmbH, Germany) which detects blaOXA-58, blaOXA-40 and blaOXA-24 genes.

Results: CR-AB has been sporadically isolated from 2005 to 2007, resistance to imipenem in A. baumannii primoisolates was below 1%. The resistance to imipenem has increased to 5.3% in 2008 and to 15.5% in 2009. In the 2010 there has been a decrease of resistance to imipenem to 8.5%. Before 2008, there were 3 to 6 patients with CR-AB per year. In 2008 CR-AB was isolated from 21 patients, in 2009 there was a sharp increase in the number of patients (58 patients) with a decrease in the year 2010 (21 patients). Majority of patients (75%) were hospitalized in surgical wards, mostly in ICUs. Half of the CR-AB were isolated from the respiratory tract, 19.8% from wounds and 5% from blood cultures and urine. 60.2% of the carbapenem-resistant primoisolates were susceptible to ampicillin/sulbactam, 30.6% to cefazidime, 21.3% to amikacin with less than 2% isolates susceptible to piperacillin/tazobactam or ceftriaxone. The presence of carbapenemases was confirmed in 13
of 14 isolates using molecular testing. One isolate was positive for NDM-1 carbapenemase (2008), 7 isolates were positive for OXA-58 and 5 for OXA-40 carbapenemases (2008–2010). Molecular mechanism of carbapenem resistance for the remaining isolate has not been determined yet.

**Conclusions:** Our retrospective analysis has indicated a possibility of a silent outbreak of CR-AB in our tertiary hospital which began in 2008 and has peaked in 2009. Carbapenemase characterization of CR-AB in our study has shown the predominance of OXA-58 and OXA-40 carbapenemases and a surprising sporadic occurrence of NDM-1 carbapenemase in 2008.

**Methods:** Carbapenem resistance was also analysed.

**Objectives:** Acinetobacter baumannii is a ubiquitous pathogen able to cause colonization and both community and healthcare-associated infections. Our purpose was to evaluate the genetic relatedness of carbapenem-hydrolysing class D b-lactamase (CHDL) producing MDR-Ab from different geographic regions of Portugal. The correlation with carbapenemase in 2008.

**Results:** Our retrospective analysis has indicated a possibility of a silent outbreak of CR-AB in our tertiary hospital which began in 2008 and has peaked in 2009. Carbapenemase characterization of CR-AB in our study has shown the predominance of OXA-58 and OXA-40 carbapenemases and a surprising sporadic occurrence of NDM-1 carbapenemase in 2008.

**Discussion:** This study provides updated data regarding the molecular epidemiology of MDR-Ab in Portugal. Here, we report the first appearance of two epidemic ST OXA-23-producers in the country, expansion. Indeed, they seem to be replacing the only ST described so far as endemic in Portugal (ST98).

**Paper:**

**Title:** Phenotypic and molecular characterisation of carbapenem-hydrolysing class D b-lactamase-producing Acinetobacter baumannii isolated in Portugal

**Authors:** V. Manageiro*, D. Jones-Dias, E. Ferreira, D. Louro, M. Canica on behalf of the Antibiotic Resistance Surveillance Program in Portugal (ARSIP)

**Objectives:** Acinetobacter baumannii is a ubiquitous pathogen able to cause colonization and both community and healthcare-associated infections. Our purpose was to evaluate the genetic relatedness of carbapenem-hydrolysing class D b-lactamase (CHDL) producing MDR-Ab from different geographic regions of Portugal. The correlation with carbapenem resistance was also analysed.

**Methods:** The study included 127 clinical A. baumannii isolates recovered in Portuguese hospitals (116 from Apr 2009–Apr 2010); 11 from 2005–2008 were included for genetic evolution comparison. The identification of strains was confirmed by PCR (based on the presence or absence of OXA-51-like genes) and antimicrobial susceptibility was screened by TSA and E-test. PCR and sequencing were applied to detect and identify genes encoding CHDLs, class B metallo-b-lactamases (MBL) (blaIMP and blaVIM), and class A b-lactamases (blaTEM, blaSHV and blaCTX-M). Genetic relatedness of MDR-Ab was examined by PFGE and MLST (typing of 25 isolates representative of different PFGE-clusters).

**Results:** All isolates were MDR but susceptible to colistin. However, the MICs of colistin confirmed one isolate as nonsusceptible. Overall, 77% of the isolates carried blaOXA-23, 18% blaOXA-24, 28% blaTEM-1, 2% blaCTX-M-15-type and 1% blaTEM-110 genes. None of the isolates carried OXA-58 or MBL-encoding genes. All isolates carried the blaOXA-51-type gene, and expressed OXA-66 (98%), OXA-71 (1%) or OXA-104 (1%) CHDL. The blaOXA-104-CHDL gene carries 182 synonymous mutations comparing with the first-described b-lactamase.

**Discussion:** This study provides updated data regarding the molecular epidemiology of MDR-Ab in Portugal. Here, we report the first appearance of two epidemic ST OXA-23-producers in the country, expansion. Indeed, they seem to be replacing the only ST described so far as endemic in Portugal (ST98).

**Objective:** To report the most recent full-year of linezolid (LZD) resistance surveillance (ZAAPS Program) monitoring European (EU) medical centers for 2010. A total of 5,532 Gram-positive organisms have the highest LZD-R occurrences and no mobile cfr genes were identified among 3,045 staphyloccocci screened in EU.

**Paper:**

**Title:** Erythromycin resistance of clinical isolates of Streptococcus pneumoniae isolated over a thirty-year period in Barcelona, Spain (1979–2008)

**Authors:** L. Calatayud, C. Ardanuy, F. Tubau, M.A. Dominguez, I. Grau, R. Pallarès, R. Martín, J. Liñares* (Hospital de Llobregat, ES)

**Objectives:** The aims of this study were to analyze trends of Erythromycin resistance (EryR) in S. pneumoniae (Spn) over thirty years in a hospital in Barcelona. The distribution of serotypes, genotypes and macrolide resistance genes were analyzed.

**Methods:** Among 8620 pneumococci collected from 7585 adult patients in 1979–2008 period, 2030 (23.5%) were EryR. Of them, 1686 (83%) were serotyped by Quellung reaction or PCR, 1082 (53%) were genotyped by PFGE/MLST and the erm(B) and mef(A) genes were detected by PCR in 1098 (54%) of the strains.

**Results:** The overall EryR rate was 23.5% (2030/8620). EryR rates progressively increased from 0% in 1979 to 35.3% in 2001, thereafter EryR rates ranged from 25 to 30%. EryR rates were higher among non-invasive 26.9% (1638/6099) than invasive isolates 15.5% (3922/2521), p < 0.001. The 94.2% of Ery-R pneum strains had MLSB phenotype. The most frequent serotypes were: 19F, 6B, 23F, 14, 19A, 15A, 23A, and 6A. Among 1013 MLSB phenotype strains 956 (94.4%) had erm(B) gene, 22 (2.2%) had both erm(B) and mef(A) genes and 35 (3.5%) had neither
genes. All 85 M phenotype isolates had mef(A/E) genes (92% mefE and 8% mefA). Among MLSB strains, eleven clones (Spain23F-ST81, Spain6B-ST79, Sweden15A-ST63, ST422A3, ST819F, Denmark14-ST230, Spain14-ST18, ST3016F, Poland8B-ST315, Spain9V-ST116 and ST71733F) accounted for the 62% of the strains. The predominant clones among M phenotype strains were Spain9V-ST116, England14-ST9 and ST6211. Three clones related to PCV7 serotypes decreased: Spain14-ST18, Spain23F-ST81 and Spain6B-ST79 (p < 0.05), whereas Denmark14-ST230, ST3016F, ST42 and ST717 increased (p < 0.05).

**Conclusions:** EryR rates progressively increased in Spain in the late 80s coinciding with the introduction of long-acting macrolides in the clinical practice, and were associated to the spread of few multidrug-resistant clones that decreased after PCV7 introduction. However, EryR rates did not decrease due to both spread of minor macrolide resistant clones and the emergence of new resistant clones such as Denmark14-ST230.

P1369 **Serotype distribution of nasopharyngeal pneumococci among children having acute otitis media before and after the introduction of a pneumococcal conjugate vaccination programme**

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**Objectives:** *Streptococcus pneumoniae* is the main pathogen in acute otitis media (AOM). Pneumococcal serotypes are determined according to the composition of capsular polysaccharides. More than 90 different serotypes known to date differ in their ability to cause diseases. In Finland, the 10-valent pneumococcal conjugate vaccine containing serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F, has been included into the national vaccine program in autumn 2010. The aim of this study was to investigate the serotype distribution of pneumococcal isolates from nasopharyngeal samples of children having AOM, during the years 2006–2008, before the era of nationwide pneumococcal vaccination.

**Methods:** Nasopharyngeal samples were taken from children (6–35 mo) at entry visit of an AOM treatment trial. Thus far, 96 pneumococcal isolates from nasopharyngeal samples have been serotyped in the Bacteriology Unit of the National Institute for Health and Welfare. Results: The most common pneumococcal serotypes were 6B, 19F, 14, 23F and 6A, in decreasing order of incidence. These serotypes covered nearly 90% of all studied isolates. Four of these serotypes: 6B, 19F, 14, and 23F are included in the 10-valent pneumococcal conjugate vaccine, which cover altogether 82% of pneumococcal serotypes detected in the nasopharyngeal specimens. Pneumococcal serotype 6A, which is not covered by this vaccine, was detected in 5% of all studied isolates.

**Conclusion:** Our results confirm that the most common serotypes of pneumococci isolated from the nasopharyngeal samples of children with AOM are covered by the 10 valent pneumococcal conjugate vaccine currently in nationwide use in Finland. This indicates that the vaccine program might decrease the number of pneumococcal AOM cases. However, the possible increase by non-vaccine serotypes like 6A already present in the nasopharynges of children calls for careful monitoring of the serotype distribution during the coming years.

P1370 **Prior use of fluoroquinolones, fluoroquinolone resistance and gyrA/parC mutations in Streptococcus pneumoniae in Ontario**

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**Objectives:** Use of FQ is known to be associated with selection for FQ resistance (FQR) in SPN. We assessed the extent to which prior FQ use was associated not only with increases in FQR but also with increases in parC and gyrA mutations in FQ susceptible SPN.

**Methods:** TiBDN performs population-based surveillance for invasive pneumococcal disease (IPD) in Toronto/Peel (pop. 4M); respiratory isolates are also collected. Prior antibiotic use is collected via chart review and patient/physician interview. Broth microdilution susceptibility testing to CLSI standards is performed. QRDR were sequenced for all FQR isolates (Cip mic: >2 μg/ml or Lev R or Mosi R), all SPN isolates from patients (PTs) with FQ exposure (FQEXP=any FQ in prior 3 months), and a sample of other isolates from 2000 to 2007. Population FQ use was obtained from IMS Health.

**Results:** From 2000–2009, FQ use increased from 68–97 scripts/1000 pop/year. Levo from 0–9.3 scripts/1000 pop/year, Mosi from 0–18 scripts/1000 pop/year. 4071 respiratory isolates and 4657 IPD cases were identified. FQR decreased significantly from 2002 to 2007, then remained stable: Levo R for IPD was 1.1% (2002), 0.46% (2007), 0.78% (2009) and for respiratory isolates was 5.9% (2002), 1.9% (2007), 1.6% (2009). Overall, Mosi R was 0.49% (2002), 0.46% (2007), 0.60% (2009). Of 97 Levo R isolates with QRDR sequencing, 89 had mutations in gyrA and 6 in gyrB only, and 2 in parC only. Of 3098 lev S/I isolates sequenced, 2 had both parC and gyrA mutations, 48 parC mutations only, 2 a gyrA mutation only, and 3046 had no mutations. Complete data on prior FQ use was available for 70% of cases. 957/7517 (13%) PTs reported FQEXP. Of the 106 Levo R isolates, 63 (59%) occurred in PTs with FQEXP; while 43 (41%) occurred PTs without FQEXP. PTs reported FQEXP. Of the 106 Levo R isolates, 63 (59%) occurred in PTs with FQEXP; while 43 (41%) occurred PTs without FQEXP. Of the 106 Levo R isolates, 63 (59%) occurred in PTs with FQEXP; while 43 (41%) occurred PTs without FQEXP. Of the 106 Levo R isolates, 63 (59%) occurred in PTs with FQEXP; while 43 (41%) occurred PTs without FQEXP.

**Conclusion:** Despite increased use of FQs, FQR decreased. Most FQ isolates with QRDR mutations are found in PTs with recent FQEXP. Exposure to more active FQ may be associated with reduced risk of development of QRDR mutations.
Prevalence and characteristics of pneumococci colonising adults older than 60 years in Portugal: the PneumoEL project

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Objectives: The PneumoEL project was launched in 2010 in two areas of Portugal, and aims to gain insights on pneumococcal carriage patterns among the elderly.

Methods: Between April and November of 2010, adults (aged >60 years) were enrolled. The participants lived either in the urban area of Oeiras (n=650), or in the rural area of Montemor-o-Novo (n=650). Swabs of the nasopharynx (NP) and oropharynx (OP) were obtained. Identification of pneumococcus was done by optochin susceptibility, solubility in bile salts, and detection of lytA (major pneumococcal autolysin) and cpsA (conserved capsular gene) genes. Isolates were tested by disk diffusion for susceptibility to erythromycin (Ery), clindamycin (Cc), chloramphenicol (Chl), tetracycline (Tet), and co-trimoxazole (SXT), and by E-test to ciprofloxacin (CIP) and penicillin (PG). Serotyping was done by multiplex PCR and/or the Quellung reaction.

Results: Sixty-three (4.8%) adults were pneumococcal carriers. Carriage rates were 4.0% and 1.5% in the NP and in the OP, respectively. Pneumococci were isolated from both sites in seven adults (0.5%). Additionally from one NP sample, two types of colonies were detected. A total of 71 isolates were obtained. Serotyping and antibiotyping revealed that six of the seven adults that carried pneumococci in both sampling sites carried a single strain. Co-colonization was detected in two adults only. The 65 strains were of types 11A (n=4), 22F, 23B, 18A, 15B, 15A, 36, 31, 9L, and 21 (1 each); 32 isolates were non-typeable (NT). Six isolates (9.2%) had a CIP MIC \( \leq 1 \) mg/L, and seven (10.8%) had a CIP MIC \( >2 \) mg/L. Rates of resistance to Ery, Cc, Tet, SXT, and Chl were 15.4%, 4.6%, 10.8%, 9.2%, and 0%, respectively. Multidrug resistance was found in five isolates associated with NT (n=3), 15B, and 19A. The potential coverages by the 23-valent pneumococcal polysaccharide vaccine and by the 13-valent pneumococcal conjugate vaccine among capssulated isolates were 36.4% and 21.2%, respectively.

Conclusion: This is the first data from Portugal on pneumococcal colonization among the elderly. Carriage rates in this age group are low. There is a great variability of serotypes. Moderately high rates of resistance to antimicrobial agents were found. Our results provide an important baseline to monitor the impact of upcoming pneumococcal vaccines in patterns of colonization among the elderly.

Penicillin non-susceptible pneumococcal invasive clones circulating in Italy in the vaccine era (2006–2010)

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Objectives: The aim of this study was to assess the impact of the 7-valent conjugate vaccine (PCV7) on the serotypes and the population structure of penicillin-non susceptible pneumococci (PNSSP) in Italy.

Methods: All pneumococcal invasive isolates recovered in an Italian nation-wide surveillance in 2006–2010 were studied. All isolates were serotyped and examined for susceptibility to penicillin, cefotaxime, erythromycin, clindamycin, tetracycline and chloramphenicol following the CLSI procedures. Clonal groups were defined on the basis of both Pulsed-Field Gel Electrophoresis and Multilocus Sequence Typing (MLST). Isolates were also genotyped by restriction analysis of \( \phi pb2x \) and \( \phi pb2x \) genes.

Results: Among 778 invasive isolates, from both adults and children, a total of 118 PNSSP isolates were found, with 67 and 51 isolates being vaccine serotypes (VS) and non-vaccine serotypes (NVS), respectively. Differences were observed about the relative frequencies of VS, NVS, and clones between vaccine and pre-vaccine (1999–2003) eras. Among PNSSP VS, serotype 14 increased in the vaccine era, while serotypes 9V, 19F and 6B significantly decreased. Within NVS, a large increment in the rates of serotypes 19A, 24F, 15A, and 6A was noted. Conversely, serotype 35F, that was quite represented in the pre-vaccine era, completely disappeared. Among 92 PNSSP available for genotyping, 15 different CC/PFGE combinations were found. Sweden15A-22F/PFGE 2 (23 isolates, 25%), Spain23F-1/CC81/PFGE 3/CC156/PFGE 1 (22 isolates, 23.9%), Denmark14–32/CC230/PFGE 3 (15 isolates, 16.3%), and Spain23F-1/CC81/PFGE 34 (7 isolates, 7.6%) accounted for 85.9% of the genotyped PNSSP. Clones Sweden15A-22F/PFGE 2 and Denmark14–32/CC230/PFGE 3 largely increased in the vaccine era. Other clones, previously encountered, completely disappeared in the vaccine period (i.e., ST16/4/4/PFGE 5). New CF/PFGE combinations were also observed, as in the case of Taiwan19F-14/CC271/PFGE 85. A high correspondence between \( \phi pb2x \) profiles and specific clones was found. New associations of serotypes with specific clones were also observed, compared to those found in the pre-vaccine period.

Conclusion: Differences in the prevalence of serotypes and clones among PNSSP have been observed if compared to those found in the pre-vaccine era. Not all the differences can be directly attributed to the vaccine impact, but rather reflect the continuous antibiotic pressure.
**P1375** Phenomenal increase in fluoroquinolone-non-susceptibility among clinical group A streptococci in Belgium during 2007–2010

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Objectives: Fluoroquinolones (FQs) are not recommended for Group A Streptococcus (GAS) infections, however resistance to FQs is frequently reported in GAS. We evaluated the impact of a reportedly high FQ use in Belgium on the evolution of FQ non-susceptibility (FQ-NS) in GAS during 2007–2010.

Methods: A total of 4495 confirmed GAS isolates recovered from patients suffering from pharyngitis or invasive infections during 2007–2010 in Belgium were screened for ciprofloxacin susceptibility on disc diffusion. MICs to ciprofloxacin were determined for GAS with a zone diameter <20 mm. FQ-NS strains (MIC ciprofloxacin ≥2 μg/ml) were further screened for norfloxacin MICs and resistance-sensitve efflux by agar diffusion. Emm typing of FQ-NS GAS was done using PCR and sequencing. Quinolone resistance determining regions (QRDR) of topoisomerase genes, parC and gyrA, were screened for mutations in selected FQ-NS GAS (n = 44). Pearson’s Chi square test was used for statistical analysis.

Results: A total of 499 (11.1%) of the 4495 GAS collected during 2007–2010 were FQ-NS. Prevalence of FQ-NS GAS increased significantly from 4.4% to 10.9% between 2008 and 2009, and more than doubled to 22.1% in 2010 (P < 0.01 for both) (Table). During 2008–2010, non-susceptible GAS recovered from both children and adults showed a significant increase (P < 0.01). Majority of FQ-NS GAS belonged to emm6 (n = 329, 65.9%) and emm75 (n = 87, 17.4%), and proportion of emm6 strains increased significantly during 2008–2010 (P < 0.01). Ciprofloxacin MICs for 497 FQ-NS GAS ranged from 2–8 μg/ml and 2 strains (emm89 and emm11) exhibited high-level resistance with MICs of 32 μg/ml. Ciprofloxacin MICs of 2–8 μg/ml correlated to ParC substitutions at the Ser79 hotspot (substituted by Ala, Phe, or Tyr). Other changes in ParC were noted at Asp83 (to Asn, Gly), Asp91 (to Asn), and Ser140 (to Pro). Interestingly, the 2 FQ-NS GAS with ciprofloxacin MICs 32 μg/ml also showed second-step substitutions in GyrA at Ser81 (to Tyr) or emm89, and Phe in emm11. Reserpine-sensitive efflux was not observed.

Conclusions: We recorded a phenomenal increase in FQ-NS among GAS that has not been reported before and is of concern. Reassuringly, FQ-NS is still primarily confined to emm6 GAS that have consistently exhibited low-level FQ resistance linked to first-step ParC substitutions.

**P1376** Unexpectedly high prevalence of spa type t008, SCCmec type IV MRSA clone in Russian paediatric hospitals

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is shown to have a clonal population structure, and is characterized by limited number of clonal groups distributed worldwide and having different pathogenicity and antimicrobial resistance patterns. The data about molecular epidemiology of MRSA in Russian paediatric hospitals are sparse.

Objectives: To determine molecular types of *S. aureus* strains causing nosocomial infections in children in different regions of Russia.

Materials and Methods: A total of 112 isolates (67 MSSA and 45 MRSA) collected from 28 hospitals of 23 geographically distinct cities of Russia in 2002–2008 were studied. The isolates were selected to represent unique resistance patterns within one ward of a given hospital and were typed by: (i) automated fluorescent multilocus VNTR analysis (MLVA) of 6 loci (SIRU01, -05, -07, -13, -15, -21) described by Ikawaty et al. (2008); (ii) spa sequence typing; and (iii) multiplex PCR SCCmec typing. Assignment and cluster analysis of spa types as well as clustering of MLVA profiles were performed using BioNumerics software, v6.1 (Applied Maths).

Results: Based on results of MLVA typing, MSSA isolates were clustered into 28 distinct groups encompassing 58 types while all but two MRSA isolates were distributed into 17 related types differing at no more than 2 VNTR loci. Spa sequence typing further divided MRSA isolates from major MLVA cluster into two groups: one including t008 (n = 20) and highly related t024 (n = 1) types, and the other including t037 (n = 7), t030 (n = 5) and 6 related (n = 10) types. SCCmec III and SCCmec IV were harbored by 21 and 24 isolates, respectively. All MRSA of spa type t008 had SCCmec IV. Interestingly, this major group of MRSA included isolates originating from 7 different cities and exhibiting variable susceptibilities to ciprofloxacin, gentamicin, erythromycin and clindamycin.

Conclusions: Epidemic spread of MRSA isolates causing nosocomial infections in children in Russia involves a limited number of genetic groups. Of notice, spa type t008, SCCmec type IV reported in the late 1990’s as community acquired MRSA was shown in our study to be predominant among nosocomial pediatric isolates in Russia.

**P1377** Clonal dissemination with particular phenotypes of methicillin-resistant *Staphylococcus aureus* isolates from patients diagnosed with mastitis in Central Taiwan

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Background: Mastitis, frequently seen in puerperal and breast-feeding women, is often caused by *Staphylococcus aureus*. Methicillin-resistant *S. aureus* (MRSA) accounts for 70% to 80% of *S. aureus* isolates in Taiwan.

Objectives: The goals were to delineate the clinical aspects and molecular and phenotypic characters of MRSA isolates that caused mastitis.

Materials and Methods: Demographic data of patients diagnosed of mastitis (ICD:611) from Jan 1st, 2008 to Jun 30th, 2010 were analyzed. *S. aureus* isolates from mastitis patients and environmental sampling were collected from Oct 1st, 2009 to Jun 30th, 2010. Molecular types were assigned with PFGE, SCCmec, MLST, spa, agr, and dru. Phenotypes were assigned with disc diffusion method, MIC to vancomycin (VA) by agar dilution, pvl, and superantigenic toxin profiles by multiplex PCR. M-W/L and X tests were used to test accordingly.

Results: The episodes of total and breast-feeding related mastitis in 30-month period were 240 and 220, respectively. The prevalence of total and MRSA mastitis increased significantly after Sep 2009 (0.95 vs. 1.42 and 0.05 vs. 0.40/1000 patient-day, respectively, p < 0.05; 6.42 vs. 13.01 and 0.32 vs. 3.70/100 delivery, respectively, p < 0.01). All patients were females; with mean age of 30.4; and 98 (40%) with right, 84 (35%) with left, and 18 (8%) with both sides; the mean duration from delivery and onset of symptoms was 25.3 days. Totally 26 isolates (MRSA:25, MSSA:1) from mastitis patient and 15 isolates (MRSA:10, MSSA:5) from sampling of healthcare-givers were collected. All 25 clinical MRSA were typed as SCCmecIV-TS59-spa t437-agr-dr16, 7, 8. Genetic variation was seen in 10 MRSA colonized isolates (each 3 of 6 for SCCmecIV-TS59-spa t437 and spa t529, respectively; 4 isolates for SCCmecIV-TS59-spa t437). All except 2 clinical MRSA were resistant to 3 antibiotics (erythromycin, clindamycin, and tetracycline); all with pvl (+) and VA MIC of 1 μg/ml except 1 (MIC:1.5); and all but one
with seb-selk-selr. Thirteen pulsotypes were assigned, which 5 major types including pulsotype A (15 isolates) belonged to clinical isolates and pulsotype B (2 clinical and 3 screening isolates) were characterized. **Conclusions:** A particular MRSA clone with unique molecular and phenotypic characteristics was identified in mastitis patients in central Taiwan. Horizontal transmission of MRSA between patients and hands of healthcare givers was demonstrated and further control maneuvers should be implemented.

**Background:** Several Department of Health high profile hand hygiene (HH) campaigns have made HH compliance results a required item of trust board, divisional, hospital infection control meetings, and trust induction day. HH audits are conventionally conducted monthly by infection control/link nurses. The results are fed back to the board for discussion of intervention effectiveness and action plans. Historically, compliance rates of above 90% have led to a culture of ignorance. We present an innovative reaudit by junior doctors (JD). The initial 2009 audit reported a 37% (667/1878) compliance in HH throughout the trust. The aim of the reaudit was to assess the effectiveness of the intervention proposed by the divisions, and to provide prospective, unbiased, sizable data on HH compliance in 2010. Secondary outcome was to raise awareness and engage JDs in the hospital hand hygiene program (HHP).

**Methods:** Data from 1840 minutes (92×20-min observational (HHA)) performed by randomly selected 6 JDs across 19 wards. Standard HHA tool (as used by nurses for monthly HHA) was used. Each JD had an audit induction explaining the procedure. The observer selected any clinical areas and observed each area at least 3 times at different times and different days (to eliminate ‘bad-day’ effect). Fly-on-the-wall’ [un-noticed] technique was used during observations (to eliminate observation related change in behavior). Definitions: Opportunity (O) for HH – touching patient or immediate surrounding (bed frame, med equipment, curtains, notes/trolley on bedside, etc). Hand hygiene (H) with alcohol gel or washing-soap & water. HH compliance = (total H ÷ total O) × 100.

**Results:** Overall HH during the 2010 study period was 35% (535/1015). There was no statistical difference between total HH compliance during 2009 and 2010 (p > 0.05).

**Conclusions:** Poor hand hygiene compliance can potentially undermine the hospital HCAI program. This study has revealed a detailed picture regarding hand hygiene compliance. Poor past performance coupled with lack of initiative results in no significant change in HH culture. Recommendations from this audit will be to completely abandon the HH audit and focus on infection intervention and nosocomial hospital infection rates. It is stipulated that outcomes of nosocomial infections rather than audit results will encourage greater compliance rates to HH and other infection interventions measures leading to an environment of accountability and ownership.
Reliability testing of the WHO Hand Hygiene Self-Assessment Framework

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Objectives: The Hand Hygiene Self-Assessment Framework (HHSF) was developed by the WHO 1st Global Patient Safety Challenge as a systematic self-assessment tool to provide a situation analysis of hand hygiene (HH) resources, promotion, and practices within healthcare facilities. It is comprised of a questionnaire with 27 indicators arranged in 5 components. Our aim was to assess the inter-rater reliability of this tool, i.e. the degree to which independent testers within a single facility give consistent answers.

Methods: National or sub-national HH campaign coordinators in 13 countries were each asked to nominate 10 appropriate facilities. In addition, facilities participating in earlier stages of testing of the HHSF were invited. Inclusion criteria were: 1) implementation of a local HH promotional strategy, and 2) the availability of two individuals with good knowledge of the local HH promotional activities and resources. The two testers in each facility were instructed to independently complete the HHSF and to provide basic demographic details. Using the variance components model (with raters nested within hospitals), reliability was estimated by the ratio of (true) between-hospital variance over total variance in ratings. This was performed for each indicator, each component sub-total and the overall score of the HHSF. By convention, results with a reliability <0.4 were considered as having poor reliability and examined individually with regard to the need for modification.

Results: Complete responses were received from 41 facilities in 16 countries, representing all 6 WHO regions. The reliability results for the total score for the HHSF and the sub-total of each of the five components ranged from 0.54 to 0.86. Seven of the 27 indicators had poor reliability, with a percentage true variance <0.4 for either the entire indicator or a part thereof. These indicators were examined by experts for potential sources of unreliability and modified accordingly.

Conclusion: The reliability testing demonstrated that this tool can be confidently used by different infection control professionals within an institution and also drew attention to several indicators with poor reliability that were examined for appropriate modifications. These results support the repeated use of the HHSF to document progress in HH promotion within a facility.

The Feedback Intervention Trial: a randomised controlled trial to improve hand-hygiene compliance in ITUs and acute elderly wards in 16 hospitals

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Objectives: Although randomised controlled trials (RCT) show feedback improves hand hygiene (HH) compliance, implementation of best practice, effects are modest & most trials don’t use psychological theory to design interventions. Systematic review suggests feedback may improve hand hygiene compliance but needs regular repetition with studies being small, short term & poorly designed. We therefore performed a national 3 year RCT of the effect of a psychological-theory based feedback intervention on hand hygiene compliance.

Methods: Stepped Wedge cluster RCT in 60 wards (16 ITUs & 44 acute care of the elderly [ACE] wards) in 16 English/Welsh hospitals (Oct 2006-Dec 2009) NRR Web site N0256159318

The intervention was based on Goal-setting & Control theories & comprised a repeating 4 week cycle (20–30 mins/week) of observation, feedback & action planning, with HCWs & groups, recorded on forms. Randomisation – computer generated step wise entry of hospitals. Primary outcome – observed hand hygiene compliance (%) with blinded observers. Secondary outcome – soap & alcohol hand rub (AHR) procurement (mls/bed day).

Fidelity to intervention (forms used/month) & Confounders (staffing levels, skills mix, agency rates) measured. Mixed effects regression analysis, accounting for confounders & temporal trends.

Results: All 60 wards randomised & analysed; 8 closed during study. 33 wards implemented intervention (11 ITU, 22 ACE).

Intention to treat analysis (ITTs): estimated odds ratio (OR) for compliance rose post-randomisation (1.44; 95% CI 1.18–1.76; p < 0.001) in ITUs but not ACE.

Per protocol analysis (implementing ward): OR for compliance rose for both ACE wards (1.67 [1.28–2.22]; p < 0.001) & ITUs (2.09 [1.55–2.81] p < 0.001), equating to a 10%-13% & a 13%-18% absolute rise in compliance in ITU & ACE wards respectively (Figure). OR for non-implementing wards fell (ACE) or was unchanged (ITU). Fidelity to intervention closely related to compliance on ITUs. OR for compliance 1.12 [1.04, 1.20] p = 0.003 per completed form.

Conclusions: Despite difficulties in implementation, ITT & per protocol analyses showed a feedback intervention based on behavioural theory significantly improved hand hygiene compliance. The effect increased with fidelity to intervention. In any one month, the greater adherence the higher the compliance. The effect was greater on ITU than ACE wards. A study of the barriers & facilitators of implementation is needed to improve this & maximise the intervention’s effect in different settings.
Hand hygiene

Linear correlation between the compliance with hand hygiene and glove using without indication

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Objectives: Although hand hygiene is usually considered as the cornerstone of the prevention of micro-organism transmission, the overall compliance for this practice is often low. According to certain studies, failure to change or remove contaminated gloves could represent an important component in this poor compliance. Therefore, a growing number of experts consider that non-sterile glove usage should be restricted to limited indications. Our object was to study the correlation between the compliance with hand hygiene and glove using without indication.

Methods: The study was conducted in 11 healthcare settings including rehabilitation units, acute geriatric wards, and nursing homes. The compliance with hand hygiene and gloving practices were recorded by observers specifically trained for the study. The observations were performed between the 15th and the 30th October 2010. The compliance for hand hygiene was recorded before and after a unique contact with a patient or its environment, before and after a sequence of successive contacts in the same room, and lastly between contacts in the same sequence. Glove usage was defined as indicated if the healthcare workers had contacts presenting a risk of exposure to body fluids. Otherwise, glove usage was considered as not indicated. The proportion of glove usage without indication was defined as the ratio “number of contacts in which gloves were used without indication/total number of contacts with gloves” (N1/N2). The linear correlation between hand hygiene compliance and N1/N2 was studied with the Pearson’s coefficient (r).

Results: During the study, 848 contacts representing 1252 opportunities of hand hygiene were observed. Gloves were used for 344 (39.2%) contacts. The compliance with hand hygiene varied from 36.5% to 84.7% according to the healthcare settings. The ratio N1/N2 varied from 7.7% to 52.8% according to the healthcare settings. There was a significant linear correlation between the compliance with hand hygiene and the ratio N1/N2 (r = −0.74, P < 0.01), showing a decrease of compliance with increasing N1/N2.

Conclusion: This study confirms the deleterious effects of the glove misuse on the compliance with hand hygiene. Interventions for improvement of glove usage including education, evaluation and feedback should be included in programs of infection control, particularly when they concern hand hygiene.

Sustaining hand-hygiene compliance following implementation of the World Health Organization Hand Hygiene Improvement Strategy in a tertiary hospital in Saudi Arabia

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Objective: King Abdul Aziz Specialist Hospital (KAASH), Taif, Saudi Arabia is a participating hospital in World Health Organization (WHO) SAVE LIVES: Clean Your Hands Initiative. This study aimed to assess hand hygiene compliance in KAASH and determine appropriate strategies for improvement.

Methods: Hand hygiene compliance using the WHO Hand Hygiene Observation Form was assessed in the Intensive Care Unit (ICU), Neonatal ICU (NICU), Kidney Center (KC) and Burns Unit (BU) in KAASH over three study periods in 2010: A hand hygiene intervention campaign was a symposium/workshop which took place on 8th May. Data was collected during the pre-intervention phase (April 24-May 06) and post-intervention phase (May 29-June 09). During the post-intervention period, ongoing intervention measures including feedback, reminders and direct observation were in place. The third period from 01–15 October 2010 was a follow-up phase with no intervention measures.

Results: A total of 1425 hand hygiene observations were carried out and compliance increased from 67.5% pre-intervention to 80.5% post-intervention but was only 59.5% in the follow-up phase. The highest compliance rate was in NICU (from 87.6% to 90.4%) followed by BU (from 70% to 78%) and ICU (from 38.85% to 56.8%) pre to follow-up phase respectively. In the post-intervention period, the improved compliance rate in KC was statistically significant (pre:43.37%; post: 70.8%, p = 0.0001). Nurses showed the highest compliance rate (63% and 73.5%) followed by physicians (56.5% and 67.9%) in pre and post intervention phases respectively. Five months after the intervention, hand hygiene compliance rate remained sustained in the NICU (90.4%) but declined in other units (ICU 53%; BU 78%; KC 36%). The main sources of missed action were before patient contact with/without donning gloves, after touching patient surrounding and before aseptic techniques when they don gloves directly without hand hygiene.

Conclusion: Our preliminary data indicates improved hand hygiene compliance after a using a combination of direct supervision and feedback. To sustain high rates of hand hygiene compliance, ongoing hand hygiene observations with continuous feedback are needed.
After a modification of the currently used alcohol formulation, tolerability and acceptance decreased inadequately at our tertiary care hospital with 700 beds. The aim of this study was to evaluate different hand disinfectants in the hospital daily routine.

**Methods:** Selection of hand disinfectants: The following preconditions for testing had to be fulfilled: Official approval for hand disinfection, effectiveness against norovirus, no colorants or perfume additives. Disinfectants without activity tests against norovirus had to have at least identical concentration of the disinfectant ingredient. After internal evaluation, 4 products (one gel formulation) were selected (Products A-D, concentration of alcohols see table).

Hospital-wide testing: 18 of the 34 wards from medicine, surgical and obstetric departments including intensive care units were chosen to participate. Health care providers were informed in written and oral form. Participation was voluntary.

All 4 selected products were tested non-blinded sequentially for 3 weeks each from July to September 2010. All the dispensers and personally used hand disinfectant bottles were equipped with the test product. After every test phase, an electronically generated questionnaire had to be completed by the test persons. Questions included tolerability, appearance of redness, dryness, effect on skin care and breakoff for any reason.

**Results:** Response rates per disinfectant varied from 225 to 290 providers. No product showed eminent tolerance results, but two products rated clearly better than the others (see table). Among these, product A was equal or slightly superior to product C for all evaluated factors. Product D, a gel formulation, was not accepted with a high break off rate.

**Conclusion:** Even the best-rated product only showed “sufficient” tolerability expressed by dryness, redness, skin care effect and general impression. Tested products showed congruent results for the different evaluated factors.

The hospital-wide aim of a norovirus-effective hand disinfectant is probably associated with reduced tolerability. Therefore, it is important to make available alternative disinfectants in personally used pocket-sized bottles for providers intolerant to the used disinfectant.

**Legend:** HD: alcoholic hand disinfectant; PD: patient-day

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**P1386** Increase of alcoholic hand disinfection performance due to new touchless dispensers

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**Background:** Hand hygiene is considered to be the single most effective tool to prevent healthcare associated infections. Despite several recommendations and efforts undertaken, compliance still remains low. In order to evaluate a new touchless dispenser for hand disinfection (HD) with an alcoholic solution, we conducted an observational study at the University Hospital Aachen, Germany.

**Methods:** Baseline usage of mechanical dispensers with counters were documented on an medical ICU over a 8 weeks time period from June to July, 2010 and compared to data with a new touchless dispenser over a 12 weeks time period from August to October, 2010. In total the ICU was equipped with 28 dispensers in each period. During the study the personnel/patient ratio and the number of patient days (PD) were comparable.

**Results:** The performance with the hygienic HD increased on average by 53% from 34 to 52 HD/PD (see Figure 1). It is of note that the operations next to the patients beds increased from 42 to 82 and on the corridors from 22 to 40 HD/PD, respectively. During the time period the touchless dispensers were used a steady increase of mean HD/PD could be observed.

**Conclusion:** Utilization of a dispenser with a new touchless mechanism for hygienic HD with an alcoholic solution resulted in an increase of 53% from 34 HD/PD to 52 HD/PD) on average. This observation showed at least a sustained effect, since the mean values for HD/PD increased steadily during the 12 weeks study period with the touchless dispenser.

In conclusion, the use of a touchless dispenser might contribute to increase the compliance with hygienic hand disinfection in the setting of an ICU.

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Healthcare workers’ perceptions of a programme inviting patients to report staff hand-hygiene compliance

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Objectives: Patient involvement in hand hygiene (HH) promotion is a novel strategy to prevent healthcare-associated infections. Patients could play a role in improving healthcare workers’ (HCWs) HH practices in two different ways: 1. by participating actively in reminding HCWs to cleanse their hands (Patient participation [PP]); 2. by observing and assessing HCWs’ HH practices (Patient observer [PO]).

Some experts suggest that patients could be invited to prospectively evaluate HCWs’ hand hygiene compliance. However, little is known about HCWs’ views of such strategy.

Methods: Cross-sectional study in 700 randomly selected registered nurses and physicians actively engaged in direct patient care at a public teaching hospital. Respondents were asked about their knowledge and perception on HH and PO. Independent predictors of acceptance and rejection of PO were assessed by multivariable logistic regression.

Results: Response rate was 40.5% (284 respondents: 64%, female; 56.5%, nurses). Overall, 108 respondents (39%) had a favourable opinion of such PO program while 90 (32%) did not, others being neutral. 165 (58.5%) believed that it would help them improve their HH compliance. 93 (33%) feared that, if a patient noticed that they forgot to cleanse their hands, it could interfere in their relationship; moreover 78 (28%) would feel uncomfortable knowing that patients could potentially assess them. Independent factors associated with the acceptance or rejection of PO, adjusted for sex, age, profession, years of work experience and work experience at HUG, are presented in Table.

Conclusion: Although the concept of PO is less appealing to HCWs that PP, more than a third of HCWs are favorable, which is not negligible. This study identifies several potential barriers to the implementation of a PO program that could guide the development of future strategies and highlights the importance of reassuring HCWs upon the implementation of such strategy.

Trends in antimicrobial susceptibility among bacterial isolates from UTI of Japanese hospitals participating in the Levofloxacin Surveillance Group during 1994–2010

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Objectives: Multidrug resistance Gram-negative pathogens are serious problem worldwide. In Japan, the prevalence of FQ resistant Escherichia coli and Proteus mirabilis has been increased from 1994 to 2007 however FQs retained activity for Klebsiella pneumoniae, Serratia marcescens and Acinetobacter species during this period. We present here the nationwide surveillance data from 1994 to 2010 for the above five causative pathogens of UTI.

Methods: A total of 17,086 isolates belonging to 5 species were collected from 92 centers participating in the Levofloxacin Surveillance group in Japan from 1994 to 2010. Antimicrobial susceptibility testing by broth microdilution methods followed the Clinical and Laboratory Standards Institute (CLSI) guidelines. ESBL phenotypic confirmatory testing for E. coli, Klebsiella pneumoniae and P. mirabilis was performed by the disk diffusion method following the CLSI document. ESBL typing was performed by PCR analysis.

Results: In E. coli, the percentage of levofloxacin susceptible isolates decreased from 97.9% (1994) to 71.5% (2010). The susceptibility of P. mirabilis isolates also decreased from 97.9% (1994) to 78.6% (2010). On the other hand, levofloxacin susceptible K. pneumoniae slightly increased from 99.7% (1994) to 98.2% (2010). Acinetobacter spp. susceptibility also slightly decreased from 90.4% (1996) to 86.2% (2010). Interestingly, the percentage of susceptibility to levofloxacin in S. marcescens increased dramatically from 86.5% (1994) to 96.9% (2010). In 2004, 3.3% of E. coli isolates produced ESBL however 8.6% of E. coli produced ESBL in 2007. The frequencies of CTX-M-1 group, CTX-M-2 group and CTX-M-9 in E. coli were 25% (16 isolates), 3.1% (2 isolates) and 64.1% (41 isolates), respectively. Interestingly, 73.4% of ESBL producing isolates show resistance to levofloxacin. The percentage of levofloxacin resistance in ESBL producing E. coli was higher than non ESBL producing E. coli (18.7%). In 2002, levofloxacin resistance percentages in ESBL producing and non ESBL producing E. coli were 15.4% and 8.5%, respectively.

Conclusions: The percent susceptibility of levofloxacin against Gram-negative organisms from UTI was maintained at 70% to 97%. Levofloxacin still retains activity for K. pneumoniae, S. marcescens and Acinetobacter spp. On the other hand, the isolation frequency of ESBL producing E. coli is increasing in Japan and the predominant type of ESBL was CTX-M-type including the CTX-M-9 and CTX-M-1 groups.
Clinical impact of fluoroquinolone resistance in acute pyelonephritis caused by Escherichia coli

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Objectives: Escherichia coli is the most common etiology of uncomplicated urinary tract infection. As fluoroquinolone (FQ) resistance rate of E. coli has been increasing, concern about possible poor outcome due to inappropriate empirical therapy has been arisen. We determined the impact of FQ resistance on the treatment outcome of acute pyelonephritis by E. coli initially treated with FQ.

Methods: From the databases of Korean multicenter surveillance studies for bacteremia and urinary tract infection from 2006 to 2009, a post-hoc analysis was conducted. Cases of community-onset acute pyelonephritis caused by E. coli, initially treated with FQ, were included. Primary outcome was defervescence within 3 days and length of hospital stay of 7 days or less.

Results: A total of 191 cases were included. FQ resistance rate was 21.5%. The mean age (63.0±12.5 vs. 56.8±17.0, P = 0.030) and the proportion of healthcare-associated infection (39.0% vs. 17.3%, P = 0.003) was significantly higher in FQ resistance group. In univariate analysis, the rates of patients achieved defervescence within 3 days did not differ between two groups (80.4% vs. 78.9%, P = 0.839). More patients stayed longer than 7 days in hospital in FQ resistance group (25.3% vs. 43.9%, P = 0.021). In-hospital mortality was observed in only 3 cases, all of which were FQ susceptible. In multivariate analysis, FQ resistance was not associated with fever beyond 3 days or hospital stay longer than 7 days (OR 0.774, 95% CI 0.257–2.328; OR 1.580, 95% CI 0.579–4.315). Severe sepsis was only significant risk factor for longer hospital stay (OR 5.828, 95% CI 1.367–24.860).

Conclusion: Fluoroquinolone resistance did not show any association with fever beyond 3 days or hospital stays longer than 7 days in acute pyelonephritis by E. coli, initially treated with FQ. Severe sepsis was a risk factor for hospital stay longer than 7 days.

Clinical predictors for the acquisition of fluoroquinolone-resistant uropathogens among patients with community-onset complicated acute pyelonephritis

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Objectives: The emergence and spread of fluoroquinolone-resistant Gram-negative uropathogens (FQR-GNUP) is already a serious problem in the community of Korea. The aim of this study is to find out clinical predictors for acquiring FQR-GNUP among patients with community-onset complicated acute pyelonephritis (CAPN) in Korea.

Methods: Clinical data of all APN patients who visited 14 hospitals during 2008 were collected retrospectively. Among them, cases having underlying systemic diseases or urinary conditions were included as CAPN cases. Information on fever duration before visit and histories of previous antibiotics, urinary tract infections (UTIs), and hospitalization was also inputted. Covariates found to be associated with FQR-GNUP on univariate analysis at a level of significance p < 0.2 were eligible for inclusion in a multivariable logistic regression model using a backward selection procedure.

Results: Total 890 cases of CAPN were included for analysis. Gram-negative uropathogens were identified in 573 cases (64%). The median age was 66 years (interquartile range 53–73 years) and 68 (12%) were male. The frequent comorbid conditions were diabetes mellitus (280, 49%), cerebrovascular accident (100, 18%), and malignancy (80, 14%) and commonly combined urinary abnormalities were urinary stone (42, 7%), hydronephrosis (37, 7%), and indwelling catheter (30, 5%). Escherichia coli was the most common pathogen (491, 85.7%), Klebsiella species (36, 6.3%), Pseudomonas species (15, 2.6%), Enterobacter species (9, 1.6%), and Proteus species (8, 1.4%) were also identified. Ciprofloxacin-resistance rate of GNUP was 27.2%. The independent clinical predictors for acquiring FQR-GNUP identified were male sex (hazard ratio [95% confidence interval], 2.14 [1.26–3.64]), intermittent catheterization (3.26 [1.06–10.02]), previous UTIs more than once within 1 year (1.57 [1.03–2.39]).

Conclusion: Male, intermittent catheterization, and UTIs within 1 year were significant clinical predictors for acquiring FQR-GNUP in patients with community-onset CAPN in the country with high rate of antibiotic resistance.
obtained and the patient data was filled at the primary care centers. After incubation and isolation procedure in a research laboratory in the same city, microbiological procedures were done in the central research laboratory.

**Results:** Totally 400 patients were enrolled. In 43.8% patients, urine cultures yielded a urinary pathogen. The most encountered pathogen was *E. coli* (62.8%). Other enteric Gram negatives (5%), CNS (24.5%), other Gram positives (5.7%) and *Candida* spp. (1.7%) were isolated microorganisms. The lowest resistance rates were detected for nitrofurantoin (0.9%) and fosfomycin (3.6%) among *E. coli* isolates. Resistance rates to 3rd generation cephalosporin (3GC) was 31%. It was >20% to both TMP-SMX and quinolones and were significantly higher in 3GC resistant isolates (P = 0.04 and p < 0.001). The MIC levels for ciprofloxacin and levofloxacin were shown in Table 1. When MIC levels were evaluated with respect to the previously detected urinary concentrations of ciprofloxacin and levofloxacin, therapeutic MIC levels were provided in 83.6% and 98.2% of the strains, respectively. Although quinolone resistance was higher in patients with history of antibiotic treatment during last 3 months (p < 0.001), resistance rates did not differ in complicated and uncomplicated cases. Primary care physicians prescribed empiric antimicrobial treatment in 49.5% patients. Levofloxacin (62.6%) or ciprofloxacin (17%) were most frequently chosen antibiotics.

**Conclusions:** Preliminary in vitro data shows that nitrofurantoin or fosfomycin are appropriate choices for community acquired lower urinary tract infections, especially in patients with a history of antimicrobial treatment in the last 3 months. Quinolones especially levofloxacin may be efficacious, because of pharmacokinetic properties, but needs further evaluation on clinical basis. For rational use of antimicrobials in the primary care setting, postgraduate education on diagnosis, resistance and treatment of urinary tract infections is needed in Turkey.

**Table-1:** The MIC distribution of *E. coli* isolates for ciprofloxacin and levofloxacin (n=100)

| MIC (mg/L) | ≤0.25 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | ≥32 |
|-----------|-------|------|-----|---|---|---|---|----|----|-----|
| Ciprofloxacin | 83 | 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Levofloxacin | 80 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**P1395** *The emergence of antimicrobial resistance in various urinary pathogens in Japan*

K. Ishikawa* on behalf of the JSC Surveillance Committee

**Objectives:** An alarming trend of resistance development among common pathogen caused urinary tract infection (UTI) has been observed worldwide. In order to investigate the emergence of antimicrobial resistance in various urinary pathogens, the Japanese Society of Chemotherapy (JSC) established the first nationwide surveillance network in 2008. I demonstrate other data from some international surveillance.

**Methods:** The first survey was conducted during the period from January to June 2008. With the cooperation of 28 medical institutions throughout Japan, a total of 688 strains belonging to six clinically relevant bacterial species were collected from adult patients with well-diagnosed complicated UTIs. The minimum inhibitory concentrations of 39 antibacterial agents were determined according to the Clinical and Laboratory Standards Institute manual.

**Results:** All *E. faecalis* strains were susceptible to ampicillin and vancomycin. Although a majority of them were susceptible to linezolid, 11 strains (7.8%) were found to be intermediate resistant. The proportions of fluoroquinolone-resistant *E. faecalis*, *E. coli*, *P. mirabilis*, and *S. marcescens* strains were 35.7%, 29.3%, 18.3%, and 15.2%, respectively. The proportions of *E. coli*, *P. mirabilis*, *K. pneumoniae*, and *S. marcescens* strains producing extended-spectrum β-lactamase were 5.1%, 11.9%, 0%, and 0%, respectively. The proportions of *P. aeruginosa* strains resistant to carbapenems, aminoglycosides, and fluoroquinolones were 9.2%, 4.4%, and 34.8%, respectively and 2 strains (1.8%) were found to be multidrug resistant. By other three studies, it has been reported that fluoroquinolone-resistant *E. coli* accounted 8% of isolates from patients with uncomplicated UTI in Japan. However, in South Korea, it has been shown that the resistance rate of *E. coli* isolated from acute uncomplicated UTI to ciprofloxacin increased from 15.2% in 2002 to 24.8% in 2009. From European reports, *E. coli* caused uncomplicated UTI showed the highest rate of susceptibility to fosfomycin (98.1%) followed by mecillinam (95.8%), nitrofurantoin (95.2%), and ciprofloxacin (91.8%).

**Conclusions:** Surveillance data of the current antimicrobial agents are essential for the optimal management of patients with UTI. These data will be a useful reference for future periodic surveillance studies, as well as for investigations to control antimicrobial-resistant pathogens.

**P1396** *Urinary pathogens in Greek children: prevalence and antibiotic resistance trends over a 5-year period (2005–2009)*

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**Objectives:** Urinary Tract Infections (UTIs) are a serious cause of morbidity for infants and young children. The aim of this study was to evaluate the isolation rates and trends in antimicrobial resistance for pathogens associated with UTI in Greek children over a 5-year period (2005–2009).

**Methods:** All urine cultures obtained from children referred to the outpatient clinics or hospitalized in the pediatric wards, were reviewed by using WHONET system. Patients hospitalized in surgical wards, oncology and nephrology department, PICU and NICU were excluded from the study. WHONET data were analyzed, considering only the first isolate per patient. Identification of the isolates was performed by classical methods and susceptibility to antimicrobials was tested by the disk diffusion agar method according to the CLSI guidelines. Resistance mechanisms were researched by phenotypic methods.

**Results:** A total of 4,008 isolates associated with UTI were recovered. The isolation rates were as follows: Escherichia coli 65.42%, Proteus spp 12.05%, Klebsiella spp 6.46%, Enterococcus spp 4.64%, Pseudomonas aeruginosa 3.94%, Enterobacter spp 1.97%, Staphylococcus spp 1.67%, Citrobacter spp 0.70%, Serratia spp 0.57%, Candida spp 0.43% and other bacteria 2.15%. During the study period (2005 through 2009) antimicrobial resistance rates for *E. coli* isolates showed a significant increase to ampicillin (41.4% to 58.6%), 3rd generation cephalosporins (2.7% to 8.6%), cotrimoxazole (25.3% to 37.9%), nalidixic acid (4.2% to 10.9%), ciprofloxacin (1.7% to 5.1%) and aminoglycosides (0.8−2.9% to 2.5−8.4%). Similar trends in resistance were observed for Proteus mirabilis to cotrimoxazole (11.2% to 18.4%), nalidixic acid (1% to 6.1%), and less to ampicillin (26.9% to 28.6%). Concerning Klebsiella spp isolates, a random annual variation for ESBL producers was noted (18.6%, 5.7%, 17.8%, 7.0%, 22.2%), with similar resistance rates to aminoglycosides. *P. aeruginosa* strains were found susceptible to antibiotics, except to aminoglycosides (0–2.3% to 0–8.8%). Carbapenemases were not detected among Gram negative isolates. All *E. faecalis* isolates were susceptible to ampicillin and nitrofurantoin. VRE strains were not isolated.

**Conclusion:** *E. coli* was the most frequently isolated pathogen, as expected. The trend of resistance rates for *E. coli* isolates to most antibiotics is upwards. This phenomenon reflects the worrisome increase of resistance rates to commonly used antibiotics in the community.
P1397 The changing pattern of antimicrobial resistance within 42033 Escherichia coli isolates from nosocomial, community, and urology patient-specific urinary tract infections, Dublin, 1999–2009
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Background: Escherichia coli (E.Coli) is the most common uropathogen in community and nosocomial urinary tract infection (UTI). Knowledge of local antimicrobial resistance patterns is essential for evidence based empirical antibiotic prescribing. A cut off point of 20% has been suggested as the level of resistance at which an agent should no longer be used empirically. Antibiotic resistance rates vary depending on whether the sample represents a nosocomial, community acquired or urology patient specific UTI.

Methods: A retrospective analysis of the 42033 E.Coli urine isolates from the eleven-year period 1999 to 2009 in a single Dublin teaching hospital was performed. WHONET software was used to analyse the changing pattern of sensitivity and resistance of E.Coli to commonly used antibiotics over the study period. The origins of the urine samples were stratified into three groups – inpatients with nosocomial UTIs, urines originating from the emergency department and general practice (community UTIs), and UTIs in urology patients.

Results: Ampicillin and trimethoprim were the least active agents against E.Coli with total eleven-year resistance rates of 58.3% and 33.8% respectively. The overall gentamicin resistance rate was 3.4% and is climbing at a rate of 0.7% per year (p < 0.0001). Within the urology patient sample population the rate of resistance was 6.4%. Significant trends of increasing resistance over the eleven-year period were identified for ampicillin, trimethoprin, gentamycin and ciprofloxacin. Significant differences were demonstrated in co-amoxiclav, gentamycin, nitrofurantoin and ciprofloxacin resistance rates depending on the sample origin. Ciprofloxacin resistance approaches 20% in the nosocomial UTI population and approaches 30% in the urology population. It remains reasonable empirical antibiotic choice in this community with eleven-year resistance of 10.6%. UTIs in the urology patient population demonstrate higher antibiotic resistance rates than nosocomial or community UTIs.

Conclusion: E. coli remains the commonest uropathogen in the community and hospital setting with its incidence climbing from 50% to 60% of UTIs over the eleven-year period. Penicillins and trimethoprim no longer represent suitable empirical agents for UTI. Ciprofloxacin resistance in this Dublin based study render it unsuitable empirical therapy for nosocomial UTI and UTI in the urology population. The dramatic eleven-year rate increase in gentamicin resistance is of paramount concern.

P1398 Aetiology and antibiotic susceptibility of urinary tract infections in the elderly: a cohort study
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Objectives: Urinary tract infections (UTIs) are the most common infections in elderly (age >65 years), whether they are community dwelling, live in long term care facilities or are hospitalized. While etiology of UTIs in young and adult population is well known, less data are available on pathogens responsible of UTIs in the elderly.

Methods: Samples were obtained from a cohort of residents in 12 nursing homes in Milan and its province in the 2009. Only a single positive culture per patient was considered in the analysis, unless new pathogens from the same patient were isolated. Pathogens were identified by Gram stain and standard biochemical procedures. Antibacterial susceptibility testing was carried out by using the disk diffusion method as described by the Clinical Laboratory Standard Institute.

Results: Mean age of patients with diagnosed UTIs was 86±8 years (median 86 years): 71.5% were females and 28.5% males. Of 472 samples 328 resulted positive. Polymicrobial infection was diagnosed in 20.1% of samples. A total of 392 microorganisms were isolated: Escherichia coli was the most prevalent (45.1%), followed by Proteus mirabilis (20.7%), Pseudomonas spp. (9.18%), Klebsiella spp. (6.38%), and Enterococci (4.34%). Enterococci were the most frequently isolated Gram positives (7.14%) while staphylococci accounted for 2.8% of cases. Almost all Enterobacteriaceae were susceptible to nitrofurantoin (97.6%), carbapenems (97.2%) and amikacin (93.3%). Limited activity was observed for fluoroquinolones (41.3%), tetracycline (34.5%) and ampicillin (16.5%). ESBL were detected in 42.1% of isolates. The most active antibiotics against P. aeruginosa were colistin (100% susceptibility), amikacin and piperacillin/tazobactam (83.3%). Fluoroquinolones resulted active against 66.7% of P. aeruginosa. All Gram positives were susceptible to glycopeptides and linezolid, 90% of them were susceptible to nitrofurantoin, while only 35% of isolates was susceptible to levofloxacin. β-lactamas production and methicillin resistance occurred in 60% and 41.7% of staphylococci, respectively.

Conclusion: E. coli remains the major responsible of UTIs in elderly people, although at a lesser extent than in younger population. Polymicrobial infections were less frequent than reported in similar studies (about 30%). Antimicrobial resistance was quite spread, thus reducing therapeutic options. A careful use of antibiotics is advisable to limit further development of resistance.

P1399 The influence of menopause on the species and distribution of causative bacteria isolated from patients with acute uncomplicated cystitis and on the sensitivity to antibacterial agents
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Objectives: The influence of menopause on the species and distribution of causative bacteria isolated from patients with acute uncomplicated cystitis and on the sensitivity to antibacterial agents were studied. Risk factors for the detection of quinolone-resistant Escherichia coli were also investigated, because its frequency has increased and it is now a clinical problem in Japan.

Methods: The subjects were patients with acute uncomplicated cystitis who presented with urinary tract symptoms from 294 institutions in Japan between January and December 2008. At admission, patient characteristics (age, frequency of cystitis, history of antibacterial medication, etc.) were investigated and bacterial cultures were performed using urine samples by the dip slide method. Also, the MIC values of levofloxacin (LVFX), sitafloxacin (STFX), ciprofloxacin (CPFX), tigecycline (TLFX), cefepime/pivoxil (CFTP) and cefdinir (CFTD) were measured. The sensitivity to each antibacterial agents was calculated using the breakpoints specified by CLSI.

Results: 990 patients with acute uncomplicated cystitis were entered in this study. They included 489 premenopausal patients (mean age: 32 years) and 501 postmenopausal patients (mean age: 69 years). The major causative bacteria were E. coli, Enterococcus faecalis, and Streptococcus agalactiae, and the detection rates in premenopausal patients were 65.0%, 12.0% and 5.5%, respectively, while the detection rates in postmenopausal patients were 61.5%; 13.7% and 4.0%, respectively. Species and the distribution were the same regardless of the presence of menopause. MIC50, MIC90 and sensitivity to six antibacterial agents are shown in Table 1. Sensitivities to quinolones were significantly decreased in postmenopausal patients. As risk factors for detecting quinolone-resistant E. coli (MIC of LVFX ≥4µg/mL), significant differences were observed in patients with more than two episodes of cystitis within a year in whom quinolones were ineffective against cystitis, and who had a history of quinolones administration within 1 month.

Conclusion: Although species and distribution of causative bacteria of acute uncomplicated cystitis were the same regardless of the presence of menopause, sensitivities to quinolones against E. coli detected in postmenopausal patients were significantly decreased. The major factors

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were a history of quinolone administration and morbidity of cystitis rather than menopause.

**Table 1. Distribution of minimal inhibitory concentrations (MICs) of 345 urogenital causative bacteria**

| Bacterial strain | Minimal inhibitory concentrations (MICs) | Antibacterial agents | Sensitivity |
|------------------|------------------------------------------|---------------------|-------------|
|                  | (μg/ml) | (μg/ml) | (μg/ml) | (μg/ml) | (μg/ml) | (μg/ml) | (μg/ml) | (μg/ml) | (μg/ml) |
| *Escherichia coli* |  | | | | | | | | |
| Fluoroquinolones (412) | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 |
| Postfluoroquinolones (442) | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 |
| *Enterococcus faecalis* | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 |
| Fluoroquinolones (50) | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 |
| Postfluoroquinolones (54) | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 |
| *Proteus mirabilis* | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 |
| Fluoroquinolones (35) | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 |
| Postfluoroquinolones (26) | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 |

**Conclusions:** In the euregion Meuse-Rhine, there are regional differences in resistance for *E. coli*. Resistance was highest for most antibiotics in the Belgian part of the euregion. This may be caused by higher use of antibiotics in Belgium, which would be in line with the generally accepted belief, that high use is a risk factor for the emergence of resistance. On the other hand, the prevalence of ESBLs was highest in the German part. Carbapenemases were not demonstrated.

**Figure 1:** Antimicrobial resistance. *significant difference. SXT = trimethoprim-sulfamethoxazole, AMC = amoxicillin-clavulanate, CXM = cefuroxime, CAZ = ceftazidime, NOR = norfloxacin.

**How do we manage pre-operative quantified bacteriuria by antimicrobial administration?**

**Objective:** Bacteriuria is common in patients with complicated urinary tract condition. If such patients undergo operation without any preoperative antimicrobial chemotherapy, postsurgical infection can occur due to dissemination of bacteria. We retrospectively examined the association between preoperative bacteriuria and postsurgical infection in patients with urological surgery.

**Methods:** Medical charts were reviewed from April 2007 to December 2009, and 413 patients who underwent urological clean-contaminated surgery and were followed for at least one month were enrolled in this study. The protocol for surgical antimicrobial prophylaxis in our department is follows: a single antimicrobial agent is administered just before the start of transurethral surgery, and from just before surgery up to 48 hours after open surgery. The decision for preoperative antimicrobial administration for patients with bacteriuria depends on the results of culture and antimicrobial sensitivity tests.

**Results:** Of 413 patients, transurethral surgery was performed in 211 and open and laparoscopic surgeries in 204. Thirty-eight of the 413 patients developed infections within one month after surgery. There was no significant difference in the frequency of postsurgical infectious disease between patients with preoperative bacteriuria of $\geq 10^{4}$ colony-forming units per milliliter (CFU/ml) and those without preoperative bacteriuria (p=0.924). In the patients with preoperative bacteriuria of $\leq 10^{3}$ CFU/ml, there was no significant difference in the frequency of postsurgical infection whether or not there was preoperative antimicrobial chemotherapy (p=0.958). On multivariate analysis, preoperative antimicrobial administration and the existence of preoperative pyuria were not associated with the development of postsurgical infection in the patients with $\leq 10^{3}$ CFU/ml (p=0.958).

**Conclusions:** In this study, the frequency of postsurgical infectious disease was almost the same in the antimicrobial-treated patients with

**Antimicrobial resistance of *Escherichia coli* isolates collected from eight urology services in the EU region Meuse-Rhine, 2009–2010**

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**Objectives:** In this study the regional differences in antibiotic resistance of *Escherichia coli* isolated from eight urology services in the euregion Meuse-Rhine were assessed. This region covers parts of three countries i.e. Limburg province in Belgium and the Netherlands, and Aachen region in Germany. Differences in resistance pose a risk to cross-border patient mobility and safety. Therefore, a current overview of resistance is needed, which may enable adaptation of treatment protocols.

**Methods:** *E. coli* isolates were collected from urology services in hospitals in the euregion; two in Belgium and three in both, Germany and the Netherlands. Quantitative susceptibility testing was performed with the micro-broth dilution method. Susceptibility breakpoint were defined by the European Committee on Antimicrobial Susceptibility Testing. Putative extended β-lactamase (ESBL) producing isolates (minimal inhibitory concentration for ceftazidime or cefotaxime $>1$ mg/L) were tested with a combination disk diffusion test to confirm ESBL production. Multidrug resistance was defined as resistance to three or more classes of antibiotics.

**Results:** A total of 360 isolates were collected: 65, 119 and 176 from the Belgian, German and Dutch urology services, respectively. Resistance to trimethoprim-sulfamethoxazole, amoxicillin-clavulanate, cefuroxime, ceftazidime and norfloxacin are shown in Figure 1. Resistance to piperacillin-tazobactam varied from 1% in Germany to 6% in Belgium and 6% in the Netherlands and 6% in Belgium (p=0.03). In contrast with resistance to ceftazidime, the prevalence of ESBLs was highest in Germany (8%) compared with 2% in the Netherlands (p=0.01) and 5% in Belgium. Resistance to carbapenems was not detected. Multidrug resistance ranged from 11% in the Netherlands to 17% in Germany and 23% in Belgium (p=0.02).

**Conclusions:** In the euregion Meuse-Rhine, there are regional differences in resistance for *E. coli*. Resistance was highest for most antibiotics in the Belgian part of the euregion. This may be caused by higher use of antibiotics in Belgium, which would be in line with the generally accepted belief, that high use is a risk factor for the emergence of resistance. On the other hand, the prevalence of ESBLs was highest in the German part. Carbapenemases were not demonstrated.

**Figure 1:** Antimicrobial resistance. *significant difference. SXT = trimethoprim-sulfamethoxazole, AMC = amoxicillin-clavulanate, CXM = cefuroxime, CAZ = ceftazidime, NOR = norfloxacin.

**P1400** Antimicrobial resistance of *Escherichia coli* isolates collected from eight urology services in the EU region Meuse-Rhine, 2009–2010
significant preoperative bacteriuria ($\geq 10^7$ CFU/ml) and those without preoperative bacteriuria. If the patients with significant preoperative bacteriuria undergo a clean-contaminated operation, preoperative antimicrobial chemotherapy contributes to control post-surgical infection. When the patients with preoperative urine $\leq 10^7$ CFU/ml undergo operation, preoperative antimicrobial chemotherapy is not necessary for prevention of postsurgical infection.

**A randomised clinical trial to evaluate the preventive effect of cranberry juice (UR65) for patients with recurrent urinary tract infection**

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**Objectives:** The purpose of this trial was to examine the relapse rate of urinary tract infection (UTI) in the patients who suffered recurrent cystitis when cranberry juice (UR65) or placebo beverage was taken continuously for maximum 24 weeks.

**Methods:** The study design was a randomized, placebo-controlled, double-blind clinical trial. Outpatients aged 20 to 79 year-old with aggravation of acute uncomplicated cystitis or chronic complicated cystitis (including self-catheterization) who have a past history of cystitis and been confirmed the healing by antimicrobial chemotherpayy were included in this study. We defined the group that women aged 25 to 29 or 55 to 69 year-old who suffered relapses and healed after medical treatment before the registration of the study as the ideal target population (ITP). The participants were randomly assigned to cranberry juice (UR65) group (CBJ group) or placebo beverage group (PB group). They took in one bottle of 125ml cranberry juice (UR65) in CBJ group and 125ml placebo beverage adjusted color and taste to cranberry juice in PB group. They commonly drink it once daily before sleep for maximum 24 weeks. The relapse of cystitis was the primary analysis target for this study. Therefore, the study in each participant discontinued when the findings of cystitis were obtained microbiologically and antimicrobial agents were administered. This trial was approved by IRB and written informed consent was obtained from the participants.

**Results:** The duration of this study was from October 2007 to December 2009. Two hundred and thirty seven participants were registered in this trial and 227 could be analyzed as intention-to-treat finally. The overall ITT analysis on relapse of cystitis was not significantly difference between those 2 groups (p = 0.430, Log-Rank test). In the subset analysis of ITP group with continuous intake of more than 8weeks, there was significant difference between those 2 groups (p = 0.030, Log-Rank test) and the relapse rate of cystitis in CBJ group was lower than that in PB group.

**Conclusion:** The results of this study clearly showed that cranberry juice could prevent recurrent cystitis significantly in ITP if intake of cranberry juice could be tolerable more than 8weeks. The prevention of recurrent cystitis without using additional antimicrobial agents contributes to not only the patients distressed by recurrent cystitis but also avoiding unnecessary antimicrobial chemotherapy.

**Diagnosis of syphilis**

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**Introduction:** The diagnosis for *T. pallidum* infections is based on treponemal and non-treponemal serologic tests. Non-treponemal tests are inexpensive and easy to perform, and allow evaluating treatment response. False-positive results can be found in some situations (pregnancy, autoimmune diseases), thus treponemal-based confirmation is required. Recently, newer treponemal-based EIAs and chemoluminiscence immunoassays (CIA) have been released. These tests can be automated, and have been reported to be highly sensitive and specific. Nevertheless, the interpretation of low positive CIA results, with non-treponemal test negative, may be doubtful. The aim of this study was to compare the results obtained with one of these CIAs with FTA-ABS and a line immunoassay (LIA). RPR was also determined for all the samples studied.

**Methods:** We studied serum samples from 36 patients positive in CIA (Syphilis TP, Abbott, Diagnostic Division) (CIA). These serum samples were therefore studied by RPR (bioMerieux, France), by a classical treponemal test (FTA abs) (Trepo-Spot IF, BioMerieux), and by a LIA (INNO-LIATM Syphilis Score, Innogenetics, Belgium) (LIA). Both the CIA and the LIA detect antibodies against the same three immunodominant bacterial proteins (TpN15, TpN17, TpN47). The LIA also detect antibodies against one synthetic peptide (TmPA) derived from transmembrane protein A. CIA and LIA positivity was considered according manufacturers instructions. FTA-ABS positivity was scored as +, ++ or +++ according fluorescence intensity.

**Results:** The 36 samples positive in the CIA had been obtained from 19 men, 16 women (10 of them pregnant), and one sample was fetal blood from a fetus whose mother had been positive. Results obtained appear in Table 1. Among the 10 samples belonging to pregnant women and positive in CIA, six were positive in LIA and FTA-Abs, 2 were indeterminate in LIA and negative in FTA-Ab and 2 were negative
both in LIA and FTA. Eight samples were positive in RPR. Seven were positive in the three treponemal tests. One sample, positive in RPR only in undiluted serum, was positive in CIA, but negative in LIA and FTA-Abs.

**Conclusions:** CIA are excellent alternative to non-treponemal assays as screening tests, but are less specific than other treponemal tests. FTA-Abs or LIA tests may be recommendable for CIA positive confirmation, especially in some patients groups, such as pregnant women, in which CIA false positive results seem to be more frequent.

### Table 1. Results with LIA and FTA-Abs in 36 CIA-positive samples

| No. samples | CIA | LIA | FTA-Abs |
|-------------|-----|-----|---------|
| 26          | +   | +   | +       |
| 4           | +   | indeterminate | -       |
| 2           | +   | indeterminate | -       |
| 9           | +   | -   | -       |

**Bioplex 2200, a new treponemal assay to diagnose syphilis**

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**Objectives:** The diagnosis algorithm of syphilis has been changed recently due to the increasing number of samples. The samples are first tested by a specific treponemal assay and the positive samples are tested further by RPR or VDRL test, to establish the stage of disease. Recently a new multiplex flow immunoassay (MFI) has been evaluated for the diagnosis of syphilis, including IgG and IgM. We assess the performance of BioPlex 2200 Syphilis multiplex assay (Bio-Rad Laboratories, Hercules, California, EEUU) compared with a treponemal assay.

**Methods:** We recovered 153 serum samples belonged to 105 patients, 112 positive by a ELISA treponemal assay test (Eitmak Treponemal assay – DiaSorin, Italy) and 41 negative samples as control. All the samples had non-treponemal assay (RPR) performed. During the study all the samples were tested by the BioPlex 2200 Syphilis IgM and IgG assays. Kits uses different population of microspheres coated with recombinant proteins from *T. pallidum* Nichols strain (flagellar polypeptides TpN15, TpN17 and TpN47 for IgG and TpN17 and TpN47 for IgM).

**Results:** We grouped the samples according to the previous results in 3 groups: Group 1 – 41 samples with negative treponemal and RPR assay; Group 2 – 105 samples with positive treponemal and RPR assay and Group 3 – 7 samples with positive treponemal assay and negative RPR assay (with uncertain interpretation).

For the IgG assay the BioPlex MFI show a sensibility (S) and specificity (SP) of 100%. We could not determined the S and SP for IgM because the lack of ELISA comparative assay and lack of clinical data (Table 1).

**Conclusion:** BioPlex IgG MFI is a rapid and sensitive and specific assay to diagnosis of Syphilis compared with a reference technique. It provides additional information through the capability to analyze specific polypeptides that could be a tool to evaluate evolution of the disease. TpN 17 is responsible of the specificity of IgG assay and TpN 47 of the IgM assay.

**Evaluation of the Liaison® Treponema screen assay compared to ELISA and CLIA assays**

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**Objectives:** Syphilis is usually transmitted by sexual contact and rarely by congenital transmission or blood transfusion, and is caused by systemic infection with the bacterium *Treponema pallidum*. Syphilis can be diagnosed by different serological laboratory tests, which are also useful in establishing the disease stage, in conjunction with other clinical tests. Serological diagnosis is generally established using a standard non-treponemal antibody screening test, the Venereal Disease Research Laboratory test (VDRL) or the Rapid Plasma Reagin test (RPR) are most commonly used, in combination with a specific treponemal antibody test such as the *Treponema pallidum* haemagglutination test (TPHA) for confirmation. The aim of the study was to evaluate the performance of the LIAISON® Treponema screen assay on an automated random access platform.

**Methods:** 249 sera samples previously collected from five hospital and community labs were used. Three assays were compared for treponema total antibody detection. ELISA (Trinity, biotech) was tested on the ETI-Max 3000 analyzer. CLIA (chemiluminescent immunoassay) was tested on the Architect (Abbott) and on the LIAISON® (DiaSorin). Positive and discrepant results were confirmed at the National Reference Center for Syphilis, Ministry of Health Public Health Laboratory. Confirmation was triple checked with TPHA (Axis-Shield), fluorescence treponemal antibody absorption test (FTA-Abs, Bioméreux) and VDRL test (BBL).

**Results:** 150 samples were positive with all three assays and 94 were consensus negative for treponema antibodies. Two discrepant samples were positive by the Reference Center TPHA only, rendering a false positive result for 2 assays. One discrepant sample was negative by all reference methods, rendering a false positive result for 1 assay. Two more samples had discrepant results by all comparison and reference methods. Total agreement between the 2 CLIA was 99.2%, positive agreement 99.3% and negative agreement 98.9%. Total agreement between the LIAISON® CLIA and ELISA was 99.6%, positive agreement 99.4% and negative agreement 100%.

**Conclusion:** The DiaSorin Liaison® Treponema screen assay exhibited very good performance and was found to be suitable for random access, relatively rapid detection of antibodies to *Treponema pallidum*.

**Evaluation of two automated chemiluminescence immunoassays, the Liaison Treponema screen and the Architect Syphilis TP, as screening tests for syphilis**

N. Wellinghausen*( Ravensburg, DE)

**Objectives:** The aim of this study was to evaluate two automated chemiluminescence immunoassays (CLIA), the LIAISON Treponema Screen (LIA) and the ARCHITECT Syphilis TP (ARCH), as screening test for the diagnosis of syphilis.

**Methods:** In a prospective study 577 unselected sera submitted to our laboratory for syphilis screening, including 318 samples from pregnant women, were investigated by LIA (n = 577) and ARCH (n = 571) in comparison to a *Treponema pallidum* particle agglutination (TPPA) screen algorithm. A serum was defined as true antitreponemal antibody positive by a TPPA titer of ≥ 1:80 confirmed by fluorescent treponemal antibody absorption test (FTA-ABS), and/or recombinant
IgG avidity maturation with Liaison® IgG avidity II assay for the diagnosis of toxoplasmosis in pregnant women

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Objectives: The aim of the study was to evaluate the performance of LIAISON® Toxo IgG Avidity II kit immunoglobulin G avidity for estimating the date of toxoplasmic infection in pregnancy.

Method: LIAISON® Toxo IgG Avidity II is a chemiluminescent immunoassay for the detection of antigen-binding avidity of IgG antibodies to T. gondii antigens. Subjects were divided in 3 groups according to their clinical settings.

Group 1: Twenty pregnant women with acute infection (74 samples) treated and followed in our clinic.

Group 2: Thirty four pregnant women with infection more than 4 months determined either on the basis of kinetic of antibodies or history of positive antitoxoplasma antibody test.

Group 3: Sixty five pregnant women with a history of positive serology more than 1 year before sampling.

Results: Group 1: Eighteen samples were excluded because of negative or low IgG titers. Among the 56 tested sera, high avidity was observed only in 2 cases, 32 weeks after estimated date of infection. Five and 2 samples withdrawn more than 32 weeks after infections displayed respectively low and intermediate index.

Group 2: Thirty nine sera (66%) displayed high avidity. Eight and 7 respectively low and intermediate index.

Group 3: Avidity was performed on 55 sera. Two had an intermediate index despite a positive serum respectively 3 and 8 years before sampling.

Conclusion: In treated pregnant women avidity maturation is often delayed. Low avidity index can persist at least 32 weeks post infection. In untreated women, low avidity can be observed event more 4 months after infection and more than 1 year after infection in some rare cases. Only high avidity index can rule out a recent toxoplasmic infection. Although useful for estimating the date of maternal infection and estimating the risk of foetal contamination, avidity interpretation is limited by slow maturation even in non treated pregnant women. Additionally, even in chronic infection, low IgG titres should be interpreted carefully.

Performance of two rapid tests for the diagnosis of malaria imported in travellers: influence of anti-malarial prophylaxis

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Objectives: Rapid tests are a rapid and accurate tool for the diagnosis of malaria. However, factors as low parasitemia presented in subject who had followed anti-malarial prophylaxis may compromise its performance. Our study aims to evaluate the effectiveness of two rapid diagnostic tests (SD BIOLINE Malaria Antigen Pf./Pv. and BinaxNOW® Malaria) in this clinical setting.

Methods: From July to November 2010, blood samples from patients with clinical suspicion of malaria remitted from the emergency department of our Hospital were studied. Samples were tested by thin and thick smear and in-house real-time PCR. In addition, SD BIOLINE Malaria Antigen Pf./Pv., a rapid test for the detection of a specific protein to Plasmodium falciparum and a specific protein to P. vivax; and BinaxNOW® Malaria, a rapid test for the detection of a specific protein to P. falciparum and a pan-specific protein were performed. Real-time PCR was considered as the gold standard. Data from anti-malarial prophylaxis was recorded.

Results: A total of 52 blood samples corresponding to 52 patients were included. Thirty-three patients were positive for P. falciparum. Seventeen of these subjects had taken anti-malarial prophylaxis and the remaining had not followed prophylaxis. The overall sensitivity and specificity of both rapid tests were 100% to diagnose P. falciparum. In contrast, overall sensitivity of blood films was 60.6% and specificity of 94.7%. According to prophylaxis, sensitivity of blood films was 35.3% in subjects who underwent prophylaxis and 87.5% in subjects with no anti-malarial prophylaxis.

Conclusion: The two rapid tests showed a high sensitivity and specificity for diagnosis of malaria. In contrast, accuracy of smear blood films may be compromised in travellers who underwent prophylaxis due to low parasitemia and distorted parasites. Because PCR is not a simple procedure, rapid test can be useful in diagnosis malaria, notably in travellers with no underwent correct prophylaxis.
Detection of malaria infection in blood transfusion: a comparative study among real-time PCR, rapid diagnostic test and microscopy

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The transmission of malaria by blood transfusion was one of the first transfusion-transmitted infection recorded in the world. Transmission-transmitted malaria may lead to serious problems because infection with *Plasmodium falciparum* may cause rapidly fatal death. This study aimed to compare real-time PCR with Rapid Diagnostic Test (RDT) and light microscopy for the detection of *Plasmodium* spp in blood transfusion, both in endemic and non-endemic areas of malaria disease in Iran. Two sets of 50 blood samples were randomly collected. One set was taken from blood samples donated in blood bank of Bandar Abbas, a city located in a malarious endemic area, and the other set from Tehran, a non-endemic one. Light microscopic examination on both thin and thick smears, RDTs, and real-time PCR were performed on the blood samples and the results were compared. Thin and thick light microscopic examinations of all samples as well as RDT results were negative for *Plasmodium* spp. Two blood samples from endemic area were positive only with real-time PCR. It seems that real-time PCR as a highly sensitive method can be helpful for the confirmation of malaria infection in different units of blood transfusion organization especially in malaria endemic areas where the majority of donors may be potentially infected with malaria parasites.

Feconomics®, a simple, novel and fast technique for stool concentration in the parasitology laboratory

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**Objectives:** We have developed a simple technique and turned it into a ready-to-use kit named as “Feconomics®” for fecal concentration. Feconomics® eliminates the need for centrifugation and floation by using absorbent beads that help homogenization and concentration of the sample. These beads have pores smaller than parasite eggs and trophozoites; while the liquid is absorbed, the parasites are concentrated in a smaller volume. For application, 1–2 cm of stool samples are put into the sample cup of Feconomics® that contain sodium acetate, acetic acid and formaldehyde (SAF) solution. The absorbent beads are then poured into the cup and shaken with the sample to homogenize. The procedure takes five minutes including staining the sample with iodine.

**Methods:** To assess the efficacy of Feconomics® in the diagnosis of intestinal parasites, a comparative, double-blind study was conducted in the Parasitology Laboratory of Celal Bayar University Medical Faculty in Manisa, Turkey. Stool samples of individuals (Group I, n = 251) submitted for routine ova and parasite examination were concentrated both with routine formalin ethyl acetate concentration technique (FEAC) and Feconomics®. Intestinal parasites obtained from animal models in the laboratory (such as *Trichostrongylus*, *Hymenolepis nana*) as well as pre-diagnosed parasite-positive samples (Group II, n = 11) were also used. The iodine-stained samples were read by different microscopists and some positive samples were stained with Gomori’s trichrome and Kinyoun’s acid fast.

**Results:** In Group I, 103 of 251 samples were positive for intestinal parasites; seventy six (74%) and 97 (94%) samples were positive with FEAC and Feconomics®, respectively. While 6 FEAC-positive samples were negative with Feconomics®, 27 Feconomics®-positive samples were negative with FEAC. In Group II, the microscopic examination revealed the same results for both techniques. There was no difference between the techniques for the morphological integrity and visual appearances of the parasites. Indeed, trophozoites of *Giardia lambia* and *Entamoeba histolytica* were only identified with Feconomics® after staining with Gomori’s trichrome.

**Conclusion:** As more parasites were identified in Group I and morphological integrity of the parasites were well-protected with Feconomics®, it may be suggested as a fast and effective fecal concentration method for routine laboratories.

Reliability of immunodiagnostic tests for diagnosis of alveolar echinococcosis

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**Objectives:** Diagnosis of alveolar echinococcosis (AE) is primarily based on imaging techniques in addition to clinical criteria. These imaging techniques are useful and reasonably accurate but have sometimes limitations in the small size of visualized lesions or atypical images. Moreover, these techniques are too expensive and inaccessible in most areas where AE is endemic. Therefore the development of immunodiagnostic tests that detect species-specific antibodies or antigens in patient specimens is urgently required because of its simplicity and reliance. Our aim is to investigate the accuracy of diagnosis of AE using different serologic tests, and to use in the follow up of patients who have RO resection.

**Method:** We investigated 39 patients who diagnosed AE. The diagnosis of AE was based on history, imaging techniques and confirmed by histopathological examination. Patients were divided into three groups. Group 1 (n = 16) inoperable patients, Group 2 (n = 11) performed RO resection. From all of the patients venous blood samples were taken for serologic diagnosis of AE. The serum samples used for assessing diagnostic sensitivities of ELISA and western blot methods. We used two different commercially tests for the serodiagnosis of AE in humans (Em2plus Elisa; Border Affinity Products, Switzerland) and echinococcus western blot IgG (LDBIO Diagnostics, France). We investigated three different markers including Em2plus, Em16−18 and Em12.

**Results:** We found that sensitivity for serologic diagnosis of AE was 88% (Em2plus), 94% (Em16−18), and 69% (Em12) in group 1, 55% (Em2plus), 27% (Em16−18) and 18% (Em12) in group 2, 75% (Em2plus), 100% (Em16−18), 75% (Em12) in group 3 respectively. The serologic test of Em16−18 was found most sensitive test in group 1 and group 3.

**Conclusion:** In conclusion, the serologic diagnostic tests are easily performed, and cheap diagnostic tools are used in the diagnosis of the AE. In our study 16–18 bands shown using western blot method, are determined to be the most reliable test in the diagnosis of AE for groups 1 and 3. According to our results in group 2, these tests can be used as a marker in the following criteria.

Comparison of two methods for detection and quantification of leptospires in tissue samples: immunofluorescence-based leptospiral detection in imprint samples versus real-time PCR

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**Objectives:** To compare two methods for detection and quantification of leptospires in tissue samples. We compared immunofluorescence-based leptospiral detection in imprint samples (ILDIS), as previously described by our group, with the now widely used real time PCR (qPCR) targeting the *L. interrogans* 16S rRNA gene, which is highly conserved among pathogenic leptospires. The units are expressed as leptospires counted in ten high power fields (400x) for ILDIS and gene copies per gram for qPCR. Eleven hamsters and fifteen mice (strain A) were experimentally infected by an intraperitoneal inoculum of 500, 10⁴ or 10⁵ leptospires. The strain used in all assays was *Leptospira interrogans* serogroup Icterohaemorrhagiae Cep strain. Hamsters were necropsied eight days after infection (moribund acute phase disease) and mice were necropsied at day 28 (mirroring the chronic carrier state). Kidneys samples were obtained for the comparison of the two methods.
Results: All hamsters and mice gave positive ILDIS and qPCR results. For hamsters, the mean values of quantification (standard deviation in parenthesis) were 152 (92) leptospires by ILDIS and 21,699 (15,867) copies per gram by qPCR (spearman’s correlation, rs = 0.65, p < 0.02). For mice, the mean values of quantification were 228 (253) leptospires by ILDIS and 67,123 (167,047) copies per gram by qPCR (rs = 0.54, p < 0.03).

Conclusions: In both cases of acute and chronic infection, the correlation between both methods was strong. ILDIS is a method of detection which is cheaper, easier to put into practice in the laboratory, and that allows the morphological identification of leptospires, thus implying their viability. ILDIS should be regarded as an option for detection and quantification of leptospires particularly when the use of molecular biology is not feasible.

Serological diagnosis of infections

P1414 Performance of new ELISA IgG anti-Aspergillus (Platelial Bio-rad) using recombinant proteins

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Introduction: Aspergillus can cause several types of disease, which depends, in large part, on the underlying immune function of the host. Aspergillus fumigatus is the most commonly isolated. In the immunocompetent host, the diagnostic of aspergillosis is based on the presence of specific anti-Aspergillus antibodies (mostly par ELISA test). The detection of precipitins is confirmed by immunoelectrophoresis (IEP) or other immunodiffusion tests (ID).

Objectives: In this study, the performance of the new ELISA Aspergillus IgG (Bio-Rad, Marnes la Coquete, France), using recombinant proteins, was compared to that of 2 other ELISA: Aspergillus fumigatus IgG (IBL, Hamburg, Germany) and Aspergillus fumigatus IgG (Virion, Würzburg, Germany) and referred to IEP.

Methods: 306 unsellected sera and 108 sera with different IE results were tested with the 3 ELISA tests on ETIMAX (Diasorin). IEP was performed on all samples using aspergillus fumigatus antigen (somatic and metabolic) and catalasic activity was measured. The presence of 3 or more precipitins indicates an evolutive aspergillosis.

Results: The IBL, PLATELIA and VIRION have an overall agreement of 67%, 94% and 86% respectively versus IEP (equivocal results are excluded). The sensitivity of the IBL, PLATELIA and VIRION is respectively 80%, 92.7% and 78%. And the specificity is 63%, 93.8% and 88% for IBL, PLATELIA and VIRION respectively. In addition, the PLATELIA index versus numbers of precipitins (IEP) showed a poor correlation coefficient <50%.

Conclusion: The ELISA PLATELIA Aspergillus IgG is the first test using recombinant proteins. According to IEP results, it is the most sensitive (92.7%) and specific (93.8). As the ELISA is a semi quantitative essay, it was not possible to establish a link between the ELISA ratio and the number of precipitins in IEP. The PLATELIA Aspergillus is an excellent test for detection of antibodies to aspergillus, but does not take the place of IEP test for the evaluation of the extension of aspergillosis.

P1416 Evaluation of a new immunochromatographic assay for the detection of antibodies against Franciscella tularensis

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Objectives: To evaluate a new immunochromatographic test for the qualitative detection of total antibodies against Franciscella tularensis in both serum and plasma samples.

Methods: A new immunochromatographic test (ICA), VIRapid Tularemia has been designed for the qualitative detection of anti-Franciscella tularensis antibodies in both serum and plasma samples. This test is based on lipopolysaccharide (LPS), obtained from F. tularensis (NCTC10857 strain) grown in Müller Hinton liquid medium and extracted by the hot phenol-water method. LPS was both adsorbed on the conjugate and test line to generate a lateral flow ICA. A control line was also included to check the correct performance of the test. Results were read out after incubation for 15 minutes at room temperature (see attached figure). The assay performance was evaluated with 247 human serum samples; 134 negative (donors from Almeria and Valladolid, Spain), 102 positive (patients from Valladolid, Spain) and 11 sera from brucellosis patients (with brucella-agglutination titers above 1/320). The microagglutination test (MAT) was used as reference method. This assay was carried out with a suspension of safranin stained F. tularensis in an U-bottom microtiter plate.

Results: 101 out of 102 sera showed a distinct red test line in the ICA. 132 out of 134 negative sera showed no reactivity on the test line in the ICA. Calculated sensitivity and specificity were 99.0% and 98.5% respectively. None of the 11 brucellosis sera reacted in the test.

Conclusions: The new test has proved to be able to detect anti-Francisella antibodies in human serum samples with good sensitivity and specificity values. Since MAT is the most frequently used technique for the serological diagnosis of tularemia, this test offers a new diagnostic tool that brings together good performance characteristics with the advantages of rapid tests (easy to run, easy to interpret and easy to store). It suits the demand of a test easy to implement that gives results in a few minutes for a disease with low prevalence such as tularemia.

P1417 Neuroborreliosis: detection of intrathecally produced antibodies against Borrelia in cerebrospinal fluid using the line blot immunoassay Anti-Borrelia Euroline-RN-AT

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Objectives: The line blot immunoassay “Anti-Borrelia EUROLINE-RN-AT (IgG)” is validated for the determination of Borrelia-specific antibodies of class IgG in serum and cerebrospinal fluid (CSF). An intrathecal antibody production is assumed if the number of specific bands in CSF is larger than in serum or at least one specific band in CSF shows a 1:5 fold relative intensity compared to the corresponding band in serum.

Methods: 50 CSF/serum pairs from patients with suspected neuroborreliosis and different neurological symptoms were incubated in a modified procedure using the “Anti-Borrelia EUROLINE-RN-AT (IgG)” test system (EUROIMMUN AG). Furthermore total IgG and
Evaluation of serological tests and clinical features in New liaison® automated immunoassays for the detection of Borrelia albumin concentration were determined and the “Anti-Borrelia plus CSF ELISA (IgG)” test system (EUROIMMUN AG) was performed. For comparability, the IgG concentration in serum was equalized to the 1:4 diluted CSF. In case of an additional IgG synthesis in the CNS, the limiting quotient CSQ lim IgG, based upon CSQ alb, was calculated and considered.

The number of bands and the band intensities were automatically evaluated using a commercial computer programme (EUROLineScan from EUROIMMUN AG).

Results: 22% (11/50) of the patients with suspected neuroborreliosis showed indications of Borrelia-specific intrathecal antibody production. 54.5% (6/11) of them demonstrated more bands in CSF than in serum and 45.5% (5/11) displayed higher band intensities in CSF than in serum. There was a 98% agreement between the results of the “Anti-Borrelia EUROLINE-RN-AT (IgG)” and the CE-labelled “Anti-Borrelia plus CSF ELISA (IgG)” test systems.

Conclusion: The line blot immunoassay “Anti-Borrelia EUROLINE-RN-AT (IgG)” is valuable to confirm the presence of intrathecally produced Borrelia-specific antibodies in neuroborreliosis. The analysis of CSF/serum pairs using a line blot immunoassay also allows qualitative characterisation of intrathecally produced pathogen-specific antibody patterns in addition to quantitative determination of relative CSQ values using ELISA.

\[
\text{serum dilution factor} = \frac{4}{\text{CSQ serum}} \quad \text{or} \quad \frac{4}{\text{CSQ CSF}}
\]
\[
\text{CSQ serum} = \frac{0.93}{(\text{CSQ IgG})^2} - 6 \times 10^{-5} - 7 \times 10^{-3}; \quad \text{CSQ CSF} = \frac{\text{CSQ total albumin}}{\text{CSQ total IgG}}
\]

\[\text{P1418 Evaluation of serological tests and clinical features in patients with culture-positive brucellosis}\]

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Objective: 1. To evaluate the sensitivity and specificity of different serological tests in patient with culture positive Brucellosis.
2. To evaluate the clinical features encountered in this group of patients.

Methodology: Sera of patients with culture positive brucellosis were tested for the presence of antibodies by the following tests: (1) Microplate Agglutination test (in this method the antigens were prepared by dilution in normal saline solution (0.9%) to give a 1:20 dilution, 1% bovine albumin was prepared by adding 1.05 ml of normal saline solution to 5 ml (22%) bovine albumin, (2) Standard tube Agglutination test, (3) 2-Mercaptoethanol Agglutination test, (4) Coombs test, (5) ELISA test.

Results: The microplate agglutination test was found to be the most sensitive detecting antibodies in 91.7% of the sera where the titre range between 1/20–1/20480. The standard tube agglutination test showed positivity in 87.8% of samples with a titre range of 1/20–1/10240. The 2-Mercaptoethanol agglutination test detected antibodies in 53.2% samples with a titre range of 1/20–1/2580. The Coombs Anti-human Globulin test was positive in 62.5% of the sera at a maximum titer of 1/160. The ELISA test was significantly positive for IgG and IgM antibody in 43.6% and 89.5% of sera respectively. The most encountered symptoms were fever, sweating, weight loss, abdominal pain, joint pains and body aches. The most important physical signs were hepatomegaly in 42.8% splenomegaly in 32% and illosacular tenderness in 27% of patients.

Conclusion: We conclude that the most sensitive serological test for the serological diagnosis of Brucellosis is the Microplate Agglutination test followed by the ELISA IgM test. However, in clinical practice, the significant cut off point of Microplate Agglutination test and the ideal cut off value of ELISA test for the diagnosis of active Brucellosis should be established.

\[\text{P1419 Dimeric OspCadv – advanced recombinant OspC antigens for the sensitive and specific detection of class IgM antibodies in early stages of Borrelia infections}\]

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Objectives: In the early phase of borreliosis, antibodies of class IgM against OspC are generally the most important serological markers. In contrast to native OspC antigens, the diagnostic value of recombinant OspC used as antigenic target has been limited until now due to unspecific reactivity with sera from healthy individuals. Here we present data from three human pathogenic Borrelia genospecies, showing that recombinant homodimeric OspCadv achieves an equivalent specificity and increased sensitivity compared to native OspC antigens purified from Borrelia membranes.

Methods: A line blot immunoassay was developed containing recombinant dimeric OspCadv antigens from pathogenic Borrelia afzelii, Borrelia burgdorferi and Borrelia garinii, expressed in E. coli and purified by affinity chromatography. This assay and a commercially available line blot immunoassay (“EUROLINE Borrelia RN-AT”, EUROIMMUN AG) comprising purified, isolated native OspC antigens from the same Borrelia genospecies were used to screen for antibodies of class IgM in 150 sera from patients with borreliosis and 126 control sera (16 from patients with past infection and persisting IgM, 10 from patients with acute EBV infection, 50 from pregnant women and 50 from blood donors). Prevalence and intensity of the bands were automatically evaluated using a commercial computer programme (EUROLineScan from EUROIMMUN AG).

Results: In 95.7% (264/276) of the sera tested, the antibody reactivity to dimeric OspCadv and native OspC antigens was concordant. 3.3% (9/276) of the sera, 88.9% (8/9) of which were from the borreliosis cohort, exclusively contained antibodies against OspCadv antigens. The OspCadv antigens showed a specificity of 99%.

Conclusion: With dimeric OspCadv antigens, the assay sensitivity in borreliosis patients was increased without loss of specificity, compared to monomeric recombinant OspC. We conclude that unspecific reactions have been prevented by optimal antigen design: the immunodominant epitopes of the OspCadv antigens agree exactly with the structures of the epitopes of native OspC antigens, thus providing an excellent sensitivity and specificity.

\[\text{P1420 New liaison® automated immunoassays for the detection of Mycoplasma pneumoniae IgG and IgM antibodies in human serum/plasma specimens}\]

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Objective: To evaluate the diagnostic performance of two recently developed automated immunoassays for determination of IgG and IgM
antibodies to *Mycoplasma pneumoniae* in human serum/plasma. Automated LIAISON® *Mycoplasma pneumoniae* IgG and IgM assays were developed in cooperation between DiaSorin and Savyon Diagnostics. **Methods:** Semi-quantitative determination of specific IgG to *Mycoplasma pneumoniae* is an indirect, two steps, chemiluminescence immunoassay (CLIA). P1 recombinant antigen (in *E. coli*) is coated onto magnetic particles. A monoclonal Ab to human IgG, linked to an isoluminol derivative (isoluminol-antibody conjugate), is used to detect specific IgG antibodies present in calibrator, samples or controls. IgM to *Mycoplasma pneumoniae* are detected by a qualitative, two steps, CLIA assay. Mycoplasmal lysate enriched with P1 recombinant antigen is coated onto magnetic particles. Specific IgM antibodies are detected by a monoclonal Ab to human IgM, linked to an isoluminol derivative. Study population included 465 samples from patients with symptoms of atypical pneumonia, 49 paired samples from patients with recent *M. pneumoniae* infection and samples from subjects positive for antibodies to other microorganisms that may cause symptoms similar to *M. pneumoniae* infection (e.g. influenza virus, adenovirus), or other infectious diseases. Both assays were designed to be used on the LIAISON® instrument. **Results:** Specimens were tested using the LIAISON® *Mycoplasma pneumoniae* assays and reference ELISA tests. Overall agreement was 90.1% for IgG and 91.9% for IgM detection. Consensus with additional serological data was then applied on discrepant specimens. Diagnostic sensitivity and specificity were respectively 94.2% and 98.8% for IgG, 97.8% and 99.1% for IgM. Significantly increased (i.e. of the order of three fold or greater) *M. pneumoniae* IgG concentration between two consecutive bleeds is considered suggestive of acute infection or re-infection, even in the absence of IgM. Diagnostic concordance on the assessed paired samples was 100% on determination of acute infections, using LIAISON® *Mycoplasma pneumoniae* assays and Savyon SeroMP recombinant IgG/IgM assays. **Conclusions:** These assays shows high analytical performance in the determination of specific antibodies to *Mycoplasma pneumoniae* in human serum or plasma samples. Results obtained with paired sequential samples allow to correctly diagnose acute infections.

**P1423**

**Clinical potential of diagnostic methods for the rapid diagnosis of Mycoplasma pneumoniae pneumonia in adults**

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**Objectives:** Results from rapid diagnostic tests can be used to initiate appropriate antibiotic treatment and prevent antimicrobial resistance through the use of narrow-spectrum antibiotics. The purpose of the present study was to evaluate the accuracy and usefulness of three rapid diagnostic methods: ImmunoCard *Mycoplasma* kit, chest high-resolution computed tomography (HRCT) findings, and the Japanese Respiratory Society (JRS) scoring system, for the early presumptive diagnosis of *Mycoplasma pneumoniae* pneumonia. **Methods:** We performed three rapid diagnostic methods at the same time in four pneumonia groups: 68 cases with *M. pneumoniae* pneumonia, 133 cases with *Streptococcus pneumoniae* pneumonia, 30 cases with *Haemophilus influenzae* pneumonia, and 20 cases with *Legionella* pneumonia. **Results:** The sensitivity and specificity were 35% and 68% for ImmunoCard, 73% and 85% with HRCT, and 83% and 90% with the JRS scoring system, respectively. Among the 57 positive cases with the JRS scoring system, ImmunoCard and HRCT were positive in all 57 patients and in 39 patients, respectively. To confirm the positive reactivity of ImmunoCard, we tested the sera from 200 adult healthy volunteers, and IgM-positive cases were observed in 61 (30%) subjects. IgM antibodies of *M. pneumoniae* pneumonia persist for several weeks and months and the longest positive period was 512 days. **Conclusion:** Among the three rapid diagnostic methods, the JRS scoring system was the most useful tool for initiating the administration of adequate antibiotic therapy for probable *M. pneumoniae* pneumonia. We suggest that *M. pneumoniae* pneumonia should be suspected when there is a correlation of more than five parameters in the JRS scoring system (99% specificity). If there is a correlation of three or four parameters in the JRS scoring system, chest CT findings are helpful for the presumptive diagnosis of *M. pneumoniae* pneumonia.
reported that host antibodies against this protein can be applied in serological screening approaches.

**Aim:** The aim of this study was to produce CagA C domain in a recombinant format for incorporation into a serological kit for screening of HP infected patients.

**Methods:** Genomic DNA was extracted from Iranian Hp strains with the 3' ABCC motif. Designed specific primers were used for PCR amplification of the 3' cagA region corresponding to the triple EPIYA C domain. The amplified (400bp) fragment was cloned into pET23a, an E. coli expression vector. Expression of rprotein was induced by 0.5mM IPTG. The identity of the expressed (18kDa) protein was confirmed through immunoblotting with anti Hs IgG antibodies as well as pooled Hp negative and positive sera. The recombinant protein was used for screening patients’ sera in comparison with the obtained cagA 3’ genotyping data. The following groups of subjects were screened: (1) Hp negative subjects, or those infected with Hp strains with missing or incomplete cagA gene (lacking one of 5’ or 3’ region) or AB type cagA gene (n = 109), (2) Patients infected with Hp strains with complete cagA gene with one or more EPIYA C domains (n = 223).

**Results:** Presence of antibodies against rCagA C3 domain could significantly reflects patients infected with Hp strains with complete cagA gene with one or more EPIYA C domains as compared to the first group which were either HP negative or bar bored HP strains with incomplete cagA gene or cagA gene without EPIYA C domain (P < 0.001).

**Conclusion:** Serologic screening of populations against the recombinant CagA triple C fragment presents a non-invasive method in identification of subjects infected with the more virulent subtypes of Hp (EPIYA C positive strains).

**Objective:** Several studies have shown that beside Helicobacter pylori (Hp) and its virulence factors, host genetic and protein content as well as environmental factors are involved in gastric carcinogenesis. In this study we aimed to estimate the risk of gastric cancer development associated with serum anti-Hp and its cytotoxin associated gene A product (CagA) as well as serum pepsinogen as non-invasive screening markers.

**Methods:** 382 gastric cancer cases, 626 non ulcer dyspeptic patients and 179 asymptomatic blood donors were enrolled in the study. Fasting blood samples were obtained for measurement of serum pepsinogen as well as anti Hp IgG and anti recombinant CagA IgG antibodies using home made ELISA and Western blotting assays. PMN/neutrophil infiltrations, gastric atrophic and intestinal metaplastic changes were also graded according to OLGA staging system. Asymptomatic subjects and dyspeptic patients with low grades of atrophic changes were categorized as “none to mild” patient group whereas dyspeptic patients with high grades of atrophy and gastric cancer patients were merged as the “moderate to severe” patient group.

**Results:** Multiple logistic regression analysis adjusting for age, gender and ethnicity of subjects demonstrated that the presence of serum antibodies against CagA increased the risk of subject placement in the high risk group by 1.5 folds which did not reach statistical significance. On the other hand, serum pepsinogen levels below 25μg/L represented a significantly increased risk of “moderate to severe” gastric diseases (OR=4.54; 95%CI=2.5–8.2, P < 0.001). The joint effect of these two screening factors however represented an even higher increased risk for development of moderate to severe gastric premalignant and malignant disorders (OR=6.8; 95%CI=1.33–35.2, P < 0.05).

**Conclusions:** Our data recommends the assessment of serum anti-CagA antibodies in addition to pepsinogen levels for population screening and identification of high risk subjects whom should then be referred for routine follow-up endoscopy and detection of premalignant lesions and intervention treatments.

**Immunology, immunopathogenesis and vaccinology**

### P1425 **Time-dependent effect of MBL, TLR2, TLR4, FcgammaRIIA genes polymorphisms, treatment and sepsis in invasive pneumococcal disease mortality**

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**Objectives:** Our aim was to assess the impact on mortality of different genotypic variants of the innate immunity in patients admitted to the hospital with invasive pneumococcal disease (IPD).

**Methods:** All Caucasian adults admitted to the hospital with diagnosis of IPD were enrolled in this prospective study and SNP's in MBL, TLR2, TLR4 and Fcgamma-RIIA genes were genotyped. Underlying diseases, severity of illness, antibiotic management and other variables related to outcome were also recorded. To determine the independent effect of the variables on outcome, we calculated multivariate-adjusted hazard ratio of death using the Cox proportional hazard regression analysis.

**Results:** We included 117 patients with IPD: 98 episodes (83.6%) of pneumonia, 17 (14.5%) meningitis, and 2 patients (1.9%) with primary pneumococcal bacteremia. Polymorphisms of MBL A0/00 were present in 37 patients (32%) (individuals heterozygous or homozygous for one of the allelic variant B, C or D), TLR4 T399I in 19 (16.2%), TLR4 D299G/T399I in 11 (9.4%), TLR4 R753Q in 3 (2.56%), and FcgRIIA-H/H131 in 26 patients (23%). All genotype frequencies were in Hardy-Weinberg equilibrium. Forty patients (34%) developed septic shock secondary to IPD. We did not find difference between genotype frequencies on the occurrence of septic shock. The bivariate analysis identified that median Charlson Index (IQR) 4 (4) vs 6 (3) (p = 0.04), presence of septic shock 21 (22) vs 19 (86) (p < 0.001) and first adequate antibiotic within the first 4 hours after admission 66 (69) vs 8 (36) (p = 0.04) were significantly different between survivors and non-survivors. Factors associated independently with mortality were SNP MBL A0/00 [Adjusted hazard ratios (aHR) 3.06 confidence interval (CI) 95% 1.01–9.31] and septic shock [aHR 10.73 (CI 95% 3.14–36.70)] whereas first adequate antibiotic dose ≤4h was a protective factor [aHR 0.24 CI 95% 0.08–0.74].

**Conclusions:** After controlling for confounding variables, variant alleles in the MBL2 gene are independently associated with mortality in patients admitted to the hospital with IPD.

### P1426 **Mannose-binding lectin inhibits cytomegalovirus capture and transmission by human primary monocyte-derived dendritic cells**

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**Objectives:** Human cytomegalovirus (HCMV) infection is common, and can’t completely get rid of from the host with present anti-cytomegalovirus therapy. The research was a study on human mannose-binding lectin (hMBL)/recombinant human wild-type MBL (rhMBL) inhibiting monocyte-derived DC (MD-DC) capturing HCMV and preventing transmission to T cells, and exploring its mechanism.

**Methods:** All methods included the establishment of the Chinese hamster ovary (CHO) cell line secreting rhMBL; the preparation, purification, identification and quantification of rhMBL and rhMBL; the preparation of MD-DC; testing the inhibitory of MBL on the ability of MD-DC to capture and transmit HCMV particles by RT-PCR, immunofluorescence confocal microscopy and flow cytometry.

**Results:** Establishing a stable cell line that human wild-type MBL gene expression is 10^3 times more than that in the empty vector, preparing high purity rhMBL, rhMBL, MD-DC (expression of CD209 was higher
Mannose-binding lectin inhibits human cytomegalovirus infection in human embryonic pulmonary fibroblasts

**W Wu*, S.Q. Shang, R. Tao, Y.H. Chen, H.J. Qiao (Hangzhou, CN)**

**Objectives:** A limited number of drugs have been used for treatment of Human cytomegalovirus (HCMV), all sharing the similar antiviral mechanism of inhibiting virus replication. This study is to study the anti-HCMV activities of Mannose-binding lectin (MBL) from blocking virus entry.

**Methods:** Recombinant human MBL (rMBL) was produced in CHO cells using a PIRE2-AcGFP expression vector system. Native human MBL (hMBL) was isolated from human serum. The concentration of purified MBL was determined by ELISA. HCMV neutralization test was carried out by treating HCMV with each diluted MBL solution and then treated HCMV being inoculated onto the human embryonic pulmonary fibroblasts (MRC-5 cell). The cells were then washed and the HCMV-DNA in cells was quantified by real-time quantitative PCR (Q-PCR). To get confocal pictures, in some experiments, the HCMV were stained by PKH-26 before treated with MBL and the cells were stained by PKH-26 before treated with MBL and the cells were applied with anti-vimentin. A HCMV growth inhibition test were also done in HCMV-infected cells. After the invasion of HCMV, the cells were incubated with MBL, every 24 hour, the supernatant of cell culture was tested for HCMV-DNA by Q-PCR. At 72 hour, cytopathic effect (CPE) was observed by the inverted microscope and cells were collected for HCMV-DNA examination.

**Results:** HCMV neutralization test revealed 10 μg/ml MBL significantly decreased the HCMV invasion in MRC-5 cell. However, the activity can be blocked by incubating together with 20 μg/ml mannan. The neutralizing activity of hMBL was higher than rMBL. Confocal pictures confirmed less HCMV invasion after MBL treatment. HCMV growth inhibition test indicated at 24th hour and 48th hour after HCMV invasion, there was no difference of the HCMV-DNA levels between MBL incubated cells supernatant and control supernatant. At 72nd hour, the HCMV-DNA levels in both culture supernatant and cells incubated with MBL were lower than the control.

**Conclusion:** MBL can inhibit invasion of HCMV to MRC-5 cells and prevent viral spreading to contiguous cells. The anti-HCMV activities of MBL were blocked by mannan, suggesting binding of MBL to HCMV is through the interaction of the MBL with the glucoprotein of HCMV.

**P1422**

**P1429** IL-15 gene polymorphisms are associated with resistance to visceral leishmaniasis

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**Objectives:** Protozoan parasites of the genus Leishmania infect millions of people worldwide causing a wide spectrum of diseases collectively termed leishmaniasis that vary in their clinical manifestations. There are several reports on the importance of IL-15 in the immunity against leishmaniasis. Since the production of IL-15, like other cytokines, is under control of its gene, we tried to find any relationship between visceral leishmaniasis (kala azar) and IL-15 genetic polymorphisms.

**Methods:** One hundred and seventeen patients with kala-azar and 146 individuals who lived in the same area as patients and didn’t have any history of leishmaniasis, joined this study. DNAs extracted from samples were genotyped for IL-15 (267C/T, 367G/A, 13687C/A, and 14035A/T) polymorphisms using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

**Results:** IL-15 (267) TT and T genotype and allele were significantly higher in the patients than the controls (P < 0.001 and P < 0.003, respectively). Also IL-15 (13687) CC and C genotype and allele were less frequent in the controls than the patients (P < 0.015 and P < 0.031, respectively). Haplotype analysis showed a higher frequency of 13687C/267T/367G/14035A in the patients than the controls (P < 0.00001).

**Conclusion:** As data shown IL-15 267T allele could be considered as a susceptibility factor for kala azar. Vice versa, IL-15 13687C allele might be one of the genetic resistance factors against kala azar. In addition, we can consider the haplotype 13687C/267T/367G/14035A as a susceptibility factor for kala azar. Evaluation of IL-15 level beside genetic polymorphisms is recommended.

**P1430**

**The effects of Candida albicans cell wall protein fractions on dendritic cell maturation and cytokine secretion profile by Th1 and Th2 in mice**

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**Objectives:** Candida albicans is an opportunistic fungal pathogen that causes oral and vaginal mucosal infections as well as systemic disease. C. albicans cell wall is composed of several protein and carbohydrate components that have been shown to play a critical role in interaction with the host immune system. This major components of C. albicans cell wall can partially modulate the host immune responses. Dendritic cells
(DC), as the most important antigen-presenting cells of the immune system, play a critical role in inducing immune responses such as initiation and activation of T lymphocytes as mainly cellular immunity for elimination of different pathogens. We evaluated the effect of the cell wall protein fraction (CPF) of C. albicans on DC maturation and Th1/Th2 cytokine secretion pattern by T lymphocytes.

Methods: The CPF of C. albicans cells was extracted by a lysis buffer containing sodium dodecyl sulphate, 2-mercaptoethanol and phosphate-buffered saline. The extract was dialyzed and its protein pattern was evaluated by electrophoresis. Dendritic cells were purified from Balb/c mice spleens through a three-step method including mononuclear cell separation, as well as 2-h and overnight cultures. Different concentrations of purified CPF (5, 10, 15 μg/ml) was added to DC. The purity and maturation status of DC were determined by flow cytometry using monoclonal antibodies against CD11c, MHC-II, CD40 and CD86. T lymphocytes were purified from lymph node of C57BL/6 mice by nylon wool method and their purity were determined by flow cytometry. T cells were cocultured with DCs that was pulsed with CPF. Supernatants of culture were collected for determination of cytokine secretion (IFNγ, IL4) by ELISA method.

Results: Treatment of DC with 10 μg/mg of CPF significantly (P value <0.05) increased both the expression of maturation markers including MHC-II, CD86 and CD40 on DC and production of IFNγ cytokine by T cells compared to the control group.

Conclusion: In this study we used C. albicans CPF with the molecular weight of 40–45 kDa for pulsing and maturation of dendritic cells and cytokine pattern secretion pattern in T lymphocytes. Since according to our results CPF significantly increased the expression of maturation markers on DC and Th1 cytokine secretion, we investigated that CPF may act as an efficient immunomodulator, or may be used as a potential adjuvant to boost the host immune system against infections for immunotherapy in clinical trials.

P1431 IgG2 and IgG3 subclass response to merozoite surface protein-2 and malaria status
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Background: The role of naturally acquired human IgG antibodies in immunity against the asexual blood stages of Plasmodium falciparum has been the focus of interest of many studies. It has been suggested that total IgG may not be the most suitable measurement for the antibody responses against malaria. The current study was designed to evaluate the various IgG subclass responses over time in an age cohort and to examine the correlation with two markers of malaria morbidity, parasitaemia and haemoglobin levels.

Methods: The IgG2 and IgG3 responses of 178 individuals from a malaria endemic area of The Gambia have been analysed, at a 20-year longitudinal study, by ELISA against different recombinant domains of the merozoite surface protein-2 (MSP-2) and a crude schizont extract. The Kolmogorov–Smirnov method was used to check the normal distribution of absorbance, haemoglobin and parasitaemia, and Spearman’s correlation was applied to analyse the data.

Results: The results of the present study showed that the specific IgG2 and IgG3 levels, as well as the IgG3/IgG2 ratio against different domains of MSP-2 were increased with the age of subjects, along with the increasing of the haemoglobin and decreasing of the parasitemia levels. Similar pattern was observed for the crude schizont extract.

Conclusion: Both the frequency and concentrations of IgG2 and IgG3 either against domains 2 and 3 of MSP-2 or crude schizont extract were increased with increasing the age. At the same time the Pearson analyses showed that, at the end of the follow up, high levels of IgG2 and IgG3 to MSP2 epitopes were associated with low levels of parasitemia and high levels of hemoglobin. In conclusion, bringing together, these results favor a protective role of IgG3 and IgG2 against domains 2 and 3 of MSP-2.

P1432 Interleukin-33 induction is correlated with an efficient hepatic granulomatous response during experimental Leishmania donovani visceral leishmaniasis
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Objectives: During Leishmania donovani infection, spleen and bone marrow are not able to control the infection, whereas the liver displays a specific microenvironment allowing the development of mature inflammatory granulomas, warranting clearance of parasites. Macrophages and T lymphocytes as well as cytokines (IFN-γ, TNF-α or IL-12) contribute to the granuloma development and maturation, and thus to the control of hepatic infection. However, regulation of these cellular and soluble mediators is complex. Here, we studied the potential implication of a recently described cytokine, IL-33.

IL-33 is mainly localized in the nucleus and released by epithelial and endothelial cells in a damage context to early induce an adapted response. It is recognized by its receptor ST2 which recruits the IL-1 accessory receptor on target cells leading to pro-inflammatory gene expression. IL-33 is also induced by various cell types in a chronic pathological context such as viral or toxic chronic fibrosis. An ST2 implication has been studied during cutaneous leishmaniasis due to Leishmania major.

Methods: In this work, we studied the hepatic IL-33 and ST2 expression in vivo during visceral leishmaniasis in C57BL/6 mice infected with L. donovani and sacrificed after 15, 30 or 60 days. Granuloma foci were counted on HES stained liver sections. Parasitic loads were evaluated by microscopic examination of Giemsa stained smears. mRNA induction of IL-33, ST2, IFN-γ, TNF-α, IL-12 and IL-4 were analysed by quantitative PCR after RNA extraction of liver samples and reverse transcription. Immuno-fluorescence staining was also performed on liver thin sections in order to localize IL-33 producing cells.

Results: Histochemical and immunofluorescent staining revealed IL-33 positive cells, mainly localized in granulomas, with an increasing number of producing cells from day 15 to day 60 post-infection. In addition, a qPCR analysis showed a significant IL-33 and ST2 mRNA induction at day 60 post-infection. This concomitant induction of IL-33 and its receptor was correlated to the induction of other cytokines: IFN-γ, TNF-α, IL-12 and IL-4. IL-33 induction in the liver of infected mice was correlated to the growing granuloma number and size during the course of the disease and to the parasitic clearance.

Conclusion: We showed that the IL-33 expression is highly associated with the efficient granulomatous response in C57BL/6 mice infected with L. donovani.

P1433 Oral intake of heat-killed Lactobacillus paracasei strain b240 reduces incidence of acute upper respiratory tract infection in the elderly: a randomised, double-blind, placebo-controlled trial
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Objectives: Mucosal immunoglobulin A (IgA) secretion decreases with age, stress and intense exercise. The decline of the salivary IgA secretion could be correlated with the raising the risk of acute upper respiratory tract infections (URTIs). We have confirmed that intake of heat-killed Lactobacillus paracasei ONRICb0240 (b240) for 12 weeks significantly increased salivary IgA secretion in healthy elderly. The object of this study was to demonstrate the reduction of incidence of acute URTI in the elderly by oral intake of b240.

Methods: A total of 294 healthy elderly individuals were randomly allocated to 3 groups as follows. 1) Placebo group, 2) Low dose group (heat-killed b240: 4 × 1010 cells), 3) High dose group (heat-killed b240: 4 × 1013 cells). Those groups received their respective test food once daily for 20 weeks. Questionnaire survey was performed concerning the occurrence of the URTI and the specialist for Infectious diseases judged the episodes in consideration of all information. Saliva was collected

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at 0, 2, 4, 8, 12 and 20 weeks and salivary IgA were measured. QOL survey was performed.

Results: The incidence of URTI was significantly decreased in healthy elderly by taking high dosage for 20 weeks, and the protective effect of taking b240 on the URTI was dose dependently. In high dose group, salivary IgA secretion was significantly increased. In addition, it was suggested that the salivary IgA secretion is related to the reduction of URTI incidence. QOL was significantly increased in high dose group. No problematic adverse event was reported by taking b240 for 20 weeks.

Conclusion: Oral intake of Lactobacillus pentosus ONRICb0240 for 20 weeks reduces incidence of acute URTI in the elderly. From these results, b240 is thought to enhance the ability to protect against infection in the elderly.

P1434 Cellular immune responses in the ferret (Mustela putorius furo): tools for understanding the correlates of protection and pathogenesis for pandemic influenza and Dengue fever

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Objectives: Our laboratory utilizes the ferret (Mustela putorius furo) as a model of acute respiratory (influenza and coronavirus) infection and is actively developing the ferret as a model of Dengue fever, Dengue hemorrhagic fever, and Dengue shock syndrome. While the ferret has been demonstrated as a useful model of virus replication and pathogenesis for multiple viral pathogens of humans and animals, a paucity of ferret specific reagents has hindered detailed analysis of immune responses to infection, particularly with regard to innate and cellular effectors. We are focused on the development and utilization of reagents in understanding the potential role of innate and adaptive effectors in the context of influenza, SARS-CoV, and Dengue infections in ferrets. Advancements in the field of immunology have led to the identification of various helper T cell subsets, such as CD4+CD152 and CD8+ T cells. There is also a slight increase in the marker such as CD28 and CD80 with a significant increase in CTLA-4 expression in humans.

Methods: Primers for ferret FoxP3 (F-FoxP3) were generated from urinary tract infection (UTI) b-defensin-1 (HBD-1) is secreted in the kidneys and thought important for the urinary tract sterile. The production of HBD-1 is up-regulated during pyelonephritis, indicating its importance for protection of the urinary tract. Furthermore, knockout defb-1 mice, have shown that lymphocytes, in addition to neutrophils, infiltrate the lung in ARDS and regulatory T cell subset contribute to its resolution. Therefore our present study aims to determine the T cell and its subset distribution as well as the expression of the costimulatory markers in ARDS and compare it with those of the normal.

Methods: Bronchoalveolar Lavage (BAL) from patients with ARDS due to infection, and that of normal group was taken during the standard sterile pulmonary bronchoscopic procedures from Pulmonology Department. BAL from each patient was taken during the initial phase of infection or at the starting phase of ARDS. Cell suspension was made from BAL, red blood cells were lysed, labelled and analysed in flow cytometer. Lymphocytes were detected by CD3+CD45+ double positive cells. Activated T-cells for both CD4 and CD8 were detected by HLA-DR+, T-regulatory cells by CD25+FoxP3+. Proliferation were screened using Ki67 staining for both subsets. Costimulatory molecules CD28 and CD152(CTLA-4) along with CD80 and CD86 were also analyzed on both T-cell subsets.

Results: The data of 10 BAL from ARDS patients and 10 normals are presented in the following table. The values represent the median in percentage. The values in the bracket represents the range. p value represents non-parametric Mann-Whitney’s U test p value.

Conclusion: From the data it is clear that in ARDS their is increase in both the activated and proliferating T cells in comparison to normal in both CD4 and CD8 T cell subsets. In case of lung infection, in both the T cell subset population, there is decrease in expression of costimulatory marker such as CD28 and CD80 with a significant increase in CTLA-4/CD152 and CD86+ T cells. There is also a slight increase in the population of T-regulatory cells in case of ARDS.

P1435 Increased activated and proliferating T-cells in acute respiratory distress syndrome with altered CD28 and CTLA-4 expression in humans

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Objectives: Acute Respiratory Distress Syndrome (ARDS) is a common, devastating clinical syndrome of acute lung injury but most often those with sepsis. The pathophysiology of this disease is still unknown. Studies have shown that lymphocytes, in addition to neutrophils, infiltrate the lung in ARDS and regulatory T cell subset contribute to its resolution. Therefore our present study aims to determine the T cell and its subset distribution as well as the expression of the costimulatory markers in ARDS and compare it with those of the normal.

Methods: Bronchoalveolar Lavage (BAL) from patients with ARDS due to infection, and that of normal group was taken during the standard sterile pulmonary bronchoscopic procedures from Pulmonology Department. BAL from each patient was taken during the initial phase of infection or at the starting phase of ARDS. Cell suspension was made from BAL, red blood cells were lysed, labelled and analysed in flow cytometer. Lymphocytes were detected by CD3+CD45+ double positive cells. Activated T-cells for both CD4 and CD8 were detected by HLA-DR+, T-regulatory cells by CD25+FoxP3+. Proliferation were screened using Ki67 staining for both subsets. Costimulatory molecules CD28 and CD152(CTLA-4) along with CD80 and CD86 were also analyzed on both T-cell subsets.
AZD9773 is a novel selective anti-TNF-α ovine polyclonal immune Fab

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Objectives: TNF-α is thought to play a central role in the pathogenesis of sepsis and septic shock. AZD9773 is an ovine polyclonal anti-human TNF-α (rhTNF-α) immune Fab and comprises both TNF-α-directed and non-specific Fab populations. Here we describe the binding and functional potency of AZD9773 against rhTNF-α and TNF-α orthologues from a number of species and characterise the specificity of AZD9773 against a panel of human frozen tissues.

Methods: AZD9773 binding and functional potency (IC50) versus rhTNF-α and TNF-α orthologues was assessed using surface plasmon resonance (SPR) technology and TNF-α-mediated cytolysis in L929 and PK(15) cell lines, respectively. AZD9773 binding to a variety of fresh frozen tissues from three unrelated human donors was determined via detection of fluorescein isothiocyanate (FITC)-conjugated AZD9773 by immunohistochemical (IHC) staining. FITC sheep anti-digoxigenin Fab was used as a control.

Results: SPR revealed that AZD9773 total Fab binding to other species TNF-α was less than that obtained with rhTNF-α. AZD9773:TNF-α orthologue binding relative to AZD9773:rhTNF-α binding was approximately 50–60% for canine, equine, feline and primate TNF-α, approximately 20% for porcine TNF-α and <20% for bovine, murine, cotton rat and rat TNF-α. In cytotoxicity assays, AZD9773 potently inhibited the bioactivity of human and primate TNF-α with limited or no inhibition of canine, porcine, mouse and rat TNF-α. IHC analysis revealed specific AZD9773 staining of occasional cells in the lung, thymus and lamina propria of the ileum, and in cells within the red and white pulp of the spleen.

Conclusion: AZD9773 has limited capacity to bind and neutralise common TNF-α orthologues; consistent with the conservation of key TNF-α receptor-binding epitopes and overall sequence-identity between species. Specific binding of AZD9773 to human tissues was consistent with the theoretical distribution of TNF-α producing cells, reflecting the intended pharmacological binding. The non-TNF-α-direct Fab populations of AZD9773 do not appear to bind human antigens. Thus, AZD9773 is a potent and selective human TNF-α-neutralising ovine immune Fab. #FB Currently at Novartis, Basel, Switzerland.

Vaccines – miscellaneous

Safety of hepatitis B, pneumococcal, and meningococcal vaccines in pregnancy: evaluation of the published evidence

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Objectives: Immunization during pregnancy has the potential to protect the mother and the newborn from preventable diseases. Current recommendations suggest that inactivated vaccines might be considered during pregnancy when the benefits outweigh the risks, although the evidence supporting this statement has been questioned. We aimed to evaluate the safety of hepatitis B (HB), pneumococcal (PPV), and meningococcal vaccine (MPV) during pregnancy.

Methods: We performed a review of the available published studies.

Results: A total of 15 studies were eligible for inclusion in our review. Four studies reported data on HB vaccine, 5 on the PPV, and 3 on the MPV, while 3 additional studies compared MPV with PPV. Minor local reactions, including tenderness and swelling, were common. No serious adverse reactions or life-threatening conditions were reported among vaccinated pregnant women, and maternal immunization was not associated with a teratogenic effect on the fetus. Stillbirths, delivery complications and infant mortality were generally within expected ranges.

Conclusion: Although the published evidence regarding safety of the hepatitis B, pneumococcal, and meningococcal vaccine during pregnancy is limited, the available relevant data seems reassuring. Large, well-organized RCTs may further clarify the issue and thus aid in the implementation of a strategy to decrease the incidence of preventable diseases in the mothers and newborns.
[P1441] Development of chimeric peptides based on group B streptococcal surface proteins as potential vaccine components
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**Objectives:** Group B streptococci (GBS) causes severe neonate’s diseases. The most cost effective and less labor-intensive approach for preventing of GBS infection is the development of vaccines which use not single proteins as components but consisting of epitopes which correspond to several surface GBS antigens. The chimeric structure was created on the basis of two GBS surface proteins – SspB1 adhesin and Bac protein accordingly. Other objectives of the study were to examine the immunogenic activity of the components of the chimera – fragments of SspB1 (rSspB) and Bac (P7) employing monoclonal antibodies (Mab) and protection studies on laboratory animals.

**Methods:** All the constructs have been cloned in pQE32 vector (Qiagen) and resultant recombinant proteins rSspB, P7, and chimera SspB-Bac were expressed in E. coli after induction with IPTG. Protein purification was made on Ni-sepharose columns.

Mab were obtained according to the standard protocol. Cells of myelogenic strain 653 A were conjugated with spleen cells of mice, immunized with P7 or rSspB. Female mice of inbreeding lines F1(Sl/J×Balg/c), F1(DBA/2×Balg/c) were used. These mice, immunized with P7 or rSspB for three times (with complete, incomplete Freund’s adjuvant and without adjuvant). In vivo protection has been done after infection of mice with virulent GBS strains (O9OR, H36) after 15 months since first immunization. Detection of a chimera with Mab was made by ELISA or Western-blot.

**Results:** A chimera SspB-Bac with molecular weight of 79.3 kDa was created. It included α-helical region of SspB1 and Bac protein immunogenic region without IgA binding both fused in one molecule. Mab panels to various P7 and rSspB epitopes were obtained. The ability of long-term immune response (about 11.5 months) was noted. Immunization of mice with P7 and rSspB polypeptide resulted in their protection from systemic GBS infection. It was shown that Mab to P7 and rSspB can detect a chimera, allowing to assume that immunogenic epitopes of Bac and SspB1 were also expressed in SspB-Bac.

**Conclusion:** Antibodies to Bac and SspB1 were protective against GBS infection. Mab to GBS recombinant proteins P7 and rSspB were also able to detect a SspB-Bac. It allows assuming that a chimera, made on the basis of P7 and rSspB, has the ability to cause synthesis of specific antibodies to both Bac and SspB1 epitopes. The potential of chimeric proteins as vaccine candidates is discussed. The work was supported with RFBR grant 10-04-00750a.

[PI442] Effectiveness versus efficacy of conjugated pneumococcal vaccine: a systematic review of randomised, controlled trials with meta-analysis examining absolute risk reduction and relative risk
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**Objectives:** Use of the 7-valent pneumococcal conjugate vaccine (PCV7) resulted in reduction in vaccine serotypes invasive pneumococcal disease (IPD). However, IPD due to serotypes not included in the PCV7 increased in frequency. This prompted the introduction of a 13 valent vaccine. Previous systematic reviews have examined vaccine efficacy (odds ratio and relative risk). However, effectiveness of PCV in reducing childhood morbidity and mortality (in terms of absolute risk reduction (ARR) and numbers needed to treat (NNT)) has not been published. At the threshold of introducing the PCV13, such an assessment of the old vaccine is useful for comparison. The objective here was to evaluate the effectiveness of PCV through a systematic review of literature.

**Methods:** Systematic literature search for randomized controlled trials reporting on measures of vaccine effectiveness (invasive Pneumococcal disease, pneumonia, meningitis, all-cause mortality, Pneumococcal disease specific mortality, and systemic adverse events/effects) was undertaken and data extracted based on a priori criteria. Data were analysed to calculate odds ratio, relative risk and absolute risk reduction (ARR); and pooled through meta-analysis. Number needed to treat (vaccinate) was calculated for effectiveness.

**Results:** There were five methodologically good trials presenting data through 11 publications. There was a small but statistically significant benefit of vaccination on clinical pneumonia (OR=0.927, 95%CI 0.885–0.971, NNT=200), radiological pneumonia (OR=0.749; 95%CI 0.682–0.822, NNT=143) and invasive disease caused by vaccine serotypes (OR=0.215, 95%CI 0.149–0.311, NNN=500). The effect on all-cause mortality was OR and RR=0.88, 95%CI 0.78 to 0.99, and RD 0.00, 95% CI –0.01 to 0.00 (NNT cannot be calculated). There was no difference in invasive Pneumococcal disease caused by vaccine-related and vaccine-unrelated serotypes. There was no data on meningitis and Pneumococcal disease-specific mortality. Examination of multiple adverse events did not show a difference in risk compared to control, except for a small but statistically significant increase in risk of asthma.

**Conclusion:** PCV7 appears to have limited effectiveness against pneumonia; but does not reduce all-cause mortality. There is significant reduction in vaccine serotype IPD. There is no data to draw conclusions for other clinical problems of public health significance such as meningitis, and Pneumococcal disease-specific mortality.

| Table: Meta-analysis showing efficacy and safety data of Pneumococcal conjugate vaccine |
|-----------------|---------------------------------|------------------|-----------------|-----------------|-----------------|-----------------|
| **Outcome**     | **RCT** | **Vaccinated** | **Control** | **OR (95% CI)** | **RD (95% CI)** | **I^2** |
| Clinical Pneumonia | 5 | 45355 | 45284 | 0.927 | 0.940 | -0.005 | 200 |
| Radiological Pneumonia | 3 | 43555 | 43258 | 0.749 | 0.744 | -0.007 | 143 |
| IPD (vaccine serotype) | 5 | 46998 | 46856 | 0.215 | 0.134 | -0.081 | 500 (26.5) |
| IPD (all serotype) | 4 | 49479 | 49042 | 0.256 | 0.258 | 0.002 | 500 (26.5) |
| IPD (Vaccine related serotype) | 5 | 46497 | 46030 | 0.315 | 0.311 | 0.004 | 100 (0) |
| IPD (Vaccine unrelated serotype) | 5 | 46098 | 45886 | 1.199 | 1.191 | 0.008 | 100 (0) |
| Meningitis | 0 | 0 | 0 | 0.780 | 0.780 | 0.000 | 0 |
| All-cause mortality | 3 | 36171 | 36085 | 0.882 | 0.895 | 0.013 | 85 (59) |
| Pneumococcal disease-specific mortality | 0 | 0 | 0 | 0.780 | 0.780 | 0.000 | 0 |

**Adverse Events Mortality**

| **Event** | **Mortality** | **OR (95% CI)** | **RD (95% CI)** | **I^2** |
|-----------|--------------|-----------------|-----------------|--------|
| Hospitalization | 3 | 27162 | 26824 | 0.910 | 0.925 | -0.000 | 125 |
| Serious adverse events as defined by authors | 0 | 0 | 0 | 0.000 | 0.000 | 0 |
| Serious | 4 | 31745 | 31632 | 0.887 | 0.948 | -0.061 | 64 (21) |
| Severe | 2 | 33810 | 33804 | 1.095 | 0.857 | 0.000 | 64 (21) |

**NNT cannot be calculated:** **NNT result suggest that vaccination could benefit in some ways.

[PI443] HPV vaccine awareness and willingness of first-year students entering university in western Turkey
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**Objectives:** This study were to assess the level of knowledge on HPV and HPV vaccination, and to determine vaccination attitude among university students in Izmir, Turkey.

**Methods:** A cross-sectional survey was conducted among students of Ege University enrolled in first-year English preparatory class. A systematic cluster sampling was applied and 717 (72%) of students registered to the 54 classes selected were contacted. Students were from 17 different faculties/schools. Data were collected between April 30 and May 18, 2010, through a self-reported questionnaire including 40
Vaccines – miscellaneous

**P1444** Vaccine status of *Streptococcus pneumoniae* evaluated with a 12plex luminex based method: comparison of data from a 6plex ELISA versus a 12plex luminex method

B. Kantsø*, K. Krogfelt, C. Jørgensen (Copenhagen, DK)

**Objectives:** The level of antibodies against *Streptococcus* (S) pneumo-

Results: The mean age of participants was 19.7 ± 1.5 and 445 (62.1%) were female. Overall, 132 (18.9%) had sexual intercourse, and only 7 of them female. Among participants, 24.1% had heard of HPV and 25.1% about HPV vaccine. The knowledge item with the highest correct answer rate (32.3%) was that HPV caused cervical cancer. The mean total knowledge score was remarkably poor (1.8 ± 2.6 over 12 items), with 59.6% of respondents having zero as their score. There was no difference in mean knowledge scores between males and females. Higher income, history of sexual intercourse and higher knowledge score were significant factors increasing HPV and vaccine awareness for the whole group, adjusted for gender. Genital cancer history in the family significantly increased awareness, but only among girls. Only three students (0.4%) had already been vaccinated, all being female. Among females, 11.6% intended to be vaccinated vs. 10.1% for males, without a significant difference. Visiting a gynaecologist/urologist in the last three years, a history of genital cancer in the family, vaccine awareness, a higher total knowledge score, and being from the East of Turkey were significant predictors of a positive vaccination attitude.

**Conclusion:** HPV and vaccination still remain as a ‘hot medical topic’ in Turkey, since it hasn’t yet become a popular health issue. Based on their age of first intercourse, first year at the university seems to be appropriate timing to inform Turkish girls, whereas it is a bit late for boys. Thus, the integration of HPV education to secondary/high schools could be considered.

![Figure 1. Students’ replies to the statements questioning knowledge on HPV and HPV vaccine (%). Correct answers included in parentheses.](Image 60x250 to 291x441)

**Figure 2. Attitude of students about the HPV vaccine (%)**

| Attitude of students (in %) | Correct answers (in parentheses) |
|-----------------------------|----------------------------------|
| Strongly support vaccination | 17 (19) |
| Somewhat support vaccination | 12 (13) |
| Somewhat oppose vaccination | 8 (9) |
| Strongly oppose vaccination | 2 (3) |

**Methods:** The ELISA measures total lg antibodies against serotypes 1, 4, 7F, 14, 18C, and 19F, whereas the 12plex Luminex assay measures IgG antibodies against serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F. Samples sent to our laboratory for routine analysis of antibody levels against *S. pneumoniae* were analyzed with the new 12plex Luminex assay, and data compared with the results from the ELISA. For this study, the ELISA results (protected or unprotected) are used as the “gold standard”.

**Results:** The outcome of the ELISA is compared with the levels of antibodies found using the Luminex assay. As expected, the results from the comparison of the putative protective levels of antibodies shows that 72% of the samples from unprotected patients have GM above the protective level used for the conjugated vaccine (0.35 µg/ml) and therefore this level cannot be applied to the polysaccharide vaccine.

A protective level of 1 µg/ml is suggested, since this corresponds better with the ELISA. When applying this level, 68% of the unprotected patients are below and 72% of the protected patients is above this cut off.

**Conclusion:** The protective level from the conjugated vaccine cannot be applied to the polysaccharide vaccine due to a higher antibody level from the polysaccharide vaccine. We suggest a protective level of 1 µg/ml for the polysaccharide vaccine.

| ELISA results | n | GM > 0.35 | GM < 0.35 | GM > 1 | GM < 1 |
|---------------|---|-----------|-----------|-------|-------|
| Unprotected   | 69 | 50 (72%) | 19 (28%)  | 22 (32%) | 47 (68%) |
| Protected     | 111 | 107 (96%) | 4 (4%)    | 80 (72%) | 31 (28%) |

Table 1: Shows the number of geometric mean (GM) values above or below two different putative protective levels.

**P1445** Safety and immunogenicity of an inactivated pandemic H1N1 vaccine provided by the Thai Ministry of Public Health as a routine public health service

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**Background:** The national vaccination program against the 2009 H1N1 virus was implemented in high risk populations in Thailand, including frontline medical personnel. Therefore, the safety and immunogenicity were studied.

**Methods:** Following immunization with 15 microgram of unadjuvanted inactivated H1N1 influenza vaccine in the first 252 participants, assessment of adverse events (AEs) by interviewing was performed at Day 2, Day 7 and Day 21 postvaccination. Immunogenicity testing, measured by hemagglutination inhibition (HAI) assay, was obtained on Days 0 and 21.

**Results:** Of 252 participants, mean ±SD age was 45 ± 11 years, body weight was 62 ± 13 kilograms and 65% were female. Seventy percent were healthy personnel, 28% were patients with chronic illnesses, and 2% were pregnant women. Median (IQR) hemoglobin was 13.0 (12.3–13.9) mg/dl, aspartate aminotransferase was 18 (13–22) U/L and creatinine was 0.7 (0.6–0.9) mg/dl. No serious systemic AEs were reported after vaccination. Mild erythema and local reaction at Day 2 were reported in 9% (23 of 252). The HAI geometric mean titers (GMTs) was 6.9 at Day 0 and 33.4 at Day 21 (4.8 times compared to Day 0; P < 0.001, by repeated measurement analysis). At Day 0 and Day 21, proportion of subjects with HAI titers ≥ 40 was 7.5% (19 of 252) and 51.2% (129 of 252), respectively. Of all, four-fold rising of HAI titers was 56% (142 of 252). Of 19 subjects with Day 0 HAI titers ≥ 40, 10 (53%) had four-fold rising of HAI titers after vaccination. By multivariate analysis, a factor ‘older age’ was associated with lower Day 21 log10HAI titer (P = 0.001, β = –0.016, 95% CI of β: –0.026 to –0.007). The age ranged ‘20–34’, ‘35–49’ and ‘50–65’ years had percentage of participants with HAI titers ≥ 40 at Day 0 vs. Day 21 HAI titers of 1.8% vs. 68.8% (P = 0.041), 7.0% vs. 50.0% (P = 0.006), and 2.2% vs. 36.7% (P = 0.132), respectively.
Conclusions: Monovalent H1N1 vaccination was found to be safe and well tolerated without serious AEs. Over all antibody response rate after one dose of vaccination in this study is relatively low, especially in the old age group. Thus, a booster H1N1 vaccination is needed.

[P1446] Preclinical development of a bio-conjugate vaccine against Staphylococcus aureus disease

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Objectives: S. aureus is a leading cause of serious infections. Antibiotic therapy is compromised by the frequent and rapid appearance of resistance. Control of S. aureus relies on hygienic measures and screening. Prevention by vaccination would substantially reduce the medical and economic impact of these infections.

Methods: GlycoVaxyn has developed a proprietary technology that enables the manufacture of immunogenic glycoprotein bioconjugate vaccines in bacterial cells. In an in vivo protein glycosylation system polysaccharides are enzymatically transferred to a protein of choice. A bioconjugate containing S. aureus capsular polysaccharide CP5 and the carrier protein rEPA (nontoxic recombinant P. aeruginosa exotoxin A) was produced. The vaccine was purified, characterized, and injected into mice, rabbits and rats to demonstrate immunogenicity. Vaccine efficacy was demonstrated in in vivo mice models and in vitro opsonophagocytic assays.

Results: Immunogenicity of the CP5-EPA conjugate was observed in mice and rats with different doses of CP5. The immune response was significantly higher in rats injected with the 0.2 mcg dose compared to 2.5 mcg. An increase in the CP5-specific immune response was seen in all groups after the second injection of CP5 conjugate. Animals tested for antibodies against the protein carrier rEPA showed also a positive response.

High-titer rabbit antibodies to CP5-EPA promoted opsonophagocytic killing of all five S. aureus strains tested in an in vitro assay with human neutrophils. Passive immunization with CP5-EPA antibodies protected mice against bacteremia. Likewise, active vaccination of mice provided protection from S. aureus bacteremia, weight loss, and renal abscess formation. A significant reduction in the bacteremia level between the mice immunized with 0.2–1 mcg CP5 and the control group was observed for most of the tested S. aureus strains. Similar bacteremia levels were observed for all the vaccinated groups (0.2 and 1 mcg of CP5), and a correlation between CP5-antibody titer and protection was found.

Conclusion: An in vivo synthesized, bioconjugate vaccine containing S. aureus polysaccharides and rEPA proved to be immunogenic and protective in pre-clinical studies. The S. aureus bioconjugate will now be evaluated in clinical trials and further developed with the ultimate goal of a vaccine containing both polysaccharide and protein antigens from S. aureus.

Miscellaneous

[P1447] Chelation of nickel cations by the urinary antimicrobial nitroxoline (5-nitro-8-hydroxyquinoline) inhibits urease activity and stone formation in clinical isolates of Proteus mirabilis

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Objectives: Urease synthesis during UTI with P. mirabilis constitutes an important virulence factor, as the generation of NH4+ and CO2 will cause an increase in pH with subsequent precipitation of phosphate salts and formation of septic kidney and bladder stones. Infections are therefore often found in patients with catheters, stents or other urinary flow disturbances and are extremely difficult to clear. Apart from lithotripsy and surgical intervention, antibiotics and acetylsalicylic acid (AHA) are currently the only therapeutic options. AHA functions as an urease inhibitor by binding to the Ni2+ containing active centre of the enzyme. However, AHA is only effective when combined with antibiotic treatment and has severe side effects. In contrast, nitroxoline (NIT) is an antibiotic that functions by chelation of di- and trivalent cations, has no reported serious side effects and has been licensed for the treatment and prophylaxis of complicated UTI. We thus examined the potential of NIT as a possible alternative.

Methods: P. mirabilis UT isolates were collected during routine diagnostic procedures and MIC determined in MH or in artificial urine with 2% TSB by the broth microdilution method. Bacteria were grown in MH broth containing 12 g/L urea and late logarithmic growth cultures harvested by centrifugation. Pellets were resuspended in artificial urine with or without NIT and supplemented with or without Fe2+ or Ni2+. At regular intervals, precipitates and supernatants were collected by centrifugation and the pH measured. NH4+ in the supernatant was determined using the indophenol method and carbonate in the sediment by staining with alizarin red S.

Results: All P. mirabilis isolates tested were found NIT susceptible by the diffusion testing (breakpoint 18 mm) and MIC values lay between 2–16 μg/mL. In comparison, MIC in artificial urine were typically 1–2 titers lower. The maximal pH increase was observed after 50–55 minutes reaching a plateau at about pH 9. This was accompanied by a corresponding increase in NH4+ and the formation of a calcium containing precipitate. Inclusion of 200 μg/mL NIT (1.05 mM) resulted in calcium precipitation. In the presence of 100 μM Ni2+ this blockage was partially resolved, whereas Fe2+ was without any effect.

Conclusion: NIT completely inhibits the urease activity of P. mirabilis at concentrations found in urine by chelation of Ni2+. Further studies will be warranted to confirm its activity in vivo.

[P1448] Uncovering the mechanism of action of the phenothiazine, thioridazine, using Salmonella typhimurium as a model bacterium

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Objectives: To study the mechanism of action of Thoridazine (TZ), using Salmonella enterica serovar Typhimurium as a model bacterium.

Methods: The MIC of thioridazine (TZ) was determined using the microbroth dilution method. Analysis of growth kinetics of S. Typhimurium SL1344 in the presence of 0, 50, 100, 150 and 200 mg/L of TZ were assessed at 37°C with shaking (200 rpm). The effect of exposure of S. Typhimurium to sub-MIC concentrations of TZ was assessed using Transmission Electron Microscopy (TEM) and standard protocols. Membrane permeability on exposure to sub-MIC concentrations of TZ was measured using the H33342 bisbenzimide accumulation assay (Piddock et al., 2010). Gene expression analysis was determined using microarray and RT-qPCR analysis. Generation of S. Typhimurium mutants was carried out using the lambda Red recombination method (Datsenko and Wanner, 2000).

Results: The MIC of TZ for all Salmonella serovars tested was >200 mg/L. S. Typhimurium treated with 200 mg/L of TZ exhibited discernable changes in the morphology of the cell but in particular in the cell-envelope, while untreated cells showed normal envelope morphology. Changes to the cell-envelope were observed after only 5 min exposure. These changes to the cell-envelope increased as a function of time and showed an apparent loss of cell integrity after only 30 minutes. In the absence of TZ there is little accumulation of H33342, which increases, resulting ultimately in the loss of cell integrity. Findings that S. Typhimurium responds to the in vitro presence of TZ by activating...
genes involved in drug resistance and envelope stress responses support this hypothesis.

Fluoroquinolones induce the expression of smaqnr, a chromosome-encoded quinolone resistant determinant, in *Serratia marcescens*

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Objectives: A new pentapeptide repeat protein, named SmaqQnr, has recently been reported from the chromosomal of *Serratia marcescens*. This protein confers low level quinolone resistance when expressed in *Escherichia coli*. The up-stream sequence of this gene contains putative LexA box, similar to that found in qnrB1 whose expression is regulated by SOS system. The aim of this study was to evaluate if smaqnr gene is induced by SOS activators such quinolones.

Methods: Three clinical strains of *S. marcescens*, *S. marcescens* 257 reference strain (Institute Pasteur collection) and *Escherichia coli* 353 carrying a natural plasmid that harboured qnrB1 as control were used in this study. Expression of smaqnr gene was analyzed by real time RT-PCR and quantified in relation to that of rpoB gene for *S. marcescens* and mdh gene for *E. coli*. The fluoroquinolones (FQ) ciprofloxacin (CPX), moxifloxacin (MXF) and levofloxacin (LVX) were tested as possible inducers of the expression of smaqnr gene. Strains were grown at 37°C to exponential phase (DO620nm=0.3−0.4) and then the FQ inducers were added in function of MIC (1/2, 1/4, and 1/8 of MIC values of each one) during 45 minutes, leaving one culture as control. RNA extraction was performed using RNeasy Mini kit (Qiagen). Normalized expression levels of the target gene transcripts were calculated relative to rpoB (mdH in *E. coli*) using the 2-DDCT method.

Results: All *S. marcescens* strains were susceptible to FQ and a LexA box (CTGTAATAAAACAGG) was present up-stream of smaqnr genes. Induction was observed in two clinical isolates (Sm5 and Sm8) and *S. marcescens* 257 strain. CPX induced the expression 2- to 8-folds compared to the controls depending of induction conditions. MFX induced the expression 2- to 4-folds and LVX induced the expression 4- to 20-folds compared to the controls. For LVX the highest value was reached by Sm8 clinical isolate (up to 18-folds) while for CPX and MFX the highest value was found in Sm5 clinical isolate (up to 6-folds) compared to the controls depending of induction conditions. MFX wasperformedusingRNeasyMinikit(Qiagen).Normalizedexpressionlevels of the target gene transcripts were calculated relative to rpoB (mdH in *E. coli*) using the 2-DDCT method.

Conclusions: CPX, MFX and LVX induce smaqnr expression in *Serratia marcescens* and could participate in emergency response against these antimicrobial agents. Whether this phenomenon occurs in other species harbouring chromosomal pentapeptide repeat protein is unknown.

The biological fitness cost and mutation frequency associated with MDR development in *Acinetobacter baumannii*

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Objectives: To study the biological fitness cost (BFC) and mutation frequency (MF) associated with stepwise in vitro selection of MDR in *A. baumannii* (AB).

Methods: The ATCC 19609 strain (WT) was used to select single, double and triple MDR mutants. To determine the MF of antibiotic resistance development, ten ON cultures (WT or mutant) were plated onto antibiotic containing MH-agar plates (rifampicin (RIF, 5 μg/mL), tigecycline (TGC, 5 μg/mL) or colistin (COL, 100 μg/mL). After 24−48 h MF was determined for each selection step and drug combination. Mutants were collected and MICs for each mutant was verified by E-test. To assess changes in BFC the drug rate for all mutants was measured. Four independent cultures/mutant, were used to determine the doubling time in Mueller Hinton broth. Doubling time for each mutant was compared with WT and related antibiotic resistance mutants.

Results: MF and BFC values are summarised in the table. No 3rd step mutants were obtained even when 10^{10} cells were plated. MICs for RIF, TGC and COL mutants were >32μg/mL, 1.5−24μg/mL and 0.25−192μg/mL, respectively. The complete loss of LPS to surface properties of live *Acinetobacter baumannii* cells examined by atomic force microscopy

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Objectives: The dry antibiotic discovery pipeline has forced the revival of Col as a last line of defence for Gram-negative ‘superbugs’, such as *Acinetobacter baumannii*. The complete loss of lipopolysaccharide (LPS) arising from mutations in lipid A biosynthesis genes, mediates Col resistance in some *A. baumannii* strains; however, characterisation of Col resistance in *A. baumannii* warrants greater attention.

Methods: Two Col-heteroresistant *A. baumannii* strains ATCC 19606 and a clinical isolate (Col MICs 1 mg/L), and their paired Col-resistant strains were employed. Bacterial cells were immobilised on clean glass slides coated with gelatin. Live/Dead BacLight™ and confocal laser scanning microscopy were employed to determine the viability of cells which were untreated and treated with Col before immobilisation. AFM examination was conducted for the morphology and surface properties of hydrated Col-susceptible and resistant *A. baumannii* at mid-log and stationary growth phase, and in response to Col treatment at 1 and 32 mg/L for 20 min at 37°C. The contribution of LPS to surface properties was investigated using *A. baumannii* strains constructed with and without the lpxA gene.

Results: Superior immobilisation was achieved for Col-susceptible cells in comparison to resistant cells; the latter required a longer duration (at least 1 h) to adhere securely to the gelatin surface. Rod-like Col-susceptible cells (length 2.62±0.40 μm) were distinguished from spherical Col-resistant cells (diameter 1.72±0.25 μm); the latter aggregated in small clusters or chains. The cell membranes of strains of either phenotype remained intact following Col treatment,
and comparative measurements of surface roughness did not reveal differences between samples. Bacterial spring constant measurements revealed that Col-susceptible cells were significantly stiffer than resistant cells at both growth phases (p < 0.01), while Col treatment at 32 mg/L resulted in more rigid surfaces for both phenotypes. Multiple, large adhesive peaks frequently noted from force curves captured on Col-susceptible cell, were not evident for Col-resistant cells. Adhesion events were markedly reduced following Col exposure.

Conclusions: This study is the first to employ AFM to examine the morphological and surface properties of hydrated Col-susceptible and resistant A. baumannii cells, and provides important insights into the understanding of Col action in this problematic pathogen.

**P1452** Ribosomal mutations associated with ketolide resistance in Haemophilus influenzae found in the SENTRY Antimicrobial Surveillance Program

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Objectives: To determine the mechanisms of ketolide resistance in *H. influenzae*. Ketolide resistance is very rare in *H. influenzae* and is usually associated with a variety of ribosomal mutations. We report on the ribosomal mutations detected in 9 *H. influenzae* strains with ketolide-resistant *H. influenzae* found in the SENTRY Program (2009) and assess the activity of solithromycin (SOLI, formerly CEM-101), a new fluoroketolide in clinical development.

Methods: 1,198 *H. influenzae* isolates obtained from patients with community-acquired bacterial pneumonia in 24 countries were tested for susceptibility to TELI by CLSI methods (M07-A8 and M100-S20-U) as part of the SENTRY Program during 2009. Only nine (0.8%) isolates were found to be TELI-resistant (MIC, ≥ 16 mg/L). Extended MICs were performed by Etest and strains were screened for mutations in the 23S rRNA, L22 and L4 proteins by PCR and DNA sequencing.

Results: Seven different mutation patterns were observed in 8 of the 9 strains. No mutations were detected in the genes sequenced for one strain (Isolate 3042). The highest TELI MIC values (>256 mg/L) were found in two geographically diverse (Sweden and USA) strains with a 23S rRNA A2059G mutation. *H. influenzae* with L4 and L22 riboprotein mutations showed TELI MIC values from 32 to 256 mg/L. TELI was 1.5- to at least 4-fold more active than azithromycin (AZI); and SOLI was 2-fold more active than DOX and 4-fold more active than TELI against all 1,198 *H. influenzae* strains. No mutations were detected in the genes sequenced for one strain (Isolate 3042). The highest TELI MIC values (>256 mg/L) were found in two geographically diverse (Sweden and USA) strains with a 23S rRNA A2059G mutation. *H. influenzae* with L4 and L22 riboprotein mutations showed TELI MIC values from 32 to 256 mg/L. TELI was 1.5- to at least 4-fold more active than azithromycin (AZI); and SOLI was 2- to at least 4-fold more active than TELI. Against all 1,198 *H. influenzae* isolates, SOLI was 2-fold more active than AZI; and TELI was 1.5- to at least 4-fold more active than TELI. When GO was omitted from the formulation no antimicrobial activity was observed. Increase vacuolation of the cytoplasm and cell ghosts were also observed. E. coli appeared elongated with no visible septa, highly vacuolated cytoplasm with platted cell walls compared to controls. When GO was omitted from the formulation no antimicrobial activity or cellular damage was observed. When pMPO was omitted from the formulation, only increased vacuolation due to H2O2 was observed at the longer exposure times. High levels of H2O2 generated from GO and glucose demonstrated microbial activity with minimal cellular damage.

Conclusions: E-101 is a potent myeloperoxidase enzyme system with multiple oxidative mechanisms of action. Ultrastructural analysis following E-101 treatment showed targeted septum formation in both *S. aureus* and *E. coli*. Induction of mesosomes in *S. aureus* by E-101 is indicative of an effect on the cytoplasmic membrane.

**P1454** Antibacterial mode of action and resistance studies with the silver cation (Ag+) in Staphylococcus aureus

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Objectives: Compounds containing Ag+ are commonly used to treat and prevent bacterial infection in burns and chronic wounds. However, the antibacterial mode of action of Ag+ remains poorly characterised. Furthermore, the current susceptibility levels of clinical isolates towards Ag+ and the propensity for bacteria to develop Ag+ resistance have not been established. Here we present a series of studies to investigate these aspects in *Staphylococcus aureus*.

Methods: Membrane integrity of *S. aureus* SH1000 exposed to Ag+ (in the form of silver nitrate) at 4x the minimum inhibitory concentration (MIC) was examined using the BacLight® assay, and by measurement of potassium ion (K+) leakage from cells using atomic absorption spectroscopy. Bacteria were examined for morphological changes using scanning electron microscopy (SEM) after exposure to Ag+ at 4x MIC for 5 and 120 min. Silver nitrate MICs were determined by agar dilution for a collection of *S. aureus* isolates (n=833) collected from hospitals across Europe between 1997 and 2010. To examine the in vitro development of staphylococcal resistance to Ag+, *S. aureus* SH1000 was subjected to continuous subculture in the presence of sub-MIC concentrations of Ag+. The MIC for SH1000 following 42 days continuous challenge with sub-MIC concentrations of Ag+ was determined using the Bactec® assay.

Results: Membrane integrity decreased by 97% after 10 min exposure to Ag+ as determined by the BacLight® assay, coinciding with 96% loss of intracellular K+. SEM revealed no morphological changes in bacteria exposed to Ag+. All clinical *S. aureus* isolates were susceptible to 8–16 mg/L silver nitrate. Reduced susceptibility to Ag+ was not observed in *S. aureus* SH1000 following 42 days continuous challenge with sub-MIC concentrations of Ag+.

Conclusions: Although no overt cell lysis occurred in cells following challenge with Ag+, the rapid and extensive loss of membrane...
integrity observed strongly suggests that the antibacterial activity of Ag+ results directly from damage to the bacterial membrane. The universal susceptibility of staphylococcal isolates to Ag+, coupled with the inability to select endogenous resistance upon extended exposure to Ag+, confirm that silver compounds remain a viable alternative to antibiotics for the treatment of topical staphylococcal infections.

**P1455** Detection of lymphogranuloma venereum based on the deletion in pmhP gene may underestimate the extension of the European outbreak

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**Objective:** During the last decade, an increase number of lymphogranuloma venereum (LGV) L2b-serovar has been described in Europe. According to a low number of known sequences, different multiplex real-time PCR assays were adapted to detect simultaneously Chlamydia trachomatis and LGV. They were based on the detection of ompA gene or multicopy criptic plasmid and an internal deletion in pmhP gene, respectively. In this study we evaluate the usefulness of this deletion to detect LGV clinical strains.

**Methods:** C. trachomatis was detected in 130 rectal samples from men who have sex with men (MSM) with proctitis using BD ProbeTec-ET system and Abbott RealTime CT/NG. In order to specifically detect L-serovars, a second real-time PCR was performed based on the deletion in pmhP described in all known sequences of LGV, according to the literature. Independently of the real-time PCR result, the complete ompA gene and a fragment of pmhP gene (400bp) were sequenced. Phylogenetic analyses with clinical and reference sequences of ompA and pmhP were constructed using PhyML method.

**Results:** 32/130 (24.6%) were positive for LGV by real-time PCR based on internal deletion of pmhP gene. Sequencing of ompA gene confirmed that they are highly associated with L2b-serovar. Phylogenetic analysis with ompA sequences from clinical and reference strains revealed the differentiation of three serocluster classes, which do not correlate with disease phenotype, being all LGV-PCR positive samples included in B-cluster (next to D, B, E, L1 and L2/L2b). Interestingly 22 sequences initially LGV-negative (22.4%, 22/98), were also clustered with B-cluster. Phylogenetic tree of pmhP gene from clinical and reference sequences showed three pathobiotypes (ocular, urogenital and LGV) with bootstrap value >95%. The LGV cluster included those 32 LGV-positive based on real-time PCR but also those 22 LGV-negative. The 22 LGV-negative did not carry the deletion in pmhP widely used in diagnosis.

**Conclusion:** A high proportion of clinical strains of LGV were found without the deletion in pmhP gene widely used in the diagnosis of LGV. This result reveals that this new variant will not be detected in the clinical setting which may underestimate their detection in the L2b-serovar European outbreak. This finding has important public health consequences for the management and control of this epidemic.

**P1456** Pharmacoeconomic analysis of perioperative antibiotic prophylaxis application in Lombardy, Italy

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**Objectives:** This study analyzes the actual cost of perioperative antibiotic prophylaxis (PAP) in our region and compares it with the ideal cost of an appropriate antibiotic prophylaxis according to an evidence-based protocol.

**Methods:** We performed a cross-sectional analysis of PAP in 7 major general hospitals (986 beds each on average) in Lombardy. We evaluated the appropriateness of: the PAP prescription, the choice of drug and the PAP duration. The analysis included 2.0% of all surgical procedures performed in these hospitals in two different semesters (2007-T0 and 2009-T1). Sample selection occurred as follows: 1) each hospital analyzed the clinical records of 2.0% of surgical activity performed in periods listed above; 2) the analysis comprised 2.0% of every type of operation (according to ICD9 CM codes). The cost of each antibiotic was assumed to be half of the list price, that is the hospital price in Italy. Data regarding T1 are shown here.

**Results:** We analyzed 2285 records and identified 6 sample groups: 1. PAP not performed and not expected, 445 pts; 2. PAP correctly performed and expected, 485 pts; 3. PAP not performed, but expected, 228 pts; 4. PAP performed, but not expected, 153; 5. PAP expected but performed with an incorrect drug, 517 pts; 6. PAP expected but inappropriate prolongation of antibiotic course, 457 pts. The theoretical cost of PAP in our sample should have been €4,915,95 (mean cost per pt €2.15, 95% CI 2.08–2.23), while the actual cost of PAP plus unnecessary prolonged antibiotic courses was €22,036 (mean cost per pt €9.66, 95% CI 8.88–10.44). In 2009, the surgical interventions performed were 111,917 in the 7 hospitals under study, and 846,372 in Lombardy. According to our data we can estimate that the mean difference between real and theoretical cost per pt is €7.51 (95% CI 6.74–8.28) vs. €5.32 (95% CI 4.66–5.96) for unnecessary antibiotic therapy, and €2.19 (95% CI 1.82–2.54) for the increasing cost of inappropriate PAP. Hence, applying an appropriate PAP, we could save each year €840,440 (95% CI 753,937–926,943) in our 7 hospitals (€595,905 for unnecessary antibiotic therapy and €244,167 for inappropriate PAP) and €6,355,826 (95% CI 5,701,645–7,010,008) in Lombardy.

**Conclusion:** PAP is often inappropriate in our hospitals in Lombardy and the implementation of a correction, evidence-based protocol could significantly reduce PAP cost, as well as unnecessary antibiotic administration and therefore antibiotic resistance.

**P1457** Granulomatous diseases following treatment with anti-TNF therapies

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**Objective:** In this study, we have aimed to determine the prevalence of granulomatous diseases following anti TNF therapy.

**Methods:** We retrospectively analysed the clinical data of 142 patients who were treated by anti-TNF therapies between April, 2005 and December, 2010 in Department of Rheumatology. All of the patients underwent routine Tuberculin Skin Test (TST) before starting anti-TNF therapy. Patients with an induration of ≥5mm received a nine months preventive therapy with isoniazid. In these patients with positive TST results the anti-TNF therapy was started at least three weeks after starting preventive chemotherapy with isoniazid. A second TST was performed two weeks later if the initial TST was negative.

**Results:** Baseline characteristics, TST results, type of the anti-TNF therapy, and the preventive therapy status of the subjects are shown in Table-1. Granulomatous infections were diagnosed in two of 142 patients and both of them received preventive chemotherapy with isoniazid for nine months. One patient with ankylosing spondylitis with 13 mm induration of TST and with known previous TB contact an active TB patient received subcutaneous Etanercept therapy for six months. After starting Infliximab for recurrence of disease, TB lymphadenitis was diagnosed at the 4th month of infliximab therapy. Second patient with rheumatoid arthritis received subcutaneous Etanercept therapy for 12 months. After starting subcutaneous Adalimumab therapy for recurrence of the disease, cutaneous leishmaniasis was diagnosed at the 10th month of this therapy.

We did not observe development of TB in the subgroup that was treated by Etanercept (60% of the study patients). Additionally, we did not observe TB reactivation in two patients with rheumatoid arthritis who had been treated for previous pulmonary TB during in last 2 years before Etanercept therapy. These patients with rheumatoid arthritis has been threatened with etanercept for 30 and 42 months.

**Conclusion:** Undetection of TB reactivation after Etanercept therapy in two RA patients who were treated for pulmonary TB was evaluated as a meaningful data the drug reliability.

In our both patients, the granulomatous infection occurred after the switch of anti-TNF agent. Interestingly these patients were receiving Etanercept.
therapy before switching and did not develop TB. This data is a new finding of our series. As our study group is relatively small, further studies are warranted in larger series.

Table 1. Baseline characteristics of the study subjects.

| Diagnosis | N(%) |
|-----------|------|
| Unknown   | 6(8.3)|
| Enough     | 3(4.2)|
| E. coli    | 4(5.6)|
| Shigella   | 3(4.2)|
| Treponema  | 1(1.4)|

**P1458 Campylobacter bacteraemia: a 10-year experience in a single centre**

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**Objectives:** Campylobacter bacteraemia (CB) is uncommon and occurs particularly among patients with HIV, hypogammaglobulinemia and in extreme ages. This study was designed to evaluate the clinical characteristics and risk factors for CB in a tertiary referral center, serving over a million citizens in northern Israel and over 40,000 blood cultures taken a year.

**Methods:** Retrospective computerised data was retrieved from January 2000 to October 2010. **Results:** There were 36 patients with CB including 10 patients less than 18 years of age including only 3 neonates and 7 patients were older than 65 years (mean age was 42 years, range 1 month-91 years). Common underlying conditions were: hematological malignancy (16), solid tumor (2), organ transplantation (2), chronic liver disease (3), and diabetes mellitus (2). Three patients had underlying disease. Of notice, 23 (64%) patients were on immunosuppressive therapy at the time of bacteremia. The most common symptom among these patients was fever followed by abdominal pain, diarrhea and vomiting. CB was asymptomatic in 3 cases. Three patients had a concomitant positive stool culture. Campylobacter jejuni was the most common isolated species (15 isolates, 41.6%), followed by C. coli (4 isolates, 11%), C. fetus (2 isolates, 5.5%). Species were not identified in 15 cases (41.6%). Most isolates were susceptible to macrolide drugs (29/31 tested, 94%), Aminoglycosides (23/24 tested, 96%) and clindamycin (18/20 tested, 90%). But 14/30 tested (53%) were resistant to fluoroquinolones. Antibiotic treatment was given to 31 patients but it was adequate (according to in vitro susceptibility) in only 18 patients (58%). The different regimens included: quinolones, macrolides, penicillins, aminoglycosides, combination of aminoglycosides and β-lactam drugs. Relapse occurred in 3 patients. The thirty day mortality rate was 8.3% (3 patients); in only one case mortality was due to CB. **Conclusion:** In this study most episodes of CB occurred among severely immunocompromised patients and were caused by C. jejuni. Mortality rate was low despite the discrepancy between antibiotic regimens and in vitro susceptibility results.
Infections by Corynebacterium species in immunocompromised and non-immunocompromised patients

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Objectives: Firstly, to evaluate the clinical significance of Corynebacterium isolation from clinical samples taken to immunocompromised (IC) and non-immunocompromised (non-IC) patients. Secondly, to determine possible clinical differences among infected patients of both groups (IC and non-IC).

Methods: During a 5 years period (2000–2004) all Corynebacterium species isolated from 136 selected clinical specimens (94 blood cultures, 24 intravascular catheters, 5 vascular ulcer exudates, 1 joint fluid, 1 pacemaker and 1 sputum) were identified at species level by using API Coryne™, V2.0, Biolog™ GP2, and 16S rDNA and rpoB. The clinical significance of each isolate was categorized by means of clinical and epidemiological data as definitive or probable. All charts were examined, and the main data noted. Patients were classified as IC or non-IC, and differences between both groups were compared by the EPIDAT program.

Results: A total of 108 patients were diagnosed of significant clinical infection by Corynebacterium spp., 81 in IC (68 definitive, 13 probable) and 27 in non-IC (26 definitive, 1 probable) patients. The most frequent species identified in IC/non-IC patients were C. amylolactum (23/4), C. jeikeium (12/4), C. striatum (10/5), C. afermentans spp. afermentans (8/1), C. coyleae (7/2), C. urealyticum (3/3), C. ureascolicolorans (2/3), C. imitans (1/2), C. mucificans (1/1), C. simulans (1/1), C. xerosis (1/1). Twelve patients, all IC, had infections by rare or non-identified species: C. appendicis (2), C. aurimucosum (2), C. propinquum (1), C. pseudolipophilicum (2), C. riegelii (1), and Corynebacterium spp. (4).

Table shows clinical data of 108 patients (81 IC and 27 non-IC) with infections by Corynebacterium species.

Conclusions: 1. Infections were more common in males (IC: 66.6%/non-IC: 63.0%) than in females.
2. Most patients had suffered recent invasive procedures (88.0%) and were IC (90.1%).
3. Most patients were treated with glycopeptides, aminoglycosides and penicillins.
4. The outcome was mostly favorable (IC: 77.8%/non-IC: 74.1%).
5. The highest rate of history of recent invasive procedure (p = 0.041) and increased use of glycopeptides (p = 0.026) in IC patients than in non-IC, was probably due to the nature of the process and empirical treatment with glycopeptides (and later maintained them) in IC patients.

Table. Clinical data in 108 patients from whom Corynebacterium spp. were isolated

| Age (years) | IC patients n=81 (%) | Non-IC patients n=27 (%) |
|------------|----------------------|-------------------------|
| Range | 0-91 | 13-84 |
| Mean ± SD | 56.11±25.78 | 59.92±19.81 |

| Gender | IC patients | Non-IC patients |
|--------|-------------|-----------------|
| Men | 54 (66.6%) | 17 (63.0%) |
| Site of infection: Blood | 70 (86.4%) | 24 (88.9%) |
| Vascular catheter | 13 (16.0%) | 1 (3.7%) |
| Soft tissue | 5 (6.2%) | 1 (3.7%) |
| Urinary Tract | 4 (4.9%) | 1 (3.7%) |
| Others | 5 (6.2%) | 2 (7.4%) |

| Recent invasive procedure | IC patients | Non-IC patients |
|---------------------------|-------------|-----------------|
| Intravascular catheter | 73 (90.1%) | 22 (81.5%) |
| Major surgery | 21 (25.9%) | 8 (29.6%) |
| Biopsy | 11 (13.6%) | 1 (3.7%) |
| RPM | 10 (12.3%) | 0 (0.0%) |
| Hemodialysis | 9 (11.1%) | 0 (0.0%) |
| Others | 7 (27.0%) | 14 (51.8%) |

| Antibiotic treatment | IC patients | Non-IC patients |
|----------------------|-------------|-----------------|
| Glycopeptides | 46 (56.8%) | 8 (29.7%) |
| Aminoglycosides | 26 (32.1%) | 8 (29.7%) |
| Penicillins | 19 (23.5%) | 9 (33.3%) |
| Cefalosporins | 9 (11.1%) | 3 (11.1%) |
| Fluorquinolones | 7 (8.6%) | 5 (18.5%) |
| Macroide | 0 (0.0%) | 0 (0.0%) |
| Others | 6 (7.4%) | 3 (11.1%) |

| Outcome | Favorable | IC patients | Non-IC patients |
|---------|-----------|-------------|-----------------|
| Favorable | 63 (77.8%) | 20 (74.1%) |
| Exitus | 14 (17.3%) | 5 (18.5%) |
| Non-evaluable | 4 (4.9%) | 2 (7.4%) |

*Statistical significance. **PDM: Prolonged rupture membranes.

Viral infections and analysis of circulating immune complexes in patients with Nijmegen breakage syndrome, a cancer-prone disease with profound immunodeficiency

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Objectives: Nijmegen breakage syndrome (NBS) is an autosomal recessive chromosomal instability disorder characterized by microcephaly, growth retardation, radiosensitivity, combined immunodeficiency, increased susceptibility to infection, and a high predisposition for the development of lymphoid malignancy. Previously we reported on a high prevalence of viral infections among NBS patients caused by viruses known to be associated with lymphomas, with a high frequency of EBV followed by HBV. Although in about 35% patients these viruses were not detected or were only transiently present, formation of circulating immune complexes (CIC) or non-specific aggregates could not be ruled out. The aim of the study was to assess whether the absence of detectable genetic material of selected viruses in peripheral blood of patients with NBS may be caused by their protection within a thick layer of serum proteins or masked in the form of CIC.

Methods: A total of 52 patients from Polish registry in whom detailed investigations confirmed the diagnosis of NBS were periodically monitored for humoral and cellular immune parameters, C3 and C4 complement components, and for selected viral infections by PCR analysis. In patients with abnormally high activity or progressive disruption of complement, immune complexes were isolated and viral genetic material was analysed at the molecular level.

Results: Viral infections (EBV, HBV, HCV and/or CMV) were found in 65.2% patients. All of them were EBV DNA positive; persistent HBV DNA was present in 9.6% patients, HCV RNA in 3.8% and CMV DNA in 5.8%. “Transient” EBV DNA was observed in 3 patients and HBV
DNA in 2. During follow-up severe decrease of C4 component was found in 11 (21.2%) patients accompanied by deficit of C3 component in 4 of them. Two patients with negative and one with transiently detected HBV DNA in whole serum have had detectable HBV DNA in CIC, while EBV DNA was found in CIC from one patient.

**Conclusion:** Our results show that in patients with NBS viruses may be hidden in CIC. Progressive hypocomplementemia may result from increased consumption secondary to persistent CIC. This, in turn, may be responsible for increased susceptibility to recurrent infections and should be warning for clinicians.

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### Studies on sepsis in haemodialysis patients

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**Objectives:** According to the annual statistical survey conducted by the Japanese Society for Dialysis Therapy, the most common cause of death in total dialysis patients in 2008 was heart failure (23.7%), followed by infections (19.9%). Especially, sepsis is noted as requiring the utmost caution among other infections because of the high case fatality rate as well as the high chance of infection. The aim of this study is to summarize sepsis cases that occurred in hemodialysis patients at our institution and to investigate relevant bacteriologic background and risk factors.

**Methods:** In the 9-year period from 2001 to 2009 at our hospital, 205 patients receiving prolonged dialysis presented with fever and had blood culture tests due to suspected sepsis. The factors considered were the associated prognostic factors of age, gender, dialysis history, presence of diabetes, catheter placement, body temperature, and blood culture test results such as C-reactive protein (CRP), white blood cell count, hemoglobin, platelet count, serum albumin, presence of methicillin-resistant bacteria, and presence of Gram-negative bacilli.

**Results:** In total, 465 blood cultures from 205 cases of patients were performed, yielding a positive rate of 23.7%. Gram-positive cocci, Gram-positive bacilli, and Gram-negative bacilli accounted for 78.4%, 5.2%, and 16.4% of the isolates, respectively. Among the Gram-positive cocci, staphylococci were the most common species with a rate of 83.5%. Among the staphylococci, methicillin-resistant *Staphylococcus aureus* was the most frequent with a rate of 42.1%. As a result, 73 patients were clinically diagnosed with sepsis. Regarding outcome, there were 38 deaths during the course of treatment; methicillin-resistant bacteria were detected in 24 of these cases, reconfirming the poor prognosis. Furthermore, multiple logistic regression disclosed that both CRP and thrombocytopenia had a significant effect on mortality rate.

**Conclusion:** Hemodialysis patients are exposed to increased opportunities for infection from various reasons, including vascular access and frequent hemodialysis procedure in addition to the lowered cellular and humoral immunity. Our results indicate a poor prognosis for elevated CRP and reduced platelet count. When administering antibiotic agent, selection of vancomycin should be considered as an initial treatment by the microbiologist was adequate in 119 pts (92.2%). We did not find any microbiological differences in 30 and genus differences in 15 flasks. Cultures positive for *S. epidermidis* were identified in 44 and failed analysis in 32 cases. Identical results between 16S and traditional phenotypic identification was obtained in 185/293 (63%), species differences in 30 and genus differences in 15 flasks. Cultures were polymicrobial in 30 and yeast was cultured in 9 flasks. Either the 16S or traditional identification was negative in 24 flasks (10 double negatives).

**Results:** The results from 293 positive culture flasks were evaluated. The 16S diagnosis was found acceptable in 217 (74%), unacceptable in 44 and failed analysis in 32 cases. Identical results between 16S and traditional phenotypic identification was obtained in 185/293 (63%), species differences in 30 and genus differences in 15 flasks. Cultures were polymicrobial in 30 and yeast was cultured in 9 flasks. Either the 16S or traditional identification was negative in 24 flasks (10 double negatives).

**Conclusions:** Our study suggests that the F-18 FDG PET/CT is a useful tool for the diagnosis of brucellar spondylodiskitis, for defining the extent of the disease and for monitoring the efficacy of treatment.

### Bloodstream infections

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**Objectives:** Early and sufficient antibiotic treatment of patients with signs of sepsis is mandatory for better outcome. Rapid and precise microbiological diagnosis is believed to be of pivotal significance in this respect.

**Methods:** A RCT was performed where the results of Gram-stained smears of the blood and subsequent traditional phenotypic identification (*Vitec*, *Api*) was augmented with direct 16S rDNA PCR and sequencing. The results from 293 positive culture flasks were evaluated. The 16S diagnosis was found acceptable in 217 (74%), unacceptable in 44 and failed analysis in 32 cases. Identical results between 16S and traditional phenotypic identification was obtained in 185/293 (63%), species differences in 30 and genus differences in 15 flasks. Cultures were polymicrobial in 30 and yeast was cultured in 9 flasks. Either the 16S or traditional identification was negative in 24 flasks (10 double negatives).

**Results:** The results from 293 positive culture flasks were evaluated. The 16S diagnosis was found acceptable in 217 (74%), unacceptable in 44 and failed analysis in 32 cases. Identical results between 16S and traditional phenotypic identification was obtained in 185/293 (63%), species differences in 30 and genus differences in 15 flasks. Cultures were polymicrobial in 30 and yeast was cultured in 9 flasks. Either the 16S or traditional identification was negative in 24 flasks (10 double negatives).

**Conclusions:** Our study suggests that the F-18 FDG PET/CT is a useful tool for the diagnosis of brucellar spondylodiskitis, for defining the extent of the disease and for monitoring the efficacy of treatment.
Background: Coagulase-negative staphylococci (CNS) are frequent contaminants of blood cultures. We investigated predictors of blood stream infection (BSI) in patients with CNS bacteremia, and the usefulness of the Systemic Inflammatory Response Syndrome (SIRS) criteria.

Methods: Prospective collection of clinical and laboratory parameters in adults with ≥1 positive blood culture in a tertiary hospital between 2003 and 2007. One episode of CNS bacteremia was defined as growth of CNS only in ≥1 blood culture taken within 1 week. Diagnosis of BSI versus contamination was assessed by a trained investigator and confirmed by an infectious disease specialist. Logistic regression was used to estimate the odds ratios (OR) of BSI in patients with CNS bacteremia.

Results: Of 3,060 positive blood cultures, 673 episodes of CNS bacteremia were identified. Of these, 243 (36.1%) were considered as BSI and 430 (63.9%) as contamination. The median number of positive blood cultures was 1 (IQR 1–1) for contamination and 2 (IQR 1–4) for BSI. There was no difference in age, sex, comorbidities (diabetes, alcohol, intravenous drug use, HIV, immunodeficiency) between patients with BSI and those with contamination. BSI was associated with surgery during hospitalization (37.9% versus 22.8%, p < 0.001), central venous catheter (67.9% versus 28.8%, p < 0.001) and intensive care unit (49.0% versus 34.2%, p < 0.001). Overall, 232 patients (95.5%) with BSI and 307 (71.4%) with contamination (p < 0.001) had ≥1 SIRS criterion. Leukocytes >12 or <4 G/L was the most frequent SIRS criterion associated with BSI, followed by fever or hypothermia, tachycardia and tachypnea. The probability of BSI increased with additional SIRS criteria (test for trend p < 0.001), ranging from 19.8% in patients with only 1 criterion to 49.3% for 2, 66.4% for 3, and 80.8% for 4 SIRS criteria. Among patients with 2 SIRS criteria, probability of BSI increased to 67.0% if there was a central venous catheter.

Conclusions: Temporal cut-offs appear to have limited utility with this pathogen.
Capsular types and genetic relatedness of *Klebsiella pneumoniae* isolates causing recurrent bloodstream infection

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**Background:** Capsular types and genetic relatedness of *Klebsiella pneumoniae* (KP) causing recurrent bloodstream infections (RBSI) have rarely been previously described.

**Methods:** We enrolled all patients with positive KP bacteremia from January 2000 to December 2005 in a tertiary hospital for further analysis. RBSI was defined as 2 or more episodes of KP bacteremia occurred more than 30 days apart. All RBSI isolates were retrieved for capsular cps genotyping for seven clinical significant capsular types (K1, K2, K5, K20, K54, K57 and a new capsular type [N1]) as previously described. Isolates with the same capsular type from the same patients were further evaluated by pulsed-field gel electrophoresis for their genetic relatedness.

**Results:** There were 3067 patients with KP bacteremia during the study period and 129 patients (4.2%) with 301 episodes of bacteremia were considered as RBSI during the 9 year period. Capsular types were determined in 127 isolates (42.2%) from 73 patients by cps genotyping. Capsular type K2 was the most commonly encountered (N = 52, 17.3%) followed by K1 (N = 27, 9.0%), K54 (N = 18, 6.0%), K20 (N = 11, 3.7%), K57 (N = 11, 3.7%), K5 (N = 6, 2%) and N1 (n = 2, 0.6%). There were 27 male and 7 female patients (46.6%) infected by the same capsular type in each episode. The median age was 55 year-old (range: 27–86). Time between each bacteremia episode ranged from 35 days to 2806 days (median: 195 days). Underlying conditions include diabetes mellitus (50%), malignancies (38.2%) and liver cirrhosis (26.5%). The origin of bacteremia included biliary tract infection (N = 9, 26.5%), liver abscess (N = 8, 23.5%), pneumonia (N = 8, 23.5%), primary bacteremia (N = 7, 20.6%), urinary tract infection (N = 4, 11.8%). The PFGE results were indistinguishable for 30 patients of their isolates (N = 58). Four patients were infected by the different pulsotypes of KP with the same capsular types (one K1 and 3 K2 capsular types).

**Conclusions:** RBSI cause by *K. pneumoniae* is not uncommon and infection by the same capsular strain could be found in certain patients. Further study is mandated to identify risk factors of KP related RBSI.

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**P1470**

A 5-year retrospective review of BSI in a national bone marrow transplantation unit

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**Objectives:** Blood-stream infection (bsi) is an important cause of morbidity and mortality among patients with haematology malignancy and those undergoing bone marrow transplantation. The spectrum of infections and susceptibility patterns are extremely important in determining the empiric antimicrobial choice for febrile neutropenia. We performed at 5-year retrospective review of bsi among haematology patients in order to review causative organisms and their susceptibility patterns and compare to National and European data.

**Methods:** St. James Hospital is a 1,000 bed tertiary referral university hospital with a 21 bed Haematology ward that includes the National Bone Marrow Transplantation unit. A retrospective five-year review of all episodes of bsi between 2005 and 2009 was undertaken. An episode of bsi was defined as isolation of a micro-organism from blood cultures. All isolates recovered from a patient within 14 days of a positive blood culture were counted as a single episode. Two isolates of coagulase negative staphylococci or corynebacteria from one blood culture set were required to be considered significant.

**Results:** There were 623 episodes of bsi; 50% were caused by Gram-negative organisms (GNO) and 47% by Gram-positive organisms (GPO) (Table 1). Coagulase negative staphylococci were the predominant cause of bsi (27%), followed by *E. coli* (19%) and *Klebsiella* species (10%). 14 of 39 (36%) of *Staphylococcus aureus* bsi were caused by Metcillin Resistant *Staphylococcus aureus*. 29 of 45 (64%) of *Enterococcus faecium* bsi were vancomycin resistant. Ciprofloxacin resistance among GNO rose from 8% in 2005 to 33% in 2007 and decreased to 8% in 2009 with a similar trend in gentamicin resistance. Ciprofloxacin resistance rates reflected ciprofloxacin usage. Less than 1% of GNO displayed extended spectrum β-lactamase production.

**Conclusion:** Our study demonstrates that GNO account for slightly more bsi’s than GPO which is consistent with more recent studies. Resistance to metcillin among MRSA are similar to national data, but vancomycin resistance among enterococci is higher. It is likely that quinolone resistance among GNOs reflects quinolone usage and has a similar effect on gentamicin resistance.

| Table 1: Distribution of organisms 2005−2009 |
|---------------------------------------------|
| n (%)                        | 2005 | 2006 | 2007 | 2008 | 2009 | Total |
| GP bacteria                  | 47(48.4) | 37 (38.5%) | 73 (50%) | 69 (51%) | 80 (45%) | 306(47) |
| GN bacteria                  | 48(49.4) | 56 (58.3%) | 70 (49%) | 60 (44%) | 94 (54%) | 328(50.4) |
| Anaerobes                    | 0 | 0 | 1 | 0 | 0 | 1(0.3) |
| Fungi                        | 2 | 2 | 1 | 5 | 3 | 13(2) |
| Mycobacteria                 | 0 | 1 | 0 | 0 | 0 | 2(0.3) |
| Total                        | 97 | 96 | 145 | 135 | 177 | 650 |

n = number; GP: Gram positive; GN: Gram negative.
Can syndromic surveillance based on crude mortality serve as a tool for monitoring healthcare-associated bloodstream infections?

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Objective: French hospitals must report all deaths attributable to health care associated infections (HCAI); however, in 2006, only 300 deaths were reported. This study was performed at a 2,789 tertiary-care referral teaching center admitting yearly >120,000 patients in Marseille (France) to evaluate if syndromic surveillance of HCAI bloodstream infections (HCAI-BSI)-associated deaths could be substituted to mandatory reporting.

Methods: Attributable mortality was calculated for HCAI-BSI patients and a cohort study was performed to compare patients whose deaths were reported to patients whose deaths were not.

Results: In 2008, 1,033 HCAI-BSI were included, 115 patients died within 15 days after the onset of BSI while 10 deaths were reported. Attributable mortality was 11.89% representing an excess of 100 deaths. The cohort study showed no difference between both groups regarding age, gender, severity of illness, underlying disease, organ dysfunction, surgical intervention, time between admission and BSI onset, primary or secondary BSI, duration of bacteremia, ICU transfer, or treatment limitation. One significant difference was found: admission at hospital S following the arrival of a young hospital epidemiologist. Extrapolation of our data to the national level suggests that 12,400 patients could have died of HCAI-BSI constituting the 6th leading cause of death in France.

Conclusion: Report of HCAI-BSI-related deaths is more linked to subjectivity than to rigorous analysis with a number of HCA-BSI related deaths >10 times higher than the reported one. These results underline the necessity to switch from mandatory reporting to systematic surveillance of laboratory results of HCAI-BSI-related deaths to obtain reproducible results.

Bacterial bloodstream infections in a newly constructed medical centre in Saint Petersburg, Russia

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Objectives: The aim of the present study was to reveal the spectrum of bacteria, causing bloodstream infections, in the multidisciplinary medical center, that accumulates patients from all regions of Russia, during the first year from its foundation, with low possibility of local nosocomial strains’ formation.

Methods: The cultures were isolated from blood with BactAlert (BioMerieux, France). The identification was performed by routine methods and sequencing (ABI Prism 3130, MicroSeq ID v2.0 Software, MicroSeq ID 16s rDNA500 Library v2.0). Resistance to routinely used antibiotics was studied by dilution techniques on Muller-Hinton agar (Oxford, GB).

Results: Bacterial bloodstream infections were revealed in 101 cases: Staphylococcus spp. were responsible for 47 (46.6%) ones. S. aureus was the causative agent in 18 (17.8%), coagulase-negative staphylococci (S. epidermidis, S. capitis, S. haemolyticus, S. hominis) – in 29 (28.7%) infections. Other Gram-positive cocci were Enterococcus faecalis in 7 (6.9%), E. faecium in 4 (3.9%) and Streptococcus spp. (S. constellates, S. mitis, S. sanguis) in 3 (2.9%) cases. Acinetobacter baumannii was revealed in 12 (11.8%), Klebsiella spp. (K. pneumoniae, K. oxytoca) in 10 (9.9%), E. coli in 8 (7.9%), Enterobacter spp. (E. cloacae, E. aerogenes, E. hormaechei) in 3 (2.9%) patients with sepsis. Rarely isolated bacteria comprised Bacillus thuringiensis in 1, B cereus in 1, Stenotrophomonas maltophilia in 1, Pantoea agglomerans in 1, Corynebacterium mucificans in 1, Paenibacillus spp. in 1 and non-identified bacterium in 1 case. Resistance to antibiotics was observed in 96 (95.1%) of bacterial isolates, multi-drug-resistant (MDR) were 34 (33.7%) strains. All E. faecium and 7 (58.3%) strains of A. baumannii were resistant to 7 or more antibiotics.

Multi-drug resistant E. faecium strains were susceptible to linezolide, A. baumannii – to tigecycline.

Meticillin resistant were 2 (11.1%) strains of S. aureus and 17 (58.4%) strains of coagulase-negative staphylococci. Vancomycin-resistant were 3 strains of E. faecium.

Conclusions:
1. Gram-positive bacteria were the leading causative agents of bloodstream infections in the newly-constructed hospital in Saint-Petersburg in 2010.
2. Meticillin-resistance was observed predominantly in coagulase-negative staphylococci, vancomycin-resistance – in E. faecium.
3. The most widespread Gram-negative agent of bloodstream infections was A. baumannii. All MDR A. baumannii strains were susceptible to tigecycline.

Coagulase-negative Staphylococcus bacteremia: a prospective study of clinical and microbiological predictors of true bacteremia

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Background: Coagulase-negative staphylococci (CNS) are both an important cause of nosocomial blood-stream infections and the most common contaminants of blood cultures (BC); judging the clinical significance of CNS is vital but often difficult. A prospective cohort study of patients with at least one BC positive for CNS was performed in order to explore the clinical and microbiological predictors of true bacteremia (TB) caused CNS.

Patients and Methods: A single reviewer examined the medical records of consecutive patients (with at least 2 consecutive samples for BC) with CNS positive BC in a tertiary-care referral teaching hospital (from January through June 2010). A determination of clinical significance was made and the result was considered as a TB (according to CDC criteria) or contaminant. Data collection from clinical records has been done according to a standard protocol. We analysed epidemiological, clinical, microbiological and laboratory variables to identify clinical and microbiological predictors of CNS TB.

Results: A total of 269 cases were included, 61% of which were men; mean age was 61 year-old; 97 (36%) were considered as TB. Predictors of TB in the bivariate analysis were: BC obtained in already admitted patients (versus those obtained at the Emergency Department – AP vs ED), having >2 positive blood cultures, time to positivity <16 hours, identification of Staphylococcus epidermidis, nosocomial bacteremia, hospital admission within the previous month, renal failure, alcoholism, severe liver failure, chemotherapy, immunosuppressive therapy, Charlson score >3, acute severity of illness at onset according to Winston criteria (I, II and III) and PITT score >1, previous surgery, previous invasive procedures, ICU admission, phlebitis, total parenteral nutrition and having a central venous catheter. In multivariate analysis predictors of true CNS bacteremia were: time to positivity <16 hours (OR 5.688; 95% CI 2.030–15.933), identification of S. epidermidis (OR 1.927; CI 95% 1.357–19.199), having >2 CNS positive BC (OR 1.927; CI 95% 1.357–19.199), central venous catheter (OR 2.810; 95% CI 1.332–5.930) and APvsED (OR 8.139; 95%CI 3.450–19.199).

Conclusions: The analysis of a cohort of patients with CNS bacteremia identified as predictors of TB microbiological features (time to positivity <16 hours and identification of S. epidermidis and >2 CNS positive blood cultures) and clinical aspects (having a central venous catheter and APvsED).
Comprehensive analysis of the impact of empirical therapy in the mortality of bloodstream infections

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Objectives: The epidemiology of bacteraemia has changed during last years. We aimed at identifying independent predictors of mortality in patients with bloodstream infections (BSI) and specifically, to estimate the impact of inappropriate empiric treatment (IET) in mortality after controlling for confounding factors.

Methods: A multicenter, prospective cohort study of BSI which occurred in adult patients in 15 Spanish hospitals from November 2005 throughout March 2006 was performed. BSI were classified as nosocomial (NOS), healthcare-associated (HCA) or community-acquired (CA) according to Friedman's criteria. The outcome variables were mortality at days 14 and 30. Multivariate analysis were performed by logistic regression.

Results: 822 episodes of BSI were included; 476 (58%) were NOS, 227 (28%) HCA, and 119 (14%) CA. Mean age was 66 years. Crude mortality at day 14 and 30 were 19% (155) and 23% (190), respectively. S. aureus, S. pneumoniae, Enterococcus sp., P. aeruginosa, A. baumanii, and Enterobacter sp. were considered as high risk microorganisms because were associated with higher mortality than other microorganisms (26% vs 14%; p < 0.001). Also mortality was lower when the source of BSI was the urinary tract, the catheter or the biliary tract (14% vs 28%; p < 0.001); the rest of sources were considered “high risk source”. In the multivariate analysis, variables independently associated with higher mortality at days 14 and 30 were: age (OR=1.2 and 1.5, respectively), ICU admission (OR=2.0 and 1.9), Charlson score >2 (OR=2.1 and 2.1), high risk microorganisms (OR=1.6 and 1.7), high risk source (OR=1.6 and 2.2), presentation with severe sepsis or shock (OR=3.4 and 3), and Pitt score >2 (OR=1.5 and 1.6). IET therapy was only associated with increased risk of mortality at day 14 (OR=1.7). The type of acquisition was not associated with mortality. When specifically investigating the impact of IET therapy in the 14-day mortality, Charlson score, ICU stay, presentation with severe sepsis or shock, and high risk microorganisms were found to be confounders; after controlling for these confounders using a forward multivariate analysis, the adjusted OR for IET was 1.8 (95% CI:1.1–3.0).

Conclusion: We identified predictors of mortality in patients with BSI. After carefully controlling for confounders, IET is associated with an increased risk of early death at day 14, but we were unable to demonstrate an impact at day 30, when the impact of other baseline features was higher.

Bloodstream infections due to ESBL-producing Escherichia coli can be safely treated with active β-lactam/β-lactam inhibitors

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Objectives: Carbapenems (CAR) are considered the drugs of choice for the treatment of invasive infections caused by extended-spectrum β-lactamase (ESBL) producing enterobacteria. The clinical efficacy of β-lactam/β-lactam inhibitors (BBLI) is controversial. We compared the prognosis or patients with bloodstream infection (BSI) due to ESBL-producing Escherichia coli (ESBLEC) treated with BBLI or CAR.

Methods: A pros-hoc analysis of all episodes of BSI due to ESBLEC treated with BBLI or CAR in monotherapy included in previously published prospective cohorts developed by our group between 2001 and 2006 were included. Episodes were excluded if the isolate was resistant to the antimicrobial used. All cohorts used similar questionnaires and the same microbiologic methods; ESBL detection and antimicrobial susceptibility were performed following the CLSI recommendations. We compared mortality at days 7, 14 and 30 by chi squared or Fisher tests, and by log rank test for Kaplan-Meier curves. Multivariate analysis using Cox regression were performed.

Results: Among the 72 patients empirically treated with BBLI (37 amoxicillin-clavulanate [AMC] and 35 piperacillin-tazobactam [PTZ]) and the 31 with CAR (22 imipenem [IMP], 8 meropenem [MER] and 1 ertapenem [ERT]), mortality was: 2.8% vs 9.7% (day 7); 9.7% vs 16.1% (day 14); and 9.7% vs 19.4% (day 28), respectively (p < 0.1 for all comparisons; log rank test, p = 0.2). In Cox regression analysis, therapy with BBLI or CAR was not associated with mortality. The variables associated were (HR and IC 95%): Pitt score >2 (3.4; 1.1–10.4), severe sepsis or shock (3.7; 1.1–11.8), and source other than urinary or biliary tracts (5.0; 1.5–17.2). Among the 54 patients who received definite therapy with BBLI (34 AMC, 18 PTZ, and 2 ampicillin-sulbactam) and the 120 with CAR (84 IMP, 16 MER, 20 ERT), mortality was: 1.9% vs 4.2% (day 7); 5.6% vs 11.7% (day 14); and 9.3% vs 16.7% (day 30) (p < 0.2 for all comparisons; log rank test, p = 0.4). In Cox regression analysis, therapy with BBLI or CAR was not associated with mortality. The variables associated were: severe sepsis or shock (3.7; 1.6–8.1) and source other than the urinary or biliary tracts (2.0; 0.9–4.5). No differences were found for individual drugs.

Conclusion: Until more data are available, we advocate the use of BBLI as an alternative to CAR in the treatment of BSI due to susceptible ESBLEC. BBLI are particularly useful as definite therapy to avoid the overuse of CAR.
**P1478** Trends of nosocomial bloodstream infection incidence in a haematology department, Edouard Herriot hospital, Lyon (France), 2004–2009: a surveillance-based study

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**Objectives:** To report the trends of nosocomial blood infection (NBI) incidence in a French hospital haematology department.

**Methods:** A prospective surveillance was performed in 3 haematology units of the Edouard Herriot hospital University Hospital, Lyon (France) between 01/01/2004 and 12/31/2009. All patients hospitalized ≥48 hours were included, only the first NBI was accounted if it occurred ≥48 hours after patient entry in the department and before the end of hospital stay. The case definitions of NBI infection were: 1) at least one positive blood culture with clinical signs for non-commensal microorganisms or ≥2 at least two positive blood cultures in case of microorganisms such as: coagulase-negative staphylococci, Bacillus sp. (except Bacillus antracis), Corynebacterium spp., Propionibacterium spp., Micrococcus spp. or another commensal microorganism with similar pathogenic impact. Incidence was the number of NBI per 1000 patient-days at risk. Poisson regression with year as explanatory variable was used for trend analysis.

**Results:** Totally, 3,001 patients counting for 77,380 patient-days at risk were included. Overall, 652 (20.2%) patients had NBI, the incidence was 11.25 per 1,000 patient-days. Demographic characteristics and trends of NBI incidence are reported in Table 1. An increased proportion of patients with aplasia (P < 0.001) and with central venous catheter was observed (P > 0.001). The NBI incidence was increased 8% per year (95% confidence interval 3%-14%, P < 0.001).

**Conclusion:** An increase of the NBI incidence was observed. The recent increased proportion of patient with aplasia since 2006 and the increased proportion of patients with central venous catheter for perfusion of more aggressive chemotherapy could explained these results.

Table 1. Description of the patients and microbiological outcomes in a haematology department, Edouard Herriot hospital, Lyon (France).

| Characteristic | 2004 (n=513) | 2005 (n=597) | 2006 (n=622) | 2007 (n=638) | 2008 (n=627) | 2009 (n=488) |
|---------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Age (years) | 52.5±15.6 | 53.4±16.0 | 53.8±16.1 | 54.0±16.2 | 53.7±16.0 | 54.2±16.1 |
| Gender | Male (n=280) | 44.1% | 47.5% | 47.1% | 47.7% | 43.8% |
| BC positivity | 28.2% | 30.3% | 30.7% | 31.3% | 30.6% | 31.7% |

**P1479** Pathogenic profiles of bloodstream infections in a West China hospital

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**Objectives:** To investigate the pathogenic characteristics in bloodstream infections (BSIs) in West China hospital.

**Methods:** A retrospective analysis of the microbiological results of blood culture and clinical data of BSIs from Jan 2009 to Mar 2010 was conducted at West China hospital.

**Results:** A total of 824 organisms were identified from the 824 episodes of the bloodstream infections during the study period. The organisms were most frequently (28.2%) recovered from the blood culture of the patients admitted to the intensive care unit (ICU). Gram-negative organisms, Gram-positive organisms and fungi constituted 434 (52.0%), 342 (41.0%) and 58 (7.0%) of the pathogens, respectively. Escherichia coli (42.2%), Klebsiella pneumoniae (15.4%), Acinetobacter spp. (11.5%) were the frequently isolated Gram-negative bacteria, moreover 60.7% of E. coli isolates and 37.3% of K. pneumoniae isolates produced Extended Spectrum β-lactamase and were multidrug resistant. Multidrug resistance was observed in 77.0% of all Acinetobacter spp. isolates. Coagulase-negative staphylococci (CoNS) (40.9%), Staphylococcus aureus (17.5%) and Enterococcus spp. (17.5%), were the most prevalent Gram-positive bacteria. The percentages of oxacillin resistant CoNS isolates and methicillin-resistant Staphylococcus aureus isolates were 84.2% and 30.0%, respectively. Only one Vancocycin resistant Enterococci was found. Candida spp. accounted for 82.8% of the fungi identified and non-albicans Candida spp. was predominant (77.1%). Of non- albicans Candida spp., C. parapsilosis was the leading species (27.1%), followed by C. tropicalis (22.9%), and C. glabrata (18.3%).

**Conclusion:** Gram-negative bacteria were the predominant pathogens for BSIs in West China hospital from Jan 2009 to Mar 2010. Multidrug resistant bacteria and non-albicans Candida species should be paid more attention to during the infection control, especially for the patients admitted to ICU.

**P1480** Few blood cultures – few infections?

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**Objectives:** The frequency of central venous catheter associated bloodstream infections (CVC BSI) is an important quality indicator in many European countries. However, according to CDC definitions CVC BSI can only be diagnosed when a positive blood culture (BC) report is available. The objective of this study was to investigate the association of BC frequencies and CVC BSI rates in intensive care units (ICUs).

**Methods:** The data of the German national nosocomial infection surveillance system (KISS) for ICUs were used to investigate this association. A questionnaire asking for the frequency of BC taken was send to all ICUs participating in KISS. The ICUs were stratified in four groups by their BC frequency and BC rates were associated with CVC BSI rates using a univariable and multivariable approach. The following parameters were used for adjustment: Type of ICU and type of hospital, affiliation of the microbiology laboratory, duration of stay, device utilization rates (CVC and ventilation).

**Results:** 223 ICUs provided their data. The median number of BC pairs taken was 60 with a huge variety from 3.2 to 680 per 1000 patient days. The median CVC BSI rate was 0.75 per 1000 CVC days. An increase of the BC frequency and BC rates were associated with CVC BSI rates using a univariable and multivariable approach. The interpretation of CVC BSI rates in German ICUs in the context of quality management is only possible if BC frequencies are also considered. If an external benchmarking of ICUs according to their CVC BSI rates is intended an adjustment according to the BC frequency is necessary.

**Conclusions:** We were astonished about the very low BC frequencies in many German ICUs which may lead to inadequate antimicrobial therapy. The interpretation of CVC BSI rates in German ICUs in the context of quality management is only possible if BC frequencies are also considered. If an external benchmarking of ICUs according to their CVC BSI rates is intended an adjustment according to the BC frequency is necessary.
**P1481** Influence of the timing of therapeutic interventions on the outcome of catheter-associated *Staphylococcus aureus* bacteremia: is there a need for speed?

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Objective: The objective of this study was to evaluate the effect on attributable mortality due to catheter-associated *Staphylococcus aureus* bacteremia (CA-SAB) of its two main therapeutic measures: the rapidity with which adequate antibiotic therapy is initiated and time taken to remove the intravascular catheter.

Methods: Adult patients from eight Dutch hospitals with a first episode of CA-SAB were included in this study. Inclusion was retrospective and began between July 1st 2003 and January 1st 2005, depending on the hospital; inclusion ended on December 31st 2008. Risk factors and outcome were retrieved from the patient charts. Primary risk factors were: failure to remove the intravascular catheter within 24 hours and failure to initiate antibiotic therapy within 24 hours. The primary outcome was attributable 3-month mortality. Secondary outcomes were: all cause 3-month mortality, hematogenous complications at 3 months, and all cause 12-month mortality.

Results: A total of 268 patients were included. Median age was 60 years. Co-morbidity included cardiovascular disease (44%), heart failure (15%) diabetes mellitus (23%), malignancy (28%), hematological malignancy (5.9%), and chronic obstructive pulmonary disease (9.3%). 34% of patients had tunneled catheters. The catheter was used for chemotherapy in 11% of the patients, total parenteral nutrition in 21%, and hemodialysis in 37%. Attributable 3-month mortality was 9.0%, all cause 3-month mortality 18%, and all cause 12-month mortality 30%. 27 patients (10%) suffered hematogenous complications. The catheter was removed within 24 hours in 62% of the patients, antibiotic therapy was initiated within 24 hours in 87%.

Attributable mortality was significantly associated with failure to initiate antibiotic therapy within 24hrs and with age, but not with failure to remove the intravascular catheters within 24hrs. The use of a tunneled intravenous catheter was associated both with a delay in catheter extraction and with a rapid initiation of antibiotic therapy. Changes in the assessment of outcome (all cause mortality, attributable mortality or hematogenous complications) determined which risk factors were found significant.

Conclusion: In this study, immediate initiation of antibiotic therapy was significantly associated with a lower incidence of attributable 3-month mortality, but not with less all cause 3-month mortality. However, studies into this subject are severely hampered by selection bias.

**P1482** Healthcare-associated versus hospital-acquired *Staphylococcus aureus* bacteremia

H. Shaked*, M. Paul, J. Bishara (Petah Tikva, IL)

Objective: *Staphylococcus aureus* bacteremia is acquired mainly in the hospital or in other healthcare settings. Data indicate that patients with health-care-associated (HCA) infections have a unique epidemiology and that the causative pathogens and the outcomes related to these infections more closely resemble those seen with infections acquired in the hospital. The objectives of this study were to characterize demographic, clinical features, and outcomes of patients with HCA and HA *Staphylococcus aureus* bloodstream infections.

Methods: Retrospective cohort study conducted in a single center between 1988–2007. Patients with clinically-significant *S. aureus* bacteremia were included and classified by place of acquisition. We compared between patients with hospital-acquired (HA) and healthcare-associated (HCA) bacteremia. Risk factors for 30-day mortality were assessed using multivariable logistic regression analysis. We documented microbiological failure (defined as persistence or relapse), 1-year mortality and 5-year survival. Cox regression analysis was used to estimate the hazard ratio (HR) for 5-year mortality with 95% confidence intervals (CI).

Results: Out of 1,347 episodes of *S. aureus* bacteremia, 86 were community-acquired and 1,261 episodes acquired in hospital or health-care setting constituted the study population. The proportion of MRSA was 48.2% (354/735) in HA vs. 42.2% (222/526) among HCA bacteremias, p = 0.04 overall and similar in the last 3 study years (122/244, 50% vs. 71/147, 48.3%, respectively, p = 0.74). The proportion HCA *S. aureus* bacteremias or MRSA bacteremias did not change throughout the study period. Mortality at 30 days and 1 year was 40.2% (507/1261) and 63.4% (800/1261) and similar for HA and HCA bacteremias. Five-year survival curves in both settings followed very similar patterns (HR 1.01, 95% CI 0.89–1.15). Risk factors for 30-day mortality were similar except for primary bacteremia, female sex and heart valve disease in the HA bacteremia group, and poor functional capacity in the HCA group.

Conclusion: HCA *S. aureus* bacteremia shares many similarities with HA bacteremia, especially with respect to the prevalence of MRSA strains and mortality rates and should be managed similarly.

| Table III. Management and outcomes of health-care associated (HCA) and hospital-acquired (HA) *Staphylococcus aureus* bacteremias |
|--------------------------------|
| HCA, N=534 | HA, N=755 | P-value |
|-----------------------------|-----------------------------|
| **Antibiotic treatment** | | |
| Inappropriate empirical therapy | 189/324 (58.1%) | 228/375 (61.2%) | NS |
| Inappropriate empirical for MRSA | 34/94 (36.2%) | 54/161 (33.5%) | NS |
| Empirical vancomycin for MRSA | 62/94 (65.9%) | 85/218 (39.1%) | NS |
| Inappropriate empirical for MRSA | 125/222 (56.2%) | 231/354 (65.3%) | NS |
| **Other management** | | |
| Central catheter removal documented | 39/171 (23.0%) | 49/131 (37.4%) | NS |
| Removal of foreign body | 13/15 (86.7%) | 7/9 (77.8%) | NS |
| Dacron debonding | 89/220 (40.5%) | 92/191 (48.3%) | NS |
| Any surgical intervention | 87/193 (45.2%) | 106/187 (56.7%) | NS |
| Vascular access therapy | 142/252 (56.5%) | 219/420 (52.6%) | NS |
| **Outcomes** | | |
| Persistence of bacteremia ≥ 7 days | 56/103 (54.5%) | 51/106 (48.1%) | 0.01 |
| Relapse | 21/247 (8.5%) | 16/245 (6.5%) | NS |
| Microbiological failure (presence ≥ 7 days or relapse) | 68/232 (29.4%) | 63/225 (28.2%) | 0.566 |
| Length of stay (days), all patients, median (inter-quartile range), N | 16/12 (5.0) | 17/19 (6.2) | 0.864 |
| Length of stay (days), patients discharged alive, median (inter-quartile range), N | 12/16 (4.4) | 14/19 (6.2) | <0.001 |
| 30-day all-cause mortality | 19/57 (33.3%) | 31/70 (44.3%) | NS |
| 1 year | 336/528 (64.0%) | 476/753 (63.1%) | NS |

1 Assessed for patients with central venous catheter at onset of infection
2 Assessed for patients with foreign body at onset of infection (see Table I)
3 Assessed for patients alive at day 7
4 Assessed for patients alive at day 30

**P1483** Impact of infectious diseases consultation on outcome of methicillin-resistant *Staphylococcus aureus* bacteremia in a tertiary care centre: a ten-year experience

F. Tissot*, T. Calandra, G. Prod hom, D.S. Blanc, G. Zanetti, G. Greub, L. Senn (Lausanne, CH)

Objective: To assess the impact of infectious diseases (ID) consultation on the management and outcome of methicillin-resistant *Staphylococcus aureus* bloodstream infections.


**Objectives:** Staphylococcus aureus (SA) is one major cause of bloodstream infection (BSI) and is associated with significant morbidity and mortality. As the outcome of these infections is usually determined by virulence factors and host response, we focused on clinical and molecular characterization of all consecutive SA BSI observed in our hospital during a 2-year-surveillance.

**Methods:** Observational, prospective study. We included all the inpatients consecutively observed between 01/01/2008 and 31/12/2009 with ≥1 positive blood culture for SA in the presence of clinical signs of infection (SA-BSI). Clinical and demographic parameters of patients enrolled in the study were compared using the chi-square and t-test. In MRSA strains, the SCCmec type was determined.

**Results:** We observed 81 SA-BSI in 72 patients. 32% (25/72) of BSI were caused by MRSA (MRSA BSI). In MRSA strains, SCCmec type I was found in 45% and type IV in 55% of cases. Both microbiology and clinical data are available for 51 out of 72 patients. Median age was 68 years (range 2–99); diabetes (36%) was the most frequent underlying disease, followed by haemodialysis (21%) and cancer (18%). 37% of cases were CVC related. Overall crude mortality was 30%; MRSA BSI were associated with higher prevalence of mortality (57% vs 6% MSSA BSI, p < 0.0001). Patients with MRSA BSI were older than those with MSSA BSI (75 vs 63 years; p = 0.04) whereas the two groups were comparable for underlying diseases. As compared with MSSA BSI, MRSA BSI were more frequently related to presence of CVC (64% vs 28% MSSAB; p = 0.01) and to surgical procedures (42% vs 16%; p = 0.04).

**Conclusion:** Our findings confirm a high prevalence of mortality associated with SA BSI, mainly in MRSA BSI. Patients carrying indwelling devices, with history of surgery and older age are prevalent in MRSA BSI. Type IV represents the most common SCCmec type in our hospital.
84.4% of patients received antifungals in period A and 83.9% in period B, therapy started within 48 hours from the diagnosis in 59.2% of CA during A and 61.7% during B. It was adequate for the antifungogram in 56.6% and 89.4% of cases respectively, and for duration in 62.5% and 42.6%. During A and B fluconazole was the first employed drug (67.8% and 51.4%), followed by any amphotericin B lipid-formulations during A (44.8%) and caspofungin during B (22.9%).

**Conclusions:** CA annual incidence was high. There was a shift of CA from ICU toward surgical and medical areas, maybe for the greater number of abdominal-pancreatic surgical patients and, in general, for the increasing number of more serious patients bedridden outside the ICU. The increased frequency of CVC-related CA in part is due to the perspective way to collect data and reflects the larger use of the device (CVC presence 81.2% during A and 92.9% during B). Treatment adequacy remains a challenge in terms of starting delay (median 4 days in period A and 4.5 days in period B) and duration (median 8 days in period A and 9 days in period B).

**Results:**

Table 1. Distribution and fluconazole susceptibility of isolated Candida species.

| Species         | 2004-08 (n=78) | 2009-10 (n=56) | Fluconazole susceptibility | Fluconazole susceptibility |
|-----------------|----------------|---------------|---------------------------|---------------------------|
|                 | S   SD  D  R  | S   SD  D  R  |                           |                           |
| Candida albicans| 34 1 40 10 92 | 40 0.5 0 10 0 |                           |                           |
| C. glabrata      | 41 8 18 2 62 | 35 6 3 7 94 |                           |                           |
| C. parapsilosis  | 15 10 9 1 95 | 100 100 0 0 0 |                           |                           |
| C. tropicalis    | 1 1 1 0 1 | 0 53 67 0 0 |                           |                           |
| C. krusei       | 1 1 1 0 1 | 0 53 67 0 0 |                           |                           |
| C. kefyr         | 0.6 1 1 0 | 10.5 20.5 2.9 100 0 |                           |                           |

Table 1. Distribution and fluconazole susceptibility of isolated Candida species.

**Conclusion:** Prior azole exposure and administration of TPN were identified as risk factors for first occurrence of candidemia with FLU-NS Candida species. Echinocandins were not identified as a risk factor for isolation of FLU-NS Candida species.

**References:**

[1] Shah*, B. Yau, J. Weston, T. Lascio, M. Salazar, H. Palmer, K. Garey (Houston, San Antonio, US)

**Objective:**

Pre-exposure to azole antifungals has been identified as a risk factor for fluconazole-non-susceptible (FLU-NS) Candida species in hospitalised patients with candidaemia.

**Methods:**

Retrospective cohort study of hospitalised patients with first occurrence of candidemia from 2006-09. Susceptibility of Candida species was determined using automated susceptibility testing (fluconazole) or E-test (caspofungin). The CLSI MIC breakpoint of ≤8μg/ml of fluconazole was used to define susceptible Candida species and MIC of ≥16μg/ml defined was used to define FLU-NS. Relative risk (RR) and 95% confidence intervals (CI) were calculated for variables with significant difference between patients with fluconazole susceptible (FLU-S) vs. FLU-NS Candida species candidemia (p < 0.05 considered significant).

**Results:**

One hundred seventy-seven patients aged 59±16 years (males: 55%, Caucasian: 53%, Apache II≥15: 47%) were identified of whom 34 (19%) had FLU-NS Candida species. One isolate was non-susceptible to caspofungin (MIC=16μg/ml). Candida species included: C. albicans (49%), C. glabrata (20%), C. parapsilosis (13%), and C. tropicalis (13%). Thirteen of 143 (9%) patients with FLU-S species and 8 of 34 (24%) patients with FLU-NS species had prior azole exposure (RR: 2.29, 95%CI: 1.20–4.37, p<0.02). Ten of 143 (7%) patients with FLU-S species and 5 of 34 (15%) patients with FLU-NS species had prior echinocandin exposure (RR: 1.86, 95%CI: 0.85–4.09, p=0.15). The other identified risk factor for FLU-NS species was total parental nutrition (TPN) administered to 56 of 143 (39%) patients with FLU-S candidemia and 20 of 34 (59%) patients with FLU-NS candidemia (RR: 1.90, 95%CI: 1.03–3.51, p=0.04).

**Conclusion:** Prior azole exposure and administration of TPN were identified as risk factors for first occurrence of candidemia with FLU-NS Candida species. Echinocandins were not identified as a risk factor for isolation of FLU-NS Candida species.
length of stay (LOS) >1 week (OR: 3.72) and period of stay (OR: 1.50) were independent risk factors for NBSI.

Age 40 to 50 years (OR: 4.38) and LOS >7 days (OR: 4.42) were linked with S. aureus NBSI. Admission from long-term care facilities (OR: 4.88) or previous ICU hospitalization (OR: 3.40), LOS >7 days (OR: 8.26), elective surgery (OR: 2.23), increasing SAPS II score (OR: 3.06) and year 2005 (OR: 4.32) were risk factors of P. aeruginosa NBSI.

Previous hospital stay (long-term care OR: 20.47; acute care OR: 2.72; and ICU OR: 7.42), elective surgery (OR: 7.38) and year 2005 (OR: 10.04) were associated with P. aeruginosa NBSI rather than S. aureus NBSI.

Conclusion: Identifying risk factors of ICU-acquired BSI may help to optimize appropriate preventive procedures and some measures might have different impact regarding the causal bacteria.

**P1490 Antimicrobial resistance in ICU-acquired bacteraemias in 13 European intensive care units**

L.P. Derde, M.J. Dautzenberg, P.J. van Duijn*, C. Brun-Buisson, M.J. Bonten on behalf of the MOSAR research consortium

Objective: To quantify incidences of ICU-acquired bacteraemia caused by Antimicrobial Resistant bacteria (AMRB) in 13 European ICUs participating in the MOSAR ICU trial.

Methods: As part of a European cluster-randomized trial, a 6 month observational study was performed between May 2008 and Oct 2009. The 13 participating ICUs were in France, Greece, Italy, Latvia, Luxembourg, Portugal, Slovenia and Spain. ICU-acquired bacteraemia (MRSA, VRE and Gram-negative) was defined as occurring 21 days after ICU-admission.

Results: During the study 2854 patients were included comprising 30400 admission days. Patients had an average age of 64 years and median APACHE-II and SAPS-II scores of 15 and 57, respectively. There were 74 episodes of AMRB bacteraemia in 50 patients (3 (6%) were polymicrobial) caused by MRSA (n = 6), VRE (n = 4), Enterobacteriaceae (n = 32), Acinetobacter (n = 11), P. aeruginosa (n = 18) and “Other” (n = 3). Of non-bacteraemia patients 19% deceased in the ICU, versus 52% of bacteraemia patients. Patients acquiring an ICU-bacteraemia had a median LOS of 36 days. Patients were admitted a median of 21 days (range 3–154) before acquiring a bacteraemia. For all pathogens, the incidence rate was 25 per 10,000 days at risk with a range of 0 to 142 episodes per 10,000 patient days at risk. The incidence was 21 (range 0–143) and 3 (range 0–14) per 10,000 patient days at risk for Gram-negative and Gram-positive (MRSA and VRE), respectively. ICU-acquired bacteraemia caused by Enterobacteriaceae occurred most frequently (16 per 10,000 patient days at risk; range 0–72), followed by P. aeruginosa (6.3; range 0–25), Acinetobacter species (5; range 0–28) and MRSA (4.6; range 0–21.9).

Conclusion: In these 13 ICUs across Europe the average incidence of ICU-acquired bacteraemia was 25 per 10,000 patient days at risk, with marked geographical variance in incidence rates. Most episodes were caused by Gram-negative bacteria. This 6-month baseline period, will be followed by implementation of hand hygiene improvement and chlorhexidine body washings (period 2, 6 months) and additional rapid diagnostic testing to detect carriage with ARB at ICU-admission followed by isolation of carriers (period 3, 12 months).
Burden of resistance: mortality and hospital stay attributable to antibiotic-resistant *Staphylococcus aureus* and *Escherichia coli* bacteremia in Europe

M.E.A. de Kraker*, P.G. Davey, H. Grundmann (Billthoorn, NL; Dundee, UK)

**Objectives:** The relative importance of human diseases is conventionally assessed by cause-specific mortality, morbidity and economic impact. Current estimates for infections caused by antibiotic-resistant bacteria are not sufficiently supported by quantitative empirical data. This study determined the incident and expected excess number of deaths, bed-days and hospital costs attributable to bacteremia caused by methicillin-resistant *Staphylococcus aureus* and third-generation cephalosporin-resistant *E. coli* (G3CREC) in 31 countries which participated in the European Antimicrobial Resistance Surveillance System (EARSS).

**Methods:** The incidence of MRSA and G3CREC bacteremia per 100,000 population was extracted from EARSS prevalence data and national health care statistics. Prospective cohort studies, carried out in hospitals participating in EARSS in 2007, provided the parameters for estimating the 30-day excess mortality and extra hospital stay attributable to MRSA and G3CREC bacteremias between 2007 and 2015. Hospital expenditure was derived from a publicly available cost model.

**Results:** In 2007, 4.8 (95% CI 4.5–5.0) episodes of MRSA bacteremia per 100,000 population caused 5503 (C195 3136–8276) excess deaths and 255,683 (C195 142,934–375,880) extra hospital days in 31 countries in the European region, whereas 2.6 (C195 2.4–2.8) episodes of G3CREC bacteremias per 100,000 population led to 2712 (C195 595–5780) excess deaths and 120,065 (C195 22,722–198,338) extra hospital days. The total cost attributable to extra hospital stay was 44.0 and 18.1 million euro, equivalent to 63.1 and 29.7 million international dollars, respectively. Based on prevailing trends, the number of excess deaths attributable to MRSA bacteremias is estimated to decrease to 2075 (C195 1106–3293), but deaths attributable to G3CREC are predicted to increase to 15,297 (C195 3435–32,408) in 2015.

**Conclusion:** Mortality attributable to MRSA and G3CREC bacteremias is significant, and the attributable bed-days impose a considerable burden on health care systems. A foreseeable shift in the burden of antibiotic resistance from Gram-positive to Gram-negative infections will exacerbate this situation and is reason for concern.

**Table 1.** Main patients characteristics, colonization status, and microbiology of ICU-acquired BSI during the study period

|                  | BSI group (n=10) | Control group (n=40) |
|------------------|------------------|----------------------|
| Age, years (m±sd)| 64 (19)          | 61 (21)              |
| Male sex, n (%)  | 8 (16.5)         | 18 (45)              |
| Admission diagnosis, n (%) | 3 (23)         | 16 (40)              |
| Surgical         | 10 (77)          | 24 (60)              |
| APACHE II, m (n) | 20 (6)           | 21 (9)               |
| Colonization status, n (%) | 10 (77)         | 17 (42.5)*           |
| *Klebsiella pneumoniae* | 8 (65.5)        | 7 (17.5)*            |
| *Acinetobacter baumannii* | 8 (65.5)       | 7 (17.5)*            |
| *Pseudomonas aeruginosa* | 2 (18)          | 2 (18)               |
| BSI episodes (n=25) | 14 (56)        | 4 (16)               |
| *Klebsiella pneumoniae* | 9 (36)          | 4 (16)               |
| *Acinetobacter baumannii* | 9 (36)         | 4 (16)               |
| *Pseudomonas aeruginosa* | 2 (8)           | 0 (0)                |
| ICU LOS, days (m±sd)| 42 (26)         | 19 (14)*             |
| ICU MORTALITY, n (%) | 7 (54)          | 13 (32)              |

*p<0.05

**P1495**

Gram-negative bacterial bloodstream infections: resistance patterns and risk factors

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**Objectives:** (1) To describe the resistance patterns of Gram-negative bacilli (GNB) isolated from blood cultures and (2) identify the epidemiological characteristics associated with multi-drug resistance (MDR).

**Materials and Methods:** Retrospective study of the susceptibility profile of GNB strains isolated from bloodstream infections (BSI) from patients admitted to two tertiary infectious diseases facilities between Jan 2009–Dec 2010. Bacteria were isolated in BacT/ALERT and identified by subsequent infection, improving adequacy of early empiric antimicrobial treatment in ICU patients with MDR-GNB nosocomial bloodstream infections (BSI).

**Methods:** A retrospective cohort study on nosocomial episodes of MDR-GNB BSI in an adult population of critically ill patients was carried out (1 August-30 November 2010). BSI was defined as a positive blood culture result on a sample obtained at the onset of an infectious episode occurring after 48 hours of ICU stay. MDR *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae* bacteremic episodes were considered. For each patient, only the first episode was considered. Colonization status was detected by active surveillance on rectal swabs (once weekly), tracheal aspirates and urines (on admission and twice weekly). The isolation of microorganisms with identical antibiotic susceptibility profile from both prior (i.e. 7 preceeding days) surveillance specimens and bloodstream was defined as colonization concordance. The administration of at least one effective in vitro antibiotic against the isolated pathogen, during the first 24 hours from the onset of the infectious episode was defined as the early appropriate empiric antibiotic therapy.

**Results:** Out of 53 eligible patients, 25 BSI episodes were identified in 13 patients (Table 1). The lag time between colonization and bacteremia was 4.7 (±2) days. Colonization concordance was 68%. In particular, prior colonization accurately predicted the implicated pathogens in 17 BSI episodes. In 15/17 (88.3%) episodes the detection of previous colonization status made adequate the early empiric treatment, whereas only in 3/8 (37.5%) episodes without pathogen prediction by surveillance specimens the treatment resulted adequate (p = 0.007).

**Conclusions:** In critically ill patients, the knowledge of colonization status by MDR-GNB might act as an useful guide for empiric antibiotic selection, leading to higher rates of appropriate empiric therapy within the first 24 hours of infection. Cost-effectiveness of this strategy requires further clarification on a larger basis.
Factors associated with death or ICU admission due to Two-site intradermal influenza vaccination in elderly S425

Influenza miscellaneous

Influenza miscellaneous

Factors associated with death or ICU admission due to 2009 pandemic H1N1 infection A. Moradi*, P. Tabarsi, S.A. Naidji, M. Marjani, P. Baghaei, D. Mansouri (Tehran, IR)

Objective: To evaluate factors associated with death or ICU admission of patients who were hospitalized with confirmed 2009 H1N1 influenza.

Methods: A retrospective, cross-sectional study was conducted among patients who were hospitalized with confirmed 2009 H1N1 influenza. Their demographic, clinical, laboratory, radiological findings and epidemiological data were abstracted from medical records, using a standardized report case-history form.

Results: Since June through December 2009, 46 confirmed hospitalized cases of 2009 H1N1 influenza were admitted to an intensive care unit (ICU) and 7 (15%) died. Among various variables, opium inhalation (P-value: 0.01), having productive cough, hemoptysis, chest pain, confusion and loss of consciousness were significantly related to ICU admission (P-value < 0.05). Pleural effusion (P-value = 0.006), elevated liver enzymes, CPK and LDH level were significantly relevant to ICU admission. (P-value < 0.05) Delayed antiviral treatment was more common among patients who died and they were aged.

Conclusion: Severe pandemic H1N1 influenza necessitating admission to the ICU was associated with delayed initiation of antiviral therapy, history of opium inhalation and some symptoms including: productive cough, hemoptysis, chest pain, confusion and loss of consciousness. Mortality rate in the population study was high but compares favorably with other studies that have been published yet.

Table: Co-existing conditions, paraclinical data and treatment regimen of hospitalized patients with laboratory-confirmed 2009 pandemic (H1N1) influenza

| Characteristic | Total (n=46) | ICU (n=20) | Hospital (other than ICU) (n=26) | p-value* |
|----------------|-----------|----------|----------------|--------|
| Bacteremia (%) | 90.9 15 7.7 0.05 |
| Positive Blood Culture (%) | 10.9 15 7.7 0.05 |
| E. coli | 4.3 10 0.2 |
| Pseudomonas | 4.3 10 0.2 |
| Acinetobacter | 2.3 0 0.2 |
| Abdominal C.S/X-ray findings (%) | 3.9 95 57.7 0.006 |
| Bilateral | 90 90 66.7 0.1 |
| Ground glass | 42.9 50 33.3 0.1 |
| Miliary | 32.6 30 33.3 0.1 |
| Consolidation | 25.7 20 33.3 0.1 |
| Pleural effusion | 8.7 20 0 0.07 |
| Pneumothorax | 4.3 10 0 0.2 |
| Co-existing conditions (n=26) | | | | |
| Hypertension | 3.8 0 0.2 |
| Diabetes | 2.3 5 0 0.4 |
| Chronic renal failure | 2.3 5 0 0.4 |
| Primary Immune deficiency† | 2.3 5 0 0.4 |
| Treatment Regimen (%) | | | | |
| Oseltamivir | 100 100 100 1 |
| Tamiflu | 97.8 100 97.8 0.1 |
| Cotrimoxazole | 95.3 100 88.5 0.2 |
| Azithromycin | 95.7 100 92.3 0.4 |
| Ceftriaxone | 52.2 90 23 0.000 |
| Ceftriaxone | 75.5 95 57.7 0.005 |
| Pneumococcal vaccine | 6.3 10 0 0.1 |

**p-value**

| Lab Data (%) | | | | |
|----------------|-----------|----------|----------------|--------|
| Leukocytosis | 25.6 25 28.1 0.9 |
| Leukopenia | 11.6 20 4.3 1.0 |
| Thrombocytopenia | 41.9 55 30.4 1.0 |
| Anemia | 54.0 35 34.8 0.05 |
| Elevated LFT | 65.2 85 54.2 0.05 |
| CPK | 448.1 ± 567 639.1 ± 699 259.3 ± 542 0.02 |
| LDH | 925.0 ± 699 115.1 ± 527 559.0 ± 286 0.001 |
| ESR | 45.9 ± 15.2 47.5 ± 13.4 44.5 ± 15.5 0.71 |
| CRP | 79.3 ± 112.9 70.1 ± 122 87.5 ± 16.8 0.000 |
| | 1.11 ± 1.4 1.41 ± 1.7 0.94 ± 0.2 0.16 |

*P-value was calculated with the use of a two-sided Fisher's exact test because of the small number of patients (in one or both groups).
†The P-value was calculated with the use of a two-sided Fisher's exact test because of the small number of patients (in one or both groups).
‡The P-value was calculated with the use of a two-sided chi-square test.
§§Including common variable immunodeficiency (CVID).
§§§Including common variable immunodeficiency (CVID).

Two-site intradermal influenza vaccination in elderly S. Sibunrung* (Bangkok, TH)

Objectives: While elderly possess one of the highest risk group for serious influenza related complications but the immunosenescence results in suboptimal response to preventive vaccination. Intraderrmal (ID) approach had shown satisfactory outcomes both economics and immunogenicity in young adults. We try to optimize the advantages of ID vaccination to the aged by evaluating and comparing immunogenicity and safety of the reduced-dose two-site intradermal (ID) influenza vaccination containing all 6 and 12 micrograms of hemagglutinin antigen (HA) per strain with standard intramuscular (IM) immunization in elderly.
**Methods:** We performed a randomized, open-label study in 180 healthy community-dwelling adults age over 60 years. Subjects were randomly assigned to receive two-site intradermal 2010 Southern hemisphere trivalent inactivated split-virion influenza vaccine containing 3 or 6 micrograms of HA per strain per site into both deltoid each or standard intramuscular injection containing 15 micrograms of HA per strain (The vaccine was manufactured by Sanofi Pasteur S.A. and provided by Government Pharmaceutical Organization). Pre- and Postvaccination measurements in the strain-specific hemagglutination inhibition titers were analyzed by comparing geometric mean titers (GMTs), seroconversion factor, seroconversion rates and seroprotection rates in both ID groups were significantly less than those of the IM group as shown in table 1. However, the immune responses elicited by two-site ID vaccinations were still sufficiently met the Committee for Proprietary Medicinal Products (CPMP) criteria. Subanalysis among subset of previous influenza vaccination recipients (n=46) demonstrated antibody responses achieved by all injection methods were comparable. Local reactions at injection sites were significantly higher among ID groups but tolerable and transient.

**Conclusion:** In this study of vulnerable population, two-site ID vaccination could be an alternative dose-sparing strategies to expand annual influenza vaccine supplies as well as prepare for waves of pandemic.

**Table 1.** Geometric mean titers (GMTs) of hemagglutination inhibition titers, seroconversion factor, seroconversion rates and seroprotection rates by route of administration pre−vaccination and at 4 weeks post−vaccination.

| GMT | H1N1 | H2N2 | H3N2 | All subjects |
|-----|------|------|------|-------------|
| Pre-vaccination (GMT) | 4.25 | 10.12 | 7.26 | 5.48 | 8.31 | 9.22 | 5.80 | 9.39 |
| Post-vaccination-GMT | 17.01 | 23.94 | 52.58 | 12.80 | 18.25 | 49.02 | 13.98 | 33.66 |
| Seroconversion Factor | 2.75 | 2.41 | 2.79 | 2.20 | 2.13 | 5.40 | 2.95 | 2.02 |
| Percentage with 4-fold increase in HAI titer | 45.3 | 3.50 | 89.7 | 53.3 | 33.5 | 28.5 | 75.0 | 45.0 |
| Percentage with rising titer−A/H1N1pdm< | 12.0 | 11.7 | 10.0 | 11.7 | 8.3 | 25.0 | 13.3 | 6.5 |
| Seroconversion rates | 30.7 | 38.3 | 70.1 | 25.0 | 23.3 | 73.3 | 25.3 | 26.7 |

**Results:** Total 180 subjects aged 60−90 years (median 67±6 yrs, female: male 4:1), of which Pre-vaccination HAI titers ≥1:40 were observed for 5 (2.8%), 11 (6.1%), 11 (6.1%) of subjects to influenza A/H1N1, A/H3N2 and B respectively. Four weeks after vaccination, GMTs, seroconversion factor, seroconversion rates and seroprotection rates in both ID groups were significantly less than those of the IM group as shown in table 1. However, the immune responses elicited by two-site ID vaccinations were still sufficiently met the Committee for Proprietary Medicinal Products (CPMP) criteria. Among the 21 (30%) participants with HAI titers <1:40 before vaccination, at least 63% developed protective HAI titers, but only 20% of these had also an IgG titer >11 VE after 2nd vaccination (Figure 1). Young age was associated with seroconversion, whereas none of the 7 patients >60 years seroconverted.

**Conclusions:** We report a serological investigation on an influenza A/H1N1pdm outbreak in an Italian military ship cruising in the Mediterranean Sea.

**Background:** Clinical surveillance may have underestimated the real extent of the spread of the new strain of influenza A/H1N1, which surfaced in April 2009 originating the first influenza pandemic of the 21st century. Here we report a serological investigation on an influenza A/H1N1pdm outbreak in an Italian military ship while cruising in the Mediterranean Sea (May 24−September 6, 2009).

**Methods:** The contemporary presence of HAI and CF antibodies was used to retrospectively estimate the extent of influenza A/H1N1pdm spread across the crew members (median age: 29 years).

**Findings:** During the cruise, 2 crew members fulfilled the surveillance case definition for influenza, but only one was laboratory confirmed by influenza A/H1N1pdm-specific RT-PCR; 52 reported acute respiratory illness (ARI) episodes, and 183 reported no ARI episodes. Overall, among the 211 crew member for whom a valid serological result was available, 39.3% tested seropositive for influenza A/H1N1pdm. The proportion of seropositives was significantly associated with more crowded living quarters and tended to be higher in those aged <40 and in those reporting ARI or suspected/confirmed influenza A/H1N1pdm compared to the asymptomatic individuals. No association was found with previous seasonal influenza vaccination.

**Conclusions:** These findings underline the risk for rapid spread of novel strains of influenza A in confined environment, such as military ships, where crowding, rigorous working environment, physiologic stress

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**P4199** Pandemic influenza on a military ship: retrospective investigation of an influenza A/H1N1pdm outbreak on an Italian military ship cruising in the Mediterranean Sea, May−September 2009

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**Background:** Clinical surveillance may have underestimated the real extent of the spread of the new strain of influenza A/H1N1, which surfaced in April 2009 originating the first influenza pandemic of the 21st century. Here we report a serological investigation on an influenza A/H1N1pdm outbreak in an Italian military ship while cruising in the Mediterranean Sea (May 24−September 6, 2009).

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**Findings:** During the cruise, 2 crew members fulfilled the surveillance case definition for influenza, but only one was laboratory confirmed by influenza A/H1N1pdm-specific RT-PCR; 52 reported acute respiratory illness (ARI) episodes, and 183 reported no ARI episodes. Overall, among the 211 crew member for whom a valid serological result was available, 39.3% tested seropositive for influenza A/H1N1pdm. The proportion of seropositives was significantly associated with more crowded living quarters and tended to be higher in those aged <40 and in those reporting ARI or suspected/confirmed influenza A/H1N1pdm compared to the asymptomatic individuals. No association was found with previous seasonal influenza vaccination.

**Conclusions:** These findings underline the risk for rapid spread of novel strains of influenza A in confined environment, such as military ships, where crowding, rigorous working environment, physiologic stress...
occur. The high proportion of asymptomatic infections in this ship-
borne outbreak supports the concept that serological surveillance in
such semi-closed communities is essential to appreciate the real extent
of influenza A/H1N1pdm spread and can constitute, since the early
stage of a pandemic, an useful model to predict the public health
impact of pandemic influenza and to establish proportionate and effective
countermeasures.

Assessing antimicrobial therapy in animals

**Diffusion of ofloxacin in the endocarditis vegetation assessed with synchrotron radiation UV fluorescence microspectroscopy**

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**Objectives:** Although the diffusion of antibiotics in vegetation bacterial masses may influence their efficacy in endocarditis, it has never been studied. Synchrotron radiation UV fluorescence microscopy is a new imaging tool which detects autofluorescent molecules in cells and tissues with a 1-μm spatial resolution. Ofloxacin has interesting autofluorescence properties: it emits fluorescence between 390 and 550 nm (peak at 460 nm) after excitation at 275 nm. Here, we used synchrotron UV fluorescence microspectroscopy to study the diffusion of ofloxacin in endocarditis vegetation bacterial masses.

**Methods:** Streptococcal aortic endocarditis was induced in 5 rabbits. Three animals received an unique 1-hour IV injection of ofloxacin, and were euthanized 30 minutes after the end of perfusion. Two controls were left untreated. Vegetation cryo-sections were deposited on UV transparent slides and contiguous slices were stained with Hematoxylin and Eosin to localize bacterial masses within the vegetation. Two fluorescence microscopes were coupled to a synchrotron beam for excitation at 275 nm (Synchrotron Soleil, France). A spectral microscope collected fluorescence spectra between 285 and 550 nm. A second, full field microscope collected fluorescence light between 510 and 560 nm.

**Results:** Fluorescence emission spectra of ofloxacin-treated vegetation showed higher intensities than control between 390 and 540 nm. Furthermore, images obtained with the full-field microscope showed that ofloxacin increased fluorescence between 510 and 560 nm in comparison with control tissues. Ofloxacin fluorescence was detected as well in bacterial masses as in surrounding tissue. However, ofloxacin maximal values located in the immediate neighborhood of bacterial masses. There was no fluorescence gradient between peripheral and central areas of bacterial masses. Finally, fluorescence images of bacterial masses located in various parts of vegetation suggested than ofloxacin fluorescence was lower in vegetation central area than in its periphery.

**Conclusion:** Ofloxacin diffuses into vegetation bacterial masses, but it accumulates in their immediate neighborhood. There may be an ofloxacin concentration gradient between vegetation peripheral and central areas. Synchrotron radiation UV fluorescence microscopy is a promising tool for assessment of antibiotic diffusion in the endocarditis vegetation bacterial masses.

**Bone tissue distribution of gentamicin and vancomycin from polymethylmethacrylate intramedullary nails in experimental osteomyelitis model (MRSA infection)**

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**Objectives:** Polymethylmethacrylate (PMMA) bone cements are used for the fixation of joint prostheses and for the production of spacers as well as carrier to deliver antibiotics into infection site. We studied the local release of antibiotics from PMMA bone cement nails loaded with a combination of Gentamicin (G) and Vancomycin (V) in an experimental model of osteomyelitis (Methicillin-Resistant *S. aureus*-MRSA in rabbits) and the correlation with the effect of treatment.

**Materials and Methods:** Experimental osteomyelitis was induced by MRSA inoculum in rabbits femurs. PMMA intramedullary nails loaded with G (1.9%) and V (1.9%) were inserted for 3 weeks then explanted. Eight New Zealand rabbits (weight 3.0±0.2 kg) were assigned to the following treatments:

- **group 1 (n=3):** 4 weeks after infection nails were inserted in both femurs;
- **group 2 (n=2):** no infection but nails were inserted in both femurs;
- **group 3 (n=3):** 4 weeks after infection, Teicoplanin was given parenterally (T, 20 mg/kg, i.m., b.i.d) for 6 days. Femurs were taken 2 weeks after the end of T administration.

Five different samples from condyle and diaphysis of each femur (trabecular bone and bone marrow) and serum were obtained. Bone samples were pulverized by Mikro-dismembrator and antibiotics extracted in appropriate buffers. G and V concentrations were determined by FPIA and T by microbiological method (B. subtilis in lososenist agar).

**Results:** High concentrations of G and V diffused in different bone fractions from PMMA intramedullary nails. In all samples the concentrations of V were higher than those of G (495.6 mg/kg and 194.7 mg/kg, respectively, group 1). The distribution of V and G was irregular, being the concentrations in condyle higher (342.8 mg/kg and 148.4 mg/kg, respectively, group 1) than those found in the diaphyseal fractions (152.9 mg/kg and 46.2 mg/kg, respectively). The greatest antibiotics concentrations were found in the condyle trabecular bone fraction. The levels of G and V in infected bone tissue were lower than those in uninfected tissues (group 2). Serum levels of both antibiotics were always below sensitivity limits (traces). Local concentrations of combined G and V eradicate the infection, while systemic administration of T showed partial efficacy. These data are in accord with microbiological and histological results.

**Conclusions:** PMMA bone cement loaded with G and V can be considered an effective system for the local delivery of antibiotics to the infected bone tissue.

**In vivo efficacy of ceftaroline in a methicillin-resistant and PVL-producing *Staphylococcus aureus* pneumonia rabbit model**

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**Objectives:** Many strains of community-acquired (CA) *Staphylococcus aureus* harbour the Panton-Valentine Leukocidin (PVL) phage, which may contribute to severe necrotizing pneumonia in young immunocompetent patients. In vitro studies demonstrate that some anti-staphylococcal drugs induce or inhibit PVL production. Animal models are needed to evaluate new therapeutic strategies in such infections. We compared the efficacy of ceftaroline, the active component of the prodrug cephalosporin ceftaroline fosamil, to 3 other antibiotics in a severe model of PVL CA-MRSA pneumonia in rabbits.

**Methods:** The well-described USA300 PVL+ clone was used to induce pneumonia in immunocompetent New Zealand rabbits (inoculum = 9.5 log10 CFU/mL). Animals were administered a human equivalent dosage of either ceftaroline (CPT 600 mg/12h), clindamycin (CLI 600 mg/8h), linezolid (LZO 600 mg/12h), or vancomycin (VAN continuous perfusion 30 mg/kg) intravenously over 48 h. Infected animals that did not receive any antibiotic treatment were used as controls. Serum drug levels were measured by microbiological assay, HPLC, or Fluorescence Polarization, and pharmacokinetic data were determined. Overall efficacy of the antibiotic treatments was assessed according to bacterial counts in lungs and spleen, and residual quantity of PVL was determined by a specific Elisa method.

**Results:** They are expressed as mean ± SD. Quantitative variables were compared with an ANOVA completed after a post-hoc analysis (Bonferroni). Early mortality: While not significantly affected by VAN, the mortality rate was greatly reduced by LZO, CPT, and CLI.
Bacterial reduction: No significant antibacterial efficacy was obtained with VAN in lungs or spleen. In contrast, LZO reduced the pulmonary bacterial content by 3 log10 CFU/g. CLI and CPT were also moderately decreased relative to controls following treatment with VAN.

**Conclusion:** In this model of CA-MRSA PVL+ severe pneumonia, CLI and CPT (and to a lesser extent LZO) achieved bacteriological reduction and an anti-toxic effect (reduction of the macroscopic score and PVL production vs controls). The efficacy of ceftaroline in this in vivo model provides support for additional studies of CPT in treatment of CA-MRSA pneumonia.

**Methods:** Fifteen oxacillin- and vancomycin-susceptible Staphylococcus aureus strains (OS-MRSA) that were partially responsive to oxacillin in animal infections. Vancomycin is mainly used against MRSA infections however its bactericidal activity is usually suboptimal. We report in vivo results of oxacillin activity compared with that of vancomycin in a larger collection of OS-MRSA isolates.

**Results:** The thigh infections in murine revealed that in 10 isolates bacterial counts grown from infected thighs (P < 0.05) compared with those of untreated controls. LZO (simulating a human-equivalent (HE) dose of 10 mg/kg/12h), VAN (constant IV infusion to reach a 20xMIC serum steady-state concentration), RA (intramuscular injection of 20 mg/kg every 12h), LZO plus RA, and VAN plus RA. Surviving bacteria were counted in infected joint fluid (JF), bone marrow (BM) and bone (BO) at day 3 and at the end of 4-days treatment (day 7).

**Conclusion:** In vivo results are shown in Table. LZO plus RA demonstrated a very powerful activity in this experimental model of acute osteomyelitis.

### Experimental infections caused by oxacillin-susceptible mecA-positive Staphylococcus aureus clinical isolates treated by oxacillin versus vancomycin

**Objectives:** We previously characterized four mecA-positive oxacillin-susceptible Staphylococcus aureus strains (OS-MRSA) that were partially responsive to oxacillin in animal infections. and ineffective against the high-level MRSA control strain and treated with dicloxacillin 500mg/kg/12h intraperitoneally, cyclophosphamide. Thighs were infected by 7 log10 CFU of each strain and treated with dicloxacillin 500 mg/kg/12h intraperitoneally, vancomycin 180 mg/kg/12h subcutaneously or left untreated over a 24-h period, when animals were euthanized. Thigh muscles were homogenized, serially diluted and CFU enumerated. The mecA-negative ATCC29213 strain, a low- (MIC 0.16 mg/L) and a high-level (MIC >256 mg/L) oxacillin-resistant mecA-positive strain were used as controls.

**Results:** The thigh infections in murine revealed that in 10 isolates colonies grown from infected thighs of murine treated with dicloxacillin were significantly reduced (P < 0.05) compared with those of untreated controls, while in five isolates dicloxacillin caused a non-significant reduction of colonies (P > 0.05). Dicloxacillin was also effective against the low-level MRSA and ineffective against the high-level MRSA control infections. Vancomycin treatment caused in all isolates a significant reduction in colonies grown from infected thighs (P < 0.05). In eight study isolates, the reduction in colonies grown from murine treated with dicloxacillin did not differ significantly from that of vancomycin (P > 0.05), while in seven isolates, vancomycin had significantly higher efficiency compared with dicloxacillin (P < 0.05).

**Conclusion:** The results of the present study suggest that a considerable proportion of OS-MRSA isolates may be responsive to oxacillin in vivo in a manner similar to that of vancomycin. This could have significant implications for the treatment of MRSA infections.
Efficacy of human simulated ceftaroline exposures against phenotypically diverse Staphylococcus aureus in a mouse thigh model

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Objectives: Ceftaroline (CPT), the active component of the prodruce cetaroline fosamil, is a new broad-spectrum cephalosporin exhibiting bactericidal activity against Gram-positive pathogens, including methicillin-susceptible (MSSA) and -resistant Staphylococcus aureus (MRSA), as well as common Gram-negative pathogens. This study evaluated the efficacy of human-simulated exposures of CPT against Staphylococcus aureus in both the neutropenic (I-) and immunocompetent (I+) mouse thigh infection models.

Methods: 26 Staphylococcus aureus (4 MSSA, 22 MRSA) isolates with CPT MICs ranging from 0.125–4 mg/L were tested in the I− model. 13 of these MRSA isolates were also tested in I+ animals. Two hours after inoculation, CPT was administered SQ using a regimen that simulated free-drug steady state exposures of CPT 600 mg q12h infused over 1 hour in patients by mimicking the dosing interval that the free drug concentration remained above the MIC of the infecting organism (tT>MIC). The change in log CFU after 24 hours of treatment was analyzed relative to the 0 and 24 hour controls for I− and I+, respectively.

Results: Human-simulated exposures resulted in efficacy against all isolates tested in both mouse models. In the I− model, a greater than 1 log CFU reduction was observed when compared to the 0 hour control versus 25 of the 26 isolates (change in log CFU range: −0.95 to −3.28); whereas, for the I+ model, all isolates obtained a greater than 1 log CFU reduction (change in log CFU range: −1.06 to −2.43) in bacterial density. An additive effect due to the immune system was not observed presumably because the human-simulated regimen had already achieved maximal kill in I− animals. Irrespective of immune competency, a reduction in bacterial density was observed at the highest MIC of 4 mg/L, equivalent to a >T>MIC exposure of 27.5%.

Conclusion: Patient derived human-simulated ceftaroline 600 mg q12h exposures provided predictable efficacy against all tested Staphylococcus aureus isolates in the mouse thigh model independent of immune status. These data support the durability and clinical utility of ceftaroline against Staphylococcus aureus, including MRSA with MICs up to 4 mg/L.

Treatment of renal abscesses caused by Staphylococcus aureus MW2, using delafloxacin and moxifloxacin

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Objectives: Antibiotics are in general poorly effective in treatment of abscesses, which are a common type of infection that often involves methicillin-resistant Staphylococcus aureus. We tested the activity of delafloxacin (DFX), a new quinolone under development, in comparison to that of moxifloxacin (MXF) against renal abscesses formed by S. aureus MW2 in a murine model of systemic infection.

Methods: On day 0, 7-to-8-week old male Swiss-Webster mice were injected intravenously with S. aureus MW2. On days 4, 5, and 6, by which time renal abscesses had developed, twice daily treatment with DFX, MXF (10 and 30 mg/kg), or vehicle was administrated subcutaneously. On day 7, kidneys were harvested, homogenized, and plated quantitatively. In addition, a 2-day early-treatment regimen was begun 24 h after injection and renal CFU was measured in a similar manner.

Results: Renal abscesses formed reliably by 4 days after injection with S. aureus inocula in the range of 3–8×10⁸ CFU. Both DFX and MXF at 10 mg/kg significantly reduced CFU (1.7 and 8.1×10⁶ CFU/g kidney, p = 0.0003 and 0.0009, respectively) compared to controls (9.77×10⁷ CFU/g kidney), and the reduction of bacterial load by DFX was significantly greater than that by MXF (p = 0.0121). The bacterial load in mice given 30 mg/kg DFX was reduced significantly relative to controls (from 2.0×10⁸ to 4.96×10⁸ CFU/g kidney, p = 0.0205). MXF at this dose also reduced the CFU, but the effect did not reach statistical significance (2.0×10⁶ CFU/g kidney, p = 0.0541). In the early treatment regimen, both DFX and MXF (10 mg/kg) showed a significant reduction in bacterial load (1.14 and 5.00×10⁶ CFU/g kidney, p = 0.0019 and 0.007, respectively), compared to that in controls (2.3×10⁶ CFU/g kidney).

Conclusion: S. aureus MW2 reliably produced renal abscesses in mice when injected intravenously. Both DFX and MXF were effective in reducing the bacterial load in established renal abscesses, but DFX was superior to MXF. DFX and MXF showed similar efficacy in an early treatment regimen whereby mice were treated prior to the formation of mature renal abscesses.
Methods: Adult male cannulated rats were anesthetized and infected either in the lungs by instilling 200 ul of a bacterial suspension of K. pneu or in the thigh by placing an infected E. coli suture. Therapy was administered starting 1 hr post infection by recreating in the rats the human concentration time profiles for the following antibiotics: GSK052 repeat dose of 500 mg i.v. (b.i.d), levofloxacin (LEV) 500 mg p.o. (o.d.) and ceftazidime (CEF) 1 gm i.v. (t.i.d.). Therapy continued for 4 days. At 96 hr post infection, rats were euthanized and the infected tissue (lung or thigh) was excised to assess viable bacterial numbers.

Results: See the table.

Conclusion: The human exposure profile of GSK052 was highly efficacious against the three K. pneu and two E. coli strains tested. These studies indicate the potential benefit of GSK052 in the treatment of serious Gram-negative infections.

| Group | E. pneumoniae | A. aerogenes | E. coli | Enterobacter aerogenes |
|-------|--------------|-------------|--------|-----------------------|
| 1 hr Control | 5.5 ± 0.7 | 6.3 ± 0.8 | 4.9 ± 0.7 | 3.7 ± 0.5 |
| 96 hr Control | 6.7 ± 0.3 | 7.2 ± 0.7 | 7.5 ± 0.5 | 5.7 ± 1.2 |
| GSK052 | 29 ± 0.6 | 3.7 ± 0.2 | 3.5 ± 1.7 | 22 ± 1.9 |
| CEF | 0.1 ± 0.01 | 0.1 ± 0.01 | 0.1 ± 0.01 | 0.1 ± 0.01 |

(P1510) Broad-spectrum fluorocycline TP-434 has oral bioavailability in humans

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Background: TP-434 is a novel broad-spectrum fluorocycline antibiotic with potent activity and efficacy against multidrug-resistant (MDR) Gram-negative and Gram-positive aerobic and anaerobic pathogens, including Enterobacteriaceae expressing extended-spectrum β-lactamases (ESBL) and/or carbapenemases, MRSA, and VRE; it has limited activity against Pseudomonas spp. The IV formulation of TP-434 is currently in Phase 2 clinical development. TP-434 had 29% oral bioavailability in humans across DGs studied. The overall systemic exposure of TP-434 was dose-proportional and linear as doses increased from 50mg to 300mg.

Methods: A single-center, double-blinded, placebo-controlled single-ascending dose study with an oral solution of TP-434 was done in 24 subjects who received study drug were included in the statistical analysis. Plasma concentration data from a total of 24 subjects who received study drug were included in the statistical analysis.

Results: No serious adverse events were reported over the course of this study. In general, doses up to 200 mg of TP-434 were well tolerated; 1 subject each receiving 200 mg reported dizziness or nausea. At a dose of 300 mg, increases in partial thromboplastin time were noted (<1.5 upper limit of normal), and 1 subject each had increased alanine aminotransferase (<1.2xULN) and unconjugated bilirubin (<1.2xULN) or nausea and transient vomiting of mild severity. All AEs resolved spontaneously. Tmax was reached at approximately 2 hours for all DGs studied. The overall systemic exposure of TP-434 was dose-proportional and linear as doses increased from 50 mg to 300 mg. Exposures consistent with therapeutic efficacy were reached. Average oral bioavailability across DGs was 28%. A total of 26 samples across 10 subjects were collected for quantitation of TP-434 and its metabolites in plasma and urine.

Conclusion: The feasibility of development of an oral formulation was confirmed. TP-434 showed promising oral bioavailability in humans, reaching exposures predicted to be therapeutically efficacious. The availability of an oral step-down option with a novel broad-spectrum antibiotic would fulfill an increasing medical need.

(P1511) Phosphorodiamidate morpholino oligomer RNA therapeutics cross the blood-brain barrier of healthy volunteers

S. Shrewsbury*, P. Iversen (Bothell, US)

Objectives: The phosphorodiamidate morpholino oligomers (PMO) can be designed to inhibit gene expression by binding specifically to RNA with complementary base sequences. We have investigated the utility of these PMOs extensively as antiviral and antibacterial agents with numerous successes. We reported that AVI-4020 can cross the blood brain barrier (BBB) of individuals with neuroninvasive West Nile Virus. The goal of these studies was to evaluate the potential for PMOs with different sequence composition to cross the BBB and enter the cerebrospinal fluid (CSF) of healthy volunteers.

Methods: Three different PMOs, AVI-4020 designed to inhibit West Nile Virus (WNV), AVI-4126 designed to inhibit expression of c-myc, and AVI-4065 designed to inhibit hepatitis C virus (HCV), were administered to healthy volunteers as a single bolus i.v. 100 mg dose. Blood plasma and CSF samples were collected for quantitative determination of oligomer concentrations at 6, 12 and 18 hours post administration (4 subjects per time interval).
Results: CSF concentrations for AVI-4065 were detectable in all subjects ranging from 19.6 to 44.5 ng/mL at 6 hours post-dose (4 subjects), 13.3 to 16.5 ng/mL at 18 hours post-dose (4 subjects), and 22.1 to 24 ng/mL at 112 hours post-dose (3 subjects). CSF concentrations for AVI-4126 were below the limit of quantitation in subjects 6 hours post-dose (4 subjects), at 12 hours post-dose AVI-4126 was detected in 2 of 4 subjects ranging from 12 to 13 ng/mL, and at 18 hours the range was from 10 to 14 ng/mL for the 3 of 4 subjects with detectable AVI-4126. Finally, CSF levels of AVI-4065 were detected in all subjects ranging from 31 to 33 ng/mL at 6 hours post-dose (4 subjects), 32 to 36 ng/mL at 12 hours post-dose and 32 to 41 ng/mL at 18 hours post-dose.

Conclusions: These studies indicate phosphorodiadimidine morphilino oligomers (PMOs) cross the BBB of normal healthy volunteers. The observations suggest that the sequence composition may influence the rate and quantity of oligomer that will enter the CSF.

**Summary of Selected Plasma GSK1322322 Pharmacokinetic Parameters**

| Regimen          | N  | AUC(0→∞) (µg h/mL) | Cmax (µg/mL) | t1/2 (hr) | tmax (hr) |
|------------------|----|-------------------|--------------|-----------|-----------|
| GSK223 alone     | 20 | 25.6 (20)         | 8.02 (54)    | 8.1 (44)  | 0.50 (0.3−2.5) |
| Food             | 17 | 30.8 (20)         | 7.12 (32)    | 8.0 (20)  | 2.5 (1.5−8.0) |
| H2 Blocker       | 17 | 16.0 (40)         | 3.72 (38)    | 7.6 (40)  | 1.0 (0.5−3.0) |
| H2 Blocker and Vit C | 16 | 22.9 (20)         | 7.04 (42)    | 7.1 (45)  | 0.50 (0.3−2.0) |

1 geometric mean (CV%) 2. median (range)

**Objectives:** POL7080 is a novel PEM (Protein Eptope Mimetic) antibiotic selectively targeting *Pseudomonas aeruginosa* species with demonstrated potent in vitro activity and in vivo efficacy in murine infection models. A single ascending dose (SAD) study was conducted to evaluate safety, tolerability, plasma pharmacokinetics (PK) and urinary excretion.

**Methods:** Forty-eight healthy male subjects, aged 18−40, were randomised and participated in a double blind, placebo-controlled study with single ascending doses. Each of the 8 dose groups consisted of 4 subjects randomised to receive POL7080 and 2 to receive placebo. All doses were given as three hour infusions. Plasma concentrations of the drug were determined by LC-MS/MS analysis and interim (using nominal time) PK parameters were calculated using WinNonLin®.

**Results:** POL7080 was detected in plasma of all dose groups with Cmax at the end of infusion. PK was dose proportional and plasma exposure increased linearly with respect to AUC0 infinity and Cmax. Inter-subject variability was generally less than 20% for both parameters. Dose normalized AUC and Cmax averaged for all groups were 9.2±2.0 µg*h/kg/mL/mg and 1.9±0.4 µg/kg/mL/mg, respectively. The mean half-life (t1/2) ranged from 2.6−5.5 hours (study grand mean: SD = 4.6±1.2 h). No serious adverse events (SAEs) were reported for any dose group and all AEs were mild and not prohibitive to dose increases. Blood chemistry and clinical laboratory results were normal during dosing and at follow up, indicating that POL7080 was well tolerated in all dose groups.

**Conclusions:** Single doses of POL7080 were well tolerated at plasma concentrations expected to meet or exceed efficacious levels and no serious adverse event was reported. A multiple ascending dose study has been initiated to assess safety and tolerability of repeated dosing over 5−7 days.

**Objective:** PMX30063 is the first of a novel class of antibiotics, a non-peptidic amphiphilic compound that mimics the properties and mechanism of action of the host defense proteins. Two phase 1 clinical studies were conducted in healthy males (77 subjects). The primary adverse event was described as a transient, self-limiting, sensory syndrome that was characterized by peripheral sensations of numbness and tingling. The syndrome, defined as a paraesthesia, was fully reversible within minutes to days following discontinuation of the treatment. A neurological assessment scale was recommended by a Board Certified neurologist, and implemented in the clinical trial PMX63−103: Randomized, Double-Blind, Placebo Controlled Study to Evaluate the Safety and Pharmacokinetics of Intravenous PMX30063 in Healthy Adult Subjects.

**Method:** Ten male subjects (8 active/2 placebo) and 10 female subjects (8 active/2 placebo) were enrolled in a randomized, double-blinded, multiple-dose, safety, tolerability and pharmacokinetic study of PMX30063. Subjects randomized to PMX30063 received a loading dose on day 1 of 1.0 mg/kg administered intravenously over 60 minutes followed by 0.35 mg/kg doses on days 2−14. In addition to pharmacokinetic, adverse events, safety chemistry, hematology, urinalysis and ECGs, specific assessments of neurological symptoms and objective neurological exams were performed. The symptoms-based neurological assessment captured severity, symmetry, region and intermittency of reported sensations of tingling, burning, numbness or pain. The objective neurological assessments conducted by a neurologist consisted of 11 tests (deep tendon reflexes, pinprick pain sensation, light touch, vibration, joint position, finger-nose test, index finger
A phase 1 trial to evaluate the tolerability, safety and pharmacokinetics of multi-dose intravenous regimens of PMX30063

B. Korczak*, E. McAllister (Radnor, US)

Objectives: There is a major need for the development of novel antimicrobial agents that attack new targets to evade resistance issues which limit the usefulness of many antibiotics. PMX30063, a non-peptidic compound that mimics the properties and mechanism of action of the host defense proteins and has not demonstrated resistance development in vitro, is currently in phase 2 clinical study. We have studied safety, pharmacokinetics and pharmacodynamics of multiple doses of this novel antibiotic in healthy male volunteers.

Methods: Seventy seven subjects (55 PMX30063, 22 placebo) were enrolled in up to 12 cohorts and randomized to either PMX30063 or placebo in a 5:2 ratio. All infusions were 1 hour in duration, administered via a peripheral vein. Cohorts 1 and 2 received 0.1 mg/kg and 0.2 mg/kg, respectively every 48 hours for a total of 5 doses each. Cohorts 3–8 received once-daily (Q24H) infusions to a total of 5 infusions starting at 0.1 mg/kg and proceeding to 0.2, 0.3, 0.4, 0.5 and 0.6 mg/kg. Cohorts 10–12 received twice-daily (Q12H) infusions of 0.08, 0.16 and 0.30 mg/kg to a total of 10 infusions and doses were chosen to enable comparison with once-daily infusions of 0.1, 0.2 and 0.3 mg/kg. Assessments included adverse events, laboratory, ECG, plasma pharmacokinetics and ex vivo antimicrobial activity.

Results: There were no serious adverse events, laboratory or ECG findings of clinical concern. The most common adverse events were paraesthesia and increase in blood pressure and heart rate, all of which were transient and required no treatment. No gastrointestinal or renal effects were observed. Key pharmacokinetic parameters were determined and the terminal elimination half life varied between 16.6 and 23 hours. Bactericidal activity of PMX30063 in subjects’ serum against MSSA and MRSA strains was observed at a single dose of 0.1–0.3 mg/kg.

Conclusions: Plasma concentration of PMX30063 shows uncomplicated pharmacokinetics and dose-dependent pharmacokinetic parameters for all 3 dosing regimens. Serum antimicrobial activities showed that bactericidal concentrations can be achieved at a single and initial dose as low as 0.1–0.3 mg/kg. Overall, PMX30063 appears to show unremarkable safety profile between 0.1 and 0.3 mg/kg Q24H dosing. Increasing the frequency of administration from Q24H to Q12H did not seem to improve the safety profile of PMX30063 at comparable doses.

Assessment of the venous tolerability of torezolid phosphate infused via a peripheral catheter: a novel approach

K.A. Muñoz, P. Bien, P. Prokocimer* (San Diego, US)

Objectives: Torezolid phosphate (TR-701) is an investigational oxazolidinone antibiotic currently in Phase 3 testing for the treatment of Acute Bacterial Skin and Skin Structure Infections (ABSSSI). In one of the two Phase 3 trials an initial 200 mg dose of TR-701 will be administered intravenously through a peripheral vein followed by step-down to subsequent oral doses of TR-701 therapy. Prior to initiating the Phase 3 IV trial, Trius investigated the venous tolerability of IV TR-701 using a method allowing for a rigorous comparison vs. IV placebo administration.

Methods: The venous tolerability of an IV dose of 200 mg TR-701 was examined in a randomized, double-blind, placebo-controlled, crossover study in healthy subjects (n = 10). Subjects received either TR-701 or placebo infused over 60 minutes in 250 mL saline via a 22 gauge catheter once daily for 3 consecutive days. Each subject served as their own control and received either TR-701 or placebo for 3 days in the right arm followed by 3 days of the alternate regimen in the left arm. Venous access was evaluated for evidence of early signs of phlebitis before the start, during, at the end, and 6 hours after the end of infusion. The primary endpoint was the occurrence of early signs of phlebitis defined as a score of 2 or higher using a modified Visual Infusion Phlebitis (VIP) scale. The time to a score difference of 2 or higher was determined using all matched time points at which the modified VIP score was measured.

Results: See the Table.

| Day | N | Number of Distinct Subjects (%) | 1 | 2 | 3 | Other |
|-----|---|---------------------------------|---|---|---|-------|
| 1   | 10| 0 (0%)                         | 1 (10%) | 9 (90%) | 0 |
| 2   | 10| 0 (0%)                         | 1 (10%) | 8 (80%) | 1 (10%) |
| 3   | 10| 0 (0%)                         | 2 (20%) | 4 (40%) | 4 (40%) |
| Overall | 30 | 0 (13.3%) | 3 (10%) | 21 (70%) | 5 (16.6%) |

The VIP score results demonstrate that over 3 days, a 200 mg TR-701 IV infusion does not produce signs of phlebitis (score of ≥2) earlier or more often than placebo infusions. These data show that 200 mg of IV TR-701 can be administered safely through a peripheral vein.

The effect of food on the oral bioavailability of AFN-1252 in healthy human subjects

N. Kaplan*, B. Hafkin (Toronto, CA; Austin, US)

Objectives: AFN-1252, a new molecular entity with a novel mechanism of action, is a potent inhibitor of staphylococcal FabI, an essential enzyme in bacterial fatty acid synthesis and is in development as an oral, specific-spectrum, anti-staphylococcal antibiotic. A novel tablet formulation of AFN-1252 was better in the fasted state than in the fed state in Beagles. To study the effect of food on the oral bioavailability of the new AFN-1252 formulation in normal healthy human subjects, a traditional fast/fed study was conducted.

Methods: For the Beagle study, a cross over design with a cohort of 8 dogs was used. A single 200 mg dose of AFN-1252, as an oral suspension, was administered with fasting for 3 hours both before and after dosing. After a 1 week washout period dosing was repeated in the dogs with free access to food before and after dosing. AFN-1252 plasma levels were determined for 24 hours following each dose. For the human study, a double blind, cross over design was used. A cohort of 8 healthy volunteers was randomized to receive a single dose of two 100 mg tablets (200 mg dose) of AFN-1252 either after an overnight fast or after a high fat meal. After a 1 week washout period the subjects were dosed again in the alternate fed/fasted state. AFN-1252 plasma concentrations were determined for 72 hours following each dose. Pharmacokinetic (PK) parameters were estimated with WinNonLin v.6.1 using a non-compartmental model.

Results: In the dog study, reduced bioavailability was seen after non-fasting compared to fasting conditions. The mean Cmax and AUC values decreased by 45% and 40%, respectively, and no effects on Tmax and half-lives were observed. In the human study, all subjects showed reduced bioavailability when AFN-1252 was given after a fatty meal compared to after fasting. The mean Tmax increased from 3 to about 7 hours, and mean Cmax and AUC values decreased by 50% and 40%, respectively. No effect on terminal half-lives was observed.

Conclusions: The presence of food in the GI tract had a significant effect on the oral bioavailability of AFN-1252 in healthy human subjects, with a mean relative bioavailability of 60% compared to the fasted state. The Beagle dog model was a valuable predictor of human PK underscoring approximation, Romberg test, gait, ankle dorsiflexion strength and ankle planar flexion strength).

Conclusion: Preliminary results indicate that the most common neurological symptoms were tingling and numbness in lips, face and fingers. Most neurological tests conducted by neurologist were normal with few abnormalities noted in tendon reflexes, pinprick and light touch tests.
the merit of using an animal model in the early development of novel oral formulations.

**P1518** Pharmacokinetic and pharmacodynamic modelling of the anti-staphylococcal activity of AFN-1252 in humans

N. Kaplan*, J.R. Koup, B. Hafkin (Toronto, CA; Ann Arbor, Austin, US)

**Objectives:** AFN-1252, a new molecular entity with a novel mechanism of action, is a potent inhibitor of staphylococcal FabI, an essential enzyme in bacterial fatty acid synthesis. AFN-1252 is currently in Phase 1 studies as an oral, specific-spectrum anti-staphylococcal antibiotic. To predict the anti-staphylococcal pharmacodynamic (PD) effects of AFN-1252 in humans, pharmacokinetic (PK) and PD data from two neutropenic mouse thigh abscess infection models were used to develop a bacterial net growth PK/PD model. The primary variable between the two mouse models was drug half-life due to different oral formulations. A population PK model was constructed with AFN-1252 oral Phase 1 PK data that was then applied to the net growth PK/PD model to predict the AFN-1252 PD effects in humans.

**Methods:** Staphylococcus aureus 29213 was the test organism in both mouse thigh abscess models. Bacterial growth parameters and AFN-1252 PK effects were estimated with the bacterial net growth PK/PD model using a modification of Campion et al. (Antimicrob. Agents Chemother. 49: 209–219, 2005). Population human plasma concentrations were modelled from oral Phase 1 single ascending dose cohorts of 0.1 to 400 mg AFN-1252 (6 healthy subjects per cohort), and then expanded to predict multiple dose PK for 4 days (at steady-state). NONMEM version 6.1 was used employing a linear one-compartment disposition model with first order absorption.

**Results:** The S. aureus net growth model successfully integrated the results from the two mouse thigh abscess experiments and showed an excellent correlation ($r^2 = 0.889$) between observed and predicted log-CFU/thigh values. Simulation of the AFN-1252 antibacterial response in humans using modelled 4 days of QD dosing showed that for S. aureus at the MICo (0.016μg/ml) all subjects showed ranges of log CFU/g reduction (compared to time 0) of +2.5 to −3.5, −1.5 to −4.2, −2.2 to −5.1 and −3.5 to −5.5 for the 100, 200, 300 and 400 mg doses, respectively.

**Conclusions:** Combination of bacterial growth/kill PK/PD parameters with predicted individual plasma concentration values allowed for simulation of log-CFU/g values vs. time following single and multiple (4 day) AFN-1252 oral dose administration in humans. These simulations predicted significant antibacterial response at AFN-1252 QD doses ≥200 mg for S. aureus strains with an MIC of 0.016 μg/ml (MICo).

**P1519** Pharmacokinetics of CXA-101/tazobactam in subjects with mild or moderate renal impairment

E. Hersberger*, D. Benziger, L. Pheng, M. Trinh, J. Marier, I. Friedland (Lexington, US; Montreal, CA)

**Objectives:** To evaluate the PK of a single IV administration of CXA-101/TAZ in subjects with normal renal function (NRF) or mild or moderate renal impairment (RI).

**Methods:** Using the Cockroft-Gault formula to estimate creatinine clearance (CrCl), 24 subjects with mild (CrCl 60–89 mL/min) or moderate (CrCl 30–59 mL/min) RI and matched subjects with NRF (CrCl ≥90 mL/min) received a single 60-minute IV infusion of CXA-101/tazobactam (1000 mg/500 mg). Blood and urine samples for PK analyses were obtained prior to and at various time points up to 35 hours following the completion of drug administration.

**Results:** Negligible differences were observed in the PK of CXA-101, TAZ and metabolite M-1 in subjects with mild RI as compared to subjects with NRF. In subjects with moderate RI, AUC0-infinity and t1/2 were increased for CXA-101 (2.6 and 2.1-fold, respectively) and TAZ (2.0 and 1.6-fold, respectively). The parent-to-metabolite ratio (AUC/TAZ/AUC-M-1) in the moderate RI group was 2.6-fold higher than the NRF group. These were related to a decrease in renal clearance (CLR) of TAZ in this group. The linear equation describing the relationship between CrCl and CXA-101 PK suggested that a decrease in CrCl from 90 to 50 mL/min would decrease its plasma and renal CL by 36 and 29%, respectively, resulting in an increase of 53% in AUC0-infinity, with no change in Cmax. The linear relationship between CrCl and TAZ PK suggested that a similar decrease in CrCl would decrease its plasma and renal CL by 31% and 29%, respectively, resulting in an increase of 44% and 20% in AUC0-infinity and Cmax, respectively. These results suggest that a 50% reduction in CXA-101/TAZ doses in subjects with moderate RI would achieve plasma concentrations comparable to those observed in subjects with NRF. Additional Monte Carlo simulations showed that the proposed 50% dose reduction would achieve the desired target of 40–50% time above MIC in 100% of the subjects (MICs <8 μg/mL).

**Conclusion:** No clinically meaningful differences were observed in the plasma PK of CXA-101, TAZ, or M-1 in subjects with mild RI indicating that dosage adjustment is not required. However, based on increases observed in systemic exposure of CXA-101, TAZ, and M-1 in subjects with moderate RI, a 50% reduction in CXA-101/TAZ doses is predicted to achieve plasma concentrations comparable to those observed in subjects with NRF.

**P1520** CXA-101/tazobactam probability of target attainment using population pharmacokinetic analysis

E. Hersberger*, M. Moeskasis, J. Steenbergen, D. Benziger, F. Fenneteau, J. Marier, I. Friedland (Lexington, US; Montreal, CA)

**Objectives:** To evaluate PK-PD target attainment (TA) using different dosing strategies for CXA/TAZ against difficult to treat Gram-negative pathogens. Monte Carlo (MC) simulations were conducted taking into account between subject variability, residual variability and the covariate distribution in the population. PK-PD TA probabilities were predicted using ad hoc simulations with predicted individual plasma concentration values allowed for simulation of log-CFU/g values vs. time following single and multiple (4 day) AFN-1252 oral dose administration in humans. These simulations predicted significant antibacterial response at AFN-1252 QD doses ≥200 mg for S. aureus strains with an MIC of 0.016 μg/ml (MICo).

**Methods:** Population PK analysis of CXA/TAZ was performed using 2-compartment models with linear elimination. Monte Carlo (MC) simulations are represented in the table below. The MC-simulated PK-PD TA show that a 50% T>MIC of 8 μg/mL was achieved in 90% of subjects using a dose of 1500 mg (1000 mg CXA/500 mg TAZ) q8h infused over 60 min. Using 2008 CXA/TAZ surveillance data there is a high probability of TA for the majority of the organisms tested: E. coli (N=721, MICo = 0.25), K. pneumoniae (N=978, MICo = 2), E. cloacae (N=266, MICo = 16), P. aeruginosa (N=914, MICo = 2) and Acinetobacter spp. (N=238, MICo = 32). Based on the observed MIC distribution for P. aeruginosa and Acinetobacter spp, 50% T>MIC was achieved in 99% and 61.3% of the subjects, respectively. Over 95% of subjects achieved a 35% T>MIC for a 16 μg/mL target at 1500 mg dose infused over 3 hrs or 3000 mg (2000 mg CXA/1000 mg TAZ) infused over 60 min.

**Conclusion:** Based on recent surveillance data, CXA/TAZ 1500 mg q8h infused over 60 min is predicted to achieve excellent TA against in vitro-susceptible pathogens as Enterobacteriaceae and P. aeruginosa. Using 40–50% T>MIC as a target, CXA/TAZ is expected to display excellent target attainments up to a MIC of 8 μg/mL.
**P1521** Safety, tolerability, and pharmacokinetics of a novel Gram-negative antimicrobial, GSK2251052, in healthy subjects

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**Background:** GSK2251052 (GSK ’052) is a novel boron-based antimicrobial which specifically targets bacterial leucyl-tRNA synthetase, an essential enzyme in protein synthesis.

**Objectives:** Phase I, single-centre, randomised, placebo-controlled, double-blind, first-time-in-human study conducted to determine the safety, tolerability, and pharmacokinetic (PK) profile of GSK ’052 (formerly AN3365) in single and multiple intravenous (IV) doses.

**Methods:** Two-stage (single and multiple ascending dosing; SAD and MAD) fusion protocol using adaptive design. Each cohort had 8 young healthy men randomised to either GSK ’052 or placebo (normal saline) by 1h infusion in a ratio of 6:2. See Table 1 for cohort dosing characteristics.

**Results:** PK results demonstrated dose proportionality of AUC and Cmax across a wide dose range (Table 1). Maximum doses of 3000 mg (SAD) and 2000 mg (MAD) of GSK ’052 were well-tolerated. There were no deaths, SAEs or any AEs leading to withdrawal from the study. The 3 most common AEs reported in the trial, irrespective of stage, were headache (HA, 20% of subjects), orthostatic hypotension (OH, 18%), and cannulation site injury (CSI, 22%). There was no apparent dose response to these AEs and all were also observed in subjects receiving placebo. There were no clinically significant laboratory values or ECG findings that were considered AEs. Most notable laboratory abnormality was a reversible decrease in reticulocyte (Rc) count to approx. 20–50% of baseline values after approx. 4–8 d of repeat dosing and a return to normal levels within 6 d after end of dosing. No scheduled doses were held due to low Rc or red blood cell (RBC) count. No AEs were causally attributed to low Rc or RBC count.

**Conclusion:** GSK ’052, a novel Gram-negative antimicrobial, demonstrates dose proportionality in plasma AUC and Cmax across a wide dose range without SAEs or dose-limiting AEs. A consistent clinical AE profile was demonstrated with the most common AEs being HA, OH, and CSI. None of which was found exclusively in subjects receiving GSK ’052. No clinically significant laboratory values or ECG findings were considered AEs, though a reversible decrease in Rc count was observed.

| Table 1: PK parameters for SAD and MAD cohorts given GSK ’052 by 1 h IV infusion |
|---------------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Cohort  | SAD 1 | SAD 2 | SAD 3 | SAD 4 | SAD 5 | MAD 2 | MAD 1 | MAD 0 | MAD 3 | MAD 4 |
| Dose (mg) | 200  | 400  | 1000  | 3000  | 5000  | 1000  | 5000  | 2000  | 5000  | 2000  |
| Frequency | x1   | x1   | x1   | x1   | x1   | x1   | x1   | x1   | x1   | x1   |
| Duration (h) | 8   | 14   | 14   | 14   | 14   | 14   | 14   | 14   | 14   | 14   |
| AUC (C.g/ml) | 9.8  | 19   | 49   | 107  | 145  | 56   | 75   | 177  | 194  |
| Cmax (C.g/ml) | 2.9  | 5.9  | 14   | 32   | 42   | 9.4  | 12   | 19   | 31   |

**P1522** Antifungal activity of the Neosartorya fischeri antifungal protein against filamentous fungal isolates from clinical sources

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**Objectives:** The incidence of fungal infections has increased over the past years due to the increasing number of immunocompromised hosts and the emergence of antibiotic-resistant strains. Some of the antifungal drugs are quite toxic and have serious side effects. Thus, there is a considerable demand for new compounds with antimicrobial activity. The extracellular defensin-like antifungal peptides secreted by the Neosartorya fischeri genome (N. fischeri antifungal protein, NFAP). Experiments have been carried out for the isolation of NFAP and for the investigation of its antifungal activity against filamentous fungi.

**Methods:** N. fischeri was cultivated in antifungal peptide induction medium and the extracellular NFAP was purified from the ferment broth. The in vitro antifungal effect of NFAP on 20 fungal isolates (15 asco- and 5 zygomycetous fungi) from clinical sources representing 6 different genera and 17 species has been investigated in 96-well microtitre plate bioassay based on the recommendation of the standard Clinical and Laboratory Standards Institute (CLSI) M38-A2 microbroth dilution method.

**Results:** The purified ~6.6 kDa molecular weight NFAP exerted remarkable antifungal activity above its concentration of 12.5 μg/ml against 9 ascomycetous fungal isolates belonging to the genera of Aspergillus and Fusarium. On the other hand, all investigated zygomycetous fungal isolates were completely insensitive to NFAP. Microscopic observation revealed that germination tubes and forming hyphae from conidiospores of Aspergillus species exhibited substantial deviation from normal morphology when cultivated in presence of 100 μg/ml NFAP. They displayed abnormal and delayed germination compared to the untreated control. Treated conidiospores formed very short, swollen, curved hyphae with multiple branches and fragmented cytoplasm.

**Conclusions:** Based on the observed characteristics, NFAP would be a promising antifungal compound in the near future against ascomycetous fungal species after further in vitro and in vivo investigations.

**P1523** In vitro susceptibility of Cryptococcus neoformans preincubated with anti-Hsp90 to many antifungal agents

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**Objectives:** The present experiment examines the effect of many antifungal agents against C. neoformans isolates pre-incubated with anti-Hsp90.

**Methods:** The antifungal activity of AMB in combination with FCZ or 5-FC against eight C. neoformans isolates pre-incubated with anti-Hsp90 was evaluated using the microdilution checkerboard technique.

**Results:** Depending on the definition of the FIC used (FIC-0), a synergistic, additive interaction was found with a significant portion of the isolates. In vitro susceptibility testing of SFC in combination with anti-Hsp90 against C. neoformans isolates was determined using a checkerboard assay. Synergism, indifferent and additive interactions were observed in 37.5%, 12.5% and in 50% of isolates, respectively.

**Conclusion:** Cryptococcus neoformans cells became considerably more sensitive to the action of standard antifungal agents when grown in media supplemented with anti-Hsp90. Anti-Hsp90 at concentrations of 4–32 μg/ml significantly decreased the MIC-0 of amphotericin B, the MIC-2s of fluconazole and the MIC-2s of 5-flucytosine. Preincubation of the C. neoformans cells with Anti-Hsp90 significantly enhanced the antifungal activity of amphotericin B in combination with either fluconazole or 5-Flucytosine. The interaction between 5-Flucytosine and anti-hsp90 was synergistic, additive or indifferent. Flucytosine alone was inactive and upon combination with anti-Hsp90, the interaction was synergistic in three isolates, additive in four isolates and indifferent in one isolate.

**P1524** BC-3781: evaluation of the CYP3A interaction potential

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**Objective:** In vitro and in vivo assessment of the interaction potential of BC-3781, an investigational pleuromutilin, with CYP450 enzymes.

**Methods:** Interaction of BC-3781 with CYP450 enzymes was investigated by reaction phenotyping using human recombinant CYP450 enzymes. Inhibition and induction was investigated in vitro using microsomes and human hepatocytes. The in vivo interaction of BC-3781 on the pharmacokinetics (PK) of midazolam and the interaction of ketoconazole on the PK of BC-781 were investigated in two cross over phase I studies in healthy subjects.
Results: Reaction phenotyping using CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4 demonstrated exclusive metabolism of BC-3781 by CYP3A4 in this setting. Microsomal inhibition experiments identified CYP3A as primary target of BC-3781. While CYP3A mediated testosterone hydroxylation was very weakly affected by BC-3781 (IC50 >300 microM) midazolam hydroxylation was inhibited with an IC50 value of 5.5 microM. Induction experiments with human hepatocytes from three single donors did not reveal any induction of CYP1A2 or 3A4 by BC-3781. A clinical study investigating the effect of BC-3781 on midazolam PK identified BC-3781 as a very weak inhibitor of CYP3A. The AUC of midazolam increased by 1.17 (CI90 0.82–1.67) while Cmax was increased by a factor of 1.04 (CI90 0.82–1.30) in the presence of BC-3781. Inhibition of CYP3A with ketoconazole identified BC-3781 as a weak CYP3A substrate in vivo with geometric mean AUC ratio estimates of 1.29 (CI90 1.20–1.40). A Cmax increase of 1.06 (CI90 0.98–1.14) was below the weak CYP3A interaction threshold of 1.25 and within the no-effect boundaries.

Conclusions: While BC-3781 did not induce CYP1A2 and 3A4 it could be demonstrated that BC-3781 serves as a CYP3A4 substrate and inhibitor in vitro. Two clinical drug interaction studies with BC-3781 showed that the PK of BC-3781 is only marginally affected in the presence of ketoconazole and only a weak inhibition of CYP3A could be suggested from the study where midazolam was co-administered. The data obtained in both studies suggest that BC-3781 can be classified as having only a weak interaction with either CYP3A4 substrates or inhibitors in a clinical setting. Taken together, these results suggest that no major CYP450 mediated drug-drug interactions are expected with BC-3781.

Antimicrobial peptides with antiviral activity against a novel bunyavirus

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Objectives: To explore if any antimicrobial peptide (AMP) could effectively inhibit the novel bunyavirus and its possible mechanism.

Methods: A novel bunyavirus associated with this life-threatening infectious disease was isolated from one patient’s blood sample from eastern China by staffs from Jiangsu CDC on Oct 2010. The novel bunyavirus was discovered by China CDC in 2009. In this study, the blood sample from a single patient was inoculated into VERO cell culture to isolate the pathogen. The pathogen was identified by electron microscopy and DNA sequencing. AMP was a gift from Dr. Yong YANG which had been observed to inhibit both bacteria and cancer (results unpublished). In this study, the antiviral activity of the AMP against the novel bunyavirus was tested in the VERO cell line by antiviral activity assay.

Results: The maximal non-cytotoxic concentration of AMP in VERO cell was determined to be 16 ug ml−1. The effects of antiviral activity of 24-h pretreated and non-pretreated AMP were examined respectively and the pretreated cells showed distinct antiviral activity against the novel bunyavirus. Further more, AMP inhibited bunyavirus infection in a dose-dependent manner.

Conclusion: This study revealed the AMP with antiviral activity on the novel bunyavirus and shed light on the future application in antiviral therapy.

Fig. 1. The antiviral effects of AMP on VERO cell with or without novel bunyavirus infection were observed by microscope (×40). A. the normal cells. B. the cells with AMP pretreated for 24h. C. the normal cells infected by novel bunyavirus for 5d. D. the AMP pretreated cells infected by novel bunyavirus for 5d. The cell number was reduced but the morphologic character was same with the normal cells.

Fig. 2. The antiviral activity of AMP against novel bunyavirus was dose-dependent. Bunyavirus was respectively treated with 250, 500 and 1000 ug ml−1 of AMP for 24h, and then titrated on VERO cells. LogNI values were determined 6 dpi. Each value is the mean±SD of three independent experiments conducted in triplicates.

Phosphorodiamidate morpholino oligomer RNA therapeutics for neuroinvasive infectious disease

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Objectives: The phosphorodiamidate morpholino oligomers (PMO) can be designed to inhibit gene expression by binding specifically to RNA with complementary base sequences. We have investigated the utility of these PMOs extensively as antiviral and antibacterial agents with numerous successes. However, numerous infectious diseases present therapeutic target challenges due to the neuroinvasive character of the infectious agent. The goal of these studies was to evaluate the potential for PMOs to cross the blood brain barrier (BBB) and enter the cerebrospinal fluid (CSF).

Methods: AVI-4020 is a PMO with sequence 5'-CTTAGACATCGAGATTTTCTG-3' was designed to inhibit West Nile Virus (WNV). Non-clinical studies involved a bolus i.v. injection of 50 mg/kg into adult male Sprague-Dawley rats. CSF was collected at 8 and 24 hours post injection for quantitative determination of AVI-4020 with 4 rats per group. A multicenter, placebo-controlled single-blinded, clinical study was conducted in patients with neuroinvasive disease suspected to be the result of WNV. A total of 10 subjects were enrolled in the study, 9 received 15 mg of AVI-4020 i.v. every 12 hours for 5 days and 1 subject received placebo. Blood plasma and CSF samples were collected for quantitative determination of AVI-4020.

Results: In rats, the CSF concentrations were 204±43 ng/mL (26.8±5.6 nM), 8 hours post injection and below the limit of quantitation 24 hours post injection. Plasma concentrations in the rats was below the limit of detection at 8 hours. In humans, CSF samples from 7 subjects were obtained 18.8 hours (range 11.3 to 35.2 hours) after the final administered dose. Each subject had detectable AVI-4020 at a mean concentration of 8.1 ng/mL (range 5.4 to 22.0). Interestingly, AVI-4020 crossed the BBB regardless of clinical disease status classification or if the meninges were inflamed or not based on CSF cytology findings.

Conclusions: These studies in both rat and human provide evidence that a PMO with molecular weight of 7624 daltons can cross the BBB. The PMO is detected in CSF as early as 8 hours post a single injection in rat, and up to 35 hours post repeated injections in the human. In the human, AVI-4020 crossed the BBB regardless of disease status or meningeal inflammation.
Objective: Dengue virus infections result in a spectrum of disease, ranging from Dengue fever (DF), to Dengue hemorrhagic fever (DHF) and Dengue shock syndrome (DSS). Earlier studies utilized a phosphorodiamidate morpholino oligomer (PMO) or peptide conjugated PMO to identify the 5′-stem loop (5′-SL) and the 3′-cyclization sequence (3′-CS) as highly conserved viral targets. The purpose of these studies was to evaluate new positively charged PMO oligomers (PMOplus™) for potentially enhanced efficacy in both mouse and ferret Dengue type 2 (DV2) challenge models.

Methods: In vivo efficacy studies were conducted with AVI-6006, a combination of the two 5′-SL and 3′-CS PMOplusTM oligomers, in AG129 mice inoculated intravenously with the DEN2 S221 viral strain which causes early lethal disease in mice. In addition, efficacy studies were conducted in ferrets inoculated i.p. with DV2. The endpoints of the studies included median survival time (MST), long term survival, body weight changes, necropsy and tissue evaluation of selected organs and blood and tissue virus load.

Results: In the mouse a 3.0 mg dose of AVI-6006 administered by the intraperitoneal route on days 0, 1, 2, 4, 6 and 8 post infection provided for a MST of 14 days and 20 percent long term survival versus MST of 4 days and no long term survival in the saline control and the scramble PMOplus™ control sequence (p < 0.05). Viral load in kidney, liver, serum and small intestine was reduced in a dose dependent manner. In the ferret, a dose of 150 mg/kg (75 mg/kg each of 5′-SL and 3′-CS oligomers) was well tolerated and reduced body weight loss. Viral load was reduced to undetectable levels in the liver and small intestine. Finally, histological evaluation of liver and lung indicate AVI-6006 has a protective antiviral effect on those tissues.

Conclusions: AVI-6006 PMOplus™ oligomers targeting the 5′-SL and the 3′-CS are effective against Dengue 2 in both the mouse lethal challenge and ferret challenge models providing survival benefit, reduction in viral titer and prevention of body weight loss. The 5′-SL and 3′-CS combination provides a broad therapeutic index.

New antimicrobials: phase 2–3 studies

A successful phase 2 program studying OMC has been completed that suggested efficacy comparable to linezolid and thus supported progression to a phase 3 program in patients with serious skin infections. Because of an evolving concern that assessing outcome 10–17 days after completing therapy for complicated skin and soft tissue infections (cSSTI) may poorly correlate with historical trial data used to establish treatment effect of antibiotics in this disease, the early responses in patients with cSSTI randomized to either OMC or linezolid were further assessed.

Methods: A post-hoc analysis was conducted that was aimed at defining: 1) incidence of cessation of spread of infection (no increase in either maximum length or width of infection site inflammation) and absence of fever (core body temp <38.2 C) 1–3 days after starting therapy and 2) reduction of lesion size during the treatment course.

Results: In OMC and linezolid-treated patients respectively, the clinical response rates 10−17 days after completing therapy were 88.3% (98/111) and 75.9% (82/108) [95% CI for the difference: 1.9, 22.9] in theITT population, 98.0% (98/100) and 93.2% (82/88) [95% CI for the difference: −1.7, 11.3] in the CE population. The incidence of cessation of lesion size increase and absence of fever at day 1–3 in OMC and linezolid-treated patients respectively were 96.8% and 94.4% in the ITT and 96.4% and 93.8% in the CE population. In ITT patients in whom complete data were collected on lesion size, mean reduction of maximal lesion dimension was greater for patients treated with OMC than those treated with linezolid evaluated within 1–3 days of starting (31.8%; SE 4.6% for OMC and 6.7%; SE 15.1% for linezolid) and upon completion of therapy (81.1; SE 3.4% for OMC and 63.2%; SE 5.8% for linezolid). Among the ITT subjects who received systemic antibiotics prior to enrolment, mean reductions were 28.3% (SE 5.1%) for OMC treated and –6.7% (SE 25.4%) for linezolid treated patients at 1–3 days of starting therapy. At completion of therapy mean reduction of lesion size in these patients was 82.7% (SE 4.3%) for OMC treated and 63.0% (SE 8.0%) for linezolid treated patients.

Conclusions: Consistent with outcomes assessed 10–17 days after completing therapy, outcome assessed early in the course of therapy with OMC compared favorably to that of linezolid. This phase 2 experience strongly supports continued development of OMC as a treatment of patients with serious skin infections.
Dalbavancin versus linezolid for acute bacterial infections of the skin: a comparison of early and standard outcome measures in study VER001–009

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Objectives: FDA Draft Guidance for treatment of skin infections has recommended the implementation of an outcome assessment at 48–72 hours post baseline of the cessation of spread plus resolution of elevated temperatures as the primary point for comparisons in non-inferiority studies, rather than the test of cure historically measured post therapy. We performed a retrospective analysis of this new endpoint in a previously completed registrational trial and compared the outcome to the protocol prespecified primary endpoint of clinical response at Day 28.

Methods: The primary endpoint at Day 28 was originally defined in the clinically evaluable population which was then further analyzed in subgroups of patients meeting the newly defined FDA inclusion criteria (surface area of lesion >75cm²; one sign of either fever, elevated white blood cell count or bandemia) as well as the early response endpoint (lesion size the same or smaller relative to baseline and temperature <37.6°C).

Results: See table.

Conclusion: Dalbavancin non-inferiority relative to linezolid as assessed by the prespecified primary analysis is reinforced with an early responder analysis performed at Day 3/4, with very similar differences in the point estimate regardless of outcome measure.

| Timepoint | Analysis population | Endpoint | Dalbavancin | Linezolid | Non-inferiority
|------------|---------------------|----------|------------|----------|----------------|
| Day 3/4    | Clinically evaluable | cessation of spread + alleviation of signs/symptoms | 26/34 (76.5%) | 35/40 (87.5%) | 11.0 (0.1, 4.3) |
|            | >75cm² lesions      | cessation of spread | 21/34 (62.1%) | 18/23 (78.3%) | 6.9 (0.5, 4.0) |
| Day 28    | Clinically evaluable | cessation of spread + alleviation of signs/symptoms | 18/34 (52.9%) | 35/40 (87.5%) | 13 (1.0, 6.3) |
|            | >75cm² lesions      | cessation of spread | 13/34 (38.2%) | 18/23 (78.3%) | 5.0 (0.4, 3.0) |

P1532 Efficacy and safety of ceftazidime/NXL104 plus metronidazole vs. meropenem in the treatment of complicated intra-abdominal infections in hospitalised adults

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Objectives: This phase II study evaluated the efficacy and safety of ceftazidime/NXL104 (CAZ104) plus metronidazole (MTZ) vs. meropenem in the treatment of hospitalised adults with complicated intra-abdominal infections (cIAIs).

Methods: This was a prospective, multicentre, double-blind, randomised, comparative study (ClinicalTrials.gov Identifier: NCT00752219). cIAIs were defined as those extending into the peritoneal space and which required surgical intervention. Eligible patients (pts) from 33 sites in 8 countries worldwide were stratified according to APACHE II score and randomised (1:1) to receive either CAZ104 (2000/500 mg) plus MTZ (500 mg) or meropenem (1000 mg) IV every 8 h. Pts were treated for a minimum of 5 and maximum of 14 days. A test of cure (TOC) visit occurred 2 weeks after last treatment. A final visit took place 4–6 weeks after last treatment. The primary endpoint was clinical response at the TOC visit in microbiologically evaluable (ME) pts. Clinical response was complete resolution or significant improvement of signs and symptoms of infection, with no additional antibiotics or surgery required.

Results: 203 pts aged 18–90 years were randomised and clinical characteristics were comparable between groups. Overall, 47.3% presented with appendicitis. The ME population comprised 144 pts (CAZ104+MTZ, n = 68; meropenem, n = 76). Most infections were polymicrobial and due to Escherichia coli (CAZ104+MTZ, n = 52; meropenem, n = 53) and Klebsiella pneumoniae (CAZ104+MTZ, n = 6; meropenem, n = 11). The mean duration of therapy was ~7 days. At TOC in the ME population, 91.2% of pts treated with CAZ104+MTZ (62/68) achieved a favourable clinical response compared with 93.4% of meropenem-treated pts (71/76); difference: −2.2%; 95% CI: −20.4, 12.2; p = 0.6). Among patients with Gram-negative CAZ-resistant infection,
favourable microbiological responses were demonstrated in 25/26 pts overall in the CAZ104+MTZ group (E. coli, 19/20; K. pneumoniae, 3/3) and 17/18 pts overall in the meropenem group (E. coli, 13/14; K. pneumoniae, 3/3). The most common adverse events overall for CAZ104+MTZ were nausea (10%) and vomiting (14%). The incidence of drug-related adverse events was similar for CAZ104+MTZ (15%) and meropenem (17%).

Conclusion: In this study, CAZ104+MTZ was generally well tolerated and demonstrated efficacy in the treatment of hospitalised adults with cUTIs, with similar efficacy to that of meropenem.

Supported by AstraZeneca, Macclesfield, UK.

**P1533 Efficacy, safety and tolerability of ceftazidime/NXL104 vs. imipenem cilastatin in the treatment of complicated urinary tract infections in hospitalised adults**

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**Objectives:** Phase II study evaluating the efficacy and safety of a combination of ceftazidime plus a novel non-β-lactam β-lactamase inhibitor, NXL104 (CAZ104) vs. imipenem cilastatin in the treatment of hospitalised adults with complicated urinary tract infections (cUTIs) due to Gram-negative pathogens.

**Methods:** Prospective, multicentre, investigator-blinded, randomised, comparative study (ClinicalTrials.gov Identifier: NCT00690378) evaluating the treatment of cUTIs, including acute pyelonephritis, UTI in men, or UTI associated with obstruction, foreign bodies or urological abnormalities. 135 patients (pts) were enrolled from 5 countries and stratified based on type of infection (pyelonephritis or other cUTI).

Pts were randomised (1:1) to receive CAZ104 (500/125 mg) IV every 8 h or imipenem cilastatin (500 mg) IV every 6 h. Pts were treated for a minimum of 7 and a maximum of 14 days. Pts who met pre-specified criteria for clinical improvement after day 4 and had susceptible pathogens were switched to oral ciprofloxacin (500 mg) monotherapy every 12 h for the remaining treatment course. Efficacy was assessed at the end of IV therapy, at the test of cure (TOC) visit 5–9 days after last treatment, and during a late follow-up, 4–6 weeks after end of therapy. The primary efficacy end point was microbiological response at the TOC visit in microbiologically evaluable (ME) pts. Favourable microbiological response in a pt was defined as eradication of all infecting pathogens; eradication of a pathogen was defined as reduction of baseline urine pathogen level from $\geq 10^5$ CFU/mL to $<10^4$ CFU/mL within 5–9 days of last treatment (TOC), without evidence of the pathogen in the blood.

**Results:** The microbiological response of CAZ104 vs. imipenem cilastatin at the TOC visit, in the ME population will be presented, along with the safety and tolerability of CAZ104.

**Conclusion:** This study will report the efficacy and safety of CAZ104 relative to imipenem cilastatin in the treatment of hospitalised adults with cUTIs.

Supported by AstraZeneca, Macclesfield, UK.

**Clinical trials with antibacterials**

**P1534 Linezolid and vancomycin in the treatment of lower extremity complicated skin and skin structure infections caused by methicillin-resistant Staphylococcus aureus by vascular disease status**

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**Objectives:** Physiologic differences exist in patients with vascular disease (PVOD) that may impact drug delivery and subsequent outcomes when treating PVOD patients with lower extremity (LE) complicated skin and skin structure infections (cSSSI) caused by methicillin-resistant Staphylococcus aureus (MRSA). We sought to evaluate the outcomes between patients with and without PVOD who were treated for a LE cSSSI caused by MRSA.

**Methods:** Data from two prospective randomized, clinical trials evaluating the safety and efficacy of linezolid (LZD) and vancomycin (VAN) for the treatment of MRSA cSSSI were pooled to evaluate outcomes by vascular disease status. In each trial, eligible adults were randomized in a 1:1 ratio to receive LZD 600 mg IV or orally every 12 h or VAN 15 mg/kg or 1 g IV every 12 h (adjusted for creatinine clearance) for 7–28 d. Patients with PVOD were identified by the investigator. Only patients with a LE cSSSI caused by culture proven MRSA were included in this analysis. Clinical success rates among patients with and without PVOD receiving LZD and VAN were compared at end of treatment (EOT) and at end of study (EOS; 6–28d after EOT).

**Results:** There were 477 patients with LE cSSSI caused by MRSA (230 LZD and 247 VAN). Treatment groups were comparable for demographics and clinical characteristics for subjects with (139 LZD, 135 VAN) or without PVOD (91 LZD, 112 VAN). For those with PVOD, treatment duration was 12.7 and 11.7d for the LZD and VAN groups (p=0.07), respectively, and for those without PVOD, 11.5d and 9.5d (p=0.002), respectively. Clinical outcomes are shown in the table below. The number of subjects with treatment-related AEs (LZD=58 vs VAN=53), serious AEs (LZD=13 vs VAN=11), and study drug discontinuations (LZD=7 vs VAN=6) were comparable between LZD and VAN treated patients. Thrombocytopenia was identified in 4.3% and 0.7% of patients treated with LZD and VAN, respectively, and anemia was identified in 7.2% and 1.5% of patients treated with LZD and VAN, respectively.

**Conclusions:** Clinical success rates at EOT and EOS were statistically significantly higher with LZD compared to VAN in patients with PVOD and LE cSSSI caused by MRSA. Thrombocytopenia and anemia were identified more frequently among LZD treated compared to VAN treated patients and AE profiles were consistent with known safety profiles.

**P1535 The impact of linezolid versus vancomycin treatment on the resolution of local signs of inflammation of complicated skin and skin structure infections caused by culture proven methicillin-resistant Staphylococcus aureus**

A. Tice*, O. Equils, D. Huang, D. Stevens (Honolulu, Collegeville, Boise, US)

**Objectives:** A recent clinical trial demonstrated linezolid (LZD) to be comparable to vancomycin (VAN) in clinical outcomes (LZD 84% vs. VAN 80%; p=0.25) among patients with culture proven methicillin-resistant Staphylococcus aureus (MRSA) complicated skin and skin structure infections (cSSSI). However, the impact of treatment on resolution of local signs of inflammation have not been analyzed. We assessed the effect of LZD and VAN treatment on the resolution of local signs and symptoms of inflammation among adult patients with cSSSI due to culture proven MRSA.

**Methods:** We conducted post hoc analyses on data obtained from a recent phase 4, multicenter, open-label, 1:1 randomized, comparator controlled study conducted in adults with cSSSI due to culture proven MRSA. Adult patients were treated with linezolid 600 mg IV or orally twice a day, or VAN 15 mg/kg every 12 h adjusted for creatinine clearance. Study treatment was to be administered for a duration of 7 to 14 days. The presence or absence of purulent discharge and inflammation (tenderness, induration, local warmth, erythema, or fluctuance) was assessed and recorded by the site investigator at baseline, day 7±1, at the end of treatment (EOT; within 72hr after discontinuation of study medication), and at the end of study (EOS) visit, which occurred 6 to 28 days after treatment completion.

**Results:** Out of 654 patients who received at least one dose of study drug and had a baseline MRSA culture, 329 were treated with LZD and 325 were treated with VAN. The groups were comparable with respect to baseline demographics, clinical presentation, comorbid diseases, microbiology, frequency of incision and drainage. At Day 7, fewer patients in the LZD group had swelling (49% vs. 59%; p=0.03)
and tenderness (52% vs. 62%; p = 0.02) and, at EOT, fewer patients in the LZD group, compared to the VAN group, had erythema (28% vs. 35%; p = 0.07), discharge (27% vs. 34%; p = 0.05), tenderness (30% vs. 38%; p = 0.04), pain (23% vs. 32%; p = 0.009), swelling (22% vs. 32%; p = 0.008), and warmth (8% vs. 15%; p = 0.001). At the EOS fewer patients in the LZD group had warmth (7% vs. 12%; p = 0.03) and purulent discharge (7% vs. 14%; p = 0.006).

**Conclusion:** We observed more rapid and greater resolution of many clinical findings of inflammation in adult subjects receiving LZD for MRSA cSSSI compared with subjects receiving weight-based VAN (15 mg/kg every 12 h).

**[P1536] Linezolid versus vancomycin in the treatment of healthcare-associated pneumonia caused by culture-proven methicillin-resistant Staphylococcus aureus**

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**Objective:** Methicillin-resistant Staphylococcus aureus (MRSA) is a major cause of health care-associated pneumonia (HCAP). We describe outcomes of the HCAP subgroup from a double-blind randomized controlled trial (DBRCT) comparing linezolid (LZD) to vancomycin (VAN) in adults with nosocomial pneumonia (NP), including HCAP, caused by culture-proven MRSA.

**Methods:** We identified all patients with HCAP enrolled in an international DBRCT of LZD (600 mg IV twice daily) compared to VAN (15 mg/kg IV twice daily) for NP, including HCAP, caused by MRSA. HCAP was defined as pneumonia acquired in a long-term care or sub-acute/intermediate health care facility; or following a recent hospitalization (hospitalized for ≥48 h and discharged within 90 days of current admission); or in a subject who received chronic dialysis care within the 30 days prior to study enrollment. In this population, we examined clinical and microbiologic success at end of study (EOS, 7–30 days after the end of therapy). We also recorded rates of treatment-related adverse events (TRAEs), serious adverse events (SAEs), AEs that led to study drug discontinuation, and 28-day all-cause mortality rates.

**Results:** Among the 78 subjects with MRSA HCAP, patients randomized to LZD (n = 38) were similar to those treated with VAN (n = 40) with respect to demographics, co-morbidities (with the exception of chronic obstructive pulmonary disease 95% LZD, 77% VAN), and APACHE II score (mean LZD 17.3 and VAN 17.9). At EOS the clinical success rates were 57% with LZD compared to 44% with VAN (OR=1.7; 95% CI, 0.6–4.6) and the microbiological success rates were 66% with LZD compared to 38% with VAN (OR=3.1; 95% CI, 1.1–8.6). All-cause mortality within 28 days after randomization (LZD=8 vs. VAN=11) were comparable between LZD and VAN treated patients. The number of subjects with treatment-related AEs (LZD=9 vs. VAN=11) and study drug discontinuations (LZD=0 vs. VAN=2) were comparable. No treatment-related serious AEs were observed, and AE distribution was similar for both groups.

**Conclusions:** At EOS, LZD resulted in numerically higher rates of clinical success (although not statistically significantly different), and statistically significantly higher microbiological success in MRSA HCAP than weight-based VAN dosing (15 mg/kg IV q12 h). Both LZD and VAN had comparable 28-day all-cause mortality and frequencies of TRAEs, SAEs, and study drug discontinuations.

**[P1538] Evaluation of safety and tolerability of daptomycin doses ≥8 mg/kg/day: results from 270 patients in the European Cubicin® Outcomes Registry and Experience (EU-COREsm)**

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**Objectives:** Daptomycin (DAP) has concentration-dependent bactericidal activity. In vitro/in vivo studies and increasing clinical experience support doses up to 12 mg/kg once daily especially in difficult-to-treat infections. The objective of this study was to assess the safety and efficacy of DAP doses ≥8 mg/kg/day.

**Methods:** All pts receiving doses ≥8 mg/kg/day in this multicentre, retrospective, noninterventional registry were included in this analysis. Outcomes of efficacy (cured and improved = success, failure, non-evaluable) at the end of DAP therapy and safety up to 30 days after DAP were assessed by the investigators.

**Results:** In the reported treatment period (Jan 2006-Jun 2010), 270 pts received DAP ≥8 mg/kg (H8), while 3351 pts received doses <8 mg/kg, including 2731 pts who received the approved doses, 4 and 6 mg/kg. The frequency of H8 use increased over the 3 reporting periods (3%, 8%, and 12%, respectively); 63% of pts were male with a median age of 63y (range: 6–94). At baseline H8 pts had more frequently valve heart disease, sepsis and infections caused by MSSA than pts treated with lower doses. The most common infections in H8 pts were endocarditis, bacteraemia, SSTI and osteomyelitis/orthopedic devices. Empiric treatment was used in 42% of pts. The most common pathogens isolated in H8 pts were S. aureus (33%), coag-neg staphylococci (27%) and enterococci (11%, most E. faecalis). The mean DAP dose of 10 mg/kg was given for a median duration of 14 d. Longest treatment was 110 d for or pts and 60 d for out-pts. 8% of H8 pts were on dialysis (dosing Q48h) and 17% received statins during DAP therapy. The safety profile of H8 was good, no clinically relevant differences were detected vs lower doses. Adverse events (AEs), regardless of relation to DAP, were reported in 14% of H8 pts, incl. 2.6% with CPK elevations and 0.4% with musculoskeletal AEs. The most common AEs leading to DAP discontinuation in H8 pts were infections (1.5%). The overall clinical success rate with H8 DAP was 76% (Figure). Lower treatment failure rates in left-sided endocarditis were observed in H8 pts vs lower doses.

**Conclusion:** The use of high dose DAP, including long duration therapy for >100 days, has increased since 2006. Doses ≥8 mg/kg for a median duration of 14 days were safe and well tolerated in pts with diverse G+ infections. The role of high dose DAP seems to be promising in pts with difficult-to-treat infections and deserves further studies.

**Figure. Outcomes in patients treated with daptomycin doses ≥8 mg/kg**

**[P1539] Results from an observational study: daptomycin is safe and well tolerated in patients receiving haemodialysis**

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**Objectives:** Infections are the 2nd most common cause of death in end stage renal disease and account for ~14% of deaths in this population. Dialysis patients (pts) are at 100-fold higher risk for invasive MRSA infection and new therapeutic options are needed. In 2010 daptomycin (DAP) was approved in Europe to treat HD pts with S. aureus bacteraemia (BAC) at 6 mg/kg Q48h. As DAP levels are higher in this population, this study aimed at evaluating the outcomes of HD pts treated with DAP with focus on the drug safety profile.
**Methods:** All pts enrolled from Jan 06 to Jun 10 in EU-CORE, European CUBICIN® Outcomes Registry and Experience – a retrospective, non-interventional, multicenter study describing characteristics and outcomes of pts treated with DAP – could be included. According to investigator’s judgment outcomes are success (cure or improvement), failure and not evaluable. All pts who underwent HD during DAP treatment were selected for this analysis.

**Results:** Of 3621 pts enrolled, 207 underwent HD (64% male). Most pts had significant underlying disease including chronic renal failure, hypertension and diabetes mellitus. The primary infections included 86 cases of BAC (42%), 58 SSTI (28%), 21 endocarditis (10%) and 17 foreign body/prosthetic infections (8%). *S. aureus* was identified in 61 cases (MRSA rate = 51%). Coagulase-negative staphylococci were isolated in 32 cases and enterococci in 23, including 3 VRE. Prior to DAP 68% of pts received other antibiotics, most commonly a glycopeptide or a penicillin, and the most frequent reason for its discontinuation was treatment failure. The most frequent dosing interval for DAP in HD was Q4H. Overall, therapy duration ranged from 1 to up to 85 days. Overall clinical outcomes were 71% success, 15% failure, and 14% non-evaluable (Figure 1). Highest success rates were achieved in osteomyelitis and bacteraemia, 88 and 79%, respectively. Serum CPK values were elevated at baseline in 23% of cases. Among pts with CPK measurements during therapy normal levels were detected in 63%. There was no DAP discontinuation due to CPK elevation. Serious AEs occurred in 12.6% of pts most commonly infections — no musculoskeletal SAE was reported.

**Conclusion:** DAP was safe and well tolerated in HD pts with a variety of infections. The high success rates and favourable safety profile were comparable to those observed in pts with normal renal function. Further clinical studies on specific subsets of infections in HD might be warranted.

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**Figure 1: Clinical outcomes with DAP therapy**

| Infection Type   | Success | Failure | Non-evaluable |
|------------------|---------|---------|---------------|
| Overall (n=207)  | 138     | 40      | 29            |
| Osteomyelitis (n=8) | 6       | 2       | 0             |
| Bacteraemia (n=52)  | 10      | 15      | 27            |
| SSTI (n=47)       | 12      | 12      | 23            |
| Endocarditis (n=25) | 16     | 9       | 12            |
| Other (n=35)      | 0       | 0       | 35            |

SSTI: Skin and soft tissue infections

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**P1540 Treatment of multi-resistant typhoid fever with tigecycline: the first 10 cases**

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Typhoid fever has become more refractory to standard therapy due to increased resistance to numerous antibiotics. In addition, relapsing disease and/or chronic carriage are pivotal public health issues. Tigecycline has a favourable kinetic profile (high intracellular concentration, excellent tissue penetration) and MICs of *Salmonella typhi*/*paratyphi* isolates range 0.12–2 mg/L (data from India and Taiwan).

We describe 10 Austrian tourists returning from Asia with refractory (n = 1) relapsing (n = 4), or resistant infection (n = 5), who where treated with tigecycline 150 mg once daily for 7–21 days. In 8 patients (m/f=3/7, age=median 24 (16–47)) therapy was successful with a median fever clearance time of 66 (24–168) hours and no relapse in a follow up period of 90 days (no symptoms and repetitive negative blood cultures); in 2 patients tigecycline was stopped due to nausea/vomiting after 3 days, all other patients had no side effects. This outcome suggests that tigecycline might be a useful alternative for treating refractory, relapsing or drug resistant typhoid fever.

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**P1541 Tigecycline as a therapeutic option in Stenotrophomonas maltophilia infections**

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**Objective:** To compare the efficacy of tigecycline (TGC) treatment with trimethoprim-sulfamethoxazole (TMP-SXT) in *Stenotrophomonas maltophilia* infections.

**Methods:** A retrospective cohort study was performed in a 432-bed tertiary care hospital. Adult patients who had received more than 3 days of SXT or TGC for *S. maltophilia* infection were included in the study. 23 (42.6%) had pneumonia, 13 (24.1%) had surgical wound infection, 9 (16.7%) had primary bacteraemia, 5 (9.3%) had secondary bacteraemia due to pneumonia and 4 (7.4%) had both pneumonia and surgical wound infection. *S. maltophilia* infections occurred a mean of 28.6±13.2 days after hospital admission. APACHE II scores were as follows: <10 (n=6, 11.1%), 10–19 (n=19, 35.2%), >20 (n=29, 53.7%). Comorbidities were present in 52 (96.3%) patients. 43 (79.6%) patients were followed up in ICUs. Additional infections were found in 31 (57.4%) patients. Among all isolates, the rates of susceptibility to TMP-SXT and TGC were 98.2% and 100%, respectively. For the treatment of *S. maltophilia* infection, 35 (64.8%) patients received TMP-SXT and 19 (35.2%) patients received TGC. There were no statistically significant differences in patient characteristics between two groups, except the treatment of patients with primary bacteraemia, whom all had been received TMP-SXT. Culture positivity rate was 93.6% in TMP-SXT group and 70.6% in TGC group at 7th day (p=0.031), whereas 24% versus 18.8% at 14th day (p=0.692). Clinical improvement was observed 71.4% in TMP-SXT group and 68.4% in TGC group at 14th day (p=0.817). Mortality rates at 30 days after the infection were respectively, 28.6% and 21.1% in TMP-SXT and TGC groups (p=0.747).

**Conclusion:** There were no significant differences in mortality and clinical response rates between TMP-SXT and TGC treatment in *S. maltophilia* infections. TGC had better microbiological eradication rate at day 7. Tigecycline may be considered as alternative option beyond TMP-SXT in treatment of *S. maltophilia* infections.

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**P1542 Effectiveness and safety of colistin therapy: a study in a tertiary hospital in Korea**

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**Objective:** Colistin has re-entered clinical use by necessity. We investigated clinical features of patients who administrated with colistin, aimed to assess its effectiveness and safety.

**Methods:** This study was conducted at a 980-bed tertiary care university hospital in Korea. We retrospectively collected the data of the patients who were admitted to hospital between January 2005 and July 2010. Nephrotoxicity was defined as an increase of creatinine more than 150% from the baseline creatinine during colistin use. The primary outcome was 30 day mortality and nephrotoxicity.
Results: One hundred patients who had received intravenous colistin for at least 48 hours for microbiologically documented multidrug-resistant Gram negative bacterial infections were included. The patients’ age was 61.7±15.3 years. Almost of them (98%) had underlying diseases, such as malignancy, neurologic disease, trauma, COPD, ESRD and heart failure. Among these, 86 patients (86%) were hospitalized in ICU. Half of them (48%) underwent operations. The mean duration of hospital stay until the start of colistin administration was 44.3±57.3 days. The patients were treated with colistin for pneumonia (58%), urinary tract infection (20%), bacteremia (11%), abdominal infection (9%) and meningitis (2%). The causative microorganisms were Pseudomonas aeruginosa (56%) and Acinetobacter baumannii (44%). The duration of colistin use was 9.0±5.2days. The 30 day mortality was 39% (39/100) and all-cause in-hospital mortality was 52%. Nephrotoxicity occurred in 48 of 100 patients (48%) and 19 of them were recovered. In the multivariate analysis, no independent predictors of nephrotoxicity were observed.

Conclusions: Our study showed that incidence of nephrotoxicity was high after colistin use. They had severe underlying diseases and in-hospital mortality of them was high.

P1543 An observational, follow-up study in a clinical cohort of Clostridium difficile infection patients comparing three initial treatment regimes

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Increase in the incidence as well as a high relapse and treatment failure rate complicate current management of C. difficile infection (CDI). A prospective, observational follow-up study of a clinical cohort of patients with Clostridium difficile infection following case definition of the European CDI Working Group comparing three initial therapy regimes. All patients were treated with metronidazole 3×500mg/d p.o. for 5–14 days (group I); 42 patients were treated with metronidazole 3×500mg/d i.v. for 5–14 days (group II) and 42 patients were treated with vancomycin 4×125–500mg/d p.o. for 5–14 days (group III). Risk ratios provided with 95% confidence intervals were calculated as measures of the effect of the three therapy regimes on the endpoints comparing Group I with Group II used as reference, Group III with Group I used as reference as well as Group III with Group II used as reference. The protective effect of the therapy regimes metronidazole p.o. and vancomycin on the risk of death as compared with Group I was calculated. After four weeks of therapy, the highest clinical response was observed in the DS group.

P1544 A randomised, double-blind, placebo-controlled pilot study to assess the effect of rifaximin “chaser” to prevent recurrent diarrhoea in patients with Clostridium difficile infection

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Objectives: Uncontrolled case series have demonstrated decreased diarrhoea recurrence in patients with Clostridium difficile infection (CDI) given rifaximin after conventional therapy. However, whether rifaximin decreases recurrent diarrhoea in patients with CDI is unknown. The purpose of this study was to assess rates of recurrent diarrhoea in patients with CDI given rifaximin vs. placebo immediately after conventional therapy.

Methods: This was a randomized, double-blind, placebo controlled pilot study. Patients with CDI and a Horn’s index ≥2 were randomized to receive rifaximin 400 mg three times daily or placebo for 20 days given immediately after finishing conventional anti-CDI antibiotics. Patients were followed for three months and assessed for a composite endpoint of recurrent diarrhoea that included CDI recurrence (return of diarrhoea with a positive toxin test) and patient self-reported return of non-CDI diarrhoea after a period of wellness.

Results: Sixty-eight patients aged 61±18 years (50% male) were given rifaximin (n=33) or placebo (n=35). Twenty four of 68 (35%) patients had recurrent diarrhoea either due to recurrent CDI (23.5%) or self-reported diarrhoea (11.5%). Recurrent diarrhoea occurred in 17 of 35 (49%) patients given placebo and 7 of 33 (21%) given rifaximin (p=0.010). CDI recurrence occurred in 11 of 35 (31%) patients given placebo and 5 of 33 (15%) patients given rifaximin (p=0.087). Self-reported diarrhoea occurred in 6 of 35 (17%) of patients given placebo and 2 of 33 (6%) given rifaximin (p=0.17).

Conclusion: A rifaximin chaser regimen decreased the incidence of a recurrent diarrhoea in patients with CDI.

P1545 Comparison of doxycycline-streptomycin, doxycycline-rifampin and ofloxacin-rifampin in the treatment of human brucellosis

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Objectives: Traditional regimens for the treatment of brucellosis are associated with significant rates of relapses. A few clinical studies with quinolone containing regimens have shown conflicting results. The aim of this study was to compare the efficacy of ofloxacin plus rifampin (OR) versus doxycycline plus streptomycin (DS) and doxycycline plus rifampin (DR) regimens in the treatment of brucellosis.

Methods: In a randomized clinical trial, 191 patients with brucellosis were enrolled in the study. One of the three therapeutic regimens including DS, DR, and OR was selected for each patient randomly. All patients were assessed during the period of therapy in the second, fourth and sixth weeks of therapy by clinical course. They also were followed-up clinically and serologically for six months after the cessation of therapy.

Results: Out of 191 patients with brucellosis 64, 62, and 65 patients received OR, DR, and DS regimens respectively. After four weeks of therapy, the highest clinical response was observed in the DS group. Therapeutic failure was observed in 9% of all patients. The DR group showed the highest rate of failure (16.9%) and the least rate was observed in DS group (4.6%). Adverse reactions were seen in 16.8% of patients,
but there was no significant difference among three groups. The lowest relapse rate (4.6%) was observed in the DS group.

**Conclusion:** The DS combination is still the first line regimen for the treatment of brucellosis in our region; we recommend the DR and OR combinations as the second-line regimen.

**P1546** Single-dose doxycycline for the treatment of Mediterranean spotted fever

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**Introduction:** Mediterranean spotted fever (MSF) due to *Rickettsia conorii* is the most important tick-borne disease occurring in North Africa. Tetracycline constitutes the conventional therapy for treatment of MSF. Doxycycline has been reported as an effective alternative. We therefore did a prospective study to compare doxycycline short-course treatment with standard tetracycline therapy for patients with MSF.

**Patient and Methods:** A prospective and randomized study of 132 patients hospitalized for MSF in the department of infectious diseases, university hospital Monastir, Tunisia, during the period 1987–2006. All patients fulfilled Raoult criteria and had a positive serology of *Rickettsia conorii* (a four-fold rise in antibody titer, a single high titer ≥64 or seroconversion to specific antigen by indirect fluorescent assay (IFA)). We divided the patients into a tetracycline antibiotics-treated group (group A) and doxycycline-treated group (group B). The patients were initially randomly assigned 1 week of daily oral treatment with 2g tetracycline (A) or a single dose of doxycycline (B).

**Results:** The mean age of the 132 patients was 39 years (range, 14–80 years). Eighty-four of the 132 patients were males (63.6%). Group A included 96 patients and group B 36 patients. There was no statistically significant epidemiological difference between the two groups, particularly the age (39.5 versus 40.2 years, p = 0.81) and the sex ratio (1.52 versus 2.59, p = 0.2). All patients had fever; a generalized maculopapular rash was seen in 94 patients (98%) of group A and in all patients of group B (p = 0.38). The presence of an inoculation eschar was observed in 59 patients of group A (61.4%) and 25 patients of group B (69.4%). In group B, the average time for apyrexia was shorter (2.9±1.1 days versus 3.3±1.1 days, p = 0.03). The average time of disappearance of myalgia was also shorter in group B (3.4±1.2 days versus 4.1±1.3 days, p = 0.016). The rash disappeared after a mean of 3.68±1.45 days in the group B and 4.62±5.9 days in group A (p = 0.003). The duration of hospitalization was longer in the tetracycline-treated group compared with the doxycycline-treated group (6.45±2.73 versus 5.05±1.72, p = 0.005). We did not find any side effects in the 2 groups.

**Conclusion:** A single dose of doxycycline may be an interesting alternative to tetracycline antibiotics to treat MSF, because of its effectiveness and safety compared to tetracycline.

**P1548** Moxifloxacin versus ceftriaxone in the treatment of primary pyogenic liver abscess: a prospective, randomized, open-labelled, active-controlled trial

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**Background:** Primary pyogenic liver abscess (PPLA) is a distinct disease entity primarily caused by *Klebsiella pneumoniae*. This trial aims to determine whether the use of moxifloxacin can effectively treat PPLA and shorten hospitalization, compared to ceftriaxone. Development of antibiotic resistance to colonized bacteria in the gastrointestinal tract was evaluated.

**Methods:** Adults with clinical diagnosis of liver abscess, supported by symptoms and radiological imaging were eligible for enrollment. Patients with biliary tract stones, malignancy, septic metastatic infections to the central nervous system and the eye, APACHE II score >20, rupture of liver abscess, and resistant micro-organisms were excluded. Stool cultures were collected at entry and 3 months.

**Treatment arms:** Moxifloxacin 400 mg IV qd for 2 weeks, then 400 mg PO qd for 7 days. Ceftriaxone 2g IV q12h for 2 weeks, then cephalxin 1gm PO q6h for 7 days (ClinicalTrials.gov identifier: NCT00895089).

**Results:** Thirty-nine of 63 screened patients were excluded. Twenty-four patients (moxifloxacin 13, ceftriaxone 11) were enrolled, with no difference in age (56.0 vs 63.4 years, p = 0.32) sex (8 vs 9 male, p = 1.00), median APACHE II score (8 vs 9, p = 0.43), diabetes (63.6 vs 61.5%, p = 0.21), and clinical symptoms, except for a longer duration of fever in days (3.7 vs 1.2, p = 0.03). Abscesses were larger in the ceftriaxone group (median 5.8 vs 2.8 cm, p = 0.01). Bacteriaemia occurred in the majority (81.8% vs 69.2%, p = 0.65), all *K. pneumoniae* except for one case with *E. coli*. Percutaneous aspiration/drainage was done whenever possible (81.8% vs 51.5%, p = 0.09). Cure was achieved in the majority (81.8% vs 76.9%, p = 1.00 on intent-to-treat, and 90.0% versus 100.0% on per protocol analysis). Only one treatment failure occurred in the ceftriaxone group. All *K. pneumoniae* isolated from the stool at 2–3 months remained susceptible to most antibiotics.

**Conclusion:** Treatment with moxifloxacin is comparable with ceftriaxone in the treatment of PPLA, and hospitalization can be shortened to 2 weeks without affecting outcome.

**P1547** A randomised, placebo-controlled trial of trimethoprim-sulfamethoxazole plus doxycycline versus trimethoprim-sulfamethoxazole alone for eradication phase treatment of melioidosis

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**Background:** Recurrence infection of melioidosis occurs around 5 to 25% of patients who survive the acute phase. After recovery from the acute phase with intravenous antibiotics administration, an eradication treatment with oral antibiotics is required for at least another 20 weeks. The current recommended oral eradication regimen in Thailand is combination of trimethoprim-sulfamethoxazole (TMP/SMX) and doxycycline whereas in Australia only TMP/SMX is used.

**Objectives:** Primary outcome was to compare the efficacy (overall relapse and microbiological documented relapse) of TMP/SMX and doxycycline with TMP/SMX alone for the eradication phase treatment of melioidosis. The secondary outcomes were mortality, switched therapy and adverse drug reaction (ADR).

**Methods:** A randomized, placebo-controlled trial was conducted in 5 hospitals in Thailand during October 2005–October 2010. The dose of TMP/SMX used in the study was calculated based on patient weight (TMP/SMX 10/50 mg/kg/d). Patients received 20 weeks of treatment and then followed for another 48 weeks.

**Results:** There were 611 patients which survived from the acute phase of culture-proven melioidosis recruited. Among these, 306 cases received combination treatment and 305 cases received TMP/SMX alone. Their baseline characteristics were comparable between 2 groups. There were 20 (6.5%) of 306 cases in combination group and 16 (5.2%) of 305 cases in TMP/SMX alone relapsed (difference 1.3%, 95% CI –2.4 to 5.0). The culture proven relapse occurred in 11/306 (3.6%) in combination and 9/305 (2.9%) in TMP/SMX alone group (difference 0.6%, 95% CI 0.0–3.4). There was no significant difference in mortality (3.6% vs. 3.9%, p = 0.825). Patients received combination therapy switched their regimen more common that TMP/SMX alone group (21.2% vs 14.4%, p = 0.028). More patient in combination group experienced treatment related GI side effects (20.9 vs. 11.1%, p = 0.001), allergic reactions (17.3% vs. 11.5%, p = 0.04), photosensitivity (8.8% vs. 7.9%, p = 0.001), any ADR (7.2% vs. 2%, p = 0.002) than in the TMP/SMX alone group.

**Conclusion:** Cotrimoxazole alone with weight-adjusted dosing should be use as maintenance therapy for melioidosis.
**P1549** Outcome of initial ciprofloxacin treatment for bloodstream infections caused by extended-spectrum β-lactamase producing *E. coli* and *K. pneumoniae*

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**Objectives:** The purpose of the study was to evaluate the treatment outcome of initial ciprofloxacin for patients with bacteraemia caused by ESBL-producing *E. coli* and *K. pneumoniae*.

**Methods:** From the database of a nationwide surveillance program (from October 2006 to April 2009) for bacteraemia, we analyzed the clinical data of 1,647 patients with bacteraemia due to *E. coli* and *K. pneumoniae*. Patients with initial ciprofloxacin treatment for bacteraemia due to ESBL-producing *E. coli* and *K. pneumoniae* were compared with those with non-ESBL-producing bacteraemia.

**Results:** Of total 1647 patients with bacteraemia, 238 (14.5%) were ESBL-producing group (153 in *E. coli*, and 85 in *K. pneumoniae*), and 1409 (85.5%) were non-ESBL-producing group (968 in *E. coli*, and 441 in *K. pneumoniae*). And of all, 10.7% (177/1647) patients were given initial ciprofloxacin without combination (5.5% [13/238] in ESBL-producing group, and 11.6% [164/1409] in non-ESBL-producing group). Ciprofloxacin was susceptible in 20.2% (48/238) of ESBL-producing group (21.6% [33/153] in *E. coli*, and 17.6% [15/85] in *K. pneumoniae*), whereas 60.0% (846/1409%) was susceptible in non-ESBL-producing group (58.3% [564/968] in *E. coli*, and 63.9% [282/441] in *K. pneumoniae*).

30-days mortality rate of patients with initial ciprofloxacin treatment was significantly lower in non-ESBL-producing group (9.1% [129/1409]) than in ESBL-producing group (15.1% [36/238]) (P = 0.001). By univariate analysis, male gender, presence of hematologic disease, presence of renal disease, primary bacteraemia, higher PITT score (>4), and severe sepsis (P < 0.05 for all) were the risk factors for 30-day mortality in initial ciprofloxacin treatment group for bloodstream infections caused by *E. coli* and *K. pneumoniae*. By multivariate analysis, only higher PITT score (>4), and severe sepsis (P < 0.05 for all) were the common independent risk factors for mortality.

Whereas ESBL production was a risk factor in all patients with bloodstream infection caused by *E. coli* and *K. pneumoniae* (P = 0.005), it was not found to be an independent factor for mortality in initial ciprofloxacin treatment group (P = 0.569).

**Conclusion:** In bloodstream infection caused by ESBL-producing *E. coli* and *K. pneumoniae*, initial ciprofloxacin treatment was associated with higher mortality. However other factor such as severe sepsis rather than initial ciprofloxacin use should be considered as an important prognostic factor.

**P1551** Pivmecillinam in the treatment of ESBL-producing *Escherichia coli*

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**Objectives:** Pivmecillinam (MEC) is a β-lactam for the treatment of uncomplicated urinary tract infection. In vitro MEC is stable against most extended spectrum β-lactamases (ESBL) but so far there are no clinical data supporting its use. We here present a retrospective cohort study on the efficacy of MEC in patients with ESBL-producing *E. coli* in urinary samples.

**Methods:** All patients above 12 years of age with urinary samples positive for an ESBL-producing *E. coli* in Kronoberg County, Sweden, during 2009 were identified in the database of the Dept of Clin Microbiology, Växjö, Sweden (n = 52, 38 women and 14 men). The EUCAST method was used for susceptibility testing. A retrospective chart review was performed and the age, sex, underlying medical conditions and treatment given at the time of culture was recorded. Outcome measures were change of therapy due to culture results and recurrent UTI after 1 and 3 months after the first culture.

**Results:** Mecillinam resistance was 6% while corresponding rates for trimethoprim (TMP) and ciprofloxacin (CIP) were 73% and 77%. In 16/52 patients no empirical antibiotic treatment was administered. Four of these were treated with MEC and one with ertapenem after culture results were available. In total 17 of the 52 patients were treated with MEC (in 14 patients administered 200 mg TID for 5 days). Two had an underlying urinary tract malignancy and seven had a history of recurrent UTI. The documented diagnosis was cystitis in 12 patients. Within one month 5/17 patients treated with MEC experienced recurrent infection, compared to 13/35 patients receiving other antibiotics or no treatment. After three months additionally 3 and 8 patients, respectively, had experienced a new UTI. No empirical MEC treatment was changed due to resistance, the ESBL-production resulted in a changed therapy in 4 patients and the corresponding figure for TMP and/or CIP resistance was 8.

**Conclusion:** This study, although small, is the first to report MEC as effective treatment as other antibiotics against ESBL-producing *E. coli* if the isolate is reported susceptible using EUCAST breakpoints. Resistance to TMP and or CIP caused twice as many shifts in therapy as did the ESBL-production indicating the importance of the associated resistance to other antibiotics rather than the ESBL-production itself for treatment decisions in UTI. Pivmecillinam seems as a safe treatment in uncomplicated UTI caused by ESBL-producing *E. coli*.

**P1550** Efficacy and safety of 5-day therapy with cefixime versus ciprofloxacin for uncomplicated urinary tract infections in women: randomised, controlled study

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**Objectives:** To evaluate efficacy and safety of short course of cefixime and ciprofloxacin in women with acute uncomplicated urinary tract infections (uUTI).

**Methods:** A multicenter prospective randomized controlled trial was performed in 3 centres in Russia. Female patients 18 years or older with symptoms of uUTI and bacteriuria (≥10⁵ CFU/ml) were included in the study after giving informed consent. Patients with anatomical or functional abnormalities of the urinary tract and signs or symptoms of upper UTI were excluded. The study protocol was approved by independent ethic committee. Patients were randomized in 1:1 ratio to two study group – group 1 – treatment with cefixime (Suprax®, Gedeon Richter) 400 mg OD for 5 days or group 2 – ciprofloxacin (Ciprolet®, Dr. Reddy’s) 250 mg (before interim statistical analysis [ISA]) or 500 mg twice daily for 5 days (after ISA). Patients were evaluated on days 3–5 (visit 2) and 28 (visit 3) after end of treatment.

**Results:** A total of 104 patients were included in the study – 49 in group 1, 55 – in group 2. ISA was carried out when 42 patients had been included into the study. At visit 2 bacteriuria had been detected in 0% (0/24) and 44.4% (8/18) among patients from group 1 and 2, respectively, p = 0.0003. After ISA the dose of ciprofloxacin was increased from 250 to 500 mg twice a day. Final statistical analysis showed that clinical efficacy in group 1 was 75.5% (37/49) and in group 2 – 58.1% (31/53), p = 0.96. Eradication at visit 2 were 95.9% (47/49) in group 1 and 66% (35/53) in group 2, p = 0.0002. An adverse events during therapy had 2 patients (4.1%) in the group 1 (bacterial vaginosis) and 11 (20%) in the group 2 (7 – diarrhea, 2 – bacterial vaginosis, 1 – urticaria and 1 – pyelonephritis).

**Conclusion:** Cefixime (400 mg twice a day) is a more effective and safe than ciprofloxacin (250–500 mg twice a day) in the treatment of uUTI in women.
Comparative effectiveness of 1 dose versus 3 weekly doses of benzathine penicillin in treatment of early syphilis in HIV-infected patients

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Objective: Serologic responses of early syphilis, which includes primary, secondary, and early latent syphilis, to a single-dose benzathine penicillin G or three weekly doses of benzathine penicillin among HIV-infected patients have rarely been evaluated before, though three weekly doses of benzathine penicillin has been recommended in the US CDC guidelines for treatment of syphilis in HIV-infected patients. We aimed to compare the effectiveness of 1 dose versus 3 doses of benzathine penicillin in treatment of early syphilis in HIV-infected patients.

Methods: From January 1, 2007 to November 30, 2009, 146 HIV-infected patients with early syphilis who were treated with benzathine penicillin at 4 referral hospitals for HIV care were enrolled in a prospective observational study for occurrence Jarisch-Herxheimer reaction. Decision of giving a single-dose or three weekly doses of benzathine penicillin for these patients with early syphilis was made at the discretion of treating physicians. Follow-up of rapid plasma reagin (RPR) or Venereal Disease Research Laboratory (VDRL) titer was performed every 3 to 6 months. Serologic responses were defined as 4-fold or greater decrease in RPR/VDRL titer 12 months following penicillin therapy. For those patients with missing data during the 12-month follow-up period, the principle of last-observation-carried-forward was applied.

Results: During the 35-month study period, 146 HIV-infected patients with early syphilis were enrolled; 80 received 1 dose and 66 received 3 doses of benzathine penicillin. Serologic failure and/or reinfection occurred in 48 patients (32.9%). There was no statistically significant difference of serologic failure and/or reinfection rate between HIV-infected patients receiving one-dose penicillin and those receiving 3 weekly doses (35.0% [28/80] vs 30.3% [20/66], P < 0.60). The serologic response was not associated with low CD4 cell count, detectable plasma HIV RNA load or use of highly active anti-retroviral therapy when penicillin was administered.

Conclusion: In this multi-center prospective observational study, the difference of serologic response rate between one-dose and 3 weekly doses of benzathine penicillin could not be demonstrated in treatment of HIV-infected patients with early syphilis.
Transmission of pertussis to 2 young infants: identification of their mothers as the potential source of infection

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Objective: To identify the source of pertussis transmission to two young infants.

Methods: In July 2009, two young baby girls aged 2 months old were separately admitted to the Department of Pediatrics B at the Children's Hospital of Tunis for difficulty breathing and pertussoid cough. A nasopharyngeal aspirate was collected from each infant and was sent to the Laboratory of Microbiology at the same hospital for pertussis investigation. The diagnosis was based on real-time PCR (RT-PCR) targeting the IS481, the IS1001 and the pertussis toxin promoter gene specific of Bordetella pertussis. Afterward, the investigation revealed that the babies' mothers had a 3-week history of upper respiratory tract infection using the faeces. Each mother administered a nasopharyngeal sample for pertussis testing.

Results: The RT-PCR turned out to be positive for Bordetella pertussis for both the infants and their mothers. Precisely, the Ct (threshold cycle) values for the first couple “baby-mother” were respectively 24.18 and 33.76, and for the second couple, the values scored at 14.50 and 31.17, respectively. The Ct values were higher for the mothers than for their babies pointing out hence a relatively old Bordetella pertussis infection for the former. These assumption advance enough proofs to consider the mothers as the source of infection.

Conclusion: Understanding the source of pertussis transmission to infants may provide new approaches to prevent pertussis in the most vulnerable infants, particularly those who are too young to be immunized. Public health measures to prevent the disease could be strengthened and booster vaccinations for adults against pertussis considered.

**P1555**

**P1556**

Pertussis cases in the paediatric department of a university hospital

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Whooping cough (pertussis) is a highly contagious, acute respiratory illness of humans that is caused by the Gram-negative bacterial pathogen Bordetella pertussis. Vaccination plays an important role in protection against pertussis. Due to routine vaccination programmes, the incidence and mortality rates of the disease have decreased significantly. However, an increase in the number of cases has recently been reported, most of which were in the population aged >4 years.

155 paediatric patients, aged 0–18 years, were admitted to the Department of Pediatric at the Istanbul Medical Faculty between February-December 2010 were studied. A pertussis-specific transport medium was used for transporting of nasopharyngeal aspirates to the laboratory. The cultures were studied. Apertussis-specific transport medium was used for transporting the Istanbul Medical Faculty between February-December 2010 were an increase in the number of cases has recently been reported, most of which were in the population aged >4 years. An overall incidence of 6.05 per 100,000 population was found.

Vaccination plays an important role in protection against pertussis. Due to routine vaccination programmes, the incidence and mortality rates of the disease have decreased significantly. However, an increase in the number of cases has recently been reported, most of which were in the population aged >4 years. An overall incidence of 6.05 per 100,000 population was found.

**P1557**

Serotype distribution of Streptococcus pneumoniae isolated from Argentinian paediatric patients

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Introduction: Streptococcus pneumoniae (Spn) is a major cause of acute otitis media (AOM) and invasive infections (INV) such as pneumonia, bloodstream infections and meningitis. Identification of serotypes involved in different pathologies may help in deciding formulations of new anti-pneumococcal vaccines and is directly related to levels of resistance to penicillin.

Objectives: To record the serotype distribution in AOM and INV in pediatric patients. To evaluate the coverage of 7-valent (PCV7), 10-valent (PCV10) and 13-valent (PCV13) pneumococcal protein conjugate vaccines. To evaluate the relationship between serotypes and resistance to penicillin.

Materials and Methods: Between May 2009 – August 2010 we studied 127 Spn isolated from middle ears of <10-y-o patients by tympanocentesis and 89 Spn isolated from normally sterile fluids in <19-y-o patients with INV. They were identified by optochin susceptibility and bile solubility. Penicillin MICs were determined by the Etest and were interpreted using CLSI guidelines. Serotyping was performed by the quellung reaction.

Results: Thirty serotypes were identified. The twelve most frequently isolated serotypes were, in decreasing order, 14 (14.2%); 19A (11.8%); 9V (8.7%); 3 and 19F (7.1%); 6A (6.3%); 18C (4.7%); 23F (3.1%); 5, 6B, 7F and 33F (2.4%) in AOM, and 14 (28.1%); 1 (6.7%); 5, 12F and 19A (5.6%); 6B and 18C (4.5%); 3, 6A, 7F, 15A and 19F (3.4%) in INV.

Serotypes related to penicillin resistance were: 14, 19A; 6A y 9V. The coverage of different vaccines was 41.7% and 44.9% by PCV7, 48.1% and 60.6% by PCV10, and 73.3% and 73.0% by PCV13; for AOM and INV respectively.

Conclusion: The most frequently isolated serotype was 14 in both cases. Serotypes: 19A, 9V, 19F y 3 were isolated most frequently in AOM that in INV (p < 0.05). The highest potential coverage would be obtained with PCV13 (>70%). Non-significant differences between coverage of Spn in OMA and INV were observed with all vaccines.

**P1558**

The dynamics of Streptococcus pneumoniae nasopharyngeal carriage in mothers and children of the Warao Amerindians from Venezuela

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Objectives: To describe the dynamics of pneumococcal transmission in Warao Amerindian communities from Venezuela with emphasis for the role of the mother as carrier and source of introduction of new serotypes into the family.

Methods: Nasopharyngeal samples were obtained from 157 families, including 336 children between 2 months and 10 years old and 152 mothers. S. pneumoniae was isolated and identified according to standard microbiological procedures and serotyped with a multiplex PCR for 35 serotypes. Pneumococcal isolates of 10 families in which the mother and at least one of her children shared a pneumococcal serotype were selected for MLST analysis.

Results: The overall pneumococcal nasopharyngeal carriage rate in our study population was 60.2% (n = 924) with 239 children (n = 71.1%) and 35 mothers (n = 56.2%) colonized. The most important capsular serotypes/serogroups found were 6 (24.5%, n = 95), 19A (12.4%, n = 48), 23F (11.1%, n = 43), 4 (4.9%, n = 19), 23A (4.4%, n = 17) y 11A (3.9%, n = 15), indicating a theoretical coverage for the 13 valent conjugate vaccine of 59%. In 92 families at least two members were colonized,
with 53 of these families sharing the same serotype. 27 mothers were colonized with a serotype different of those isolated from their children. In 23 families the serotype was shared between the mother and at least one of her children and the isolates of 10 of these families were selected for MLST analysis. We detected 13 clones of which 4 never have been described. In 7 families the mother and the children were colonized with a strain of the same serotype and genotype. In 3 families mothers and children were colonized with the same serotype but a different genotype.

**Conclusions:** The high pneumococcal colonization rate found in the Warao children justifies the introduction of a conjugate vaccine in this population. Our study demonstrates that the Warao mother is an important pneumococcal reservoir sharing serotypes and genotypes with their children but also carrying serotypes or genotypes not (yet) found in the family, pointing toward a possible role of her in the transmission of the bacteria and in the introduction of new serotypes into the family. The proposed introduction of the 13-valent conjugate vaccine in this population in the beginning of the year 2011 should be used to follow up on pneumococcal carriage of the Warao mothers to study the herd effect of this vaccine.

**P1550 Nasopharyngeal carriage of Streptococcus pneumoniae in healthy Czech children: implications for clinical practice**

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**Objectives:** The aim of this study was to assess the prevalence of pneumococcal serotypes in nasopharynx of healthy children attending day-care centres, determine their antimicrobial susceptibility and compare the findings in vaccinated and non-vaccinated children.

**Methods:** Nasopharyngeal samples were collected in January-March 2010 from children attending 9 day-care centres in Prague. The specimens were selectively cultivated for pneumococci. The susceptibility testing was performed by disk diffusion method and by determination of MIC according to current CLSI standards. The serotype was identified by latex-agglutination (SSI, Copenhagen).

**Results:** The nasopharyngeal swab specimens were obtained from 230 children. *Streptococcus pneumoniae* was cultivated in 119 of them (51.7%). The age median of the study group was 57.97 months (IQR 45.82–67.32), 40 children (19%) were vaccinated with the recommended vaccination schedule (PCV7). The highest number of carriers was in the cohort of 48–59 months old children (colonization rate 60.1%). The nasopharyngeal carriage in children 60–71 and 72–83 months old remained relatively high, 54.8% and 50% respectively. In non-vaccinated children the colonization rate was 22/40 (55.0%), in non-vaccinated children during the first 6 months of life the colonization rate was 10/22 (45.4%). The nasopharyngeal carriage rate in vaccinated children was 22/40 (55.0%), in vaccinated children 103/190 (54.2%), p=1.00. The most frequently isolated serotypes were: 19F (16.3%), 23F (13.3%), 3 (12.5%), 14 (10.3%), 6B (9.2%) and 19A (10.3%). Serotypes 19A and 19F were the most frequent in vaccinated children, both were cultivated in 4/22 (18.2%). The susceptibility of isolated strains to β-lactams was high (108/115, 93.9%), 6 strains were intermediate resistant (5.2%) and only 1/115 (0.9%) resistant; 2 strains (1.7%) were resistant to erythromycin and 1 (0.9%) to clindamycin.

**Conclusion:** Relatively high colonization rate supports the importance of *Streptococcus pneumoniae* as a significant cause of community-acquired infections in children. High susceptibility of the isolates to β-lactams amplifies the importance of this antibiotic class in prescription practice in paediatric community-acquired respiratory tract infections. Vaccine and antimicrobial selective pressure represents a risk of replacement with different serotypes and bacteria and enhances the necessity of good surveillance programs.

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**P1560 Invasive pneumococcal disease in Austrian children <5 years of age (2007 to 2009)**

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**Objective:** Since 2004 vaccinations against invasive pneumococcal disease (IPD) have been subsidized for children at special risk of infection with *S. pneumoniae* in Austria. The aim of the study was to show the burden of disease in Austrian children below 5 years of age.

**Methods:** In a nationwide study including all Austrian pediatric hospital wards cases of IPD in children below 60 months of age were collected. Isolates of *S. pneumoniae* were serotyped and tested for antimicrobial resistance.

**Results:** Between 1.1.2007 and 31.12.2009 95 cases of IPD were reported. Among them there were 26 cases of meningitis and 4 deaths, giving a case fatality rate of 15.4% for pneumococcal meningitis. The average annual incidence rate for children <60 months of age was 8.0 per 100.000 for IPD and 2.2 per 100.000 for meningitis. Incidence rates for children <24 months of age were 11.8 per 100.000 for IPD and 4.5 per 100.000 for meningitis, respectively. Risk factors for IPD were present in 16.8% of the affected children; vaccine-serotypes (contained in PCV13) were identified in 57.1% of the isolates from risk-children. 18 children attended community institutions prior to the IPD. Antimicrobial resistance was tested in 49 isolates. No resistance was detected to amoxicillin, amoxicillin-clavulante or ceftriaxone. Resistance to macrolides (erythromycin and/or clarithromycin) was present in 9 isolates (6 isolates 14, 15C, 19F, 23F), 2 isolates (14 and 19A) were resistant to 3rd generation cephalosporin (cefpodoxime and/or ceftxime) and one strain (serotype 3) showed resistance to lincoasamide (clindamycin). Multiresistance, defined as resistance to two antibiotic classes or more, was present in 6 isolates. The multiresistant isolates were serotypes 1, 14 (3 isolates), 15A and 24F.

The serotype was detected in 71.6% of all IPD (68 cases). The hypothetical vaccination coverage rate for the ten-valent pneumococcal conjugated vaccine was 61.8% and for the thirteen-valent vaccine it was 76.5%.

**Conclusion:** IPD is a rare, but severe disease in Austrian children and especially meningitis has a high fatality rate. The bigger part of the circulating serotypes, in particular isolates with resistance to antibiotics, were covered by PCV10 and PCV13 justifying not only a risk-group vaccination program, but general vaccination of all children.

**P1561 Determination of immunoglobulin E and eosinophil cationic protein in children with Mycoplasma pneumoniae infection**

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**Objectives:** *Mycoplasma pneumoniae* has worldwide distribution and may cause severe respiratory infections in children. *M. pneumoniae* stimulates production of immunoglobulin E (IgE). Eosinophil cationic protein (ECP) derived from activated eosinophils in these patients may play a role in the pathogenesis of the inflammatory process. In this study we evaluated the clinical significance of IgE and ECP levels in serum of children with *M. pneumoniae* infection.

**Methods:** A total of 230 pediatric patients aged 1.5 to 14 years old were divided into three groups. Group 1 comprised of 150 patients with *M. pneumoniae* infection indicated by the presence of specific IgM antibodies and the absence of specific IgG antibodies. Group 2 comprised of 50 children suffered from pneumonia other than mycoplasma etiology. Group 3 consisted of 30 healthy children with negative serology against *M. pneumoniae* and was designated as control group. IgM and IgG antibodies against *M. pneumoniae* were determined by EIA (Plateletia – Biorad). Total IgE and ECP levels in serum were measured using a FEIA method (Immunocep 250 – Pharmacia). Statistical analysis was
performed using the chi-square test for IgE abnormal levels and the Wilcoxon signed-rank test for levels of ECP.

**Results:** Abnormal IgE levels were found in 50% (75/150) of group 1 children, in 60% (30/50) in group 2 and in 33.3% (10/30) in control group. Statistically significant difference was found between children with mycoplasma infection and control group, and also between children with, other than mycoplasma, pneumonia and control group. Conversely, no significant difference was noted between subjects including in group 1 and 2. Further, mean levels of ECP serum in children including in the first group (13.04) were statistically significant higher (p < 0.001) than those observed in both other groups; 9.11 in group 2 vs 8.33 in group 3.

**Conclusions:** Pneumonia in pediatric patients regardless the causing agent is associated with high levels of serum total IgE, suggesting that respiratory tract infection may be implicated in the initiation of asthma. The elevated ECP levels found in the serum of children with mycoplasma pneumonia may be associated with the damage to the respiratory epithelium. Potentially serum ECP levels may be a useful marker for identifying disease activity.

**Norovirus prevalence in “pathogen negative” gastroenteritis in Peruvian children**

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**Objective:** The aim of this study was to determine the prevalence of norovirus in children with gastroenteritis in whom all common pathogens had been excluded (“pathogen negative”).

**Methods:** Specimens analyzed in this study were obtained as part of a prospective, passive surveillance cohort diarrhea study in children 2−24 months of age in periurban communities of Lima, Peru. The study was conducted between September 2006 to December 2007 (1034 children) and from January to July 2008 (529 children). We used a modified Vesikari score to determine the severity of a norovirus-associated diarrhoea episode and to compare it with the clinical characteristics of rotavirus diarrhea from infants of the same cohort study. We obtained 1,102 diarrhea stool samples in the cohort study; 739 (67%) were negative for all common pathogens (Salmonella, Shigella, Campylobacter, Vibrio, diarrhoegenic E. coli, rotavirus and parasites), and were considered “pathogen negative”. From these, 224 (30%) samples were available for the current study. Viral RNA was extracted from 10% fecal suspension by a spin column technique, and was reverse transcribed using a commercial kit. Two sets of primers were used to amplify capsid region of norovirus as previously described.

**Results:** Norovirus was detected in 17.4% of 224 “pathogen negative” diarrhoeal samples. Norovirus prevalence in each age group was 8% in children 2−5 months of age (n=64 samples), 9% in children 6−11 months of age (n=80 samples), 33% in children 12−17 months of age (n=36 samples) and 34% in children 18−24 months of age (n=44 samples). Norovirus was identified more frequently in samples from children older than 12 months of age in younger children (34% vs. 8%, p < 0.001). Among norovirus-positive samples, norovirus genogroup II was identified more frequently (92%) than norovirus genogroup I (8%). Norovirus GI was the most frequent genogroup in each age group. All episodes associated with norovirus were acute. When comparing the clinical characteristics of norovirus with those of rotavirus, the former was more common in older children, while rotavirus was more common in younger children (mean 14.1 vs. 8.3 months, p < 0.001). Norovirus episodes tended to be of shorter duration and less severe than rotavirus episodes.

**Conclusion:** The role of norovirus as a cause of diarrhoea and the ascertainment of its severity in developing countries needs further confirmation by future epidemiological studies.
persistent diarrhea episodes. The frequency of the all genes was similar between severe and moderate episodes (Venkari score), except pagC which was more common in severe cases (p < 0.05).

**Conclusion:** Our findings indicate that in EPEC strains isolated from Peruvian children O122 genes are frequently present. Of these studied genes, efa1/lifA was more frequent in diarrhea caused by aEPEC strains. Additionally the EPEC strains found in diarrhea cases tended to have all 5 genes of the PAI O122. Further studies are needed to evaluate additional EPEC strains to determine the importance of these genes in the disease.

**P1556 Community-acquired respiratory tract infections during childhood**

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During childhood there is a high incidence of bronchiolitis and community acquired pneumonia due to viruses (Respiratory Syncytial Virus-RSV) and atypical bacteria (*Mycoplasm pneumoniae*), leading to an increased admission to paediatric hospitals requiring appropriate therapy.

**Objectives:** To assess the incidence of respiratory infections due to RSV and *M. pneumoniae* in children in various ages with respiratory symptoms (fever, cough) and X-ray findings (pulmonary consolidation), in order to provide appropriate antibiotic treatment.

**Methods:** A total of 1074 bronchoalveolar lavage specimens for RSV and 1149 blood samples for *M. pneumoniae* from children aged 1 month to 14 years (boys/girls:2/1). Detection of RSV was performed with rapid immunochromatography (Meridian, Bioscience). Detection of IgM antibodies against *M. pneumoniae*, was performed with rapid immunoassay, as well as with ELISA for both IgM and IgG antibodies (Vircell Microbiologists). Positive results were confirmed with indirect immunofluorescence (Bios, Germany).

**Results:** From a total of 1074 respiratory specimens, 27.1% were positive for RSV, while IgM and IgG antibodies were positive in a percentage of 24.5% and 43% respectively. RSV was the predominant pathogen causing bronchiolitis in children between 2 months and 1 year old, while *M. pneumoniae* in children aged above 4–6 years with atypical pneumonia. Morbidity was higher during winter and spring months.

**Conclusions:** RSV is the predominant pathogen in children less than 1 year causing bronchiolitis. Rapid detection of IgM antibodies against *M. pneumoniae* is a reliable diagnostic procedure during the acute phase of community acquired pneumonia in childhood.

**P1557 Neonatal bacteraemia due to Pasteurella bettysae**

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**Objectives:** To describe a case of mother-to-child transmission of *Pasteurella bettysae* through the birth canal, with occurrence of bacteraemia in the neonate. Cases of bacteraemia are rare, and neonatal bacteraemia due to vertical transmission, which may have severe effects, is infrequent.

**Methods:** Study of a case of neonatal bacteraemia by referencing the medical record and reviewing the literature published so far.

**Results:** The child was born by vaginal delivery induced at 39 weeks, with Apgar score of 7−9, head circumference of 36 cm and weight of 3,150 g (p 50–75). After delivery, he suffered from respiratory distress and was admitted after leaving the delivery room. The hemogram showed 45,490 leukocytes (77%N), the remaining parameters being normal. An empiric treatment with ampicillin and gentamicin was applied. 24 hours after admittance, Gram-negative cocobacilli were reported to have grown in hemocultures (Bact/ALERT, bioMérieux, Marcy l’Etoile, France) – suprapubic and lumbar punctures were made, without any relevant findings, and cefotaxime was added to the treatment. The bacterium was biochemically identified as *P. multocida* (Apt 20 NE, bioMérieux, Marcy l’Etoile, France) and showed sensitivity to ampicillin, penicillin, gentamicin, cefuroxime and amoxicillin/clavulanic acid. The child completed the treatment and his progress was satisfactory. It was decided to study urine and cervical-vaginal samples from the mother, in the latter of which *P. multocida* was isolated. The mother did not show any symptoms and reported not to have had contact with pets. In the national reference laboratory, identification and molecular characterization revealed that the same strain was involved in both cases, but also that it was *P. bettysae*.

**Conclusion:** Even though *P. multocida* is the species of *Pasteurella spp*. which more frequently leads to infections, sometimes different species are identified, such as *P. bettysae*, whose only reservoir known so far is human being. Species of *non-multocida Pasteurella* should be identified by molecular methods in reference centres, as conventional methods might produce wrong results. In general, this microorganism is sensitive to β-lactam antibiotics, the treatment of choice. β-lactam production is rare but should be considered – in such cases, amoxicillin/clavulanic acid, 3rd generation cephalosporins, fluoroquinolones, doxycyclin or cotrimoxazole may be used.

**P1558 Complications of bacterial meningitis in children**

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Festering inflammations of CNS still remain one of the last infectious diseases with considerably high mortality and morbidity accompanied by numerous complications and consequences even in the developed countries.

Out of a group consisting of 137 children admitted to a hospital with proven purulent meningitis in the Paediatric Infectious Diseases Clinic (PIDC) in the Brno Faculty Hospital, in the years 2001 to 2008, 96 patients have fulfilled the evaluation criteria of the study. The study carried out in the PIDC has proven that the incidence of festering affections of the CNS does not depend on the sex of the patients, and at the same time there is no difference in the frequency of complications and consequences in terms of male and female population.

We have not proven the evidence of a seasonal increase or decrease of patients with purulent affections of the CNS. After the admission to the hospital, 1/4 of paediatric patients had average up to severe consciousness disorders (GCS around 10), however only 12% of them required intubation. Almost half of the patients were of pre-school age (0–6 years). The initial phase of the disease is accompanied by vomiting in 70%, and by spasms in 23%. Bleeding symptoms into hypodermis and mucosa appeared in 34% of the patients, which correlates with the frequency of meningococcal infections (37%). In the childhood the primary forms of purulent meningitis (66%) prevail. Among very rare childhood complications we can rank the incidence of: hydrocephalus (2%), brain abscess (1%), thrombosis of cerebral sinus (3%), necrosis-gangrenes of acral parts of extremities (3%).

On the contrary, as a frequent necessary measure in case of festering affections of the CNS there seems to be a need of indication of the imaging methods (CT, MRI of brain – up to 47% of the patients have serious positive finding). In addition, there is a necessity to provide for interdisciplinary cooperation (indication of surgical intervention – up to 17% of patients require invasive intervention). In addition the execution of the EEG study (up to 17% of patients for the pathologic finding from EEG require a temporary medication and neurologist’s monitoring. Hearing impairment (up to 29%), visual impairment (8%), CNS impairment (up to 27% of the patients – subdural effuse, dilatation of chamber system, epilepsy etc.), and also incidence of profound psychomotor retardation (up to 40% of the patients) rank among the serious and frequent consequences.
Risk factors associated with mucosal colonisation by common Gram-negative species and yeasts in the neonatal intensive care unit

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Objective: To characterize the dynamics of mucosal colonization of neonates by common aerobic Gram negative microorganisms and Candida spp. and to identify independent perinatal, neonatal, and environmental risk factors influencing the colonization process in neonatal intensive care unit (NICU).

Methods: The nasopharyngeal (NP) and rectal swabs were collected on admission and thereafter twice weekly in all newborns (n = 276) admitted within the first 72h of life with risk factors of early onset sepsis. The association between colonization by different microbes and a total of 22 predefined risk factors was determined by univariate and multiple logistic regression analyses.

Results: A total of 1242 rectal and 1145 NP swabs with a per patients median of 3 (rectal and NP IQR 2/6 and 2/5, respectively) for both sites were collected. During the study about half of the patients had rectal (55.8%) or NP (42.8%) colonization with Gram-negative microorganisms and 38.8% had colonization of both sites. Colonization dynamics and interfering risk factors were similar for a given bacterial species in both mucosal sites, except that nonfermentative microbes more commonly harbored NP and Enterobacteriaceae in rectal swabs. The duration of NICU stay (median 6.7 days; IQR 3.75/17) as the factor most influencing mucosal colonization was associated with rectal and NP colonization of Acinetobacter spp. and all Enterobacteriaceae except E. coli, and rectal colonization by Candida spp. Depending on the species each day in NICU increased colonization risk by 5% to 8%. Factors interfering with the mucosal colonization were species specific depending on the origin of the particular species. While colonization by E. coli, K. oxytoca and C. albicans was most likely of maternal origin being associated with early perinatal factors like prolonged rupture of membranes, vaginal delivery and breast milk feeding, colonization by K. pneumoniae, E. cloaca, Acinetobacter spp. and non-albicans Candida spp. likely originated from hospital environment being influenced by treatment unit and period, duration of hospital stay and invasive interventions. The latter four organisms were also affected by low gestational age.

Conclusions: Risk patterns of Gram-negative colonization in NICU are bacterial species specific. The knowledge of risk factor profiles allows the development of strategies to prevent heavy colonization and subsequent invasive disease in high risk neonates.

Reduced vancomycin susceptibility in Staphylococcus capitis strains causing late-onset sepsis in intensive care neonates

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Objectives: Coagulase-negative staphylococci (CoNS) are the leading cause of neonatal sepsis in neonatal intensive care unit (NICU) setting, in which the high prevalence of methicillin resistance leads to the massive use of vancomycin. In the NICUs of Lyon, France, the majority of CoNS belong to a single pulsotype of Staphylococcus capitis (pulsotype A), also found in other French NICUs but distinct from pulsotypes isolated from adult patients. We determined the rates of resistance and heteroresistance to vancomycin (VRCap and hVRCap, respectively) among pulsotype A isolates, and searched for an association of S. capitis with persistent or recurrent bacteremia in NICU infants.

Methods: Vancomycin resistance. Vancomycin MIC was measured using E-test method in 53 S. capitis isolates (pulsotype A, n = 40), and other pulsotypes isolated from adult patients, n = 13. An isolate was VRCap if its vancomycin MIC was \( \geq 2 \) mg/l (EUCAST 2010 breakpoint), or was hVRCap if initial MIC was \( < 2 \) mg/l and if a stable resistant subpopulation was recovered after a selection step on agar containing 4 mg/l vancomycin. Persistent bacteremia. Microbiological records of neonates admitted to 2 NICUs of Lyon from 2004 to 2009, with LOS caused by either S. capitis (n = 234) or S. epidermidis (comparator, n = 176), were reviewed. Persistent or recurrent bacteremia was defined by \( \geq 3 \) distinct positive blood cultures with the same species over a period \( > 72 \)h.

Results: Vancomycin resistance. Among pulsotype A isolates, the proportion of VRCap (n = 15; 37.5%) and hVRCap (n = 14; 35.0%; total, n = 29; 72.5%) was significantly higher than in other pulsotypes (total 23.1% (p < 0.01, Z-test). Persistent bacteremia. Persistent or recurrent bacteremia were significantly more frequent during LOS caused by S. capitis (21.4%) than by S. epidermidis (9.1%) (OR=2.72 [1.49–4.96]).

Conclusion: S. capitis pulsotype A strains, which are currently endemic in several French NICUs, exhibit worrisome rates of vancomycin resistance and heteroresistance, and are associated with persistent or recurrent bacteremia in NICU infants.

Fungaemia in paediatrics patients: population characteristic, trends in infection frequency, aetiological factors and strain susceptibility. Epidemiological study 2000–2009, Poland

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Objectives: The aim of the study was to determine aetiological factors, frequency and antifungal susceptibility of clinical yeast strains isolated from blood samples of paediatric patients hospitalised in The Children's Memorial Health Institute. We also characterised the patients population according to medical records describing their age, sex, hospital ward at the time of infection diagnosis, underlying disease, agents used for antifungal prophylaxis with fluconazole alone or in combination with amphotericin B.

Methods: The clinical material was peripheral blood incubated directly in automated Bact/Alert system (bioMerieux). After receiving positive blood cultures, fungal isolates were identified by their morphological and biochemical features using ID 32C test (bioMerieux). Antifungal susceptibility was assessed using ATB Fungus tests (bioMerieux).

Results: During the 10-year period of time (from 2000 to 2009), mycological assays confirmed 118 cases of fungaemia in 107 patients. The highest number of infections occurred on the oncology ward (27,2%), nutrition and paediatrics ward (19,5%), intensive care ward (18,3%), transplantology and surgery ward (9,4% and 8,2%, respectively). The main aetiologic factor was Candida parapsilosis (39,0%) followed by C. albicans (35,6%), C. tropicalis (5,1%), C. glabrata (5,1%) Candida lusitaniae (3,4%), C. krusei (8,8%) and Saccharomyces cerevisiae (3,8%). Underlying conditions in patient population were characterized as cancer in 30 cases (28%), gastrointestinal disorders in 29 cases (27%), congenital heart disease in 13 cases (12,2%), respiratory failure in 11 cases (10,3%), preterm birth (6,5%), immunological disorders (3,7%), cardiovascular disorders (2,8%), organ transplant (1,9%), cerebral palsy (1,9%), other (1,4%). The main risk factor appeared to be central venous catheterisation (72,9%), parenteral nutrition (51,4%), immunosuppressive treatment (19,6%), broad-spectrum antibiotic therapy (60,7%), and antifungal prophylaxis with fluconazole alone or in combination with other antifungal agents (28%).

Conclusion: 1. Until 2005 C. albicans was the predominant aetiologic factor of fungemia. Since then a prevalence of non albicans candidaemiaens with a benefit towards C. parapsilosis is observed.

2. Increasing occurrence of Candida species with intrinsic resistance to fluconazole (C. dubliniensis) and amphotericin B (C. lusitaniae) and appearance of other fungal genus, such as Saccharomyces.
**Community-acquired pneumonia and its complications**

**P1571** Mycoplasma pneumoniae is not an important cause of lower respiratory tract infections in the European GRACE study: a combination of methods is necessary for early detection of cases

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**Objectives:** Diagnosis of atypical bacteria such as *M. pneumoniae* (MP) is often based on serology, which may require up to 3 weeks for a definite result. PCR based assays offer earlier diagnosis; however often respiratory specimens are not available from patients in a primary care setting. The aim of this study was to evaluate the prevalence of MP infection and compare serology and real-time PCR on both sputum and nasopharyngeal flocked swabs (NPFS) for diagnosis of MP.

**Methods:** From 10/2007 through 04/2010, a total of 3102 adult patients with lower respiratory tract infections (LRTI) in the community and 2984 controls were enrolled in a 3 year prospective study in 16 primary care networks PCNs in 11 European countries. NPFS, sputa, and paired sera were collected and sent to the local laboratory to be frozen until transport to the central lab in Antwerp. On a subset of 374 patients from whom sputa were available in the first winter period, in-house real-time PCR for MP was performed both on the sputum and NPFS. Sputa were scored according to the number of leucocytes (WBC) and squamous epithelial cells: specimens with ratios of WBC/epithelial cells ≥1 were defined as good quality sputa, ratios <1 were considered low quality sputa. IgG and IgM serology was performed using Mycoplasma pneumoniae-IgG/IgM-ELISA (Medac GmbH, Wedel, Germany) for detection of IgM or a IgG seroconversion or significant rise in anti MP IgG in sera collected 3–4 weeks apart; paired sera were available from 313 patients.

**Results:** MP was detected in 2.9% (9/313) of the studied adults with CA-LRTI by a IgG seroconversion or significant rise in IgG, only 3 (33%) tested IgM positive. In 4374 (1.1%) of the sputa MP was detected by PCR, with a mean Ct value of 19.62: only 1/374 (0.3%) was also positive in the NPFS with a Ct value of 34.27. PCR positivity in sputum was not related to sputum quality. All patients positive by PCR were also positive by IgG seroconversion; 6/9 IgG positive patients were positive in the acute phase by either IgM or PCR.

**Conclusion:** This is the largest etiologic study on LRTI in primary care ever done. Prevalence of MP infection in the first winter period was low. Detection of MP IgM antibodies in the early phase of LRTI is too insensitive. A combination of IgM and PCR on sputum is the most sensitive method for early detection of a MP infection. Bacterial loads are higher in sputum samples; these are therefore superior to NPFS for PCR detection.

**P1572** Review of positive *Chlamydia pneumoniae* PCR in a tertiary care centre over a ten-year period (2001–2010)

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**Objective:** In recent studies, the prevalence of respiratory infection due to *Chlamydia pneumoniae* was reported to be lower than previously published. Our objective was to review the prevalence of positive *C. pneumoniae* PCR results in a tertiary care center over a ten-year period (2001–2010).

**Methods:** A duplex real-time PCR for the detection of *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* has been developed (Welti et al. 2003) and applied in our routine diagnostic laboratory since October 2001. We reviewed all duplex *C. pneumoniae* + *M. pneumoniae* PCR analyses performed from October 2001 to June 2010.

**Results:** During the study period, 2244 respiratory specimens retrieved from 1583 patients were sent to the diagnostic laboratory for *C. pneumoniae* and/or *M. pneumoniae* PCR: 884 bronchoalveolar lavages, 843 nasopharyngeal swabs, 354 bronchial aspirates, 111 sputa and 52 other samples. Only four (0.2%) PCR in 2 patients were positive for *C. pneumoniae*, whereas 76 (3.4%) were positive for *M. pneumoniae* in 65 patients. The first patient with a positive *C. pneumoniae* PCR was a 48-year old man receiving immunosuppressive therapy for inflammatory bowel disease. He presented with a chronic cough initially suspected to be asthma and a febrile exacerbation. The second patient was a healthy 13-year old girl presenting with a third episode of pneumonia over a 5-month period. In both cases, the diagnosis of *C. pneumoniae* infection was not suspected by the clinician and the test was done thanks to the duplex format of our molecular test.

**Conclusion:** This study confirms that *C. pneumoniae* is rarely detected, at least in our setting. This may reflect a low prevalence of the disease and/or suggests that clinicians do not target the right population.

**P1573** Hospitalised community-acquired pneumonia: role of atypical organisms

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**Objectives:** Atypical respiratory pathogens such as *Mycoplasma pneumoniae* (MP), *Legionella pneumophila*, and *Chlamydia pneumoniae* (CP) are isolated with increasing frequency from community-acquired pneumonia (CAP). Local epidemiologic data on the etiologies of hospitalized patients aids in developing guidelines for clinical practice. This study elaborates the role of such “atypical pathogens” (AP) among adult hospitalized patients from Smolensk with CAP.

**Methods:** A prospective, observational study of consecutive adult CAP patients hospitalized during December 2009-November 2010 in Municipal Clinical Hospital was conducted. All adult patients (≥18 yr) had clinical features and radiological findings compatible to CAP. Inclusion criteria were new infiltrates on chest radiography or consolidation that could not be attributed to some other etiology and 3 or more of the following signs and symptoms: cough, acute changes in the quality of sputum (<10 epithelial cells and >25 polymorphonuclear leukocytes per low-power field in microscopy), documented fever (>38°C) or hypothermia (<36.1°C) within the preceding 24 hr, rales, leukocytosis (>1E10/L or >15% bands), malaise, myalgia or gastrointestinal symptoms. All patients were enrolled in this study before antibiotic treatment. Nasopharyngeal swabs were obtained for detection of MP, *Legionella pneumophila*, and CP by species-specific real-time polymerase chain reaction (AmpliSens, Interlabservice, Russia).

**Results:** A total of 74 patients (36 males, 38 females), averaging 44.7 yr (SD=15.8) were included in this study. Atypical pathogens were identified in 18 patients (24.3%), 15 of which were ≤40 yr (83.3%), and 3 (16.7%) more than 40 yr. The most common pathogen was CP (10 (14%), followed by MP – 8 (11%). No cases of *L. pneumophila* or mixed infections were observed. A retrospective analysis of these patients revealed an association of CAP with frequent respiratory viral infections, especially in patients working in service sectors.

**Conclusion:** Our data reflects an overall low prevalence of these atypical pathogens among Smolensk patients with CAP. Local microbiological data could be helpful in outlining therapeutic guidelines and diagnostic approaches for MP and CP species. A higher prevalence of AP in CAP is evident in young patients.

**P1574** Doxycycline versus macrolides in combination therapy for treatment of atypical community-acquired pneumonia: outcomes from ACAPS

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**Objectives:** Atypical pathogens (*Legionella*, *Chlamydophila* and *Mycoplasma* species) make up approximately 15% of the aetiology of community-acquired pneumonia (CAP) with many international and Australian guidelines recommending the use of empiric therapy with a β-lactam combined with a macrolide or doxycycline. We have attempted to determine whether there is any difference in patient outcomes when...
doxycycline or a macrolide is used in combination with β-lactams for empiric CAP treatment as little data is available comparing these combinations.

Methods: The Australian CAP Study (ACAPS) was a prospective, multicentre study of 885 episodes of CAP. Analysis of the recorded initial choice of antibiotic agents, in particular the choice of doxycycline or macrolide in combination therapy with a β-lactam antibiotic and the relevant clinical outcomes, including time to stability and length of stay (LOS), was performed. Possible confounding factors of age and severity of the presenting illness as measured by the Pneumonia Severity Index and SMART-COP scores were accounted for by the performance of multivariate regressions on the two patient groups (doxycycline versus macrolide-treated).

Results: For patient episodes due to atypical pathogens, patients treated with combination therapy containing doxycycline had a shorter median time to clinical stability (median 2.0 days vs. 2.0 days, Hazard ratio = 0.5 (0.31–0.82), p = 0.006) and LOS (3.0 vs. 6.0 days, HR = 0.32 (0.19–0.53), p < 0.001) compared to those who received macrolide containing combinations. A subgroup analysis looking at Legionella species alone still showed a trend favouring the doxycycline treatment group with a median time (interquartile range) to stability of 2.5 (2–3) days and LOS of 6 (4–6) days in comparison to 4 (2–6) days and 6.5 (4.5–13) days for the macrolide-treated group with a hazard ratio of 0.36 (p = 0.09) and 0.27 (p = 0.06) respectively. A similar trend was noted in other aetiological categories including those unknown, and without an atypical aetiology.

Conclusion: The use of doxycycline in combination with β-lactams for the treatment of CAP resulted in outcomes for time to clinical stability, LOS and, need for mechanical ventilation or vasopressor support that were at least as good as those in patients receiving macrolides. Doxycycline in combination with a β-lactam is suitable as first line therapy for the empiric treatment of CAP.

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confounders, we retrieved individual-level data on use of statins, other medications, comorbidities, and socioeconomic markers from medical databases. For the cohort outcome analysis we followed pneumonia patients for 30 days after hospital admission.

**Results:** Among the 70,914 patients hospitalized with pneumonia, 10.2% (7,223) were current statin users compared with 9.1% (64,523) among 709,140 population controls, corresponding to a crude pneumonia OR of 1.17 (95% CI: 1.14–1.21). However, current statin users had considerably more comorbidity and medication use than non-users. After adjusting for these and other confounders using conditional logistic regression analysis, the adjusted OR for a pneumonia-related hospitalization associated with current statin use was 0.80 (95% CI: 0.78–0.83). In contrast, we found no decreased pneumonia risk in former statin users (adj. OR = 0.98, 95% CI: 0.92–1.04). The prevalence of current statin use among our Danish pneumonia patients more than doubled from 4.6% in 1997–2004 (Thomsen et al., Arch Intern Med 2006) to 10.2% in the updated study period 1997–2009. Updated outcome analyses showed a 30-day mortality following pneumonia hospitalization of 11.3% among statin users vs. 15.1% among non-users. Cox’s regression analyses showed a 31% decrease in pneumonia mortality with current statin use (adjusted hazard ratio = 0.69, 95% CI: 0.64–0.74), similar to our earlier findings.

**Conclusion:** Statin use appears to be associated with a decreased risk of hospitalization for pneumonia in Denmark. Moreover, with the dramatic increase in the prevalence of statin use in Denmark’s population, the apparent pneumonia mortality reduction associated with statin use remains unchanged. Large randomized trials are needed to prove or disprove the effect of statin therapy in preventing severe pneumonia and improving prognosis.

**Keywords:** statin therapy, pneumonia, mortality, current and former statin users.

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**Management of community-acquired pneumonia and capping Clostridium difficile infections: are we falling at the CURB?**

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**Background:** Community Acquired Pneumonia (CAP), a common lower respiratory tract infection, accounts for 83,000 annual hospitalizations in the UK. BTS guidelines for management of CAP include severity assessment by calculation of CURB-65 to help guide choice, route and duration of antibiotics, investigations and discharge of low risk patients. Blackpool Victoria Hospital operates a successful Clostridium difficile infections (CDI) programme with high emphasis on antibiotic stewardship and root cause analysis (RCA) of CDI cases. We present results of audit on management of CAP and its association with findings of CDI-RCAs. These were used to inform remedial steps in the trust CDI programme.

**Methods:** Retrospective notes review of 50 randomly selected patients admitted with CAP between January and September 2009. Review of joint infection control-Microbiologist CDI-RCDA database.

**Results:** CAP AUDIT: M:F ratio[1: 1.7] with 68% [34/50] patients over 70-years & 16% over 90y. CURB65 score was documented in 32%[16/50] patients; 44% (7/16)was incorrect. 15/16 patients had a CURB 65 score of 0–1. Poor documentation limited analysis of reason for admission. 68% (34/50) of patients received antibiotics within 6 hours. Inappropriate antibiotics were prescribed in 42% (21/50) & improper route in 32% (16/50). IV to oral switch was delayed in 20% (10/50). Duration was appropriate in 90% (45/50). Mean length of stay was 13.7 days while in-patient mortality was 26% (13/50). CURB-Root cause analysis: RCA data on 113 cases revealed: avoidable cases 57% [64/113], key associations:78%[50/64] – non-compliance to antibiotic formulary & co-amoxiclav use each. Co-amoxiclav followed by quinolones and cephalosphorin use; delay in sample testing; repeated and prolonged courses of high risk antibiotics not supported by documentation, radiology/microbiology investigations.

**Conclusions:** Despite availability of updated guidelines, management of patients with CAP remains suboptimal. In a large number of patients CURB-65 is not calculated and this may lead to inappropriate management including inappropriate choice, route, duration of antibiotic use. Most of patients are elderly. Risk of CDI, complications and mortality is higher than previously reported. Findings from CDI-RCAs and this audit are associated and have been used to raise emphasis on intensive education of junior doctors, dissemination of guidelines, joint ward rounds with microbiologists, antibiotic prescribing audits & feedback. This is being done in our trust as a part of advancing quality program.

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**Epidemiology, clinical features, and outcomes of community-acquired pneumonia in patients with diabetes mellitus**

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**Objectives:** Although DM is associated with many alterations of the immune system that predispose to infection, current information regarding CAP in this population is scarce. We aimed to ascertain epidemiology, clinical features, and outcomes of CAP in pts with DM. The risk factors for mortality and the impact of immunomodulatory drugs on outcomes were also analyzed.

**Methods:** Observational analysis of a prospective cohort of nonseverely immunosuppressed hospitalized adults with CAP (1995–2008). A diagnosis of DM was based on a previous clinical and/or biochemical diagnosis of DM and/or treatment with oral anti diabetic agents or insulin.

**Results:** We documented 2407 CAP episodes, of which 516 (21.4%) cases occurred in pts with DM; 483 (97%) pts had DM type II, and 197 (40%) were on insulin treatment. The median value of HbA1c was 6.85 mmol/L. A total of 119 (23.9%) pts had DM-related complications. Compared with the remaining pts, those with DM were older (76.6% vs 51.6%; p<0.001), more often had been vaccinated against pneumococcus (67.9% vs 48.2%; p<0.001) and influenza (30.3% vs 21.4%; p<0.001), and had more comorbid conditions (70.7% vs 58.4%; p<0.001). Conversely, they were less frequently current smokers (15.2% vs 28.3% p<0.001) and heavy drinkers (12.5% vs 18.3% p<0.002). Pts with DM had more commonly altered mental status at admission (19.4% vs 14.5%; p=0.007) and were more often classified into high-risk PSI classes (groups IV-V) (77.5% vs 56%; p<0.001). In contrast, cough (79.6% vs 84.2%; p=0.01), pleural chest pain (29.1% vs 43.5%; p<0.001), multilobar pneumonia (24.8% vs 33.2%; p=0.043) and pleural effusion (12.8% vs 18.6%; p=0.002) were less frequent. No differences in causative agents were found between groups. The frequency of bacteremia was similar in both groups (11% vs 12.7%). No differences in main outcomes were found. Independent risk factors associated with mortality in pts with DM were advanced age (OR 5.29; CI 95% 1.17–23.89), DM-related nephropathy (2.50;1.07–5.84), multilobar pneumonia (2.46;1.11–5.43), shock at admission (5.05;2.05–12.45), and chronic heart disease (2.31;1.06–5.04). The use of immunomodulatory drugs (statins, ACEI, ASA, and β-blockers) was not associated with better outcomes.

**Conclusions:** CAP in pts with DM is quite similar than in pts without this condition. In our study, the use of immunomodulatory drugs has not a beneficial effect on the outcomes of CAP in pts with DM.

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**Clinical impact of combined viral and bacterial infection in patients with community-acquired pneumonia**

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**Objectives:** To compare hospitalised patients with community-acquired pneumonia (CAP) and a pure bacterial aetiology with those with CAP and findings of both bacteria and virus regarding severity of illness and length of hospital stay.

**Methods:** Adults with CAP admitted to Karolinska University Hospital were studied prospectively (n = 184). Microbiological methods included cultures from blood, sputum and nasopharyngeal secretions, sputum joint samples analysed with real-time quantitative PCR for Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, nasopharyngeal specimens analysed with PCR and serology for Mycoplasma pneumoniae, Chlamydia pneumoniae and virus common in the
The relative importance of respiratory viruses in lower respiratory tract infections in primary care

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Objectives: The role of viruses in adult lower respiratory tract infections (LRTI) in the community is not well known. We therefore investigated the viral aetiology in LRTI at the GP's office in the European GRACE primary care network using sensitive real-time PCR.

Materials and Methods: From October 2007 through April 2010, a total of 3102 adults with LRTI in the community were enrolled (P1) in a prospective study in 11 European countries. For these patients, matched controls (1198; Con.) and follow-up samples (1987; P2) were collected. Nasopharyngeal swabs were analyzed for the presence of influenza virus (INF) A/B, parainfluenza virus (PIV)1–4, rhinoviruses, human metapneumovirus, respiratory syncytial virus, adenovirus (HAdV), bocavirus (BOCA), coronavirus (HCoV) OC43, NL63, 229E, as well as the novel polyomaviruses KI and WU. Primary etiological data will be presented in an abstract by Ieven et al. Here, we investigated i. the relative importance of quantitative measurements (Ct values); ii. the aetiology of double infections and iii. the results of Con. and P2 samples.

Results:

i. DNA viruses were found at significantly lower loads (average Ct 37) than RNA viruses (average Ct 29.8). RSV and PIV3 were the only viruses for which Ct values differed significantly between P1 samples (−4 Ct) compared to Con. and P2.

ii. WU (26.7%) and KI (21.8%) were most frequently found in double infections. Of all positive V1 patients 7.1% was double-infected, in Con. 7.9% and P2 7.0%, despite lower infection rates.

iii. PIV positive patients (P1) were most prone to a positive follow up (12.7% positive P2; any other virus), for PIV3 this figure increased to 27.3%. HAdV, WU and KI are prominent secondary pathogens (48%, 40% and 42% of all positive samples, resp., are in P2). 96.6% of INF positive samples were in P1, for INF B this figure was 100%. Despite the fact that DNA viruses are detected substantially (0.5%, 1%, 1.4% and 1.5% for BOCA, KI, HAdV and WU, resp.) in respiratory specimens, their overall impact is overestimated since these viruses are largely present in Con., P1-doubled infections, or P2 (70%, 82%, 72% and 83%, resp.). On the other side of the spectrum, 90% of INF infections were detected in P1 as mono-infection (99.6% for INF B).

Conclusions:

- Quantitative viral load data will contribute little to individual patient management.
- Double infections do not affect presentation rates.
- The relative importance of DNA viruses as respiratory pathogens is low.

Value of prognostic scores in predicting mortality from community-acquired pneumonia. A prospective comparison in emergency departments

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Objectives: The routinely used of prognostic scores provide pivotal information for the management of infectious diseases, helping decisions about diagnostic and therapeutic strategies. Community-acquired pneumonia (CAP) represents the field where the clinical value of prognostic scores was better developed. Existing rules have different strengths and weaknesses, and have rarely been validated in populations different from the original cohorts. The objective of this analysis is to compare performances and concordance of two of the most used scores (CURB-65 and SCAP) and the sepsis grading, in terms of 30-day mortality, in totally different population.

Methods: Prospective enrolment and follow up of a population-based cohort of adults with pneumonia diagnosed in Emergency Departments (ED) is planned from the summer 2009 to the end of 2011. In this interim analysis, relation between prediction scores and 30-day mortality were examined using ROC curves analysis. Kappa coefficient was also calculated.

Results: Enrolled patients were 442, females were 40%, mean age (sd) was 60.6 (21.1) years (min max 17–99); comorbidities were present in 66.4%. Deaths at 30 days were 22 (5%). 64.6% of patients were in CURB65 low risk class, 68.9% in SCAP low risk, 69% in no sepsis or sepsis class. ROC curve analysis is reported in the graph. No statistically significant differences between ROC AUC were found (p = 0.21).

Kappa statistic was 0.54 between CURB65 and SCAP, 0.17 between CURB65 and sepsis, 0.21 between SCAP and sepsis.

Conclusion: The SCAP score performs better than others in predicting 30-days mortality. Of particular concern are the low level of sensitivity of sepsis score and the minimal concordance with the specific scores for pneumonia in the identification of severe disease.

The use of procalcitonin to guide antimicrobial use for respiratory tract infections in a district general hospital: a feasibility study

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Objectives: Our Trust, consisting of two large district general hospitals, admits 1700 patients with lower respiratory tract infections (LRTI) per year. A recent national audit of patients with chronic obstructive pulmonary disease (COPD) revealed our patients had a higher than average rate of antimicrobial use, including intravenous (IV) antibiotics. LRTI patients also had a longer than average length of stay. Our aim was to investigate the feasibility of using procalcitonin (PCT) in our setting to guide the use of antimicrobials in patients admitted with LRTI.

Methods: We conducted a retrospective observational study. Patients admitted to medical wards with a diagnosis of LRTI were identified...
Practical use of urine antigens and implementation of naïve PCT in the diagnosis and antibiotic therapy.

**Objectives:**
To evaluate the use of the urine antigens for Legionella pneumophila (Lp) and Streptococcus pneumoniae (Sp) done respectively, were not performed in accordance with the NR and had no impact on the antibiotic therapy for Sp. It remained the only test for an early diagnosis and treatment for Lp. The Fine score was never reported in practice. Using an a posteriori computed Fine score, based on chart records, the initial therapy would have agreed with the recommendations in only 20% (in 12% larger spectrum and in 61% antibiotics not agreed with suspected bacteria were used). The concordance between the NR algorithm based on the Fine score and the algorithm based on clinical signs, was good (kappa 0.80 to 0.61) except for 6 very old patients for whom broader spectrum antibiotics should have been given with the Fine score algorithm, but without any difference in outcome. This suggests that comorbidities are more relevant than the weighting by age beyond the 70 years old cut-off for the prognosis and first empirical therapy.

**Conclusion:** The Fine score is not used in real life emergency practice and may not be relevant for our emergency practitioners. We think that simpler clinical algorithm could be an alternative and should be prospectively evaluated.

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**N1585 Pneumonia treated in the internal medicine department: a nationwide study in Spain**

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**Objectives:** To assess the incidence, epidemiology, use of microbiological resources, management, and outcome of pneumonia treated in the internal medicine department (IMD) in Spain.

**Methods:** IMD reported all patients with pneumonia attended in their department during 1 week in January 2010 and 1 week in June 2010.

**Results:** Seventy-two IMDs from 66 hospitals submitted 1,031 patient records, of which 1,002 fulfilled the criteria for pneumonia. Patients were classified as having community-acquired pneumonia (CAP) in 60% of cases, healthcare-acquired pneumonia (HCAP) in 30%, and hospital acquired pneumonia (HAP) in 10% of cases. The incidence of pneumonia was 141 episodes/1,000 IMD admissions in January and 75.3/1,000 IMD admissions in June. According to the pneumonia severity index (PSI) score, 24% of CAP and HCAP patients were classified as having a low risk of death (classes I, II and III) and 76% a high risk (classes IV or V). According to CURB-65, 31% of patients were low-risk (<2 points) and 69% were moderate to very high risk (>2 points). Thus, according to the PSI and CURB-65 scores, 24% to 31% of admissions were not strictly necessary. A request for microbiological diagnosis (>1 sample sent to microbiology departments) was made in 87%, 78%, and 71% of CAP, HCAP, and HAP patients (p < 0.001), with an overall positivity of 29%. S. pneumoniae was the main pathogen of CAP and HCAP, while P. aeruginosa was the main pathogen of HAP. Overall, 30% of patients did not receive any antibiotic within 6 hours of evaluation. Adherence to IDSA guidelines in CAP was 70%; adherence to ATS guidelines in HCAP and HAP was 23% and 56% (p < 0.001). In patients with an etiological diagnosis, empirical antibiotic treatment was active against 73% of isolates. For CAP, HCAP, and HAP, the median length of hospital stay was 8, 9, and 11 days (0.002), and in-hospital mortality 8%, 19%, and 27% (<p < 0.001). Therapy was switched in 60% of patients and discharge delayed a median of 5 days after the patient was considered clinically stable. Only 2% of patients were vaccinated against S. pneumoniae during their hospital stay.

**Conclusions:** Pneumonia affects 7% to 14% of patients hospitalized in the IMD in Spain. Admission policy, use of microbiological resources, therapeutic management, discharge criteria, and prevention of future episodes are amenable to intervention.

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**P1584 Practical use of urine antigens and implementation of national recommendations for the management of community-acquired pneumonia (CAP): results from a retrospective study on 214 CAP hospitalised by the emergency department**

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**Objectives:** To evaluate the use of the urine antigens for Legionella pneumophila (Lp) and Streptococcus pneumoniae (Sp) in the management of CAP and the agreement of first empirical antibiotic therapy with the French NR.

**Methods:** This is a retrospective study on consecutive patients with CAP hospitalised by the emergency department in a general hospital between November 1st 2006 and October 31st 2007 (corresponding to two full resident periods). We excluded lung abscess and aspiration or nosocomial pneumonia. Diagnosis with the subsequently Fine scoring and antibiotic strategy were compared to the march 2006 French NR and to a more simple clinical algorithm intended for general practitioners and based on early clinical severity and comorbidities and/or age >70 year.

**Results:** 214 patients fulfilled the inclusion criteria. Median age was 79 years with 55% of male, 72% had at least one comorbidity and 21% were living in an institution. In-hospital mortality rate was 14%. There was only 36% of bacterial documentation among which 25 Sp and 8 Lp. Use of both urine antigens, 60% and 70% for Sp and Lp done respectively, were not performed in accordance with the NR and had
incidence and mortality are sparse. We examined temporal changes in the incidence, length of hospital stay, and patient mortality associated with empyema-related hospitalizations.

Methods: Using nationwide health registries covering all Danish hospitals, we identified all adults with a first-time hospital diagnosis of empyema between 1995 and 2009, and ascertained their comorbidity, length of hospital stay, and dates of death (if any). We computed age-standardized incidence rates and adjusted mortality rates by calendar year.

Results: We identified 6,549 patients with empyema, of whom 67% were men. The incidence rate increased from 8.7 per 100,000 person-years in 1995 to 13.3 per 100,000 person-years in 2009, representing a relative increase of 53.5%. During the same period, the median length of hospital stay for empyema decreased slightly, from 17 days (interquartile range (IQR): 9–31 days) to 14 days (IQR: 7–22). Mortality among patients diagnosed during the most recent 5-year calendar period (2005–2009) was only slightly lower than among patients diagnosed during the earliest period under study (1995–1999): 9.0% vs. 9.6% after 30 days. This corresponds to an adjusted 30-day mortality rate ratio of 0.76 (95% CI: 0.62–0.93) after controlling for changing age and comorbidity over time. Advanced age and high comorbidity level were poor prognostic factors.

Conclusion: Similar to previous findings for pneumonia, the incidence of empyema-related hospitalizations among adults in Denmark has increased substantially during the last 15 years. In the same time interval prognosis has improved modestly.

Bacteraemia, sepsis and endocarditis

Trends in the incidence of bloodstream infections and sepsis management organisation in Emilia-Romagna, Italy

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Objectives: Bloodstream infections are frequent and severe infections, with an incidence on the rise. To face this problem, as regional campaign to fight sepsis (LaSER – Lotta alla Sepsi in Emilia-Romagna) was launched in 2007. We report the trend in blood-stream infections (BSI) in the period 2005–2009, and an analysis of the organization of sepsis mangement in Emilia-Romagna, Italy.

Methods: Data regarding BSI and antibiotic resistance were obtained from the regional public health system data-bases, merging data from 5060 regional hospitals. BSI rates were calculated on the regional population. Coagulase-negative staphylococci and other possible skin contaminants isolate form blood-cultures were excluded, due to limitation in the interpretation of these blood cultures results of the system. Data regarding sepsis management were obtained from the LaSER project data.

Results: Between 2005 and 2009 the incidence of BSI increased from 78 to 120 cases/100,000 inhabitants (+55%, p < 0.0001), with Escherichia coli and Staphylococcus aureus being the most common germs isolated: 51.8 and 25.1 cases/100,000 inhabitants/year in 2009, respectively. Striking increases were observed for Klebsiella pneumoniae (from 5.2 to 10.2 cases/100,000 inhabitants, +97%), E. coli (from 28.2 to 51.8, +84%), Enterococcus faecium (from 3.4 to 5.8, +71%), and Enterococcus faecalis (from 7.4 to 11.5, +55%) (see table). Antibiotic resistance increased significantly both for E. coli and K. pneumoniae. Antimicrobial resistance increased strikingly among Enterobacteriaceae (see table), and it remained stable among Gram-positive cocci and non-fermenting Gram-negative rods. The LaSER project educated 5,800 health-care workers on sepsis diagnosis and management, made drawing blood-cultures possible in the casualty ward in 13/18 trusts, made urgent lactate dosage available in 16/18 trusts and a sepsis team has been organized in 13 trusts.

Conclusion: A steady increase in BSI was observed between 2005 and 2009, slightly more evident from 2008 after the implementation of the LaSER project. The increase observed among Enterobacteriaceae was mostly due to the emergence of antimicrobial resistant isolates. The

LaSER project favored improvement in the organization regarding sepsis management, of primary importance in this epidemiological setting. Evaluation of efficacy need to be performed in order to permit more appropriate analysis of the impact of the project.

The impact of diabetes and poor glycaemic control on risk of bacteraemia with haemoletic streptococci groups A, B and G in adults: a 15-year population-based case-control study

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Objectives: Diabetes has been associated with bacteraemia due to haemoletic streptococci (HS) but epidemiological evidence is limited.

Methods: We conducted a population-based case-control study of all adults with a first-time diagnosis of bacteraemia with HS groups A, B, and G and matched population controls. The study setting was Northern Denmark between 1992 and 2006. We computed odds ratios (ORs) for streptococcal bacteraemia according to diabetes and glycaemic control, using regression analysis for confounder adjustment.

Results: We identified 397 adult patients with HS bacteraemia (median age = 67 years, 51% women), of which 63 (17%) had diabetes. Persons with diabetes had a 2.1-fold increased risk of streptococcal bacteraemia (+84%), compared with population controls (adjusted odds ratio (OR) = 2.1; 95% confidence interval (CI): 1.5–2.9). For persons with type 1 diabetes, the adjusted OR was 14.8 (95% CI: 2.4–91.2). Longer diabetes duration and poor glycemic control conferred higher risk estimates: adjusted OR = 1.5 (95% CI: 0.8–3.0) for HbA1c level <7%, and OR = 3.6 (95% CI: 1.6–8.1) for HbA1c level ≥7%. The association between diabetes and HS bacteraemia was independent of the underlying foc of infection and was strongest for HS group B bacteraemia (OR = 3.5; 95% CI: 1.8–7.0) and for HS group G bacteraemia (OR = 2.6; 95% CI: 1.6–4.4). There was no clear increase in risk for HS group A bacteraemia (OR = 1.2; 95% CI: 0.7–2.2).

Conclusions: Diabetes is a strong risk factor for group B and group G, but not group A, HS bacteraemia. The risk increase is particularly high for type 1 diabetes, long diabetes duration, and poor long-term glycemic control.

Delayed appropriate therapy in Staphylococcus aureus bloodstream infection does not influence outcome

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Objectives: Delayed treatment with appropriate antimicrobials has been shown to augment mortality in patients with septic shock. Therefore early, aggressive treatment has been advocated for all bloodstream infections. However, clinical experience suggests that a delay of therapy is not as critical in patients with Staphylococcus aureus bloodstream infections (SAB). Therefore, we conducted a prospective epidemiological study to test whether treatment delay influences outcome parameters in patients with bacteraemia and SAB.

Methods: 258 patients with SAB from 10 study centers were enrolled in the prospective preSABATO study. All patients were followed for 3 months and predisposing factors, clinical features, diagnostic
measures, antimicrobial therapy, and outcome were recorded. Therapy was considered delayed when appropriate therapy was initiated ≥48h after the initial positive blood culture; antimicrobial therapy was considered appropriate when in-vitro activity against S. aureus was demonstrated and the correct dosage was given.

Results: In 48 (18.6%) patients SABU was classified as community-acquired, while 70 (27.1%) patients were community-acquired and in 140 (54.3%) cases SABU was nosocomial infection. The most prevalent portals of entry were catheter-related infections (78, 30.2%), skin and soft-tissue infections (25, 9.7%), pneumonia (23, 8.9%), and endocarditis (21, 8.2%). In 48 (18.6%) cases the infection was caused by a methicillin-resistant strain. Antimicrobial therapy was administered delayed in 73 (28.3%) patients. Between patients with timely vs. delayed therapy, no significant difference in crude mortality (in-hospital, 30-day, 90-day mortality) and late complications was noted (Table).

Conclusion: In our study, delayed appropriate antimicrobial treatment did not adversely affect crude mortality in SABU.

| Total (n=298) | <48h (n=185) | ≥48h (n=113) |
|--------------|-------------|-------------|
| Hospital mortality | 48 | 16 | 32 |
| S. aureus related | 21 | 8 | 13 |
| 30 day mortality | 15 | 5.5 | 10 |
| S. aureus related | 13 | 7.5 | 6 |
| 90 day mortality | 13 | 7 | 6 |
| S. aureus related | 12 | 6.7 | 6 |
| Late complications | 12 | 6.7 | 6 |

*P=0.001

Clinical significance of Staphylococcus aureus bacteriuria in patients with S. aureus bacteremia

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Objectives: S. aureus bacteriuria (SABU) in association with S. aureus bacteremia (SAB) is well documented while its clinical significance is not well understood. The study objective was to evaluate the relationship between SAB and concomitant SABU and assess its clinical significance.

Methods: This population based retrospective cohort study included all individuals ≥18 years old being diagnosed with SAB in Iceland between December 1st 2003 and November 30th 2008. Concomitant SABU was defined as growth of S. aureus in urine sample taken within 24 hours of the index blood culture. SABU was defined as being of urinary tract origin if there was clinical suspicion of genitourinary tract infection with known structural abnormality or recent instrumentation, and no other identified source for the SAB. Complicated SABU was defined as one with secondary haematogenous or unknown focus; three day persistence on active treatment; or signs of metastatic infection remote from initial focus.

Results: Concurrent urine culture was done in 199 of 300 SAB cases (66%), but in 33 a negative result was unreliable as antibiotics had already been administered. SABU was seen in 27 of 166 episodes (16.3%) of whom 11 were of urinary tract origin. Concomitant SABU was thus seen in 16 of 152 cases (10.5%) of non-urinary tract origin. In this group it was correlated to community acquisition (68.8% vs. 33.8%, p=0.006), having complicated bacteraemia (87.5% vs. 53.3%, p=0.009) and endocarditis (18.8% vs. 3.7%, p=0.01), while a trend was seen with being admitted to intensive care unit (ICU) (37.5% vs. 17.6%, p=0.06). By using logistic regression analysis concomitant SABU was shown to be independently associated with having endocarditis [risk ratio (RR) 6.68; 95% confidence interval (CI) 1.53–17.3; p=0.01] and ICU-admissions [RR 2.84; 95% CI 1.25–4.44; p=0.02], with a trend for having complicated SAB [RR 1.56; 95% CI 0.96–1.80; p=0.06]. These associations were however not observed when SAB cases to urinary tract origin were included in the analysis. There was no correlation to 30-day mortality (6.3% vs. 16.9%, p=0.27) or relapse rates (12.5% vs. 6.6%, p=0.39).

Conclusion: Concomitant SABU appears to be secondary to SAB in some patients while primary urinary tract infection causes the SAB in others. In patients with SAB of non-urinary tract origin, concomitant SABU should probably be regarded as distant haematogenous spread and the bacteremia therefore regarded as complicated one.

Community-onset ESBL E. coli bacteraemias: prevalence, risk factors and outcomes in a London tertiary hospital

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Objectives: Community onset infections caused by extended spectrum β-lactamase (ESBL) producing E. coli is a growing problem. The primary aim of this study was to determine prevalence and risk factors for community onset bacteraemia due to ESBL producing E. coli (ESBL-EC).

Methods: We conducted a retrospective study of patients who presented to Accident and emergency department at Royal London Hospital with laboratory confirmed E. coli bacteraemia between 1 February 2005 and 31 December 2007. Demographic, clinical, laboratory data, length of stay and 14-day mortality data were collected from medical records.

Results: Of the 162 patients admitted with community onset E. coli bacteraemia, 21 of 162 (13%, 95% CI: 8.2 to 19.1%) E. coli isolates were ESBL producing. Those with ESBL producing isolates were on average 9 years older (p = 0.077), more likely to have been hospitalized in the previous 3 months (OR 4.9; 95% CI, 1.9 to 12.8; p = 0.001). They were also marginally more likely to die within 14 days, 19.1% and 6.4% respectively (OR= 3.9; 95% CI, 1.1 to 14; p = 0.052). Among patients with accessible medical records (95), those with ESBL-EC bacteraemia were more likely to present with hypotension (OR 2.7; 95% CI, 0.5 to 8.6; p = 0.092). Patients with ESBL producing isolates were less likely to be treated initially with an appropriate empirical antibiotic 11% vs 95% (p < 0.001).

Conclusions: Community onset ESBL-EC bacteraemia is a significant problem in tertiary care hospitals and current empirical β-lactam based antibiotic regimens may be inadequate to treat suspected Gram negative sepsis. There is an urgent need to establish antibiotic resistance threshold at which empirical antibiotics should be changed.

Laboratory predictors of mortality in community-acquired Escherichia coli bloodstream infection

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Objectives: As laboratory clinicians with the telephone as a key means of initial clinical liaison, it may be difficult for the Clinical Microbiology (CM) team to help identify for physicians those at most risk of death from an infection or to prioritise those for a CM bed-side review. Laboratory data on the Laboratory and Hospital Information Systems (LIS & HIS) provide objective readily available information for the CM team. The aim of this study was to determine laboratory predictors of 7-day in-hospital mortality in community-acquired Escherichia coli bloodstream infection (CA-E. coli BSI).

Methods: A retrospective analysis of CA-E. coli BSIs in St. James's Hospital, Dublin (SJH) from March 2004-December 2009 was performed using the CM laboratory BSI database, the LIS, and the HIS. CA-E. coli BSI was defined as infection where E. coli was cultured from a blood culture taken within 48 hours of presentation and the patient was not an in-patient in SJH for the preceding 3 months, had not attended SJH out-patients or Emergency Department within the previous month, and had not been admitted from another healthcare facility. For each haematological and biochemical parameter analysed, the first available result within 48 hours of presentation was recorded. Laboratory parameters were analysed for an association with in-hospital mortality within 7 days of presentation. Only those parameters for which results were available in ≥90% of cases were included.

Results: There were 172 CA-E. coli BSIs during the time period. In 11 (6.4%) of these, death occurred within 7 days of presentation. ESR, CRP coagulation profiles and glucose data were not available in ≥90% of cases and were excluded. The following were significantly associated with 7-day in-hospital mortality: white cell count <4×10⁹ cells/L, urea
Clinical characteristics and outcome of *Haemophilus* bacteremia in the post-vaccination era

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**Objectives:** Despite the epidemiology has been extensively studied, little information is still available about clinical characteristics of *Haemophilus* bacteremia in the post-vaccination era.

**Methods:** All episodes of positive blood cultures with *Haemophilus* species identified at the Department of Clinical Microbiology, Herlev Hospital during the time period 2000–2009 were included in the study. Clinical and laboratory information were collected retrospectively from medical records.

**Results:** 105 consecutive episodes (median age: 69 years) with only 4 children <16 years were identified (see Figure). 72% of the *Haemophilus* bacteremic episodes were due to non-typeable *H. influenzae* (NTHi), 16% to typeable *H. influenzae* type b (serotype b and f) and 11% to other *Haemophilus* species. 58% of episodes were community-acquired, 30% hospital-acquired, and 22% healthcare-related. Healthcare-related infections were predominantly caused by NTHi (87%), and 82% of episodes due to typeable *H. influenzae* were community-acquired.

Pneumonia was the most common primary focus (in 48%), and these episodes were mainly caused by NTHi (94%). Contrary, foci in the upper respiratory tract (83%) and meningitis (75%) were predominantly due to typeable *H. influenzae*, whereas all 3 episodes of endocarditis were due to *H. parainfluenzae*. 58% of the patients in the study population scored “medium” or “high” in the Charlson comorbidity index, 48% were smokers, 21% were immunosuppressed, and 23% had an alcohol abuse. The 30-day CFR was 22% (24% female, 20% male), highest for NTHi (25%) and lowest for typeable *H. influenzae* (12%). Risk factors for death in the unadjusted analysis were hospital-acquired bacteremia, alcohol abuse, altered mental status, development of septic shock, need for assisted ventilation, temperature <38 degrees, low P-sodium, and therapy with benzylpenicillin (P < 0.05), whereas altered mental status was the only significant risk factor in the multivariate analysis (P = 0.01).

**Conclusion:** *Haemophilus* bacteremia still carries a high mortality and is predominantly caused by NTHi among elderly having comorbidity and with a primary lung focus. Benzylpenicillin should not be considered as therapy for *Haemophilus* bacteremia.

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**P1594** Five cases of *Campylobacter fetus* infection detected at a university hospital in Japan

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**Objectives:** *Campylobacter fetus* is a rare pathogen causes bloodstream infection presenting variety of clinical features. We investigated clinical and bacteriological characteristic of the recent five cases at Kyushu University Hospital and summarize regional overview of this infection in characteristics.

**Methods:** We reviewed medical records of the five cases admitted to Kyushu University Hospital from January 2005 to December 2010. *C. fetus* isolated from blood, drained liquid from abscess or subcutaneous puncture, and bone marrow were analyzed by clinical microbiological methods and pulse field gel electrophoresis.

**Results:** Three of the five patients were male and their age ranged from 35 to 69 years old. The *C. fetus* infections of all cases were community acquired. Except for a one previously healthy male aged 32, the patients had underlying diseases causing significant immunocompromised condition, such as diabetes mellitus, post hematopoietic stem cell transplantation, and cardiovascular disease. Major symptom was fever and clinical presentations of the infection were bacteremia, cellulitis, osteomyelitis and arthritis. All *C. fetus* strains isolated were sensitive to antimicrobials generally recommended for treatment. Patients were treated by ampicillin, carbapenems, fluoloquinolones, or cefetriaxone with or without gentamicin or clindamycin. The duration of treatment for bacterial infection and underlying condition ranged from 21 days for the case with cellulitis and 3 months for the case with osteomyelitis.

**Conclusion:** *C. fetus* infection in immunocompromised patients. We experiences five cases in 13 year including one previously healthy patient. Choosing antimicrobial drugs guided by sensitivity analysis of the isolated strain is a key to effective treatment.

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**P1595** *Abiotrophia* endocarditis and bacteremia – an 8-year experience in a tertiary care centre

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**Background:** *Abiotrophia defectiva* and *A. adiacens* are rare causes of endocarditis (IE). Previous publications suggest that morbidity and mortality exceed those of other forms of viridans streptococci IE with bacteriological failure in 41% of patients. More than 30% of strains of *Abiotrophia* sp. were found to be resistant to penicillin. We report our 8 year experience with *Abiotrophia* endocarditis and bacteremia.

**Methods:** All cases (n = 36) of *Abiotrophia* bacteremia diagnosed at our institution from 2002–2010 were included in the study. Medical records of all patients were reviewed. IE was diagnosed according to the modified Duke criteria. Frozen bacterial isolates were available in 25 (69%) cases. Isolates were thawed and bacterial identification was repeated using API and classical methods. Antibiotics susceptibility was assessed using Etest. Fishcr exact tests were used when appropriate.

**Results:** IE, was diagnosed in 13/36 (36%) patients (9 definite and 4 possible). Most cases involved the mitral valve (n = 7). Primary bacteremia was diagnosed in 23 (64%) patients. *A. adiacens* was more common than *A. defectiva* in both groups (7 and 4 in IE patients and 14 and 1 in bacteremia patients, respectively). The median age was 57 years in IE patients and 48 years in bacteremia patients. Underlying heart disease was found among 9/13 (69%) patients with IE and none of those with bacteremia (P < 0.0001). 9/23 (39%) patients with bacteremia were immuno compromised in contrast to none among those with IE (P = 0.014). Hemiparesis or other neurological signs were the presenting symptom in 4/13 (31%) IE cases. The mortality rates among patients with IE and bacteremia were 3/13 (23%) and 7/23 (30%), respectively.

In bacteremic patients there was a trend towards increased mortality for bacterial infection and underlying condition ranged from 21 days for the case with cellulitis and 3 months for the case with osteomyelitis.

**Conclusion:** *A. defectiva* and *A. adiacens* are rare causes of IE. IE caused by these two species is associated with higher mortality than IE caused by other viridans streptococci. Most patients are treated with a combination of penicillin and an aminoglycoside. More studies are needed to establish the optimal treatment for these infections.

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**Bacteraemia, sepsis and endocarditis**

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>20 mmol/L, creatinine >150 μmol/L, bicarbonate <21 mmol/L, bilirubin >60 μmol/L, LDH >600 IU/L, AST >400 IU/L, albumin <35 g/L, time to detection of blood culture isolate <0.5 hours, the presence of another significant isolate in the blood culture, ciprofloxacin resistance, and gentamicin resistance.

**Conclusion:** Laboratory parameters provide objective data to identify those at risk of in-hospital death within 7 days of presentation with a CA-E. coli BSI. These data may help prioritise those in need of an urgent CM bed-side review. Further studies are needed to determine if such CM bed-side reviews for these selected high-risk patients have any impact on mortality.
Significance of troponin-I value in infective endocarditis

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Objectives: To assess significance of Troponin-I values in Infective Endocarditis for its association with different variables and complications.

Method: We analyzed data of 78 patients from the hospital registry. There were 46 Males & 32 Females. Mean age was 52.5 ± 31.1 years. Mean BMI was 28.3 ± 3.4. Our study population included a diverse population with 77.6% African Americans, 18.4% Caucasians, and 2.6% Hispanics. Since some patients had ultra-sensitive Troponin-I value and the rest had conventional Troponin-I value, we considered the percentage rise of the Troponin-I values from the reference high values. 33 patients had increased Troponin-I levels values (Mean % form reference high 141.79 + 598.52) and 45 patients had normal Troponin-I levels (Mean % to reference high 41.39 + 7.74). We analyzed the Troponin-I values for association with incidence of death, embolic event, intracranial hemorrhage, intracardiac abscess, rise in creatinine value, ICU admission, left-sided endocarditis, congestive heart failure, pulmonary embolism, IV risk factor (IV drug abuse or permanent IV line placement), history of prosthetic valve, prior endocarditis, prior hospitalization in 1 year and need for cardiovascular surgical intervention for treatment.

Results: Increased Troponin-I values were significantly associated with death (p < 0.001, HR 22.84, 95% CI 4.7–110.2), embolic event (p = 0.003, HR 5.86, 95% CI 1.7–20.4), intracranial hemorrhage (p = 0.003, 95% CI 1.04–1.44), intracardiac abscess (p = 0.048, HR 4.78, 95% CI 0.9–25.4), rise in creatinine value (p < 0.001, HR 7.66, 95% CI 2.5–23.5), ICU admission (p = 0.004, HR 4.0, 95% CI 1.5–10.3), left-sided heart disease (p < 0.001, HR 16.1, 95% CI 3.0–84.3). No significant association was found between Troponin-I and congestive heart failure (p = 0.066, HR 2.7, 95% CI 0.9–8.0), pulmonary embolism (p = 0.072, HR 0.17, 95% CI 0.2–1.5) IV risk factor, history of prosthetic valve, prior endocarditis, prior hospitalization in 1 year and need for cardiovascular surgical intervention for treatment.

Conclusion: Increase in Troponin-I serum level can serve as a sensitive prognostic tool for patients with infective endocarditis.

The broadening spectrum of septic pulmonary embolism: a retrospective review

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Objectives: Septic pulmonary embolism (SPE) is a rare manifestation of systemic infections most commonly associated with right sided infective endocarditis (RIE) in intravenous drug users (IVDU). Prompted by recent clinical experiences, we undertook a review of the epidemiology of presumptive cases of SPE seen at our hospital.

Methods: Cases of SPE were identified by a retrospective computer assisted search of the chest CT scan reports at our hospital between January, 2000 and June, 2010. Electronic charts of patients were studied to establish the diagnosis and to follow their course of illness and treatment. A “definite” case of SPE was defined as having chest CT findings of focal or multifocal lung infiltrates; an extrapulmonary infectious source for emboli; exclusion of other explanations for the lung infiltrates; and resolution of the infiltrates with antibiotic therapy. A “probable” case had typical CT findings but lacked one or more of the other features.

Results: 34 patients (25 definite and 9 probable), 24 male and 10 female with ages ranging from 1 to 71 years (median – 45 years) were identified. The most common presenting complaint was fever in 31 patients while 3 patients presented with dyspnea and cough. Sources of infectious emboli were identified in all but 6 cases. These included – 12 peripheral (skin; soft tissue deep venous plexus); 7 cardiac (4 RIE); 7 long term intravenous devices; and 2 dental/throat foci. 26 patients were bacteremic and 8 had pathogenic bacteria isolated from other sites. Staphylococcus aureus (MSSA in 20 and MRSA in 5) was the predominant pathogen. 24 (70%) had echocardiograms and valvular vegetations were identified in 6 patients. All received antibiotic therapy for 2 to 8 weeks (mean duration 27 days). The most common associated comorbidities included IVDU in 8, end stage renal disease (on hemodialysis or with transplant) in 7 and diabetes mellitus in 6 patients. Follow up imaging was performed on 16 patients. 5 patients died and 4 were lost to follow up.

Conclusions: The epidemiology of SPE has broadened over the past decades with an increase in non cardiac sources related to contiguous infections. Radiologic studies can identify possible venous embolic sources. Prolonged antibiotic therapy is required. A clearer understanding of the etiologies of SPE will help facilitate the diagnosis and management of this potentially fatal condition.
The role of imaging of the urinary tract in patients with urosepsis

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Objectives: The study aim was to provide recommendations for imaging of patients with urosepsis in order to detect urological complications that need intervention as well as conditions that predispose to renal infection.

Methods: We conducted a retrospective, observational study of adult patients admitted to medical wards at a Danish university hospital. Eligible patients had community-acquired bacteremia with a urinary tract focus in 2005 through 2009. Electronic admission files, laboratory data and radiological data were reviewed. Outcomes of ultrasonography and/or x-ray computed tomography were classified as "major abnormalities", "minor abnormalities" and "normal". A major abnormality was defined as a finding that should influence subsequent treatment. Data were analysed with χ² or Fisher's exact test or Wilcoxon, as appropriate.

Results: A total of 225 patients were included in the study. Radiological imaging had been performed in 116 patients (52%), 74 females and 41 males. Major abnormalities were found in 50 of the 116 (43%) patients. The three most common major abnormalities were signs of acute pyelonephritis (n=21, 18%), hydronephrosis (n=20, 17%) and urolithiasis (n=9, 8%). The following biochemical and clinical parameters were statistically significant predictors of a major abnormality: C-reactive protein (P<0.002), p-creatinine (P<0.002), diabetes with complications (P=0.04), known kidney disease (P=0.03) or alternatively, a history of kidney disease, ureolithiasis, congenital or acquired structural abnormality (combined, P=0.005). Other clinical parameters such as gender, age, blood pressure, fever, malignant disease, liver disease, neurological disease, prostate disease, chronic indwelling urinary catheter, white blood cell count and neutrophils, did not show a statistically significant association with the finding of major abnormalities.

Conclusion: A large fraction (0.43) of the subset of patients scanned in this study had a clinically important finding as a result of the radiological evaluation. It indicates that a visible renal infection or an abnormality of the kidney or the urinary tract that might predispose to renal infection is common in medical patients with urosepsaemia.

Elevated C-reactive protein, p-creatinine and a positive history of diabetes with complications, kidney disease, ureolithiasis or structural abnormality may be particularly helpful in clinical decision making in this group of patients.

Evaluating the importance of defining healthcare-associated bloodstream infections

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Background: Bloodstream infections have been traditionally classified as either community or nosocomial in origin. However, more recently the category of healthcare-associated community onset disease has been recognized. The objective of this study was to evaluate characteristics of adults with bloodstream infection according to acquisition setting.

Methods: All first episodes of incident bloodstream infections (BSI) occurring among adults admitted to hospitals in a large health region in Canada during 2000–2007 were included. BSI cases were classified as either nosocomial (NA), community acquired (CA), or community onset healthcare associated (HCA) infections. NA BSI was defined as first culture positivity ≥48h following hospital admission or within 48 h of discharge. HCA BSI was defined as (i) discharge from an home parenteral therapy clinic within 2–30 days before BSI (ii) attendance at a hospital clinic or emergency room within 2–30 days before BSI; (iii) hospital admission for two or more days within 90 days before BSI; (iv) sample sent from a nursing home or a long-term-care facility resident; and (v) outpatient haemodialysis. CA BSI were defined as those with first culture-positivity within <48h of admission or ≥48h after discharge from hospital, provided no HCA criteria was met.

Results: A total of 7,712 patients were included; 2,132 (28%) NA, 2,492 (32%) HCA, and 3,088 (40%) community-acquired infection. Patients with CA BSI were significantly younger and less likely to have co-morbid medical illnesses than patients with HCA or NA disease (p<0.001). The proportion of cases in males was higher for NA (60%; p<0.001 vs. others) as compared to HCA or CA (52% and 54%; p=0.13). The proportion of cases that had a poly-microbial etiology was significantly lower for CA (5.5%; p<0.001) compared to both NA and HCA (8.6 vs. 8.3%). The microbiology of infections differed between acquisition setting, with the distribution of organisms causing HCA intermediate in the spectrum between NA and CA. The median length of stay was 29.9 days for NA, 9.1 days for HCA, and 7.5 days for CA (p<0.001). Thirty-day all cause fatality rates were 26%, 19%, and 10% for NA, HCA, and CA disease, respectively (p<0.001).

Conclusion: Healthcare-associated infections are distinctly different from CA and NA infections. These data support the classification of community-onset disease into separate CA and HCA categories.
Respiratory pathogens

**[P1602] In vitro activity of antimicrobial agents against Streptococcus pyogenes obtained in four different regions of Turkey**

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**Objectives:** Although penicillin-resistant isolates of *Streptococcus pyogenes* have not been established to date, non-β-lactams such as macrolide and quinolone resistance among *S. pyogenes* have emerged in recent years. Surveillance studies are important to identify any changes in antimicrobial susceptibility rate.

**Material and Methods:** From January 2007 and May 2009, 395 consecutive clinical isolates of *S. pyogenes* were obtained from 4 different regions of Turkey were tested. Isolate identification was confirmed using standard methods, PYR test and/or by a latex agglutination assay. The isolates were stored at −80°C and sent to a central laboratory for MIC testing. The susceptibility to penicillin, cephalosporin, vancomycin, erythromycin, azithromycin, clindamycin and levofloxacin were determined by the agar dilution method according to Clinical and Laboratory Standards Institute.

**Results:** The MIC50 for penicillin, cephalosporin, vancomycin, erythromycin, azithromycin, clindamycin and levofloxacin were 0.007, 0.06, 0.5, 0.125, 0.125, 0.5, and 0.05 mg/L, respectively. The MIC90 for penicillin, cephalosporin, vancomycin, erythromycin, azithromycin, clindamycin and levofloxacin were 0.03, 0.25, 0.5, 0.25, 0.5, 0.25, and 2 mg/L, respectively. All isolates of *S. pyogenes* tested were susceptible to penicillin, cephalosporin, and vancomycin. Erythromycin, azithromycin, clindamycin and levofloxacin resistance were 6.4%, 3.1%, 5.6% and 5.1%, respectively.

**Conclusions:** These results show that antimicrobial resistance against *S. pyogenes* is not yet a problem in Turkey but epidemiological studies for antimicrobial resistance profiles of *S. pyogenes* must be go on periodically.

**[P1603] Susceptibility of respiratory pathogens to less common agents**

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**Objectives:** Over the past two years, swine flu incidences have increased in the UK. There is concern that supplies of the most commonly used antibiotics to treat secondary bacterial infections may become low, especially after stockpiling in 2009. We studied the susceptibility of likely secondary bacterial pathogens to less common agents to establish additional potential empiric therapies.

**Methods:** Over a 3-month period laboratories across Wales were asked to submit 10 isolates each of *Staphylococcus aureus* (MRSA), and Group A streptococi (GAS) from any community specimens. Agar dilution MICs were performed by BSAC methods for flucloxacillin (FLU), co-amoxiclav (COA), cefuroxime (CXM), erythromycin (ERY), clarithromycin (CLM), clindamycin (CLD), tetracycline (TET), chlorotetracycline (Cте), oxytetracycline (OXY), lymecycline (LYM), doxycycline (DOX), chloramphenicol (CHL). D-tests were performed on staphylococci resistant to ERY but sensitive to CLD.

**Results:** 252 isolates from 10 laboratories were tested. Geometric mean MIC (mg/L) and % sensitivity (using BSAC/EUCAST breakpoints) are shown in the table. Tetracyclines had good activity against all pathogens, although DOX was less active against *H. influenzae*. CLD showed poor activity against *H. influenzae* and *S. pneumoniae* and FLU had potentially useful activity against some *H. influenzae* (62.8% had MIC ≤ 2 mg/L).

**Conclusions:** Some agents not usually used for respiratory infections may have useful activity against respiratory pathogens. In the event of a shortage of the most commonly used antibiotics during 'flu outbreaks their potential use should be evaluated.

|          | FLU (n=8) | SPN (n=16) | MRS A (n=20) | MRSA (n=55) | GAS (n=55) |
|----------|-----------|------------|--------------|-------------|------------|
| Mean     | 3.05      | 0.05       | 0.05         | 0.05        | 0.05       |
| % Sens.  | 100%      | 100%       | 100%         | 100%        | 100%       |
| Max.     | 0.05      | 0.05       | 0.05         | 0.05        | 0.05       |

- No breakpoints exist

**[P1604] Nationwide surveillance of bacterial pathogens isolated from patients with lower respiratory infections in 2010 in Japan**

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**Objectives:** The surveillance committee has been operating a nationwide surveillance study of the major bacterial pathogens of lower respiratory infections in Japan since 2006. We report here the results of our etiological and antibiotic susceptibility studies conducted in 2010.

**Methods:** Bacterial strains reported here were *S. aureus*, *S. pneumoniae*, *H. influenzae*, *K. pneumoniae* and *P. aeruginosa* and they were isolated from patients who were firmly diagnosed as respiratory infection. A total of 1002 clinical isolates were obtained from April through Sep. 2010 from 33 hospitals throughout Japan. The antibiotic susceptibility of was tested by the broth-microdilution method.

**Results:** Among 201 strains of *S. aureus*, 170 and 31 were isolated from in-patients (IP) and out-patients (OP), respectively, and of 57.6 and 25.8% respectively, were classified to the meticillin-resistant *S. aureus* (MRSA). The MIC of vancomycin, teicoplanin and linezolid in the MRSA strains tested were within the susceptible range by the CLSI criteria without exception and their MIC90 were 1, 2 and 2 mg/L, respectively. The penicillin resistant *S. pneumoniae* was 63.9% in the strains from IP and that in OP was 58.2% as judged by the CLSI criteria 2007. The penicillin resistant *S. pneumoniae* in 2006 through 2009 was in the range of 35–47% and that in 2010 was 60.9%. Among 169 *H. influenzae* strains the amoxicillin resistant strains was 67.8% in the strains from IP and that in OP was 60.8%. The penicillin G resistant *S. pneumoniae* and the ampicillin resistant *H. influenzae* showed high susceptibility to meropenem, garenoxacin, and zefloxacin (MIC90, less than 0.5 mg/L). Among 139 strains of *K. pneumoniae*, three were found to be the extended spectrum β-lactamase (ESBL)-producing strains. Among 156 strains of *P. aeruginosa*, we found only one metallo-β-lactamase (MBL)-producing multi-drug resistant strain. The fact that only a few ESBL- and MBL-producing strains were isolated was a relief to the surveillance committee.

**Conclusion:** This study revealed the followings. (i) The frequency of MRSA from IP was considerably high compared with that from OP. However, the ratio of penicillin resistant *S. pneumoniae* was comparable between IP and OP and this tendency was also found in the ampicillin resistant *H. influenzae*. (ii) The MBL-producing *P. aeruginosa* and ESBL-producing *K. pneumoniae* were very few.

- No breakpoints exist
**P1605** Antimicrobial resistance of respiratory pathogens in Finland, 2005–2009

M. Bergman*, P. Huocinen, A. Hakonen and the Finnish Study Group for Antimicrobial Resistance (FiRe Network)

**Objectives:** Respiratory tract infections are the most common indication for antimicrobial treatment. The most important respiratory bacterial pathogens are Streptococcus pneumoniae, Streptococcus pyogenes, Haemophilus influenzae, and Moraxella catarrhalis. In this study, we present the latest resistance results of these pathogens in Finland.

**Methods:** The resistance data was collected by the Finnish Study Group for Antimicrobial Resistance. It is a national network of 24 laboratories which collects resistance data from all central hospital districts in Finland. The resistance data was collected during the years 2005–2009. The number of studied strains per bacterial species per year varied from ~700 to ~18000. Only one strain per patient per year was included. A harmonised FiRe standard (based on the CLSI standard) was used in the susceptibility testing.

**Results:** Macrolide resistance in invasive *S. pneumoniae* isolates increased during the study period from 19.0 to 27.1% (Table 1). Ampicillin resistance in *H. influenzae* increased from 13.4 to 25.6%. Sulfamethoxazole resistance in *M. catarrhalis* increased from 2.1 to 4.4%. Macrolide resistance in *S. pyogenes* throat isolates decreased from 4.9 to 2.2%.

**Conclusion:** Macrolide resistance in *S. pneumoniae* in Finland is at a high level and is increasing while in other European countries the trend seems to be decreasing. Also penicillin non-susceptible pneumococci are prevalent. This development has major clinical consequences for the treatment of respiratory tract infections. Pediatric pneumococcal vaccine was introduced in Finland in 2010, and hopefully it has an effect on the resistance trends. Macrolide resistance of *S. pyogenes* is at a low level and has not changed markedly during the study period. Ampicillin resistance in *H. influenzae* will be carefully monitored, because of the increasing trend.

![Table: Resistance of *S. pneumoniae* to Antimicrobials](image)

| Antimicrobial     | Resistance (%) | 2005 | 2006 | 2007 | 2008 | 2009 |
|-------------------|----------------|------|------|------|------|------|
| *Streptococcus pneumoniae* blood isolates | Penicillin (R) | 0.8 | 1.1 | 1.2 | 0.4 | 1.4 |
|                   | Penicillin (R) | 6.8 | 10.3 | 7.0 | 10.6 | 13.1 |
|                   | Erythromycin   | 19 | 23 | 22.8 | 23 | 27.1 |
|                   | Clindamycin    | 5.8 | 8.2 | 6.3 | 6.7 | 5.7 |
|                   | Sulfamethoxazole | 14.2 | 19.8 | 20.2 | 20.1 | 25.2 |
| *Haemophilus influenzae* | Amoxicillin | 13.4 | 12.4 | 13.1 | 19.7 | 25.6 |
|                   | Amoxicillin/clavulanate | 0.9 | 1.3 | 2.6 | 3.7 |
|                   | Sulfamethoxazole | 18.9 | 21.6 | 21.1 | 18 | 15.5 |
| *Moraxella catarrhalis* | Amoxicillin | 0.2 | 0 | 0 | 0.1 |
|                   | Erthromycin    | 1.6 | 0.8 | 1.7 | 2.1 | 2.7 |
|                   | Sulfamethoxazole | 2.1 | 4.2 | 3.6 | 3 | 4.4 |
| *Streptococcus pyogenes* throat isolates | Erythromycin | 4.9 | 2.7 | 1.4 | 2.1 | 2.2 |
|                   | Clindamycin    | 1.2 | 1.3 | 1 | 1.4 | 1.8 |

**P1606** Risk factors for fluoroquinolone-resistant *Streptococcus pneumoniae* bacteremia

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**Objectives:** Resistance of *Streptococcus pneumoniae* to fluoroquinolones is rare but it is increasing. The aim of this study is to determine risk factors associated with development of resistance against fluoroquinolones among *S. pneumoniae* isolated from blood cultures.

**Methods:** Review of cases of bacteremia caused by FQRSP from January 2007 to April 2010 according to a previously designed protocol. Susceptibility was studied following the CLSI standards. A randomly selected group of patients with bacteremia caused by fluoroquinolone-resistant *S. pneumoniae* (FQSSP) was reviewed with the same protocol for comparative purposes as control. Fisher’s exact test and logistic regression was used for statistical analysis.

**Results:** 17 cases of FQSSP bacteremia were found. An increasing number of cases was observed during the study period. No differences were found regarding age, sex or presence of chronic cardiac and respiratory diseases. FQRSP isolates were commonly resistant against other antimicrobials (macrolides, tetracyclines, chloramphenicol and cotrimoxazole). Most isolates belonged to serogroup 8. Statistically significant risk factors for FQRSP were previous admission to hospital (RR 3.66; IC95 1.06–12.62; p = 0.036); reside in homeless shelters (RR 4; IC95 1.40–11.42; p = 0.0080893321); previous treatment with fluoroquinolones (RR 7.23; IC95 1.57–33.37; p = 0.01) HIV infection (RR 6.67; IC95 1.62–27.38; p = 0.012); chronic B or hepatitis C infection (RR 5.51; IC95 1.54–19.71; p = 0.006) and alcohol intake (RR 4.34; IC95 1.23–15.36; p = 0.019). No differences in mortality between both groups of patients were detected.

**Conclusions:** Resistance of *S. pneumoniae* to fluoroquinolones is an emerging problem. Previous admission to hospital, fluoroquinolone treatment, reside in homeless shelters, HIV and Hepatitis B or C infections and alcohol intake may be risk factors for FQRSP bacteremia.

**P1607** Activity of ceftaroline and comparator agents tested against organisms responsible for community-acquired respiratory tract infections in Europe (2009)

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**Objective:** To evaluate the activity of ceftaroline (CPT) and comparators against isolates from patients with community-acquired respiratory tract infections (CARTI) in European (EU) medical centres. CPT, the active component of the prodrug CPT fosamil, exhibits broad-spectrum activity against Gram-positive organisms, including resistant (R) subsets of methicillin-R *S. aureus* (MRSA) and penicillin (PEN)-R *S. pneumoniae* (SPN).

**Methods:** 1085 consecutive, non-duplicate isolates from RTI (n = 942) and blood cultures (n = 143, including; SPN and *H. influenzae* [HI]) were collected in 2009 from 25 hospitals located in 13 EU countries. Isolates included: SPN (n = 581; 16.4% PEN-R [MIC ≥2 mg/L]), HI (n = 292; 16.1% β-lactamase [BL] producers), M. catarrhalis (MCAT, n = 134) and *S. aureus* (n = 78 [44.9% MRSA]). All isolates were susceptibility (S) tested using reference CLSI broth microdilution methods against CPT and comparators for RTI treatment.

**Results:** CPT inhibited all SPN, MAC and HI isolates at 0.25, 0.25 and 0.03 mg/L, respectively. CPT was the most active β-lactam tested against SPN (MIC<sub>50</sub> = 0.008/0.12 mg/L), exhibiting 8-, 16- and 64-fold lower MICs than ceftriaxone (CRO; MIC<sub>50</sub> = 0.25/1 mg/L), amoxicillin/clavulanate (A/C; MIC<sub>50</sub> = 0.125/2 mg/L) and cefuroxime (MIC<sub>50</sub> = 0.125/2 mg/L), respectively. Against PEN-R SPN (n = 95), CPT (MIC<sub>50</sub> = 0.25/0.25 mg/L) was at least 4- and 8-fold more potent than CRO (MIC<sub>50</sub> = 2.5/0.5 mg/L) and A/C (MIC<sub>50</sub> = 2/0.5 mg/L), respectively. CPT was very active against HI (MIC<sub>50</sub> = 0.015 mg/L) regardless of BL production. BL-producing HI isolates showed CPT MIC values slightly higher (MIC<sub>50</sub> = 0.015/0.03 mg/L) than those of non-BL-producers (MIC<sub>50</sub> = 0.008/0.015 mg/L). CPT was very active against MAC isolates (MIC<sub>50</sub> = 0.03/0.02 mg/L), most (>90%) BL-positive. All methicillin-S *S. aureus* (MSSA) were inhibited by CPT at ≤0.5 mg/L and the highest CPT MIC among MSSA was only 2 mg/L (MIC<sub>50</sub> = 1/2 mg/L). Against MSSA, CPT (MIC<sub>50</sub> = 0.25/0.5 mg/L) was 8- to 16-fold more potent than CRO (MIC<sub>50</sub> = 4/8 mg/L) and ceftizoxime (CPEP; MIC<sub>50</sub> = 0.25/0.4 mg/L), respectively.

**Conclusion:** CPT was the most active β-lactam agent tested and demonstrated good coverage against contemporary (2009) CARTI organisms recovered from EU hospitals. CPT showed excellent in vitro activity against all PEN-R SPN, BL-producing HI and MAC, MSSA and MRSA isolates tested.
Macrolide resistance in European isolates of \textit{Streptococcus pneumoniae}, 2009–2010

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Background: \textit{S. pneumoniae} continues to be an important cause of community acquired pneumonia and bacteremia globally. Macrolides constitute an important first or second line antibiotic choice for community acquired SPN infections. This study describes the prevalence of macrolide resistance in European SPN 2009/2010 and the activity of relevant comparator antibiotics against \textit{Streptococcus pneumoniae} of various phenotypes.

Methods: 1086 clinical isolates of SPN were collected from European countries between 2009 and 2010 from a variety of clinical sources including blood and the lower respiratory tract. MICs were determined and interpreted by broth microdilution according to EUCAST guidelines.

Results: The following table reports the percent susceptible and MIC\textsubscript{90} (mg/L) of SPN including resistant phenotypes.

| Drug     | % S | MIC\textsubscript{90} (mg/L) |
|----------|-----|---------------------------|
| Azithromycin | 75% | 0.5 ± 1.34% |
| Clarithromycin | 75% | 0.5 ± 1.34% |
| Clindamycin | 87% | 0.5 ± 1.34% |
| Levofloxacin | 98% | 0.01 ± 0.03% |
| Erythromycin | 85% | 0.01 ± 0.03% |

Conclusions: Linezolid, meropenem, tigecycline and levofloxacin were the most active agents against SPN including both penicillin and macrolide resistant phenotypes with % susceptible >98%. In Europe in 2009–2010 28.8% of SPN were resistant to the macrolides azithromycin or clarithromycin.

Trend of antimicrobial susceptibility among bacterial isolates from RTI of Japanese hospital participating in the levofloxacin surveillance group during 1994–2010

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Objectives: Fluoroquinolones (FQs) are widely used as antimicrobial agents to treat respiratory tract infections (RTI). FQs resistance is still very rare in \textit{Streptococcus pneumoniae}, \textit{Streptococcus pyogenes}, \textit{Moraxella catarrhalis} and \textit{Haemophilus influenzae}. In the past several years, however, FQs resistance in these organisms has been sporadically reported. We have already been taken nationwide surveillance for FQs and other antimicrobial resistance against many bacterial clinical isolates in Japan since 1994. In this study, we report surveillance data between 1994–2010 for the above four causative pathogens of RTI.

Methods: A total of 12,269 clinical isolates in 4 species were collected from 92 centers participating in the Levofloxacin Surveillance group during 1994–2010 in Japan. Antimicrobial susceptibility testing by broth microdilution methods was based on CLSI guidelines that were updated annually as revised documents were published.

Results:

1. \textit{S. pneumoniae}: The increase of resistance rate for levofloxacin was not recognized and fluctuated at a low level of 1.03±1.34%. Interestingly, MIC mode of ‘levofloxacin-susceptible’ isolates was 0.5 \textmu{g}/mL until 2004 but was 1.0 \textmu{g}/mL since 2007. However, the rate of isolates with one mutation in QRDRs did not differ between isolates with MIC of 1.0 \textmu{g}/mL in 2004 and 2007. The rate of ‘Susceptible’ isolates for macrolides has rapidly decreased since 2002.

2. \textit{S. pyogenes}: A high susceptible level to levofloxacin, sitafloxacin, penicillin and cefdinir has been maintained during 1994–2010, in contrast the susceptibility to macrolides has rapidly decreased since 2002.

3. \textit{M. catarrhalis}: ‘Susceptible’ rate of almost 100% was maintained to levofloxacin, the advanced-generation cephalosporins, carbapenems and macrolides during 1994–2010.

4. \textit{H. influenzae}: Resistant isolate for levofloxacin was not found or very few if any. Macrolide resistance was slightly-increased since 2007. The rate of BLNAR has rapidly increased from 20% in 2002 to 50% in 2010.

Conclusions: Levofloxacin continued to have still a high level activities against \textit{S. pneumoniae}, \textit{S. pyogenes}, \textit{M. catarrhalis} and \textit{H. influenzae}, and maintained one’s position of useful therapeutic medicine in RTI. It is deeply concerned that the resistant rate of \textit{S. pneumoniae} and \textit{S. pyogenes} for macrolides is rapidly increasing, because fluoroquinolones and \beta-lactams are not available for child and for patients with penicillin-allergy, respectively.

Serotype distribution and antimicrobial susceptibility of IPD-causing \textit{Streptococcus pneumoniae} in the Comunidad Valenciana, Spain during the winter of 2009–2010

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Objectives: \textit{Streptococcus pneumoniae} is a bacterium that can cause serious invasive pneumococcal disease (IPD) such as meningitis and pneumoniae and is cause of morbidity and mortality worldwide. The bacterium produces a capsule, of which 90 serotypes exist. After the introduction of the vaccine PCV7 that protects against 7 serotypes, a shift in IPD-causing serotypes has been observed worldwide towards serotypes not included in the conjugate vaccine. Furthermore, penicillin intermediate resistance increased in the years following PCV7 introduction, especially in these non-PCV7 serotypes. The first objective of this study was to determine the serotype distribution of IPD-causing \textit{S. pneumoniae} in Valencia, Spain, and thus determining the coverage rates of the two new vaccines PCV10 and PCV13. The second objective was to measure the antimicrobial resistance of these strains.

Materials and Methods: During September 2009 until March 2010, 285 \textit{S. pneumoniae} IPD strains were collected in the 22 participating hospitals in the Comunidad Valenciana and sent to the University hospital La Fe. Serotyping was performed by serum slide agglutination (Denka Seiken, Tokyo, Japan). Antimicrobial resistance profiles were determined using E-tests.

Results: The serotype of 273 IPD strains of \textit{S. pneumoniae} was determined; 12 strains were not typable. The most common serotypes in order of prevalence were: 19A (17.9%), 7F (13%), 1 (12.3%), 3 (9.1%), and 22F (5.3%). The antimicrobial resistance profile was determined of 203 strains. The majority of the strains (70.4%) was penicillin sensitive, 24.1% was intermediate resistant, and 5.4% was resistant. Seventy five percent of the strains was erythromycin sensitive, and 10.8% of the strains was resistant to levofloxacin. None of the strains was resistant to vancomycin, teicoplanin or linezolid. Compared to 2008, the number of multi resistant strains has risen. Currently the genetic determinants and molecular mechanisms underlying the antimicrobial resistance are determined.

Conclusions: The serotype coverage of the newly developed vaccines in Valencia (Spain) are: PCV7 9.9%, PCV10 35.9% and PCV13 71.8%. The majority of the antibiotic resistant strains belongs to serotypes, which are included in PCV13. The increase in multi resistant strains might indicate possible increasing therapeutic difficulties These data implicate that PCV13 might be a good option to include in the vaccination calendars in the Comunidad Valenciana.
**Biomarkers for infections**

**P1611** Measurement of C-reactive protein velocity in febrile bacterial infections and non-bacterial febrile illnesses

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**Objectives:** C-reactive protein (CRP) is one of plasma proteins known as acute-phase proteins (proteins whose concentrations increase during inflammatory disorders). We hypothesized that measuring the velocity of this biomarker instead of its absolute serum concentration could enhance its ability to distinguish febrile bacterial infections from non-bacterial febrile illnesses.

**Methods:** We prospectively studied 357 adult patients who presented to the emergency department with fever. CRP velocity (CRPv) was defined as the ratio between CRP on admission and the number of hours since the onset of fever. Patients were diagnosed by clinical symptoms, blood cultures and imaging studies.

**Results:** A total of 357 patients met the inclusion criteria for the current study, 236 (66%) of which were classified as having bacterial infection and 121 (34%) as having a non-bacterial febrile illness. The mean age of patients with bacteria infection was higher relative to patients with non-bacterial febrile illness (62.6±18.4 vs 32.2±14.1 years, p<0.001) and 61% were women. Patients with bacterial infection had significantly higher CRP levels than those with non-bacterial infection (72.6 mg/L vs 25.3 mg/L, respectively p<0.001). The febrile illness CRPv was also significantly higher in the bacterial group compared to the non-bacterial (4.32 mg/L/hour vs 0.56 mg/L/hour, respectively p<0.001).

**Conclusion:** CRPv is possible to improve the differentiation between acute bacterial infections and non-bacterial febrile illnesses.

**P1612** CSF LDH estimation to differentiate pyogenic and viral meningitis and its role in tuberculous meningitis

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**Background and Objectives:** Acute infections of the central nervous system are among the most important problems in medicine because early recognition, efficient decision making, identifying the responsible pathogen and rapid institution of therapy can be life saving.

**Aim** was to find out the role of CSF LDH in differentiating viral and purulent meningitis and its role in TB meningitis.

**Materials and Methods:** CSF and serum LDH was estimated for all patients admitted with a clinical triad of fever, headache and neck stiffness with biochemical evidence of meningitis from 1/10/2008 to 1/09/2009. 150 patients were included of which 50 had viral, 50 had bacterial, 7 had tuberculous and 43 had partially treated meningitis. Repeat CSF LDH estimation was done to assess the prognosis. Patients with AIDP, ADEM, Carcinomatous meningitis, hemorrhagic lumbar puncture, other organ dysfunction like hepatitis, hemolysis and renal failure (which can cause a false elevation in serum LDH) were excluded from the study.

**Results:** In bacterial meningitis, mean CSF LDH was 96.5 IU/L (normal value was 20–30 IU/L) and corresponding serum LDH was normal. In viral meningitis the mean CSF LDH was 31.5 IU/L and when compared to bacterial meningitis the mean value showed a standard deviation of 65.7 IU/L and this was statistically significant. In tuberculous meningitis, mean CSF LDH was 110 IU/L. This high value in TBM was statistically significant when compared to the viral meningitis and not statistically significant when compared to bacterial meningitis. The mean CSF LDH in partially treated meningitis was 28.9 IU/L. A reduction CSF LDH of 24.8 IU/L from the mean value was seen after treatment for 3 days and this change was statistically significant.

**Conclusion:** CSF LDH >35 IU/L has a high negative predictive value to exclude the diagnosis of aseptic meningitis and even in aseptic meningitis, a value above 35 IU/L has a high predictive value of 75% to predict the chance of ependymitis. CSF LDH alone cannot differentiate pyogenic and tuberculous meningitis. Reduction in CSF LDH after treatment can be used as a prognostic marker, along with CSF glucose.

**P1613** Establishing the optimal breakpoints in cerebrospinal fluid cell counts for the diagnosis of secondary meningitis

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**Background:** Secondary meningitis is a frequent complication of neurosurgical procedures. The diagnosis relies on clinical symptoms, cerebrospinal fluid (CSF) cell counts and biochemistry and CSF culture results, all of which have limitations. In practice, the diagnosis is established based primarily on CSF culture results and white blood cell (WBC) counts, but no consensus exists on the adequate cut-off point for the latter. This study intended (1) to establish an evidence based WBC cut-off point for the diagnosis of secondary meningitis, and (2) to determine the optimal CSF WBC correction factor for red blood cells (RBCs) in the CSF.

**Methods:** Cases were patients with positive CSF cultures and a diagnosis of secondary meningitis, controls were patients with >4 negative CSF cultures, no positive CSF cultures at any time and no suspicion of secondary meningitis. Receiver operating characteristic (ROC) curves were constructed to determine the optimal WBC cut-off point to diagnose secondary meningitis. The correction factor for RBCs was determined from CSF samples with >1000 RBCs/mm³, drawn from patients who did not suffer meningitis. Data were extracted from the microbiology laboratory management and information system, from the Utrecht Patient Oriented Database (UPOD), and from the patient charts.

**Results:** 134 cases and 34 controls (with 227 control CSF samples) were included in the study. Median CSF WBC count was 380 in cases with secondary meningitis, and 10 in controls. The WBC count was significantly higher in cases where secondary meningitis was caused by Gram-negative micro-organisms. A sensitivity of 70% for secondary meningitis could be achieved with a specificity of approximately 80% applying a cut-off for CSF WBC counts of 90–100 WBCs/mm³. A median of 2 WBCs/443 RBCs was found in patients with an initial CSF sample with >1000 RBCs/mm³; this ratio, however, declined in subsequent CSF samples. Correction of the WBC count based on the RBC count changed the cut-off points, but did not improve the test characteristics.

**Conclusion:** WBC counts are useful in diagnosing secondary meningitis, but understanding of the predictive value of different cut-off points is necessary for adequate evaluation of patients.

**P1614** CSF lactate level in diagnosis of bacterial meningitis following neurosurgical intervention

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**Objectives:** Post-neurosurgical bacterial meningitis (BM) causes a high mortality and morbidity rate. Diagnosis of bacterial meningitis is complicated by several factor related to neurosurgical intervention. The aim of this observational study is to investigate the value of cerebrospinal fluid (CSF) lactate level for the diagnosis of post-neurosurgical BM.

**Methods:** Fifty of CSF samples were collected from 23 patients with clinical suspicion of post-neurosurgical bacterial meningitis for bacterial culture, leukocyte count, glucose, protein and lactate level. Simultaneous serum glucose concentration was also measured for determination of CSF-serum glucose ratio. All patients were evaluated by Infectious Diseases (ID) physicians for bacterial meningitis. Eleven of 23 patients were diagnosed with BM and received anti-bacterial treatment. In the study, we used a cutoff of >4 mmol/L for the lactate level and <0.5 for CSF-serum glucose ratio. BM was identified as pleocytosis and <0.5 of CSF-serum glucose ratio in patients diagnosed with BM by ID physician in the study.
Results: Mean lactate levels were 4.28±2.08 and 2.94±1.00 mmol/L among patients with BM diagnosis and no BM diagnosis, respectively. The difference of CSF lactate level between BM diagnosed and no BM diagnosed patients was statistically significant (p<0.008). CSF lactate level had 88% sensitivity and 81% specificity in the diagnosis of post-neurosurgical BM. Positive predictive value was 50% and negative predictive value was %97. In addition, CSF lactate level had positive correlation with leukocyte count in CSF (r=1.00) and negative correlation with CSF/serum glucose ratio (r=-0.527).

Conclusion: Determination of lactate level in CSF is a sensitive, specific test in diagnosis of post-neurosurgical BM.

Blood cultures and diagnosis of sepsis

Predictive value of soluble urokinase plasminogen activator in ventilator-associated pneumonia and sepsis

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Objectives: Urokinase plasminogen activator (uPAR) is a receptor expressed on neutrophils and NK cells. The role of its soluble form, namely suPAR, as a predictor of sepsis outcome was investigated.

Methods: Blood samples were collected from 180 patients with ventilator-associated pneumonia (VAP) and sepsis for seven consecutive days; 47 with sepsis; 49 with severe sepsis; and 84 with septic shock. suPAR was measured in serum by an enzyme immunoassay and PCT by an immuno-time-resolved amplified cryptate assay.

Results: ROC analysis of day 1 measurements for prediction of death is shown in the Figure. Mean suPAR of days 1, 2, 3, 4, 5, 6 and 7 among survivors was 9.1, 10.5, 10.7, 10.6, 10.5, 11.2 and 10.9 ng/ml respectively; respective values among non-survivors were 14.1, 14.7, 15.1, 16.1, 18.1, 19.3 and 19.3 ng/ml.

Conclusion: suPAR is a strong predictor of unfavorable outcome in VAP and sepsis probably superior than PCT. Serum concentrations remain constantly elevated among non-survivors.
Biomarker levels were significantly higher in bacteraemic patients compared with those in non-bacteraemic patients (p < 0.001). PCT had the best predictive value for bacteraemia with an AUC of 0.80 (95% CI: 0.75–0.84), sensitivity 89% (95% CI: 78–96%), specificity 58% (95% CI: 52–64%), and an optimal PCT cut-off value of 0.253 ng/ml (Figure 1). The predictive value of a combination of PCT plus a panel of the other biomarkers (sensitivity 91%, specificity 63%), or clinical signs (sensitivity 91%, specificity 57%), or analysis of serial PCT levels (sensitivity 64%, specificity 73%) did not lead to a significant improvement of the predictive value of PCT alone.

Conclusion: The value of PCT to predict bacteraemia in patients with sepsis does not further improve when combined with other biomarkers, clinical signs, or serial measurements.

Pre-interventional phase from January 2006 to October 2006 consisted of 3572 adult and 823 paediatric sets. Interventions were introduced from 1st of November 2006. Disinfectant swipes, blood culture bottles and 20ml syringe were distributed as a pack with an instruction leaflet on proper blood culture collection techniques. Junior doctors, nurses and phlebotomists were actively and individually informed of their short comings with feedback sent to each unit. Post interventional phase from November 2006 to December 2007, consisted of 4724 adult and 977 paediatric sets.

Results: Though an increase from 21% to 24% among positive yield was observed before and after intervention this was not statistically significant. However, a reduction of potential contaminants (62% vs 56%, p < 0.05) and an increase in yield of probable pathogens (27% vs 33%, p < 0.05) for the adult sets were observed and shown to be statistically significant. The monthly average of adult blood culture bottles submitted was reduced from 357 to 337 and the paediatric samples from 82 to 70 post intervention. These indicates better patient selection according to the provided guidelines. The drop in numbers of paediatric samples (31% vs. 9.5%, p < 0.001) may be explained by more accurate sample collection post intervention by avoiding contamination.

Conclusion: The intervention adopted by James Paget hospital demonstrates that inexpensive interventional methods will be adequate to increase the yield of blood cultures and reduce the contamination by normal skin flora. It also shows that in adults where sample collection is relatively easy, improving standards will increase the pathogen isolation. The findings to contrary in paediatric group suggest that more difficult sample collection may overestimate the pathogen positivity due to contamination.

Objectives: To measure and analyze transport times for blood cultures, identify the factors for long transport time, and study which possible consequences the transport time may have on detection of the sepsis pathogens.

Methods: A total of 909 blood cultures from 10 clinics from 3 hospitals were included in the study. Only one blood culture per patient was included. Transport time was defined as the time between the sampling and the insertion of the bottle into the blood culture system. The impact of the following factors on transport time were analyzed: i) time and day of sampling ii) clinic and hospital iii) the number of transports per day and iv) laboratory working hours. Further analysis of effects of long transport time on time to detection (from incubation to positivity in the system) and on total time to detection (from inoculation at RT to positivity in the system) were done by performing in vitro experiments using standard inocula and 5 ml human blood. Fifteen clinical isolates were inoculated in BacT/ALERT system after various holding times of 2h, 9.5h, 19h at room temperature (RT).

Results: The average transport time for blood cultures was 9,37h (1,48h, 10%tile and 18,35h, 90%tile). The hospital where the microbiology laboratory is located had the shortest transport time compared to others (p < 0.0007). There was no difference in transport time between the clinics in the same hospital. Similarly no difference was observed between the days of the week. There was significant difference depending on sampling time on the same day. Samples taken between 16:00–24:00 had 6–9h longer transport time compared to samples taken between 8:00–16:00 or 24:00–08:00. Both the numbers of transports per day and laboratory working hours were significantly related to transport time. In vitro experiments showed that the average time to detection is shorter for the samples that were preincubated at RT for 19h compared to the ones preincubated for 2h or 9.5h (7,6h vs. 16,7h and 15,6h respectively). In contrast total time to detection was significantly longer for the samples that were preincubated at RT for 19h compared to the ones preincubated for 2h or 9.5h (27,9h vs. 18,8h and 25,0h respectively).

Conclusion: Off-site location, time of sampling, numbers of transports per day and laboratory working hours were significantly related to
Differential time to positivity (DTTP) for the diagnosis of catheter-related bloodstream infection: do we need to obtain one or more peripheral vein blood cultures?

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Differential time to positivity (DTTP) for the diagnosis of catheter-related bloodstream infection (CRBSI) requires blood cultures taken from each catheter lumen to be compared with a blood culture obtained by direct puncture of a peripheral vein.

Objectives: Our objective was to determine whether it is necessary to obtain more than 1 peripheral blood culture in order to not miss CRBSI episodes.

Methods: We performed a retrospective study in patients with microbiologically proven CRBSI in whom catheter lumen cultures and 2 or more peripheral blood cultures were performed simultaneously. We calculated the number of episodes that would have been recovered if the culture of 1 or more peripheral blood samples had been eliminated.

Results: We collected 60 episodes of proven CRBSI from 58 patients (Jan 1, 2006 to Jul 31, 2010). If 1 peripheral vein culture had been eliminated in the patients with 2 or 3 peripheral blood cultures, we would have recovered 91.8% (p = 0.362) and 96.9% (p > 0.999) episodes of CRBSI, respectively. If we had eliminated 2 peripheral blood cultures in patients with 3 peripheral blood cultures, we would have recovered 90.8% (p > 0.999) of episodes.

Conclusions: When performing DTTP to confirm CRBSI, a single paired blood culture obtained through a peripheral vein was not associated with a significant number of missed CRBSI episodes.

Bloodstream infections related to Gram-negative rods: Usefulness of the Vitek2C System for direct identification and susceptibility testing from positive Bact-Alert blood cultures

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Objectives: To compare the result of identification and antimicrobial susceptibility testing obtained directly from the positive bottle of the blood culture with those performed according to manufacturer indications from isolated colonies and to determine the usefulness of these results on the antibiotic initial election.

Methods: A prospective observational study was conducted in two hospitals of Buenos Aires city (Argentina); 191 clinically significant monomicrobial Gram-negative bloodstream infections were included, corresponding to 189 patients. The median patient age was 72.5 years (range 1–94), 51% were males and 49% females.

Organism identification and susceptibility results directly from the bottle using serum separator tube (Becton Dickinson) to prepare the inoculum were compared with those obtained from cards inoculated with a standardized bacterial suspension obtained following subculture to agar.

Species identification was carried out with the GNI card. Antibiotic susceptibility testing was determined by the AST-82 card of the Vitek 2C System (Biomerieux, Marcy, France).

During the study period, a total of 191 clinically significant isolates of Gram-negative rods including 157 Enterobacteriaceae and 34 non-fermenting rods were tested.

Results: The blood culture positivization median time was 14.1 h (range: 2–71 h) and the median time to obtain the final result of identification and susceptibility testing directly from the blood culture bottle by Vitek 2C was 8.2 h (range: 3.75–18.8 h); by using the identities obtained from pure cultures with the Vitek 2C System as reference method, the agreement between the reference method and the Vitek 2C system tested directly by using cultures of blood from patients was 99%. By antimicrobial susceptibility test the overall categorical accuracy was 99%, with 0.21% very major errors, 0.17% major errors and 0.61% of minor errors.

The overall 14-day mortality rate for all patients was 24.3%. Of the 189 bloodstream infections episodes, 108 (57%) received appropriate initial empirical antibiotics.

After the results obtained directly from the bottle were reported, antimicrobial therapy was changed in 116 (60.7%) of the bloodstream infections episodes.

Conclusion: The identification and antimicrobial susceptibility testing results obtained directly from the blood culture bottles were reliable and had an important impact in the election of the initial antimicrobial treatment.

Comparison of three blood culture systems (Bactec 9240, BacT/ALERT 3D, and Versatrek) for blood cultures involving delayed vial entry

S.Y. Ng*, S. Teh, T. Tan (Singapore, SG)

Objective: Accurate and timely detection of bacteraemia is important in determining appropriate treatment of systemic sepsis. The use of automated continuous monitoring equipment for blood cultures is the current gold standard. This study simultaneously evaluated the performance of three blood culture systems.

Methods: The equipment and blood culture media were provided by representing vendors, and consisted of Bactec™ 9240 (Becton Dickinson), BacT/ALERT 3D (bioMérieux) and Versatrek (Trek Diagnostic). Multiple vials from each system were inoculated with standard dilutions containing ATCC strains of Escherichia coli, Staphylococcus aureus and Streptococcus pneumoniae, and wild-type strains of Klebsiella pneumoniae, Streptococcus intermedius, methicillin-resistant Staphylococcus aureus, Bacteroides fragilis, Candida glabrata, and Candida albicans. The performance of each system was evaluated for multiple variables: 2 ml inocula, 0.5 ml inocula, delayed entry of vials following retention of inoculated vials at 4°C, 22°C, and 35°C for 14 hours. The time-to-detection (TTD) was monitored for all inoculated vials.

Results: Some inoculated vials failed to flag positive after incubation for 5 days (Bactec 2%, BacT/ALERT 3%, Versatrek 3%). For vials that were incubated immediately following incubation, average TTD was lowest for the Versatrek system (9.2 hours for Gram-negative bacilli, 12.6 hours for Gram-positive bacteria, 18.0 hours for Candida spp. and 38.6 hours for Bacteroides spp.). The Bactec system significantly outperformed the BacT/ALERT system for detection of Candida spp. (TTD 36.1 hours compared with 68.7 hours), but performed worse for Bacteroides spp. (TTD 95.7 hours compared with 43.3 hours). For vials with delayed entry, the Versatrek maintained a lower TTD for all tested organisms. Vial management and workflow was easiest for BacT/ALERT, followed by the Bactec system.

Conclusion: The Versatrek system had the lowest TTD for all test organisms in this evaluation for both immediate and delayed vial entry.

Performance analysis of blood culture and frequency of medically important bacteria in 9 university hospitals in Korea

S. Kim*, J. Shin, N.Y. Lee, E. Kim, M.N. Kim (Inju, Busan, Seoul, KR)

Background: Optimal blood culture performance is critical for successful diagnosis and treatment of sepsis. To understand the status of blood culture, we investigated several issues regarding the procedure at nine university hospitals.

Methods: Ordering blood culture sets and sampling volume for adults and children were investigated in Jan. through Apr. 2010, while positive rate and growth of skin contaminants were compared in 2009. Microbial growth in aerobic and anaerobic bottle was investigated prospectively. The frequency of common pathogens was analyzed.

Results: Most of the hospitals used two sets of bottles in adults and one bottle in children. The average blood volume of each set was 7.7 mL in adults and 2.1 mL in children. The positive rate of microorganisms...


**P1625** Nationwide survey of blood culture performance in Korea, 2010

S. Kim*, J. Shin, M.N. Kim, N.Y. Lee (Jinju, Busan, Ulsan, Seoul, KR)

Objectives: Blood culture is essential in the diagnosis of sepsis. Although many laboratories have instituted the use of automated blood culture systems, adequate skin disinfection and optimal volume of sampling are major factors for successful blood cultures. Information on positive rates and skin contamination rates, as well as knowledge of optimal procedures, are requirements for the medical personnel.

Methods: The questionnaires included details on disinfection materials, sampling intervals, and recommended blood volumes. Laboratory personnel were asked about the equipment used and the storage place of the samples before installation. For the quality control, the positive rates and skin contamination rates were recorded.

Results: Answers to the survey were collected from 74 hospitals across the country. Povidone iodine with either isopropl alcohol (31.1–44.6%) or ethanol (14.9–20.3%) was the most widely used skin disinfectant. Sampling of the 2nd specimen was performed simultaneously in 38%, within 30 min in 50%, and within 1 h in 5%. The recommended blood volume was predominantly 10 mL (69%), whereas 24% of hospitals use 20 mL. BioMerieux 3D was used at 54.1%, Becton Dickinson 9240 at 23.0%, and both at 10.8%. The bottles were stored at 37°C in 23% hospitals and at room temperature in 16% before installation, whereas 57% practice direct installation into the equipment. Positive rates were 8–10% at 32%, 5–8% at 23%, and <5% at 12%. Skin contamination rates were 2–3% at 32%, 1–2% at 27%, and >3% at 13%.

Conclusion: Skin decontamination, including the type of disinfectant used and the waiting time, varies among the hospitals. Sampling intervals and volumes should be standardized. Positive growth rates and skin contamination rates were acceptable.

**P1626** C-reactive protein is not a clinically useful predictor of outcome in prosthetic joint infection

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Objectives: Prosthetic joint infection (PJI) complicates up to 2.5% of arthroplasties. Management is usually with surgical removal or debridement followed by antibiotics. Measurement of C-reactive protein (CRP) is often used to monitor response to treatment. However, there are no data on its use in this context. In this study, we analysed CRP data from patients treated by debridement, antibiotics and implant retention (DAIR) or by two-stage revision for PJI, to determine its usefulness in predicting treatment failure.

Methods: Retrospective data were collected for patients with PJI managed either by DAIR (n=109) or by two-stage revision (n=151). CRP data were analysed in four datasets: (1) DAIR patients during the first 180 days; (2) DAIR following the first 180 days, using time to treatment failure or to the end of follow up; (3) two-stage revision patients during the first 120 days after the 1st stage and (4) two-stage revisions after re-implantation, excluding the first 28 days post re-implantation.

Results: During the first 180 days after DAIR, CRP readings were 57% higher (95% CI 7% to 200%, p=0.02) in the group who ultimately experienced treatment failure. There was no significant change in CRP over time in patients without failure, but a significant increase over time in subjects who did experience failure (15% per month, 95% CI 6% to 25%, p=0.006).

During two-stage revision, the CRP between 1st stage excision and re-implantation was not significantly different between patient groups, but there was a 2% increase per month following re-implantation (95% CI −3% to +7%) in patients with treatment failure. However, there was wide scatter of CRP results within all datasets, and hence CRP was poorly predictive of outcome despite the association observed (areas under the receiver operator curve (AUROC) were 0.6, 0.65 and 0.55 for the first 180 days of DAIR, DAIR after 180 days and two-stage revision after re-implantation respectively).

Discussion: CRP weakly predicted eventual treatment failure of DAIR but not two-stage revision in the immediate post-operative period. However, the sensitivity and specificity reflected by AUROC analysis suggest that the overall association with outcome is weak. Caution should be exercised when interpreting results: a high CRP in the absence of other clinical signs is not an important measure of treatment failure and conversely, clinicians should not be falsely reassured by a falling CRP when other features suggest complications.

**P1627** Is prosthetic joint vortexing/sonication effective in aseptic failure?

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Background: It has been shown that sonication of prosthetic joints is more sensitive than peri-implant tissue culture for diagnosis of prosthetic joint infection (PJI). Herein, we study whether sonication improves the microbiological diagnosis in implants removed due to aseptic failure (AF). We also evaluated the relative frequencies of definitive diagnosis of PJI in the postoperative period among patients with pre-and intraoperative diagnosis of AF.

Methods: From February 2009 to November 2010 all consecutive patients undergoing partial or total removal of hip or knee implants due to AF were included. Pre-operative AF was defined when the patient had radiological signs of loosening without symptoms or signs of infection; intraoperative diagnosis was confirmed in the absence of purulence or sinus tract. Definitive PJI was based on histopathological criteria. The retrieved implants were vortexed, sonicated (40 kHz, 5 minutes) in 400 mL Ringer solution and concentrated. Microorganisms were defined as causative if ≥2 peri-implant tissues were positive for the same organism and sonicate fluid culture ≥100 cfu on either plate.

Results: A total of 139 patients were studied; 20 had PJI (Hip 18, Knee 2) and 119 AF (Hip 73, Knee 46). All pre-and intraoperative AF patients with definitive PJI had ≥10 cells per high power field in frozen sections. 75% (15/20) of PJI and 55% (66/119) of AF were partial removal (p=0.1). The median age was 69 years. Sensitivities of peri-implant tissue and sonicate fluid cultures were 40% and 75% (p=0.025), respectively, and the specificities were 98% and 97%, respectively. Coagulase negative staphylococci was the most frequent microorganism detected by both peri-implant tissue 62.5% (n=5) and sonicate fluid 73% (n=11) cultures.

Conclusion: Fourteen percent of the patients with pre- and intraoperative suspicion of AF had a definitive diagnosis of PJI. Patients undergoing partial removal due to AF were more likely to have definitive PJI. Implant sonication followed by culture was more sensitive than peri-implant tissue culture for the microbiologic diagnosis of PJI.
Improving the diagnosis of prosthetic joint infection by prolonged culture of periprosthetic tissues

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Objective: To clarify if prolonged bacterial culture from 5 to 12 days of periprosthetic tissue samples from prosthetic joint revision surgery would result in a better bacteriological diagnosis of prosthetic joint infection (PJI).

Methods: All patients from the departments of Orthopedic Surgery at Bispebjerg and Hvidovre Hospitals, Copenhagen DK, from the period June 2008 to July 2010 with positive periprosthetic tissue samples were included in the study. The patients had undergone revisions of hip or knee arthroplasty and 5 sterile tissue samples were taken during operation. Tissue samples were immediately transported to the Department of Clinical Microbiology, Hvidovre Hospital, servicing both orthopedic departments, and processed: Gram stain, aerobic and anaerobic cultures on 5% horse blood agar, anaerobic agar and in semi-solid thioglycolate, which were inspected daily for five days or until growth whereupon classic microbiological diagnosis and sensitivity testing were made. Positive results were immediately reported to the clinicians. Negative final results were reported on day 5 and the samples from the semi-solid thioglycolates were subcultured onto 5% horse blood agar and anaerobic agar on day 7. The plates were evaluated on day 12 and only positive growth results were reported to the clinicians. Two or more samples with growth of identical isolates were used as a definition of infection.

Results: During the two year period the two orthopedic departments did 371 revisions of hip and knee arthroplasty suspected for PJI. A total of 317 revisions (279 patients) were positive with growth of one to five tissue samples. 237 revisions of hip arthroplasty were positive and of these 153 in two or more samples: 136 (89%) were positive on day 5 and 17 (11%) on day 12. Of 80 revisions with positive samples after revisions of knee arthroplasty 60 had growth of two or more samples: 55 (92%) on day 5 and 5 (8%) on day 12. The most often isolated bacteria after 12 days were slow growing and low pathogenic bacteria as coagulase-negative staphylococci, enterococci, Corynebacterium species and anaerobes but also S. aureus and Streptococcus species.

Conclusions: The prolonged culturing of periprosthetic tissue samples from revision of hip and knee arthroplasty increased the number of patients with a confirmed bacteriological diagnosis with approx. 9%. The strategy is easy to implement, it improves the diagnosis of PJI and makes the choice of antimicrobial treatment more relevant.

Three different ways of processing samples from sonication.

Which is the best to improve diagnosis of orthopaedic devices?

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Background: Diagnosis of infection in orthopedic implants remains difficult. Sonication of orthopedic devices (OD) seems to improve the isolation of bacteria causing infection.

Objective: To evaluate 3 different methods of processing samples obtained from sonication compared to standard cultures of tissue samples (TS).

Methods: All patients who had orthopedic sugery for removal of an OD were included (Oct 2009-July 2010). None of the patients received antibiotics in the previous 14 days. Standard samples from bone, synovial fluid, synovial membrane, bone interface and cement were collected in the surgical procedure, as well as OD (prosthesis, spacers, internal fixation devices) and they were sent for sonication and for conventional cultures. Standard TS were inoculated in solid aerobic and anaerobic media and thioglycolate broth was incubated for 5 days in standard conditions; systemic subculture of thioglycolate broth in solid media was done for another 5 days.

Sonication: OD were sent in sterile containers to the lab, where 500 cc of Ringer lactate solution was added; samples were vortexed for 30′, then sonicated at 50 kHz for 5′ and vortexed for 30′. The solution obtained after sonication was processed in 3 different ways: A, 10 mL were centrifuged at 2500 rpm for 5′ and the cented was cultured in the same way as standard TS; B, 10 mL were incubated for 24 hours at 37°C and were processed as A, and C,10 mL were incubated in a blood culture bottle (BactaR®) for 5 days.

Diagnosis of infection was based on clinical data and 2 standard positive intraoperative cultures for the same bacteria; results of sonication were not known by the orthopedic surgeon.

Sensitivity, specificity, positive predictive value and negative predictive value were calculated.

Results: 34 surgical procedures in 29 patients were analysed [17 knee prosthesis, 5 hip prosthesis, 6 cement spacers and 6 internal fixation devices]. 224 TS were processed (median 7.7 samples/surgery), and 46 were from sonicated devices. 12/34 (35%) episodes had diagnosis of infection (9 knee arthroplasty, 3 hip arthroplasty) with a median number of positive cultures of 4.9. 22/34 (65%) of episodes had no infection (median number of positive cultures 0.8). Results are summarized in the table below.

Conclusions: (1) NPV of direct sonicated samples are higher than standard culture TS. (2) Additional methods of processing sonicated samples do not contribute to improve the diagnosis of infected OD.
The role of Gram staining in the rapid diagnosis of Nosocomial infections, emerging MDR pathogens and Skin soft tissue infections

Conclusions: Periodic surveillance cultures of the skin and hubs of patients with hemodialysis catheters can identify a population at higher risk of infection amenable to preventive or anticipative therapeutic measures.

**P1631** The role of Gram staining in the rapid diagnosis of tunneled central venous catheter colonisation

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Gram staining of catheter tips from withdrawn non-tunneled central venous catheters (CVCs) is very useful for anticipating catheter colonization or central line-associated bloodstream infection (CLABSI) (Bouza et al., DMID, 2006, 56 [255–256]). No similar data are available for tunneled catheters.

Objectives: Our objective was to define the validity values of Gram staining for the prediction of colonization and CLABSI in patients with tunneled CVCs.

Methods: All tunneled catheters removed in our institution were prospectively and routinely sent to the microbiology laboratory for Gram staining (first) and tip culture (Maki and sonication in a random order). Breakpoints for positive culture were as follows: roll-plate technique (Maki), ≥ 15 cfu/plate; tip sonication ≥ 100 cfu/plate. CLABSI was defined as isolation of the same microorganism in both peripheral blood culture and catheter tip culture.

Results: We received 111 tunneled catheters of which 25.2% were colonized. The microorganisms isolated in the colonized catheters were Gram-positive (66.7%), Gram-negative (24.2%), and fungal (9.1%). Of all withdrawn catheters, 8 (7.2%) were from bacteremic patients. The validity values of Gram staining for detecting colonization and CLABSI were as follows: sensitivity, 32.1% and 50.0%; specificity, 100% and 95.1%; positive predictive value, 100% and 44.4%; and negative predictive value, 81.4% and 96.1%.

Conclusion: Gram staining of catheter tips from withdrawn tunneled CVCs is highly predictable of colonization. Negative results rule out CLABSI.

**P1632** Is it possible to know whether candidemia is caused by catheter-related infection without having to remove the catheter?

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CDC guidelines recommend withdrawing the catheter in patients with candidemia when there is evidence that the catheter is the source of infection. However, data regarding minimal time to positivity (MTTP), proportion of positive blood cultures (BC) (in patients with two or three BC drawn), and differential time to positivity (DTTP) from BCs obtained from the catheter and peripheral veins have not been properly assessed in patients with candidemia.

Objectives: Our objective was to compare MTTP, the proportion of positive BCs, and DTTP in patients with well-demonstrated catheter-related candidemia (C-RC) and non-catheter-related candidemia (NC-RC).

Methods: We included patients with a catheter tip culture performed within 7 days after the episode of candidemia (July 2005 to August 2010). An episode was considered C-RC only when there was a positive catheter tip culture (≥ 15 cfu/plate) with the same *Candida* species. An episode was considered NC-RC when there was no evidence of *Candida* species in catheter tip culture. For each variable evaluated, a ROC curve was created to determine the best cut-off for predicting C-RC.

Results: We retrospectively collected a total of 108 episodes of candidemia (84 in adults and 24 in children; 67 C-RC and 41 NC-RC) fulfilling our enrolment criteria and caused mainly by *C. albicans* (49.1%) and *C. parapsilosis* (30.6%). Values for the different tests are shown in the table.

Conclusions: None of the tests evaluated allows for a clear-cut prediction of C-RC, and criteria accepted for bacteremia should not be automatically extrapolated to candidemia. In our study, a low proportion of positive blood cultures with *Candida* had a high negative predictive value for a catheter origin.

**P1633** Nosocomial infections, emerging MDR pathogens and antibiotic prophylaxis in cardiothoracic surgery: clinical surveillance study at Lancashire cardiac centre, UK

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Background: Guidelines recommend use of prophylactic antibiotics to decrease the risk of post operative infections. Lancashire cardiac unit is a tertiary centre located in Blackpool. We present findings from a comprehensive retrospective surveillance study over 12-months to examine the association between emergence of multi-drug pathogens, apparent rise in post operative infections, *C. difficile* infections and variations in practices of antibiotic prophylaxis between cardiac surgeons.

Methods: Retrospective review [April 2009 – March 2010] of cardiac surgery and pathology database to establish patients with clinically significant post operative infections. Further case notes review of randomly identified 50 patients with prolonged infections, re-do operations due deep infection, and death with infection.

Results: 1096 patients underwent elective/urgent cardiac surgery during Apr09-Mar10. 280 patients [electives – 160; urgent – 120] had 503 specimens cultured for suspected infection. 58% [294/503] samples had significant pathogen. Mortality was 5% [4/80]. 8 cases of CDI and nil MRSA bacteraemia. Details of infections [UTI, sternal/soft tissue infections, line infections, pneumonia, bacteraemia & others] with etiological agents to be presented. Multidrug resistant *Pseudomonas aeruginosa*, *Serratia marcescens* and *Stenotrophomonas maltophilia* isolated from cases with pneumonia and empyema.

Antibiotic prophylaxis: The eight surgeons vary in the choice, single/combination and duration of prophylactic antibiotic. Cefuroxime at 1.5g x 3 doses to removal of central line/d3. Teicoplanin [800 mg x 3 doses till d3] used in combination by one surgeon.
Conclusions: Most guidelines on antibiotic prophylaxis in surgery recommend single or 24h use of antibiotic. The choice is based on type of surgery, likely pathogens, and local resistance.

A recent audit on antibiotic prophylaxis in cardiac surgery across cardiac units in UK revealed a shift away from use of cephalosporins to a single dose or 24h use of Flucloxacinil±gentamicin in most centres. This change in practice may be secondary to increased concern about emerging multi-resistant pathogens, CDI rates, antibiotic stewardship and surgical site infections linked to surgeon performance indicator. The findings from this study have been used to inform an action plan & strategy including revision of antibiotic prophylaxis guide, skin prep audit, microbiologist ward rounds, etc.

Results: Among cases, 71 had an UTI, 15 had a SSI, 10 developed pneumonia, 1 patient had a SSI plus UTI, while the remaining 3 cases experienced other kind of infection.

The univariable logistic regression model revealed the following significant predictors (p < 0.10) of post-operative infection: age, obesity, ASA score, the duration of urinary catheterization, the anatomical location of the surgical procedure, the type of anesthesia and the kind of the chemoprophylactic regimen. However, in multivariable modeling, only the type of chemoprophylaxis retained the statistical significance. More specifically, cases were almost 60% less likely (OR: 0.41; 95% CI: 0.19–0.89) to have received vancomycin as prophylaxis rather than cephalosporin compared with the control population.

Conclusion: UTI was the most frequent infection after orthopaedic procedure in our study following by SSI. Although many factors were significantly associated with post-operative infection in univariate analysis, only the kind of chemoprophylaxis remained a statistically significant predictor in multivariable modeling. More specifically, the use of vancomycin, instead of cephalosporin, is associated with a lower risk of infection.

Objective: Correlation between operative site skin bacterial counts in neurosurgical procedures and the development of surgical site infections (SSI) has not been proven. We evaluated the association of bacterial Colony Forming Units (CFU), type of procedure and development of SSI in a prospective pilot study.

Methods: Skin swab cultures were obtained within 1 cm from the incision, pre-post-preparation and preclosure samples were obtained. Bacterial counts were enumerated and the most prevalent organisms were recorded. Procedures were classified as clean, clean with a foreign body, clean contaminated, contaminated and dirty.

Results: 157 procedures (43.9% clean, 19.1% clean-contaminated, 26.1% clean with foreign body, 7.6% contaminated and 3.2% dirty) in 118 patients (62.4% male) were evaluated prospectively. 38.9%patients were hospitalized in the ICU. Oncology and trauma were the most common reasons for surgery (28% and 26.8% respectively). 157 sample sets were cultured. 85.4% pre-prop,27% post-prop and 43.9% of the preclosure samples tested positive. Coagulase-negative staphylococci (CoNS) were the most frequently isolated organisms irrespectively of sampling time (65%, 17.8% and 27.4% for samples 1, 2 and 3 respectively) and independently of procedure classification followed by P. acnes. The median cfu count for CoNS were 2.97log (IQR 2.47–3.73), 2.48 (range 2–7.67) and 2.47 (IQR 2.2–6.9) respectively for each sampling. P. acnes was the second most frequent pathogen isolated. The median cfu count for P. acnes were 4.25log (IQR 3.4–5.8), 2.87 (IQR 2.48–3.43) and 4.3 (IQR 3.35–4.6) respectively for each sampling. There was a significant difference in rates of pathogens in preppe samples for the head compared to other sites (OR 2.9;p = 0.028). The same was true for the P. acnes isolated from head versus other sites for preppe and postprep samples (OR 5.2, p = 0.01 and OR 1.16, p = 0.024 respectively). Procedure classification or prolonged surgery duration were not associated with microbial counts irrespectively of sampling time. SSI development was not associated with bacterial CFU at any sampling but with ICU stay (p < 0.001).

Conclusion: CoNS and P. acnes were the most frequent bacterial pathogen cultured irrespectively of sampling. The pathogen cfu log did not significantly differ among the samples. P. acnes isolation correlated with head specimens. Procedure classification was not associated with microbial skin counts at any sampling. SSI was associated with ICU stay.
**P1637** Gram-negative bacillary meningitis in post-neurosurgical patient associated with high mortality
C. Moon*, J. Lee, J. Shin, M. Kim (Busan, KR)

**Objectives:** Widespread use of antibiotics and the increasing frequency of neurosurgical procedures have altered the epidemiology of post-neurosurgical meningitis in recent years. Gram-negative bacillary meningitis (GNBM) has become increasingly common. We investigated the changing etiology and outcome of GNBM in post-neurosurgical patients at our center.

**Methods:** All patients with culture-proven post-neurosurgical meningitis diagnosed between January 2006 and November 2010 were retrospectively reviewed.

**Results:** Fifty-four episodes of culture-proven meningitis were reviewed. A total of 66 isolates were identified from cerebrospinal fluid. Gram-negative bacilli (41 isolates) were Acinetobacter baumannii (16, 39.0%), Pseudomonas aeruginosa (8, 19.5%), Stenotrophomonas spp. (4, 9.8%) and Klebsiella pneumoniae (3, 7.3%). Glucose non-fermenting organisms accounted for 78.0% of the total Gram-negative bacilli. Gram-positive cocci (25 isolates) were coagulase-negative staphylococci (10, 40%) and Staphylococcus aureus (9, 36.0%). The resistance rate of Gram-negative bacilli to cefazidime, ceftazime and meropenem were 70.7%, 48.8% and 41.5%. Compared to the patients with meningitis caused by Gram-positive cocci, those with GNBM had a higher incidence of neurologic complication (74.2 vs. 30.4%, p=0.001) and in-hospital mortality (54.8 vs. 30.4%, p=0.065). The mortality from GNBM was significantly associated with the carbapenem resistance (p=0.044) and inappropriate antimicrobial therapy (p=0.042).

**Conclusion:** GNBM in post-neurosurgical patient showed a higher mortality than previous studies. Carbapenem resistance of GNBM is significantly increasing during relatively short period. Adequate use of antibiotics and effective infection control program is needed in post-neurosurgical patients.

**P1638** Risk factors for mesh-related infections after hernia repair surgery: a meta-analysis of cohort studies
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**Objective:** Mesh infection, although infrequent, is a devastating complication of mesh hernioplasties. We aimed to systematically review and synthesize the available evidence on risk factors for synthetic mesh infection post-hernioplasty.

**Methods:** A systematic search was performed in PubMed and Scopus databases. The extracted data were synthesized with the methodology of meta-analysis.

**Results:** We identified 6 eligible studies, reporting on 2418 mesh hernioplasties. The crude mesh infection rate was 5%. Statistically significant risk factors were smoking (risk ratio [RR]=1.36 [95% confidence intervals: 1.07, 1.73]), American society of anesthesiologists (ASA) score [≥3 (RR=1.40 [1.15, 1.70]), and emergency operation (RR=2.46 [1.56, 3.91]). A trend towards higher mesh infection rates was observed in obese patients (RR=1.56 [0.96, 2.55]) and in patients operated by a resident (in contrast to a consultant: RR=1.18 [0.99, 1.40]). Moreover, mesh infections were significantly correlated to patients’ age (weighted mean difference [WMD]=3.29 [0.59, 5.98]) and ASA score (WMD=0.23 [0.08, 0.38]), as well as the duration of the hernioplasty (WMD=−0.20 [28.64, 71.76]).

**Conclusion:** Patient’s age, ASA score, and smoking, as well as the duration and emergency setting of the operation were found to be associated with the development of synthetic mesh infection. The heterogeneity of the available evidence should be taken under consideration. Prospective studies are warranted to further investigate mesh-related infections.

**P1639** External ventricular drain-related ventriculomeningitis – a five-year, single-centre survey
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**Objectives and Background:** External ventricular drainage (EVD) is frequently used in neurosurgical and neurological intensive care. Central Nervous System (CNS) infections related to EVD, namely ventriculitis and menigitis, have been well described and pose a dangerous threat to critical care patients. In this report data from one single institution collected prospectively from 2005 to 2009 is presented, giving detailed information on the incidence of infection and pathogens involved.

**Methods:** Description of prospectively collected data, including 371 patients with a total of 481 EVD between 2005 and 2009. Detailed information for each patient was recorded in a database as part of an active infection surveillance program and evaluated continuously in the KISS-infection surveillance system. Handling of EVD is standardized, and ICU personnel is well trained. Cerebro-Spinal Fluid (CSF) diagnosis including cell count, protein-, glucose- and lactate concentration, as well as Gram stain is performed routinely on a daily basis. CSF microbiological cultures are sent to the laboratory twice weekly as routine, or immediately anytime if infection is likely. All relevant laboratory tests and microbiological findings are recorded. Weekly conferences have been established, with close and prompt information exchange on any change of status of patients under surveillance.

**Results:** 57 infections meeting the KISS criteria of ventriculomeningitis were diagnosed. In 51 cases infectious microorganisms could be cultured from CSF. The overall device-related infection rate was 11.63 per 1000 EVD-days (57/4901).

**Conclusion:** This survey demonstrates the value of an active surveillance program for reducing device-related ventriculomeningitis in neurosurgical critically ill patients and gives insight in the changing epidemiology of microorganisms associated with EVD-related infections.

**P1641** Hardware-related infections in patients subjected to deep-brain stimulation
K. Chang*, A. San Gil, M. Virela, E. Calbo, M. Garcia-Bach, V. Pascual, J. Garau (Terrassa, Barcelona, ES)

**Background:** Deep brain stimulation (DBS) is a commonly performed procedure for intractable movement, psychiatric disorders and pain. As with any implanted device, hardware for DBS (H-DBS) constitutes a new risk of infection.

The aim of our study is to describe 3 cases of H-DBS infection from a single center and another 178 cases found in the review of the literature.

**Methods:** From 2004–2010, 3 cases of infection related to DBS (performed by a single surgeon team and one centre) were included. An exhaustive literature review was conducted to identify any possible reported cases. Data on incidence, DBS indication, time to initiation of symptoms, diagnoses, site of infection, clinical features, causative microorganism, antimicrobial therapy and surgical management were recorded.

**Results:** Between 2004 and 2010, 102 H-DBS were implanted. Hardware related infection was diagnosed in 3 patients. 178 other cases were found in the literature review. Incidence of infection ranged from 0.62% to 15% for patients or 1% to 9% for electrodes. In only 92 cases a microorganism was reported. The most common isolated microorganism was S aureus.
Surgical wound infections after median sternotomy: clinical and microbiobiological preliminary results of a large multicentre study in Italy

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Objectives: Sternal wound infection (SWI) after cardiac surgery is a rare complication associated to high mortality and costs. Few SWIs are treated in every single center and the approach is often empiric. Multicentric studies are needed to evaluate the optimal management.

Methods: Twenty divisions of Cardiac Surgery spread all over Italy participated in this observational study. All patients developing SWI in two periods (from Oct. 2005 to July 2006 and from March to Sept. 2008) were included. SWI was defined according to the CDC diagnostic criteria. Patients characteristics, comorbidities, ASA score, pre-operative risk factors, S. aureus nasal colonization, modulation of depilation, perioperative prophylaxis, clinical manifestations of SWI, microbiological results, therapeutic approaches and outcome were recorded, inserted in a data base and analysed by a central supervisor.

Results: Out of 4711 pts undergoing cardiac surgery, 131 (2.7%) developed SWI and were included. The mean age was 68.3 years. The mean time of pre-operative hospital stay was 5.3 days. Eighty-one percent of pts had ASA score 2. The most frequent pre-operative risk factors were: obesity (32.0%), hyperglycemia (31.3%), COPD (24.4%) and cigarette smoking (19.8%). Out of 69 pts investigated for S. aureus nasal colonization (17.3%) were positive. Shave of the operative site was performed in 88.5%. Ninety-one SWIs (69.4%) were incisional, 33 (25.1%) organ/space involving and 7 (5.3%) not classified. Dehiscence was present in 87.7%, sternal instability in 33.5%, fever in 35.8%. Cultures from SWI were obtained in 114 pts (82%). Blood cultures were performed in 86 pts (61.8%) and were positive in 29 (32.5%). Pathogens were identified in 96 pts (73.2%): CNS (n. 36) and S. aureus (n. 26) were the most important microbiological isolates. Systemic antibiotics were frequently required to cure the infection.

Conclusion: Surgical wound infection is an uncommon event and it occurs early after implantation mainly due to patient’s skin flora. It is rarely accompanied by intracranial abscesses. Complete hardware removal is frequently required to cure the infection.

Procedure specific surgical site infection rates in a tertiary care hospital in Turkey

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Objectives: To describe the incidence of procedure specific surgical site infections (SSI) in a tertiary care hospital in Turkey and compare it with National Nosocomial Infections Surveillance (NNIS) system rates.

Methods: We conducted a prospective surveillance study among 8204 patients who underwent surgery during a period of 42 months. SSI rates were calculated using US Centers for Disease Control and Prevention and NNIS definitions. The SSI rates were computed and benchmarked with the US rates by using standardized infection ratio (SIR).

Results: We observed 8204 operations and 309 SSIs. Overall crude SSI rate was 3.76%. Most SSIs were classified as deep incisional SSI 55.6%, followed by organ/space SSI 25.9% and superficial SSI 18.5%. Table 1 shows the crude infection rates (CIR) and SIR for each procedure and risk category. The most commonly isolated pathogens were E. coli (34%), A. baumannii (12%), K. pneumoniae (8%) and Gram-positive microorganisms (21%). The most common frequently performed operative procedures were appendectomy, herniorrhapsy and knee prosthesis.

Conclusion: We found SSI rates higher in our hospital than those reported by the NNIS system. Benchmarking infection rates with NNIS data can be misleading where the number of operative procedure is limited. Comparing infection rates with SIR may offer a resolution to this issue. To reduce the rate of infection, it must be kept in mind that the most important aspect of this effort is to comply with strict infection control practices.

Impact of post-discharge surveillance on the incidence of surgical site infections in cardiac surgery in a Swiss tertiary care hospital

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Objectives: To demonstrate the impact of formal post-discharge surveillance (PDS) on the incidence of surgical site infections (SSIs) in cardiac surgery.

Methods: We performed a prospective surveillance of cardiac-surgery patients (316 valve replacements, 221 aorto-coronary bypass procedures, 96 combined interventions) from June 1, 2009 to May 31, 2010 regarding the incidence of SSIs. CDC-Definitions were used to diagnose and classify SSIs in superficial, deep and organ-space infections. PDS was performed by standardized telephone-telephone of patients, 1 and 12 months (in case of implantation of prosthetic material) after intervention.

Results: Among a total of 633 patients (69% male, mean age 66.2±10.9), 49 developed a SSI within 12 months of surgery at the chest site (7.7%). To distinguish the impact of PDS on SSI-incidence, we compared the in-hospital infection rate with the infection rates 1 and 12 months after
Incidence and risk factors for surgical site infection in
Surgical site infections after elective craniotomy: a review of
Postoperative bacterial meningitis after trans-sphenoidal
The fact that most SSIs are diagnosed after discharge illustrates that
The data suggest that PDS is not essential for the detection of DSWI.
rehospitalisation, but 37% would have been missed without formal PDS.
Conclusion: Our results demonstrate the impact of formal post-discharge
surveillance (PDS) on the detection of SSIs. Formal PDS increases
sensitivity for superficial SSI but has a lesser impact on DSWI detection.
The data suggest that PDS is not essential for the detection of DSWI.
the most SSIs are diagnosed after discharge illustrates that
a comprehensive comparison of incidence data between countries and
institutions requires standardized surveillance programs including a 12
months follow-up period.

Table 1: Occurrence and detection of chest SSIs during follow-up period

| Site of SSI | In-hospital | Post-discharge 1 month* | Post-discharge 12 months* | In-hospital and rehospitalization episodes | Non-total excluded SSI without formal PDS |
|------------|-------------|-------------------------|--------------------------|------------------------------------------|------------------------------------------|
| Chest      | 0.7% (9/1218) | 3.6% (43/1184) | 0.4% (5/1249) | 4.3% (53/1249) | 0.6% (7/1184) |
| DSWF       | 4.5% (53/1184) | 4.1% (49/1218) | 0.2% (3/1249) | 0.2% (3/1249) | 0.1% (2/1184) |
| SupraTotal | 5.2% (68/1295) | 7.7% (92/1218) | 0.6% (8/1249) | 4.5% (57/1249) | 0.7% (9/1184) |

*In-hospital refers to the period of hospital stay. Post-discharge period refers to the period after discharge. The incidence of SSIs was calculated as the number of SSIs divided by the total number of patients at risk during the specified period.

Fifty-five (63.2%) SSI occurred post-discharge. Higher incidence was observed in colon-surgery (8.6%) and appendectomy (3.9%). Overall, risk factors found to be significantly associated with SSI were age (OR=1.2 for each 10yrs increase), NNIS score (>2 (OR=3.0) and non elective admission (OR=2.0). These findings did not substantially change when only in-hospital SSI were considered.

Conclusions: The study is consistent with national data and stresses the relevance of the 30-day post-intervention SSI survey. Future surveys should auditing the application of preventive bundle for SSI, in order to verify the observed associations adjusted for this important variable.

Postoperative bacterial meningitis after trans-sphenoidal surgery: a retrospective study of 99 interventions over a 10-year period
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Objectives: Bacterial meningitis is a rare but severe complication of trans-sphenoidal surgery, associated with high morbidity and mortality. The optimal type and duration of perioperative antibiotic prophylaxis respective preemptive treatment has not yet been defined. Recommendations vary from a single-dose antibiotic prophylaxis to a one-week treatment. We evaluated the incidence and risk factors for early bacterial meningitis after trans-sphenoidal surgeries at our institution.

Methods: We reviewed all trans-sphenoidal surgeries performed at our institution over a 10-year period (2001–2010). Cases with early bacterial meningitis (within 30 days of surgery) were compared to controls without early postoperative meningitis. Bacterial meningitis was defined according to the CDC definition of nosocomial meningitis.

Results: Among 99 trans-sphenoidal surgeries performed during the 10-year study period, 3 cases of early bacterial meningitis occurred in 3 women aged 29, 59 and 79 years. The meningitis was diagnosed 1, 8 and 10 days after surgery. The following pathogens were identified in the cerebrospinal fluid (CSF): Streptococcus pneumoniae in 2 cases and Proteus vulgaris in 1 case. One patient died, while the other two patients recovered without sequelae. All 3 cases of bacterial meningitis occurred as a cluster during early 2010, resulting in this investigation. The following risk factors were identified: visible CSF leakage during surgery, which was immediately closed (present among all 3 cases compared to 23% in controls), trans-nasal endoscopic approach (used in all 3 patients compared to 23% in controls) and a short (single dose) prophylaxis compared to a prolonged prophylaxis in controls (mean, 5.5 days, range 1–15 days). Cases received cefazolin (n = 2) or amoxicillin/clavulante (n = 1), whereas controls received mainly amoxicillin or amoxicillin/clavulante (78%). The identified pathogens were susceptible to the antibiotic they received as prophylaxis in 2 cases and intermediate resistant in 1 case.

Conclusions: We describe a cluster of 3 cases with early bacterial meningitis after trans-sphenoidal surgery, which occurred in 2010. The following risk factors were identified: visible CSF leakage during surgery, trans-nasal endoscopic approach and short antibiotic prophylaxis (single dose). Further studies are needed to evaluate if longer prophylaxis for patients with intraoperative CSF leakage may prevent meningitis.

Surgical site infections after elective craniotomy: a review of 362 procedures performed in a four-year period (2006–2010)
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Objectives: Nosocomial infections of the central nervous system are a serious complication contributing to morbidity, prolonged length of stay in the ICU and/or hospital, and mortality of neurosurgical patients. The aim of this study was to provide an overview of surgical site infections after elective craniotomies in a tertiary hospital.

Methods: Retrospective study of adult patients who had surgical site infection after elective craniotomy. The study was performed at Hospital
del Mar, Barcelona, between July 2006 and June 2010. Collected data: demographic characteristics, Charlson score, operation type, type of infectious cranial complication, bacterial findings and final outcome. All patients had a minimum follow up of 1 year. In 2009 a bundle of preventive measures was implemented.

Results: During the period of study 362 craniotomies were performed, 38 of which presented an infectious intracranial complication (10.5%): 13 out of 112 in 2007 (11.6%), 10 out of 82 in 2008 (12.2%), and 5 out of 88 in 2009 (5.7%). Among the infectious intracranial complications, 14 (36.8%) had meningitis, 11 (28.9%) surgical wound infection, 8 (21.1%) subdural empyema, 3 (7.9%) epidural empyema and 2 (5.3%) presented cerebral abscess. In patients with meningitis, the most common identified microorganisms were: S. aureus (28.6%), P. aeruginosa (14.3%) and Enterococcus spp. (14.3%). In patients with surgical wound infection, S. aureus (45.5%) was also the most frequently isolated microorganism. All S. aureus isolated were methicillin susceptible. The crude mortality in our study was 5.2%, but the related mortality was 0%.

Conclusions:
1. A high incidence of infectious intracranial complications after elective craniotomies was found in our center. This incidence decreased over this 3-year period due to the implementation of a bundle of preventive measures.
2. Our microbiological data are different from those reported by other studies because we had fewer infections due to S. aureus and P. acnes but we found more infections due to P. aeruginosa, Enterococcus spp. and Enterobacter spp.
3. In our series there were not postoperative infections due to MRSA. This could suggest that the protocol of empirical antibiotic in our institution, which includes MRSA coverage, could be reviewed.

P1649 Analysis of risk factors for surgical site infections after gastric surgery in the Korean Nosocomial Infections Surveillance System (KONIS)
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Background: A nationwide prospective multicenter study using web-based report system was performed to evaluate the risk factors for surgical site infections (SSIs) after gastric surgery in Korea.

Methods: SSI was assessed according to the methods of National Nosocomial Infection Surveillance (NNIS) system. Demographic data, host factors, and perioperative risk factors were collected in 21 hospitals during 2007.7–2009.12. Statistical analyses were conducted to identify variables related to SSIs using MedCalc Ver 11.2.

Results: Among a total of 4,240 cases monitored, 1,486 (35%) were females and median age was 60 years (range 0–96). The 75th percentile (T75) of durations of the operations was 260 minutes. The SSI rates based on NNIS risk index scores were as followings: 2.11% (19/900) in score 0 (laparoscopic surgery), 3.26% (70/2,149) in score 0 (laparotomy), 6.45% (71/1,100) in score 1, and 10.99% (10/91) in score 2 or 3. The univariate analysis showed that longer operative duration (>T75), diabetes mellitus (DM), emergent operation, higher classes of wound, multiple procedures in the same operation, combined infections, perioperative transfusion, reoperation, smoking, recent steroid use, and male gender were significant risk factors for SSIs after gastric surgery. The multivariate analysis revealed that significant risk factors were reoperations, longer operative duration, male gender, DM, and multiple procedures.

Conclusions: Host factors (male gender and DM) and operation-related factors (longer duration of operation, reoperation, and multiple procedures) were significantly associated with SSIs after gastric surgery in Korea.

P1649 Using a data warehouse to predict rates of external ventricular and lumbar drain-related meningitis
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Objective: Use of external ventricular (EVD) and lumbar (ELD) drains is frequently complicated by the development of drain-related meningitis (DRM). Monitoring of DRM rates is important for infection control, but manual surveillance is time-consuming and susceptible to error. We aimed to develop a prediction model to retrospectively detect DRM using data stored in a clinical data warehouse.

Methods: As part of the hospital infection control programme all patients receiving an EVD or ELD (2004 to 2009; n = 742) had been evaluated for the development of DRM. Two infection control professionals performed manual chart review, with adjudication through review in case of disagreement. This categorization was used as reference standard. Children, patients dying <24 hours after drain insertion or with <1 day follow up and patients with infection at the time of insertion or multiple simultaneous drains were excluded. All data on demographics, microbiology reports, clinical chemistry results of blood and cerebrospinal fluid (CSF) and antibiotic use present in the clinical data warehouse were used to develop a model predicting DRM (logistic regression, stepwise backward selection). Missing data were imputed using single imputation. Shrinkage was applied to increase generalizability.

Results: 82 out of 537 included patients developed DRM (13.5/1000 days at risk). The final model used the number of drains placed during admission, drain type (EVD or ELD), blood leukocyte count, C-reactive protein, CSF leukocyte count and culture result, number of antibiotics started during admission and empiric antibiotic therapy. Discriminatory power of the model was excellent (area under the ROC curve 0.97, 95%CI 0.95–0.99). A predicted probability cut-off of 0.10 achieved sensitivity and specificity of 98.8% and 87.5% respectively. Positive and negative predictive values were 58.7% and 99.7% respectively. Overall, predicted yearly infection rates (summed predicted probabilities) concurred with observed infection rates without the need for manual confirmation.

Conclusion: A prediction model based on multi-source data stored in a clinical data warehouse can identify the occurrence of DRM in patients with an EVD or ELD and can accurately quantify incidence rates. This method allows DRM surveillance at the patient level with a 75% workload reduction when limiting manual review to patients identified by the model.
Methods: Prospective cohort study of 2,393 patients who underwent colon surgeries performed by 31 surgeons in 9 secondary and tertiary-care public Swiss hospitals, recruited from a surveillance program for SSI between March 1998 and December 2008 and followed-up for one month after their operation. Risk factors for surgical site infection were identified in univariate and multivariate analyses that included the patients’ and procedures’ characteristics, the hospitals, and the surgeons as candidate covariates. Correlations were sought between surgeons’ individual adjusted risks, their self-reported adherence to guidelines, and the delay since their board certification.

Results: 428 surgical site infections were identified (17.9%) with hospital rates varying from 4% (95% CI: 0 to 8) to 25% (21 to 29) and individual surgeon rates varying from 4% (0 to 8) to 36% (27 to 46). Features of the patients and procedures associated with SSI in univariate analyses were: male gender, older age, higher ASA score and contamination class, longer operation duration, and emergency procedure. Correctly timed antibiotic prophylaxis (within 1 hour) and laparoscopic approach were protective. Multivariate analyses adjusting for these features and for the hospitals found 4 surgeons with higher risks of SSI (OR, 95% CI: 2.37, 1.51 to 3.70; 2.19, 1.41 to 3.39; 2.15, 1.02 to 4.53; and 1.97, 1.18 to 3.30), and 2 surgeons with lower risks of SSI (OR, 95% CI: 0.43, 0.19 to 0.94 and 0.19, 0.04 to 0.81). No correlation was found between surgeons’ individual adjusted risks and their adherence to guidelines or their experience (Spearman coefficients: −0.16, P = 0.39 and −0.20, P = 0.30).

Conclusion: For reasons beyond his adherence to guidelines or his experience, the surgeon may constitute an independent risk factor for SSI after colon surgery. Reliable assessment methods of surgical skills are needed.

**P1651** Persistently bacteremic infective endocarditis

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Objectives: Persistent bacteremic infective endocarditis (PBIE) is defined as persistence of positive blood cultures for 3 or more days despite antibiotic therapy. The reason for persistent bacteremia is deficient or inappropriate selection of antimicrobials, resistant organism, foreign body or prosthetic endocarditis.

Methods: Within 606 cases of infective endocarditis within last 25 years in Slovakia, 85 (14%) of infective endocarditis fulfilled this definition and had positive blood culture for 3 and more days (3 to 7 positive blood cultures). We have compared 85 cases of PBIE to 606 cases of all infective endocarditis from the database of the national survey.

Results: Several risk factors were more frequently observed among persistent infective endocarditis: elderly age (60% vs. 33%; p < 0.01), diabetes mellitus (26.9% vs. 11.4%; p < 0.01), prior cardiac surgery (20% vs. 9.9%; p < 0.05), prior surgery (19.4% vs. 42.7%; p < 0.001), right side (17.6% vs. 11.4%; p < 0.001) and prosthetic valve (32.9% vs. 2.4%; p < 0.001).

Conclusions: In conclusion, diabetes or elderly patients after prior surgery as well as those with prosthetic or right sided infective endocarditis may suffer on PBIE with multiple positive blood cultures. However, risk of embolisation as well as mortality was surprisingly lower among persistent infective endocarditis.

**P1652** Drainage days – an independent risk factor for serious sternal wound infections after cardiac surgery

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Objectives: Postoperative organ/space surgical site infections (SSIs) are potentially devastating, complication following cardiac surgery. Sternal wound infections lead to increased morbidity and mortality. The aim of this study was therefore to determine risk factors associated with the patient’s baseline characteristics, the peri- and postoperative management for the development of SSIs after cardiac surgery involving sternotomy.

Methods: Since 2009, the University Hospital of Basel participates in the national SSI surveillance program with post discharge surveillance, using the Centers for Disease Control and Prevention (CDC) definitions of SSIs. To determine risk factors for SSIs, a retrospective cohort study from 2011 ending in May 2019 was conducted. 30 consecutive patients were included as cases after development of an organ/space SSI after cardiac surgery from January, 2009 to May 2010 involving sternotomy. Control patients after heart surgery involving sternotomy without development of a SSI were matched to cases in 2:1 ratio, according to age, gender, identical kind of heart-surgery, and preoperative length of hospital stay, adjusting for time at risk before surgery. The groups were compared by univariate and multivariable logistic regression analysis.

Results: There were no significant differences between the 30 cases and 60 matched controls regarding age and underlying diseases. Univariate analyses revealed receipt of antibiotics prior to operation and elevated levels of C-reactive protein (CRP) on admission as significant patient-associated risk factors (p = 0.032 and 0.018, respectively). The number of drainages inserted at the operation site, and the duration of their retention, as well as central, peripheral and urinary catheter days, ventilation days, days to the first change of dressings, days on the intensive care unit, and resuscitation during hospital stay, were all significantly associated with SSIs. Drainage days (3.7 versus 2.08) remained highly significant in multivariable logistic regression analysis (p < 0.001).

Conclusion: Receipt of antibiotics prior to cardiac surgery and elevated CRP levels on admission, predispose for the development of a SSI. The duration of retention of drainages (>2 days) at the operative site is an independent risk factor for organ/space SSI after cardiac surgery, involving sternotomy.

**P1653** Shifting sands: a multicentre survey of changes in practices of antibiotic prophylaxis in cardiac surgery across cardiac centres in United Kingdom

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Background: The use of prophylactic antibiotics is shown to decrease the incidence of surgical site infection in several previous studies. However, the lack of national guidelines for cardiac surgery has resulted in marked variation in the choice of agent/s and duration of antibiotic usage across cardiac centres in United Kingdom. Antibiotic stewardship programmes in institutions for reducing healthcare associated infections including MRSA, *Clostridium difficile* and multiresistant organisms have dictated prudent antibiotic use. This survey [joint initiative Cardiac centres Plymouth & Blackpool] included cardiac centres in UK to assess changes in practice of prophylactic antibiotics compared to the previous audit two years ago. Also assess *C. difficile* and MRSA rates and quality of data collection for cardiac centres.

Method: A telephone survey across 36 cardiac surgical units in the UK was conducted. The information regarding the choice, duration and dose of prophylactic antibiotic used for cardiac surgery; MRSA and *C. difficile* infection rates were obtained either from microbiologist or the cardiac surgeon. This was compared to information carried in 2008.

Results: Data from 36 centres is presented. 32 (88.88%) hospitals had unit protocols for antibiotic prophylaxis in cardiac surgery; 12 (37.5%)
units use single agent and the rest 20 (62.5%) use dual antibiotics. The different antimicrobial agents used in varying combinations include flucloxacillin (56.25%), cefuroxime (34.37%), gentamicin (59.37%), co-amoxiclav (9.37%) and teicoplanin (3.12%). The prophylactic antibiotic is administered at induction in 84.37% units and within 1 hr before skin incision in the rest (15.63%). The duration of antibiotic usage was 48hrs (3.12%), 24hrs (75%), 12hrs (6.25%), single dose (9.37%) and during surgery (6.25%) [3 doses given at induction, during bypass and post bypass]. The comparison of antimicrobial use between 2008 and 2010 is shown in the graph below. During 2009–10 the cardiac centres had between 0–2 MRSA bacteraemias and 0–21V. difficile infections. Details to be presented.

Conclusions: There is some evidence of streamlining in the choice of prophylactic antibiotic/s in cardiac surgery with a shift away from broad spectrum cephalosporins. This may be attributed to the concerns and efforts in reducing cephalosporin use across healthcare institutions to contain MRSA and C. difficile infections rates. A marked variation in data collection for cardiac centres was evident.

Chart 1: Comparison of antimicrobial use between 2008 and 2010

Prophylactic antibiotic usage in Cardiac Surgery

| Year | Amoxiclav | Teicoplanin | Cefuroxime | Gentamicin | Co-amoxiclav | Flucloxacillin |
|------|-----------|-------------|------------|------------|--------------|---------------|
| 2008 | 10%       | 5%          | 30%        | 20%        | 15%          | 25%           |
| 2010 | 5%        | 10%         | 20%        | 15%        | 30%          | 15%           |

Prosthetic joint infections

Prosthetic joint infections (PJI) are the most devastating complication of joint replacement surgery and report a considerable disability and cost. Aim of this study was to evaluate the characteristics of patients with prosthetic joint infection and their outcome after treatment.

Methods: In an observational study we included the cases of prosthetic joint infection referred to our division during the last 5 years. PJI was defined by local pain, erythema or tenderness, by radiologic findings, and by positive cultures obtained from a sinus tract or from pus collected from the periprosthetic tissue. Epidemiological, laboratory and microbiologic findings were considered. Cure was defined by clinical and microbiologic evidences, coupled with a negative imaging study or with the absence of scintigraphic examination, as assessed 6 months after the end of therapy.

Results: Seventy-three cases (median age 64 years, range 48–82, males 45%) were observed (hip replacement 33 cases, knee replacement 40 cases). Co-morbidity were reported in 38 (52%) patients, diabetes mellitus, cardiovascular co-morbidities, and chronic liver disease were retrieved more frequently. Twenty-five cases were observed within 3 months from surgical procedure (early infection), and 48 cases were observed after >3 months (late infections). Staphylococcus aureus was identified in 26 (36%) cases (18 strains were merticillin resistant), 16 (22%) cases were sustained by coagulase negative Staphylococci, 6 (8%) by Enterococcus spp, 4 (5%) by Pseudomonas aeruginosa, and 11 (15%) by other bacteria. Polymicrobial infections were reported in 2 (3%) cases. No microbiologic evidence was reported in 8 (11%) cases. All early infections received debridement followed by antibiotic therapy (success rate 96%). Late infections were treated with a two-stage exchange in 14 cases (success rate 86%). The remaining cases received long-term suppressive antibiotic treatment without prosthetic joint replacement, because of refusal of further surgical procedure (12 cases), or because of co-morbidity (22 cases). On treatment infection suppression was reported in 23 (68%) cases.

Conclusion: Prosthetic joint infections are frequently sustained by multi-drug resistant bacteria. Debridement followed by antibiotic treatment is a successful procedure for early infection. Patients with late infections have to be evaluated on the basis of underlying conditions to establish the surgical and antimicrobial approaches.

P1655 Outcome of periprosthetic joint infection caused by rifampin-resistant staphylococci

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Objectives: Periprosthetic joint infection (PJI) caused by rifampin-resistant staphylococci are considered difficult to treat. Their optimal treatment approach is not universally accepted, but most experts recommend a 2-stage exchange of the prosthesis with a long interval (>6 weeks) and without the use of spacer. We investigated the characteristics and outcome of PJI caused by rifampin-resistant staphylococci.

Methods: In a multicentre study (01/2000−12/2010), PJI caused by rifampin-resistant Staphylococcus aureus or coagulase-negative staphylococci (CNS) were retrospectively included. PJI was defined as previously published by Zimmerli et al. (NEJM 2004). Patients were regularly followed at the outpatient orthopedic clinic or were contacted by phone. A relapse was defined as recurrence of PJI with the same organism. A Kaplan-Meier survival and Cox proportional regression analysis were performed.

Results: We included 47 patients (17 females; median age 67y; range 39−88y) with PJI involving the following joints: hip in 28 (60%), knee in 13 (28%), elbow in 4 (9%), shoulder and ankle each in one patient (2%). S. aureus was isolated in 9 (19%), CNS in 38 (81%) cases. Debridement and device retention was performed in 13 cases, 1-stage exchange in 9 cases, 2-stage exchange with short interval (≤6 weeks) in 4 cases, 2-stage exchange with long interval (>6 weeks) in 16 cases, a resection arthroplasty in 4 cases and no surgical intervention in 1 case. The median duration of intravenous antibiotic therapy was 27 days (range 0–102
Multicentre review of prosthetic joint infections treatment and outcomes

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Objectives: Prosthetic joint infection (PJI) remains a devastating complication of arthroplasty. There is significant heterogeneity in treatment approaches to these infections and information on their efficacy relies on single-centre studies. This multi-centre study examines current treatment approaches and outcomes of patients with PJI.

Methods: A retrospective cohort study was conducted over a 3-year period (January 2006–December 2008) involving 10 hospitals in Victoria, Australia. Cases of prosthetic joint infections of hips and knees were identified using an established statewide nosocomial infection surveillance network. Individual medical records were accessed to describe the management and record the outcomes of these patients.

Results: Initial analysis from five hospitals revealed 100 patients with PJI. Polymicrobial infections occurred in 44% of cases. Staphylococci species was the most common pathogen in monomicrobial infections accounting for two thirds of these infections. Debridement and retention (DR) was the most common treatment modality (73%), followed by resection arthroplasty without reimplantation (12%), superficial debridement and antibiotics (8%), two-stage exchange (4%) and one-stage exchange arthroplasty (3%). The timing and number of surgical interventions was however highly variable. Of the patients with staphylococcal infections, two-thirds received a rifampicin-containing regimen. Overall 72% of patients remained infection-free when median follow-up was 12 months, and in almost 2/3 of patients the prosthesis can be retained. There was no significant difference in survival according to treatment modality.

Conclusions: This multi-centre study demonstrates that DR is the favoured treatment modality in Australia with reasonable success. Of note, the management and outcomes of patients markedly differed between hospitals. This study reports real-life management and outcomes from patients at several centres, including many that do not have dedicated research interest in PJI.

Gram-negative prosthetic joint infection

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Objectives: To describe the characteristics of Gram negative prosthetic joint infections (GN PJI).

Methods: All patients with documented GN PJI were analysed from 2003 to 2009. PJI was diagnosed if the same microorganism was recovered from at least 2 joint aspirate or intraoperative tissue cultures or if one intraoperative culture was positive plus clinical evidence of infection. Acute PJI (APJI) was considered if it was diagnosed before 1 month from surgery. The rest were chronic (CPJI). Demographic data, comorbidities, local complications of wound, clinical symptoms, fistula formation, lab and radiologic findings and treatment were collected. An accurate follow up was performed to evaluate the outcome.

Results: 145 episodes of PJI were reviewed. 17 were GN PJI (11.7%). 10 were APJI (6 knee prosthesis and 4 hips) and 7 were CPJI (6 knee and 4 hips). Median age was 71.2 vs 66.8 years. Charlson index of comorbidity was 1.4 in APJI vs 1.5 in CPJI. There were no patients with rheumatoid arthritis nor taking immunosuppressive therapy. Complications of the wound were present in 80% of APJI vs 28% CPJI. Pain was present in 1% APJI vs 71.4%. In CPJI, pain was the main reason for revising the joint. Abnormal radiological findings were seen in 1 patient of each group. Infection was polymicrobial in 40% APJI vs 57% CPJI. Most frequent microorganisms were Enterobacter spp (60% APJI vs 40% CPJI), E. coli (20% APJI vs 14% CPJI), Klebsiella spp (20% APJI vs 28% CPJI) and Pseudomonas spp (10% APJI vs 28% CPJI). None of them were resistant to quinolones. Quinolones were the most used antibiotic in the two groups with a median length of treatment of 5.5 months. 40% APJI need 2-stage replacement vs 57% CPJI. The median of follow up was 2.5 years in the two groups. Only one patient with CPJI was considered as a failure after a 2-stage replacement and a Girldestone was performed. None of the APJI had pain in the follow up vs 70% CPJI. Joint function was normal in both groups. One aseptic loosening was detected in the follow up with scintigraphy in one CPJI patient. No altered lab parameters were detected in the time of follow up after stopping antibiotic.

Conclusions: In GN APJI the first alert symptoms are local complications of the wound. APJI have a good functional outcome in all cases, and in almost 2/3 of patients the prosthesis can be retained. First alarm symptom in CPJI is pain but it doesn’t disappear when the infection does. More studies are needed to understand GN PJI.

Healthcare-associated pneumonia: an attempt to refine the concept

M. Salvadó*, V. Pascual, E. Calbo, N. Freixas, M. Riera, M. Xercavins, J. Garau (Martorell, Terrassa, ES)

Background: A reassignment of the criteria for health care associated pneumonia (HCAP) has been proposed recently in order to reconstruct the classical triad of community acquired pneumonia (CAP), hospital acquired pneumonia (HAP) and pneumonia in immunosuppressed patients (PISP). (Ewig S et al. Rethinking the concepts of community-acquired and health-care-associated pneumonia. Lancet Infect Dis 2010). The aim of our study was to revise the new criteria in a cohort of patients with nosocomial pneumonia.

Methods: From Jan 2004 to Feb 2010 consecutive adult patients with pneumonia were identified in a 500-bed acute care teaching hospital. Patients who resided in a nursing home and those receiving any sort of home care were included in the group of CAP. Any patient with a previous hospitalization was considered as HAP except for immunosuppressed patients that were always categorized in the PISP group. PISP included patients on haemodialysis, with chemotherapy, neutropenia, stem-cell transplantation, HIV/AIDS or iatrogenic immunosuppression.

Data obtained included demographics, co-morbidities (Charlson score), aetiology, severity of disease (Pitt score) and in-hospital mortality.

Results: 306 episodes of BP were identified. CAP was diagnosed in 195 (63.3%) patients, PISP in 84 (27.3%) and HAP in 27 (8.8%). Mean age was 62 in CAP, 64 in PISP and 71 years in HAP (NS). Charlson score was 1.66 in CAP vs. 2.7 in PISP (p<0.001) and 2 in HAP (HAP vs. HAP, NS). Pitt score was 1.22 in CAP vs. 1.3 in PISP (NS) and 1.83 in HAP (NS). S. pneumoniae was isolated in 91%, 62% and 48%, S. aureus in 1%, 5% and 15%, P. aeruginosa in 1%, 20% and 18.5% in CAP, PISP and HAP respectively. The in-hospital mortality rate was 16.5% in CAP vs. 28% in PISP (p=0.03) and 22.2% in HAP (NS).

Conclusions: PISP is similar to HAP in terms of presence of co-morbidities, pathogens and in-hospital mortality.
Ventilator-associated pneumonia rates in three different intensive care units in a tertiary care hospital

M.A. Yetkin, D. Kanyilmaz, P. Onguru, E. Atinci, A. But, F. Alaca Coskun, N. Aksu, H. Bodur* (Ankara, TR)

Objective: Ventilator associated pneumonia (VAP) is the most frequent healthcare associated infection in intensive care units. The aim of this study was to assess the ventilator associated pneumonia (VAP) rates and frequency of nosocomial pathogens in our intensive care units.

Methods: Patient-based active nosocomial infections surveillance in medical intensive care unit (M-ICU), surgical intensive care unit (S-ICU) and medical/surgical intensive care unit (M/S-ICU) has been performed in our hospital since January 2007. Hospital acquired infections were diagnosed according to CDC criteria. Conventional methods and automated systems were used for the identification and detection of the antimicrobial susceptibility pattern of the microorganisms.

Results: In 2007 a total number of 517 patients with a 2164 patient days were hospitalized in M-ICU. Detected VAP rate was 18.22/1000 ventilator days and ventilator utilization rate was 0.4 in this year. Ventilator utilization rate increased and VAP rates decreased in this ICU in the following years and in 2010 the same ratios were 14.87/1000 ventilator days and, 0.51 respectively. During the study period in M/S-ICU, the highest VAP rate (26.70/1000 ventilator days) was detected in the year 2007 whereas the same ratio was decreased to 19.69/1000 ventilator days with approximately the same ventilator utilization ratios. Same as the other ICUs, the highest VAP rate (24.87/1000 ventilator days) was detected in year of 2007 and VAP rates decreased in the following years in S-ICU also.

The most frequently isolated pathogens were Staphylococcus sp., Pseudomonas species and Acinetobacter sp. among patients with VAP all through four years, but significant changes had been detected among the frequencies of the microorganisms. There were a statistically significant increment in the isolation frequencies of Acinetobacter spp. (p < 0.001), whereas isolation rate of Staphylococcus spp. was decreased (p < 0.0001) in 2010 when compared to isolation rates of the same microorganisms in 2007. Isolation frequencies of Pseudomonas sp. did not change through the years.

Conclusion: Although some seems to be a decrease in VAP rates during the following years in all three ICUs, these rates are still higher than NHSN rates. Effective strategies such as continuing educational programs and applying strict infection control practices should be developed to reduce the rate of infection.

Candida sp. colonisation could promote antibiotic-resistant bacteria selection within the airways of the patients with clinically suspected ventilator-associated pneumonia

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Objective: Candida sp. airways colonization has been shown to promote the development of VAP in patients colonized by Pseudomonas aeruginosa, a bacteria prone to become resistant to antibiotics. We address therefore the question of the risk of selecting bacterial resistance within the airways of the patients undergoing mechanical ventilation whether Candida sp. was present or not.

Methods: A prospective one-center observational study including all the patients with clinically suspected VAP over a 5-year period. Every episode was assessed according to the Clinical Pulmonary Infection Score (CPIS). Airways’ specimen were systematically collected for culture on specific medium for yeast isolation.

Results: 323 clinically suspected VAP were recorded. Among these patients, 192 (59.4%) were simultaneously colonized by Candida sp. No difference was found between colonized and not colonized patients regarding baseline characteristics including severity score and prior exposure to antibiotics. The VAP current episode was also found to be similar with regard to clinical data. However, in proportion, P aeruginosa was more frequently encountered in the colonized patients than in the not colonized ones (67% vs. 33%, respectively; p < 0.05). In addition, regardless of the bacterial species, antibiotic-resistant strains were more likely to be recovered from the airways of the patients with Candida sp. colonization than in those without (76% vs. 24%, respectively; p < 0.05). This could account for the increased mortality rate met in this subset of patients.

Conclusion: In our cohort of patients with clinically suspected VAP, airways colonization with Candida sp. is associated with an increased risk of P aeruginosa as well as other antibiotic-resistant bacterial strains isolation, which could participate to worsen the outcome.

Hospital-acquired bacteraemic pneumonia in non-ventilated patients

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Objectives: To evaluate the characteristics, factors related to etiology and prognostic factors of hospital-acquired bacteraemic pneumonia (HABP) in non-ventilated patients.

Methods: Prospective study of 90 consecutive cases of HABP outside the Intensive Care Unit, from 1984 to 2009, in a 350-bed teaching hospital.

Results: Of the 90 patients with HABP, there were 68 males (75%) and 22 females (25%), aged 68±13 yr. The mean length of hospital stay (LOS) until the occurrence of pneumonia was 16±11 days. Chronic obstructive pulmonary disease (43%), malignancy (39%), diabetes mellitus (16%), chronic renal failure (14%) heart failure (13%), and HIV infection (11%) were the main underlying conditions. There were ultimately fatal in 42 cases (47%) and rapidly fatal in 11 cases (12%). The main causative microorganisms were Pseudomonas aeruginosa (39 cases) and Streptococcus pneumoniae (24), followed by Klebsiella pneumoniae (6), Escherichia coli (6), Staphylococcus aureus (5), and Haemophilus influenzae (4). The LOS until the pneumonia was similar in cases caused by P aeruginosa (19±10 days) and S pneumoniae (16±10 days). P aeruginosa infection was associated with previous antibiotic treatment, COPD, and neutropenia (P < 0.001). The empiric antibiotic treatment was appropriate in 75 cases (83.3%). The crude mortality was 48.8%. The factors significantly associated with a worse prognosis were the presence of shock (OR 2.3; 95%CI 1.5–3.3), disseminated intravascular coagulation (OR 1.9; 95% CI 1.4–2.8) and inappropriate antibiotic treatment (OR 1.7; 95% CI 1.1–2.5).

Conclusion: Most cases of HABP in non-ventilated patients are due to P aeruginosa or S pneumoniae, irrespective of the LOS. Previous antibiotic treatment, COPD and neutropenia are significantly associated with P aeruginosa pneumonia. Mortality is high. Shock, disseminated intravascular coagulation and inappropriate antibiotic treatment are adverse prognostic factors.

A study on etiology and drug resistance pattern of ventilator-associated pneumonia in an Iranian 1.000-beds tertiary care hospital

M. Rahbar*, H. Bahrami (Tehran, IR)

Objectives: Ventilator associated pneumonia (VAP) is the most common nosocomial infection in ICUs and making up one-third of the total nosocomial infections. The aim of this study was to determine etiology and drug resistance pattern of most frequency isolates in an Iranian 1000-bed tertiary are hospital in Tehran.

Methods: VAP was defined as any lower respiratory tract infection that developed 48 hour after mechanical ventilation. The criteria for clinical suspicion of pneumonia were as follows: presence of a new or persistent lung opacity on chest radiographic plus two of the following items: fever >37°C, WBC count >10,000/mm³ and Purulent tracheal aspirate. Tracheal Specimens were collected and processed according standard microbiological methods. Bacterial identification and susceptibility testing were performed using standard methods. Demographic date of patients abstracted from their files.
Results: One hindered and one patients developed at least one episode of nosocomial pneumonia. Of 101 patients 61 patients were male and 40 patients female. The mean time for hospitalization in ICUs and ventilation were days were 16 and 9,5 days respectively. Old age, History of previous use of antibiotics and duration of ventilation times were the most important risk factors for VAP. In total 126 microorganisms were isolated from VAP cases. Acinetobacter baumannii with 46 (36.5%) isolates was the predominant organism followed by Staphylococcus aureus with 31 (24.6%). Pseudomonas aeruginosa were accounted 19 (15%) isolates. Other isolated organisms were Klebsiella pneumoniae and E.coli. The majority isolated organism including Acinetobacter baumannii and Pseudomonas aeruginosa were resistant to many antibiotics including third generation of cephalosporins and nearly 50% isolates were resistant to Amikacin. Colistin was the most effective antibiotic against multidrug resistant (MDR) isolates. We found a high rate of resistance among isolates of S.aureus (93.54, %) and S. pneumoniae were MRSA. All isolates of S. aureus were susceptible to vancomycin.

Conclusion: Our study revealed that A. baumannii, S. aureus and P. aeruginosa were the major etiological agents of VAP in our hospital. The majority isolates were resistant to routinely used antibiotics including third generation of cephalosporins. We also observed a high rate of MRSAs among our isolates.

Objective: To identify risk factors for carbapenem-resistance and predictors for mortality

Methods: Retrospective cohort study. Results: Between January 1, 2006 and December 31, 2008 there were 510 patients with K. pneumoniae bacteremia in our hospital. Of this, 317 patients, including 103 patients with carbapenem-resistant K. pneumoniae (CRKP), fulfilled our inclusion criteria and were evaluated. As compared with 214 patients with carbapenem-susceptible K. pneumoniae (CSKP), several characteristics (hematological malignancy, renal failure, chronic liver disease, previous bone marrow transplantation, mechanical ventilation, central vein catheterization, urinary catheterization, hemodialysis, stay in the ICU or in the hematological department and prior antibiotic use) were significantly more common among CRKP. On multivariable analysis, prior use of macrolides (OR 3.3, 95%CI 1.295–8.45; P = 0.012), any antibiotic exposure for ≥14 days (OR 17.3, 95%CI 4.568–65.5; P < 0.001) remained independent factors associated with CRKP. Mortality rate among patients with CRKP was significantly higher than that found for patients with CSKP (45 of 103 patients, 43.7% vs. 62 of 214 patients, 29% respectively, P < 0.001). On multivariable analyses, bed ridden status (OR 2, 95%CI 1–3.8; P = 0.044), chronic liver disease (OR 4.8, 95%CI 1.3–12.8; P = 0.003), Charlson Comorbidity Index ≥5 (OR 6.7, 95%CI 2.4–18.9; P < 0.001), mechanical ventilation (OR 4, 95%CI 1.2–7.2; P < 0.001), and hemodialysis (OR 2.7, 95%CI 1.2–6.2; P = 0.019) remained independently associated with mortality among patients with K. pneumoniae bacteremia.

Conclusion: This study confirms that previous antibiotic exposure is a risk factor for severe infections due to CRKP. Mortality among patients with K. pneumoniae bacteremia is associated with severity of illness and many other well known serious co-morbidities, but not with carbapenem-resistance.

Objective: To assess the frequency, risk factors, and outcome of ventilator-associated pneumonia (VAP) due to methicillin-resistant S. aureus (MRSA) in adult intensive care units (ICUs) in a large teaching institution.

Methods: Comparative study carried out over 4 years in 3 adult ICUs at our hospital. All mechanically ventilated patients with suspicion of VAP were prospectively followed. Clinical and microbiological data were recorded. Patients with confirmed MRSA-VAP were compared with those with bacterial VAP caused by other microorganisms.

Results: Overall, 474 episodes of confirmed bacterial VAP were collected during the study period. The frequencies of pathogens were as follows: P. aeruginosa, 29%; MRSA, 23%; Enterobacteriaceae, 23%; MSSA, 10.5%; Haemophilus spp., 6%; A. baumannii, 4%; S. pneumoniae, 2.5%; and S. maltophilia, 2%. Overall, 32% of VAP were polymicrobial. Differences between MRSA-VAP patients (111) and those with VAP due to other microorganisms (363) were found for median age (68 vs. 62 y, p = 0.003), median Apache II score (12 vs. 11, p = 0.006), previous abdominal surgery at the present admission (35% vs. 19%, p = 0.001), receiving any antibiotic treatment at the present admission before VAP (82.9% vs. 64.5%, p < 0.001), receiving imipenem (24% vs. 11%, p = 0.001), and evidence of pleural effusion on x-ray (12% vs. 5%, p = 0.01). A multivariate analysis of risk factors for MRSA adjusted for age, McCabe/Jackson, Charlson comorbidity, number of antibiotics before VAP, Apache II score, and days from hospital admission to VAP diagnosis showed that emergency surgery during the present admission, prior treatment with imipenem, high Apache II score, and pleural effusion were independently associated with MRSA. As for treatment and outcome, the differences between MRSA-VAP and other VAP were empiric treatment that was not effective against the causative agent of VAP (70% vs. 53%, p = 0.001), severe sepsis (43% vs. 31%, p = 0.04), median cost of antibiotic treatment per episode (€974 vs. €726, p = 0.05), and in-hospital mortality (60% vs. 47%, p = 0.02). In the multivariate analysis, however, MRSA was not found to be an independent risk factor for mortality.

Conclusions: MRSA is the second most common cause of VAP in our hospital. High Apache II score, prior emergency surgery, and antibiotic treatment, especially with imipenem, are predisposing factors. The high mortality of MRSA-VAP was not attributed to the microorganism itself but to underlying conditions.

Objective: Healthcare-associated pneumonia requiring hospitalisation

Methods: All consecutive nonimmunocompromised adults hospitalized with pneumonia were prospectively included from 2001 to 2009. Patients who had recent contact with the health system through nursing homes, home health care programs, hemodialysis clinics, or prior hospitalization were considered to have HCAP.

Results: A total of 2245 patients with pneumonia were hospitalized through the emergency room, of whom 577 (25.7%) had HCAP. Significant differences in causative pathogens were found between...
groups (table). The presence of antibiotic-resistant organisms, including methicillin-resistant Staphylococcus aureus, resistant strains of Pseudomonas aeruginosa, and extended-spectrum β-lactamase producing Enterobacteriaceae, was scarce in all groups. In contrast, aspiration pneumonia was particularly frequent. No differences were found regarding inappropriate initial empirical antibiotic therapy between groups. Overall mortality was higher in patients who attended a hospital or hemodialysis clinic or received intravenous chemotherapy in the 30 days before pneumonia and among patients who resided in a nursing home or long-term care facility.

**Conclusions:** According to our study, most HCAP patients could be treated in the same way as patients with CAP, after carefully ruling out the presence of aspiration pneumonia.

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**Table 1:** Molecular detection of antibiotic resistance

| Group | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 | Group 7 | Group 8 |
|-------|---------|---------|---------|---------|---------|---------|---------|
| CAP   | WAP     | CAP     | WAP     | CAP     | WAP     | CAP     | WAP     |
| Group 1 | 0.125 | 0.0625 | 0.03125 | 0.015625 | 0.0078125 | 0.00390625 | 0.001953125 |
| Group 2 | 0.25 | 0.125 | 0.0625 | 0.03125 | 0.015625 | 0.0078125 | 0.00390625 |
| Group 3 | 0.5 | 0.25 | 0.125 | 0.0625 | 0.03125 | 0.015625 | 0.0078125 |
| Group 4 | 1 | 0.5 | 0.25 | 0.125 | 0.0625 | 0.03125 | 0.015625 |
| Group 5 | 2 | 1 | 0.5 | 0.25 | 0.125 | 0.0625 | 0.03125 |
| Group 6 | 4 | 2 | 1 | 0.5 | 0.25 | 0.125 | 0.0625 |
| Group 7 | 8 | 4 | 2 | 1 | 0.5 | 0.25 | 0.125 |
| Group 8 | 16 | 8 | 4 | 2 | 1 | 0.5 | 0.25 |

**Objectives:** Several carbapenemases, such as the Ambler class A KPC, the class D OXA-48, or the metallo-enzymes (IMP, VIM or NDM-1) have now widely emerged. The latest, NDM-1 is probably the most worrisome, in part due to its gene that is largely encountered in the archetypal F plasmid, indicating p3452 is a member of the IncF group. The remainder of the plasmid encodes a putative novel type IV secretion system, which appears to have been mobilised onto p3452 via a composite IS911 transposon. There are also putative novel fimbrial genes, located between identical IS1 elements, implicating involvement of IS1 in their acquisition. The presence of putative fimbriae indicates a possible role for p3452 in virulence. p3452 could be transferred by conjugation into E. coli DH5α, demonstrating its transfer system is functional. PCR showed a loss of the plasmid in variants of 345−2 carrying both silent antibiotic resistance genes encoded on the IncN plasmid pVE46.

**Results:** DNA sequencing revealed that p3452 is 87.6 kb in size and encodes 84 putative open reading frames. The core replication, transfer and stability functions of the plasmid are encoded in a region approximately 50 kb in size with more than 90% nucleotide identity with the archetypal F plasmid, indicating p3452 is a member of the IncF group. The remainder of the plasmid encodes a putative novel type IV secretion system, which appears to have been mobilised onto p3452 via a composite IS911 transposon. There are also putative novel fimbrial genes, located between identical IS1 elements, implicating involvement of IS1 in their acquisition. The presence of putative fimbriae indicates a possible role for p3452 in virulence. p3452 could be transferred by conjugation into E. coli DH5α, demonstrating its transfer system is functional. PCR showed a loss of the plasmid in variants of 345−2 carrying both expressed and silent versions of the antibiotic resistance plasmid pVE46 indicating that p3452 had become unstable. Simultaneous instability and silencing of unrelated, normally stable plasmids in the same strains suggests the two phenomena may be linked.

**Conclusion:** The cryptic plasmid p3452 from E. coli 345−2 is an F-like plasmid encoding putative virulence functions. It may be linked to the phenomenon of antibiotic resistance gene silencing observed in the strain.

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**Table 1:** Molecular detection of antibiotic resistance

| Group | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 | Group 7 | Group 8 |
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| CAP   | WAP     | CAP     | WAP     | CAP     | WAP     | CAP     | WAP     |
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| Group 2 | 0.25 | 0.125 | 0.0625 | 0.03125 | 0.015625 | 0.0078125 | 0.00390625 |
| Group 3 | 0.5 | 0.25 | 0.125 | 0.0625 | 0.03125 | 0.015625 | 0.0078125 |
| Group 4 | 1 | 0.5 | 0.25 | 0.125 | 0.0625 | 0.03125 | 0.015625 |
| Group 5 | 2 | 1 | 0.5 | 0.25 | 0.125 | 0.0625 | 0.03125 |
| Group 6 | 4 | 2 | 1 | 0.5 | 0.25 | 0.125 | 0.0625 |
| Group 7 | 8 | 4 | 2 | 1 | 0.5 | 0.25 | 0.125 |
| Group 8 | 16 | 8 | 4 | 2 | 1 | 0.5 | 0.25 |

**Objectives:** We have previously reported the naturally occurring silencing of antibiotic resistance genes encoded on the IncN plasmid pVE46 in Escherichia coli 345−2, a wild-type strain of porcine origin. In affected isolates transcription of adaA1, blaOXA-2, sulI and tet(A) was absent but the wild-type genes were retained, following passage through the pig gut. The mechanism by which silencing occurs is unknown. E. coli 345−2 also carries a large cryptic plasmid, named p3452. The aim of this study was to obtain the DNA sequence of p3452, and investigate its possible association with the observed silencing phenomenon.

**Methods:** The complete DNA sequence of plasmid p3452 was obtained from small insert libraries using dye terminator chemistry on ABI3700 automated sequencers and analysed using Artemis and BLAST. The mobility of p3452 was investigated by conjugation using the agar mating method following insertion of a kanamycin resistance marker onto the plasmid using the Tn5 transposon. The presence of p3452 in different variants of E. coli 345−2 was investigated by PCR.

**Results:** DNA sequencing revealed that p3452 is 87.6 kb in size and encodes 84 putative open reading frames. The core replication, transfer and stability functions of the plasmid are encoded in a region approximately 50 kb in size with more than 90% nucleotide identity with the archetypal F plasmid, indicating p3452 is a member of the IncF group. The remainder of the plasmid encodes a putative novel type IV secretion system, which appears to have been mobilised onto p3452 via a composite IS911 transposon. There are also putative novel fimbrial genes, located between identical IS1 elements, implicating involvement of IS1 in their acquisition. The presence of putative fimbriae indicates a possible role for p3452 in virulence. p3452 could be transferred by conjugation into E. coli DH5α, demonstrating its transfer system is functional. PCR showed a loss of the plasmid in variants of 345−2 carrying both expressed and silent versions of the antibiotic resistance plasmid pVE46 indicating that p3452 had become unstable. Simultaneous instability and silencing of unrelated, normally stable plasmids in the same strains suggests the two phenomena may be linked.

**Conclusion:** The cryptic plasmid p3452 from E. coli 345−2 is an F-like plasmid encoding putative virulence functions. It may be linked to the phenomenon of antibiotic resistance gene silencing observed in the strain.

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| Group 2 | 0.25 | 0.125 | 0.0625 | 0.03125 | 0.015625 | 0.0078125 | 0.00390625 |
| Group 3 | 0.5 | 0.25 | 0.125 | 0.0625 | 0.03125 | 0.015625 | 0.0078125 |
| Group 4 | 1 | 0.5 | 0.25 | 0.125 | 0.0625 | 0.03125 | 0.015625 |
| Group 5 | 2 | 1 | 0.5 | 0.25 | 0.125 | 0.0625 | 0.03125 |
| Group 6 | 4 | 2 | 1 | 0.5 | 0.25 | 0.125 | 0.0625 |
| Group 7 | 8 | 4 | 2 | 1 | 0.5 | 0.25 | 0.125 |
| Group 8 | 16 | 8 | 4 | 2 | 1 | 0.5 | 0.25 |
Multicentre evaluation of a new DNA microarray for Enterobacteriaceae

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Objectives: To evaluate the performance of a new commercial ESBL/procarba test (Check-KPC, Check-Points, Wageningen) against a large set of Enterobacteriaceae isolates with characteristic mechanisms of resistance to b-lactams.

Methods: 207 characterized (including bla gene sequencing) Enterobacteriaceae isolates (89 E. coli, 67 K. pneumoniae, 10 C. freundii, 8 P. mirabilis, 7 E. aereogenes, 5 E. cloacae, 21 others) were selected according to their known resistance genes (86 AmpC, 91 non AmpC and non NDM (various ESBLs and KPCs), 15 NDM-1 (including 10 concomitantly NDM-1 and pAmpC-positive isolates), 25 pAmpC progenitors (strains harbouring natural chromosomal AmpC sharing very similar DNA sequences with plasmidic AmpCs). Samples were blindly processed and analyzed for the presence of pAmpC (CMY-2 like, DHA, FOX, ACC, ACT/MIR, CMY-1 like), NDM and also for TEM, SHV, CTX-M groups and KPC which were validated previously. Results were interpreted according to the manufacturer's instructions and they were compared with those obtained by PCR and sequencing.

Results: All of the 86 AmpC-positive isolates (64 CMY-2-like, 3 CMY-1-like, 9 DHA, 7 FOX, 3 ACT/MIR, and the 15 NDM-1) of these co-expressing CMY-2-like were correctly identified by the array (100% sensitivity). Regarding specificity, all 91 strains harbouring resistance mechanisms other than, and not including, pAmpC or NDM-1 (55 TEM; 37 SHV, 19 CTX-M, 3 LEN; 16 KPC), did not yield any positive signal for plasmidic AmpCs nor for NDM-1 (100% specificity). Cross-reactivity was nevertheless observed with chromosomal AmpCs harbouring species known to be progenitors of the AmpCs by escaping the chromosome (cross reactivity for 4/10 C. freundii (CMY-2), 4/4 M. morganii (DHA), 5/6 E. cloacae/asburiae (MIR/ACT), 1/1 chromosomal AmpC gene from E. cloacae cloned in E. coli and 1/2 H. alvei (ACC), 0/2 A. hydrophila (CMY-1)).

Conclusion: The new Check-Points array appears as a robust tool for the detection of pAmpCs and of NDM-1 in addition to other ESBL and KPC encoding genes in Enterobacteriaceae. Detecting these novel resistance determinants in addition to other ESBL encoding genes in complex genetic backgrounds (large number of different b-lactamase genes) provides significant versatility to this system. Cross-reactivity is observed with natural chromosomal AmpC genes of species known as pAmpC progenitors.

Evaluation of a DNA microarray for the rapid detection of extended-spectrum β-lactamases (TEM, SHV and CTX-M), and carbapenemases KPC, OXA-48, VIM, IMP, and NDM

T. Naas*, G. Cuzon, P. Bogaerts, Y. Glupczynski, P. Nordmann (Le Kremlin-Bicêtre, FR; Mont-Godinne, BE)

Objectives: Extended-spectrum β-lactamases (ESBLs) and carbapenemases (CARBA) are reported increasingly in Gram-negative bacilli (GNB) and represent an emerging public-health concern. Laboratory detection of ESBL- and CARBA-producers remains a challenge for the microbiology laboratory and is important to avoid clinical failure due to inappropriate antimicrobial therapy and to prevent nosocomial outbreaks. We evaluated a new molecular diagnostic test for specific identification of TEM-, SHV- and CTX-M-type ESBLs, and of NDM, KPC, OXA-48, VIM and IMP carbapenemase producers.

Methods: We evaluated the “Check-Points ESBL/CARBA array” test (Check-Points, Wageningen, Netherlands) that employs highly specific DNA markers to identify the β-lactamase genes of TEM, SH, CTX-M, NDM, KPC, OXA-48, VIM and IMP, and discriminates between ESBL and non-ESBL TEM and SHV variants. 144 well-characterized Gram-negative rods (Enterobacteriaceae, Pseudomonas sp., Acinetobacter sp.) isolates possessing different bla genes were tested. Several wild-type isolates or isolates harbouring other β-lactamase genes were used as controls. Total DNAs were extracted using Qiagen DNA mini kit.

Results: The “Check-Points ESBL/CARBA array” system identified correctly representatives of the three ESBL gene families tested, including differentiation between non-ESBL and ESBL TEM and SHV variants. In addition, the clinically relevant carbapenemases were also reliably detected. Specificities and sensitivities of 100% were recorded for the blaKPC, blaVIM, blalMP, blaNDM, and blaoXa-48 genes, whereas the sensitivities for blakPC genes was 98%, likely as a result of plasmid instability.

Conclusion: The “Check-Points ESBL/CARBA array” is a powerful high-throughput tool for rapid identification of ESBLs and carbapenemase-producers such NDM in cultures. Due to its rapid performance, this platform could be used in epidemiological or infection control studies in which large collections of isolates need to be characterized.

Bacteriology highlights

Bacteriophage and Helicobacter pylori: an underestimated phenomenon

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Introduction: The recently sequenced strain B45 isolated from a gastric MALT lymphoma patient has a prophage sequence in its genome with 26.6 kb, highly similar to Helicobacter acynonymychsus prophage II. Until now Helicobacter pylori has been described has a species without prophage with the exception of the strain B38 (Thibeje JM et al., BMC Genomics 2010) which contains some prophage remnant sequences. Moreover, the number of reports of H. pylori phases is sparse in the literature.

E. coli cloned in E. coli and 1/2 H. alvei (ACC), 0/2 A. hydrophila (CMY-1)).

Conclusion: The Check-Points array appears as a robust tool for the detection of pAmpCs and of NDM-1 in addition to other ESBL and KPC encoding genes in Enterobacteriaceae. Detecting these novel resistance determinants in addition to other ESBL encoding genes in complex genetic backgrounds (large number of different b-lactamase genes) provides significant versatility to this system. Cross-reactivity is observed with natural chromosomal AmpC genes of species known as pAmpC progenitors.

Evaluation of a DNA microarray for the rapid detection of extended-spectrum β-lactamases (TEM, SHV and CTX-M), and carbapenemases KPC, OXA-48, VIM, IMP, and NDM
IS-pro: fully automated molecular analysis of the human intestinal microbiota

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The human large intestine is one of the most densely populated microbial ecosystems on earth, with bacterial cell counts of up to $10^{11}$ 1/g luminal content. An especially intriguing feature of this ecosystem is its commensalism with the human host. It has been shown that the intestinal microbiota is strictly species specific, implying that host and microbiota have co-evolved for a long time. The recently acquired evolution of a modern ‘westernized’ lifestyle, coincides with a dramatic increase in a number of previously rare diseases, including inflammatory bowel disease, asthma, diabetes mellitus, rheumatoid arthritis, multiple sclerosis and others. Many of these diseases have now been shown to be characterized by an altered intestinal microbiota. Analysis of the exact nature of the often complex changes in the intestinal microbiota may greatly enhance our understanding of the etiology of these diseases. Moreover, as these changes are often disease specific, analysis of fecal microbiota may be used as a non-invasive diagnostic tool. Currently however, techniques employed for these analyses are typically expensive, laborious or both. This has restricted research in this field to small patient numbers and has prohibited implementation of microbiota analysis in clinical diagnostics.

We developed IS-pro: an inexpensive and fast method for high-throughput analysis of the human intestinal microbiota which has been validated in silico, in vitro and in vivo in human samples. The method combines species identification by 16S-23S interspace (IS) length with phylotyping by colour labelling of primers. The entire process of IS-pro consists of a single PCR followed by fragment analysis by capillary gel electrophoresis and automated analysis of digital profiles. For the automated analysis we developed a web-based software tool that first calibrates profiles and identifies peaks and then translates profiles into a list of bacterial species by means of a large library of IS sequence data that we built. An internal amplification control consisting of multiple DNA fragments of varying lengths is used for quality control of the PCR process over the entire range of fragment lengths. IS-pro is currently optimized for the human intestinal microbiota, but may easily be adapted for use in other microbial communities.

P1673 Evolution of a high-prevalent clone of Pseudomonas aeruginosa in a cystic fibrosis patient during chronic infection

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Objectives: Adaptation of Pseudomonas aeruginosa (PA) during chronic lung infection in cystic fibrosis (CF) patients includes conversion to a mucoid phenotype, increased antibiotic resistance and reduced virulence (ref 1). We reported previously on the existence of a high prevalent PA clone, ST406, among Dutch CF patients (ref 2). To investigate adaptation of this clone to the CF lung during chronic infection two ST406 isolates, recovered from the same patient three years apart, were analysed.

Methods: The first ST406 strain was obtained one month after first colonization (2004). The second isolate was obtained after mitomycin or UV induction. On liquid media, mitomycin has a selective advantage over UV, which may explain the diversity of PA strains isolated from chronic CF patients. We developed IS-pro: an inexpensive and fast method for high-throughput analysis of fecal microbiota may be used as a non-invasive diagnostic tool. Currently however, the techniques employed for these analyses are typically expensive, laborious or both. This has restricted research in this field to small patient numbers and has prohibited implementation of microbiota analysis in clinical diagnostics. IS-pro is currently optimized for the human intestinal microbiota, but may easily be adapted for use in other microbial communities.

Methods: The aim of our study was (1) to test the inducibility of the B45 prophage and using mitomycin C or UV, (2) to investigate the prevalence of H. pylori prophage carrying isolates. The reference strain H. pylori 26695 was used a negative control, and the JPI strain which contains an inducible filamentous phage, as positive control (Vale FF et al., Microbe Microanal 2008).

Results: On agar plates, no significant lysis plaque was observed after mitomycin or UV induction. On liquid media, mitomycin has a toxic effect on bacterial cells even at low concentration (0.1 μg/mL). Finally, good results were obtained after UV irradiation of a 24h inoculated broth followed by 24h more incubation. Phages particles were obtained after polyethylene glycol precipitation followed by transmission electron microscopy using negative staining or fixation embedding and sectionning. Phage particles having a structure compatible with the Siphoviridae phage family were observed. Circular phage DNA was recovered after alkalin extraction and detected by PCR. Using a PCR strategy expectantly sensitive, laborious or both. This has restricted research in this field to small patient numbers and has prohibited implementation of microbiota analysis in clinical diagnostics. IS-pro is currently optimized for the human intestinal microbiota, but may easily be adapted for use in other microbial communities.

Conclusion: The B45 strains harbours a temperate phage. This study shows for the first time that bacteriophage can play a significant role in H. pylori genetic diversity. It also opens new exciting ways in the comprehension of the interaction between the bacteria within its environment.

P1674 Identification of a novel Brachyspira species in human intestinal spirochaetosis

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Objective: Infections by the spirochaete Brachyspira are a common cause of intestinal disorders in animals and consequently have been studied extensively in veterinary science. Knowledge of human intestinal spirochaetosis is scarce. It is thought to be caused by two Brachyspira species: Brachyspira aalborgi and Brachyspira pilosicoli. Prevalence rates are estimated between 1–32% in the general population and up to 63% homosexuals. Diagnosis of intestinal spirochaetosis is based on histopathology of colon-biopsies and does not allow for species identification. The aim of this study was to use molecular techniques to identify the infecting Brachyspira species in human intestinal spirochaetosis.

Methods: A specific primerset was designed against a region of the 16S rRNA gene that was both specific for, and conserved within all Brachyspira species. Formalin fixed colon-biopsies were included from histopathology spirochaete positive patients (n=25 patients, n=50 biopsies) and spirochaete negative patients (n=19 patients, n=23 biopsies). All samples were revised by a gastrointestinal pathologist to confirm the diagnosis. DNA was isolated from the biopsy samples with
a Roche MP96 and real-time PCR and SYBR-green melt-curve analysis of the PCR products was performed on a Roche LC480. Specificity of the amplification was confirmed by DNA sequence analysis of the PCR fragments.

**Results:** All colon samples from histopathology positive patients showed amplification of the predicted 136 bp 16S rRNA amplicon, whereas all negative controls did not generate a PCR product. SYBR-green based melt-curves indicated that next to the two Brachyspira species known to infect humans, a third Brachyspira species was occasionally present. Sequence analysis confirmed the presence of single infections with *B. aalborgi* (12/25), *B. pilosicoli* (3/25), and a thus far unreported Brachyspira species (3/25). In addition, there were seven double infections: 3/25 *B. aalborgi* together with the novel Brachyspira species, 2/25 *B. pilosicoli* with the novel Brachyspira species and 2/25 *B. aalborgi* with *B. pilosicoli*.

**Conclusions:** To our knowledge this is the first real-time PCR that allows for immediate detection and species discrimination of *Brachyspira* on routine biopsy materials. The PCR revealed that in human splanchnococci a surprisingly high number of double infections is present, and a thus far unreported Brachyspira species is involved.

**P1675**

**Genotypic detection of rifampicin- and isoniazid-resistant Mycobacterium tuberculosis strains by the pyrosequencing method**

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**Objectives:** Tuberculosis (TB) is a major global health problem, worsened by the emergence of isoniazid (INH) and rifampin (RIF) resistant strains (multi-drug resistant, MDR). The objective of our study was to evaluate a method for molecular monitoring of the most common mutations conferring resistance to INH and RIF in clinical isolates of *Mycobacterium tuberculosis*, based on pyrosequencing techniques.

**Methods:** 72 *M. tuberculosis* (MTC) strains isolated from clinical samples of different patients were selected. The source of the bacteria was either a colony grown on Löwenstein-Jensen or from pelleted MB/BacT® broths. DNA was extracted using a Capture Column DNA extraction kit (QIAGEN) and the NucliSens® easyMAG® system (BioMérieux). For the detection of INH and RIF resistance, we studied mutations in different genes (katG, inhA, ahpC and rpoB) by pyrosequencing and the MTBDR Assay (Hain Lifescience GmbH, Germany). Pyrosequencing was performed in the automated PSQ 96MA 96-well pyrosequencer (Biotage). Preparation of templates and sequencing reactions was performed according to the manufacturer. A cyclic nucleotide dispensation strategy and a sequence analysis (SQA) software were used. The sequences deemed acceptable by the software were submitted as BLAST queries through NCBI.

**Results:** The concordance between the MTBDR assay and the pyrosequencing results was 100%. All susceptible strains showed a wild-type MTBDR hybridization pattern and no mutations were found at the codons studied by pyrosequencing. Three isolates were resistant to RIF and INH (MDR-TB), 2 were high-level INH mono-resistant and 6 were low-level INH mono-resistant. 64 were susceptible to both drugs. For the three isolates resistant to RIF, the rpoB gene mutations found were Ser531Leu, His526Tyr and Asp516Val. A Ser315Thr mutation in katG was identified in 4 of the 8 isoniazid-resistant isolates. Two of these isolates presented mutations in both katG and inhA. The other 6 isolates presented a mutation only in the inhA gene. The inhA promoter gene was the most frequently mutated gene. No mutations in the ahpC gene were detected.

**Conclusions:** Pyrosequencing is a real-time sequencing technique that is fast, reliable and cost-effective for the detection of resistance to RMP and INH in clinical *M. tuberculosis* isolates. Both the MTBDR Assay and pyrosequencing are useful tools for the diagnosis of MDR-TB, but pyrosequencing is more versatile and allows the detection of new mutations.

**P1676**

**Detection of epidemiologically significant organisms (ESO) from surveillance specimens using pooled ESwabs compared to swabs in Amies transport medium**

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**Objectives:** A pilot study comparing 254 single ESwabs to mattress swabs in ATM for detection of methicillin-resistant and -susceptible *S. aureus* (MRSA, MSSA) from nares and axilla indicated that yield from eSwabs fell in between ATM with direct plating and ATM broth enrichment culture. This study compared the yield from pooled nasal (NAS) and rectal (REC) eSwabs (PeS) to individual swabs in ATM.

**Methods:** From Aug-Dec 2010, with ethics approval and informed consent, single NAS and REC swabs in ATM were collected in parallel to PeS from Emergency Room and Inflammatory Bowel Disease Clinic patients. The ATM swabs were plated manually as per laboratory protocol to selective agars for MRSA, MSSA, resistant (R) Gram-negative bacilli (R-GNB) species, and enterococci (VRE). The automated WASP instrument (Copan) plated 30uL of liquid from each PeS to the same media. Incubation and identification methods were standardized.

**Results:** In total, 475 patients consented to parallel specimens. MRSA was identified from 7 patients, of which 5 (11.1%) were detected from NAS-ATM, 4 (0.8%) from REC-ATM and 6 (1.3%) from PeS. Due to the low MRSA prevalence among this population, comparison of swab types was performed using MSSA detection as a proxy. As such, MSSA was identified from 102 (21.5%) NAS-ATM, 37 (7.9%) REC-ATM (112 patients with the NAS-ATM and REC-ATM swab sets combined), while 107 (22.5%) were detected by the PeS, from a total 118 MSSA cases. The difference in overall number of MSSA patients detected between the combined ATM and the PeS was not statistically significant (107 PeS MSSA+ versus 112 ATM MSSA+: Fisher's exact test/two tailed P value = 0.7079). Similarly, there was no statistical difference between detection for R-GNB by the 2 swab systems: REC-ATM and PeS both detected 49 R-GNB, respectively, from 51 positive patients. Of the 51 patients with R-GNB (1 C. freundii, 42 E. coli, 4 K. pneumoniae, 4 P. mirabilis), 47 were identified from both REC-ATM and PeS, while E. coli from 4 remaining patients were identified from REC-ATM (2 isolates) or PeS (2 isolates) only. No VRE were identified from any swab.

**Conclusions:** This study found the WASP planted PeS (nosal plus rectal) to have an equivalent yield of ESO compared to NAS-ATM and REC-ATM separately.

**P1677**

**Stability of Bordetella pertussis and Bordetella parapertussis in the ESwab transport system for culture and PCR**

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**Objectives:** Pertussis (whooping cough) is caused by *Bordetella pertussis* and *Bordetella parapertussis*, with several states in the USA reporting an increased incidence. Laboratory detection methods include culture, direct fluorescence assay, and PCR methods; Successful culture and PCR detection requires preservation of culturable bacterial cells and transport. This study examined the effectiveness of the ESwab system (consisting of liquid Amies transport medium and a flocked nasopharyngeal swab, Copan Diagnostics, Inc.) for maintenance of viability of *B. pertussis* and *B. parapertussis* for culture and preservation of nuclear material for detection by PCR.

**Methods:** Seven Bordetella isolates were tested: including 4 *B. pertussis* isolates (ATCC 9340 strain and 3 recent clinical isolates of *B. pertussis*) and 3 *B. parapertussis* isolates (ATCC 15237 strain and 2 recent clinical isolates). Test methods were based on CLSI M40 guidelines.
Three Bordetella saline suspensions (108, 106, and 104 CFU/mL) were prepared. Each suspension was used to inoculate ESwabs in triplicate. The inoculated ESwabs were stored refrigerated (2–8°C) for up to 96 hours prior to plating. Bacteria from the ESwabs were cultured on Regan Lowe agar plates at 0, 24, 48, and 96 hours of refrigerated storage post-inoculation; plates were then incubated at 37°C in ambient air for a minimum of 4 days. The numbers of colonies at 24, 48, and 96 hours were compared to the 0 hour count to determine percent recovery (viability). Real-time PCR, using primers targeting the IS481 gene of *B. pertussis* and the IS1001 gene of *B. parapertussis*, was performed on 24-hour and 96-hour inoculum in the ESwab.

**Results:** Bordetella was isolated from all of the ESwabs after 96 hours of refrigerated storage. Percent recovery ranged from 36.6% to 96.3% for *B. pertussis*, and from 32.7% to 74.5% for *B. parapertussis*, and was similar across the three inoculum densities for all isolates tested. PCR detected *B. pertussis* and *B. parapertussis* in all of the 24- and 96-hour ESwabs, regardless of initial inoculum concentration (104 or 106).

**Conclusion:** The ESwab maintains sufficient viability of *B. pertussis* and *B. parapertussis* to permit detection in bacteria cultures and preserves DNA integrity for PCR detection, even after 96 hours of refrigerated storage.

**P1678** Preliminary evaluation of a Copan ESwab system for lower respiratory tract samples for microbiological culture

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**Objectives:** To evaluate the ability of a preservative impregnated sponge to preserve the quality of lower respiratory tract samples for accurate quantification and species identification in the microbiology laboratory.

**Methods:** 78 samples (67 bronchial aspirate and 10 bronchoalveolar lavage) were evaluated. Specimens collected into screw cup containers were inoculated over the routine culture solid medium according to laboratory-defined standard procedures. The preservative sponge (Copan Liquid Amies Elution Swab Collection and Transport System, Brescia, Italy) was dipped into the remaining sample to adsorb it, and then the swab applicator was inoculated into Copan liquid amies, mixed using a vortex for 5 seconds and then 10 l of this suspension were inoculated over the routine culture solid medium.

**Results:** Significant growth of pathogens bacteria (\( \geq 10^{5}\-) and \( \leq 10^{5}\) colony-forming units/mL for bronchoalveolar lavage and bronchial aspirate respectively) were evaluated. 77 of the cultures (98.7%) showed equivalent results for both methods. Among the 14 positive case, 92, 8% showed concordance in terms of significant growth and species identification. Only 1 case was not detected in significant growth by Copan ESwab system thus generating a major error in the final result.

**Conclusion:** In this study for detection of patients with potentially significant lower respiratory tract infections, the two system showed a concordance of 92, 8%. Differences in volume inoculated or sampling error might account for the quantitative variation observed in 1 positive culture. The preservative impregnated sponge may offer a simple alternative method for the transport of lower respiratory tract samples for microbiological culture. Nevertheless several studies are needed to address this issue.

**P1679** Comparison of the Copan fecal swab to dry containers for collection and transportation of stool samples for detection of bacteria causing gastrointestinal infections

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**Objectives:** Collection, preservation and transportation of stool specimens is important for the diagnosis of microbes causing gastrointestinal infections. Liquid Base Microbiology (LBMI) is a new concept of liquid specimens transport used with the Walk Away Specimens Processor (WASP). The Fecal Swab (a tube with 2 ml of Cary Blair medium and a flocked Swab) and 2 enrichment broth, a Selenite (SE) and a Rappaport Vassiliadis (RVS) broth, both available in 2 ml tube, are part of the LBM media. The objective of this study was to compare the Copan Fecal Swab (FS) device to dry containers (DC) for the collection, storage and transportation of stools (S) specimens for detection of bacteria, toxins and nucleic acids of gastrointestinal bacteria. To compare the Copan SE and RVS broth to SE broth currently used.

**Methods:** Stool samples (N = 125), collected in FS and in DC, were analyzed as per current laboratory procedures. For the DC 4–5 mg of sample were transferred in SE broth and processed on the ROBOBACT (Diesse) for the detection of *Salmonella* (SA) and *Shigella* (SH). For FS samples, after vortexing, 300 ul of each sample was inoculated into each of 2 ml tube of Copan SE and RVS broths and incubated at 37°C for 18–24h. Both broths for SA/SH culture were loaded on the WASP and inoculated with the 10ul loop on SS, McConkey and SM2 chromogenic *Salmonella* agar plates. Both DC and FS samples for Campylobacter, were passed through a Campylocel microfilter on Skirrow agar. Detection of *Clostridium difficile* (CD) toxin A/B was tested with the Xpect CD Test A/B test (Remel) as per kit procedure. For *C. difficile* culture, samples were shock treated with an ethanol and seeded on CLO selective agar. CD toxin was also tested with the Xpert CD (Cepheid) assay as per kit method.

**Results:** Of the 125 S samples, 72 were negative, 35 were positive for SA group, 7 positive for *C. jejuni*, and 11 positive for CD toxin (11 cultures positive) for the DC samples. For the FS samples, 64 were negative, 40 were positive for SA group, 10 positive for *C. jejuni*, 10 positive for CD toxin (10 cultures positive). Concordant results were found in the detection of SA with the Copan SE and RVS broths.

**Conclusions:** More positive were detected by all testing methods in S samples collected with the Copan FS and shown to be a better device for transporting and storing gastrointestinal tract pathogens. RVS can be used instead of SE, eliminating the problem of SE disposal problems for the laboratory.

**P1680** SL-Solution for pre-treatment of mucus-rich respiratory specimens for culture and molecular assays

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**Objectives:** Respiratory samples represent a significant proportion of routine microbiological specimens and are very important for management of critically ill patients. Copan has developed the SL-Solution (SL); a ready to use mucus dissolving solution that comes in tubes with 1.0 ml. The objective of this study was to compare the performance of Copan SL-Solution to Sputasol (SPU; Oxoid) with microscopical, culture and molecular assays.

**Methods:** In this study were compared 100 specimens, 81 bronchoalveolar-lavages (BAL), 3 bronchial-aspirates (BA) and 16 sputum (SP) for microscopy, UFC and culture inoculation with the Walk Away Specimens Processor (WASP). Forty-five (15 BAL, 15 BA and 15 SP) were also tested for: \( \beta \)-actin gene and 16S rDNA by real time PCR, *C. pneumoniae*, *M. pneumoniae*, *L. pneumophila* with the Duplicate Real Time CP; MP, LP on the Smart Cycler, (Euroclone); *M. tuberculosis* complex with the MTB GeneXpert (Cepheid); MOTT (Mycobacteria other than *tuberculosis*) with the Genotype Mycobacteria Direct (Hain Life Science) Each sample was treated in duplicate, one with SPU after 30 minutes incubation at 350C, the other with SL, 1 ml specimen was added to a SL tube to obtain a 1:1 ratio (sample/SL, with the exception of samples tested on GeneXpert for which a ratio 2:1 is recommended by the manufacturer), vortexed and used immediately. Smears of SL treated samples were prepared immediately (time 0) and after 15 minutes, to evaluate if time could affect the microscopic results. Nucleic acid was extract from all samples with the easyMag (Biomerieux) and the EZ1 Robot (Qiagen). Nucleic acid obtained from all samples was tested for \( \beta \)-actin gene, a portion of the 16S rDNA and for the agents listed in the above assays.

**Results:** SL-samples showed 100% concordance with culture results on the WASP as SPU-samples. 34 samples were positive with significant pathogens and 66 negative. SL microscopy results had 100%
concordance at time 0, but 30% discrepancies in smears prepared after 15 minutes. The UFC results, had 7 discrepant, 6 SL-samples had lower UFCs than same SPU-samples, one had greater UFCs with SL. The molecular results had 100% agreement and no inhibition was present in both samples pre-treatments.

Conclusion: The SL is excellent for recovering microorganisms by culture on the WASP and with molecular assays. Its user friendly, enables rapid and uniform fluidization without pre-incubation, but smear must be prepared immediately.

Cryopreservation of Blastocystis: significance of foetal calf serum
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Objectives: Cryopreservation is defined as keeping living cells frozen with the chance of regaining cellular viability, functions and antigenic structures whenever required, after heating. Blastocystis is a common intestinal protozoon infecting people worldwide with a high prevalence especially among patients with irritable bowel syndrome. Owing to its easy cultivation in vitro, Blastocystis is both an in vitro and in vivo model for intestinal protozoa in laboratory studies. Our aim is to conduct cryopreservation of Blastocystis with different solutions to identify the most appropriate option for the procedure.

Methods: Blastocystis-positive stool samples of six patients were cultivated in Jones medium at 37°C for 48 hours, followed by successive passages every 48 hours. The growth rates and viabilities of Blastocystis were determined by counting on hemocytometer and staining with eosine, respectively. Positive cultures were then centrifuged at 1000 RPM for 10 minutes, and the pellets containing 2 × 10⁷ parasites/ml were suspended in equal volumes of dimethyl sulfoxide (DMSO) and Jones medium, or glycine and Jones medium (50%/50%), or DMSO and fetal calf serum (FCS) (10%/90%), or glycine and FCS (10%/90%). All tubes were kept for 1 hour at +4°C, 2 hours at −20°C, overnight at −86°C and then taken to liquid nitrogen tank for cryopreservation. Control examination was done after 3 months; the ingredients were thawed at 37°C, inoculated in tubes containing fresh Jones medium and their growth rates and viabilities were checked every 48 hours.

Results: A total of 5 × 10⁷ viable Blastocystis were counted both in DMSO/FCS (10%/90%) and glycine/FCS (10%/90%). It was also detected that Blastocystis isolates maintained their viabilities even in subcultures.

Conclusion: Cryopreservation with DMSO/FCS (10%/90%) or glycine/FCS (10%/90%) seems to be an optimal protocol for Blastocystis.

Molecular diagnosis of urogenital infections

P1681 Preliminary evaluation of the HB&L system for the microbiological screening of storage liquids of preserved corneas at 31°C
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Objectives: Aim of this study is to compare the performances of the HB&L with the Bactec system, validated and used in the Veneto Eye Bank Foundation to identify microorganisms contaminating storage liquids of human corneas preserved at 31°C for transplantation.

Methods: Corneas collected within 24 hours from the death after disinfection of the ocular surface are stored at 31°C in preserving liquid. Suitable tissues are transferred into a deturgescent liquid to restore physiological thickness. The microbiological tests by inoculating a sample of liquid in Bactec Peds Plus and Bactec Plus Anaerobic bottles are performed after six days of storage, at the end of storage and after 24 hours of permanence of the cornea in deturgescent liquid. For this preliminary study 1476 samples of preserving and deturgescent liquids were subjected to microbiological control in parallel using the Bactec system and the HB&L system. Samples were incubated for 24 hours in HB&L and for 6 days in Bactec. The positive results were subjected to standard identification procedures.

Results: 23 positive samples (1.6%) and 1453 negatives (98.4%) were detected. Among the positive samples, 7 samples were positive for HB&L+/BACTEC+, 14 samples HB&L+/BACTEC−, 2 samples HB&L−/BACTEC−, 2 samples HB&L+/BACTEC+. Samples HB&L+/BACTEC− are represented by streptococci (3), Candida sp. (2), enterococci (1) and Kocuria sp. (1), in samples HB&L+/BACTEC− have been identified staphylococci (13) and Candida sp. (1) and then the samples HB&L+/BACTEC+ showed the presence of Fusarium (1) and Bacteroides (1).

Conclusion: HB&L+/BACTEC− samples should be considered bacteria present in the cornea, because the organisms isolated were the same in the two diagnostic systems. Samples HB&L+/BACTEC− should be considered as contamination of the sample inoculum, whereas staphylococci were not found in the preserving liquid analyzed with traditional culture. The only exception is the detection of Candida found in HB&L and the preserving fluid, but not in Bactec bottles. The 2 samples resulted BACTEC+/HB&L− were a filamentous fungus and an anaerobic bacterium; this was a supposed limit of the HB&L system because the broth is suitable for aerobic bacteria. Further investigation will be done with new broths that are under development especially formulated for anaerobic bacteria and fungi. The HB&L system could be proposed as a fast test for the detection of the absence of microorganisms in liquid storage of corneas as supporting the existing method.

Molecular diagnosis of urogenital infections

P1683 Diversity of human vaginal bacterial communities in healthy women and bacterial vaginosis
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Background: Balance of microbial flora in the vagina plays an important role in preventing genital tract infections in women, thus an accurate understanding of the composition and ecology of the ecosystem is essential for understanding the aetiology of related diseases. Traditional cultivation technologies are limited for most of the microbial species that reside in the vagina, while the method of construction 16S rRNA library is credible for illuminate the diversity of human vaginal bacterial communities.

Methods: Broad-range polymerase-chain-reaction (PCR) amplification of 16S rRNA and construction of 16S rRNA library were used to analysed the vaginal flora diversity of 20 healthy women and 20 bacterial vaginosis (BV), and Fluorescence in situ hybridization (FISH) performed directly on the vaginal fluid to illuminate the relationship between bacteria and epithelial cells. Clinical manifestation, Amsel criteria and Nugent score were used to strictly distinguished the healthy and BV positive women.

Results: The diversity and kinds of organisms that comprise the vaginal microbial community varied among women. Women without bacterial vaginosis had 1 to 5 vaginal bacterial species (phytotypes) in each sample, as detected by broad-range PCR of 16S rDNA, and lactobacillus species were the predominant bacteria. Women with bacterial vaginosis had greater bacterial diversity, where Gardnerella, Atopobium, Prevotella, Leptotrichia/Sneatha were strongly associated with BV. FISH confirmed that BV associated bacteria detected by PCR corresponded to specific bacterial morphotypes visible in vaginal fluid.

Discussion: Culture-independent methods can provide new insights into the diversity of bacterial species found in the human vagina, and this information could prove to be pivotal in understanding the etiology of BV.

P1684 Diagnostic accuracy of molecular biology in the diagnosis of syphilis: a meta-analysis
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Objectives: Syphilis remains an important sexually transmitted infection (STI) worldwide. However, the diagnosis of syphilis is based on
imperfect serological tests and/or darkfield microscopy which require skilled personnel. Trepomonema pallidum gene amplification by Polymerase Chain reaction (TpPCR) is viewed as an interesting alternative. We conducted a systematic review of published studies assessing the diagnostic performance of TpPCR.

**Methods:** We searched bibliographic databases (Medline, Embase, ISI Web) and abstracts from the main infectious diseases and STI congresses between 1999 and 2009 (ICAAC, ECCMID, ICID, IUSTI). We included all cross sectional and diagnostic accuracy studies with data on the diagnostic performance of TpPCR compared to traditional syphilis tests. Pooled sensitivities and specificities were assessed by syphilis stage and biological specimen using systematically random effects models. Heterogeneity was explored.

**Results:** We identified 55 studies of primary syphilis (1379 cases, 2676 controls), secondary syphilis (411 cases, 296 controls), early syphilis (including primary, secondary and early latent) (670 cases, 1737 controls), neurosyphilis (106 cases, 24 controls), latent syphilis (292 cases, 40 controls) and congenital syphilis (89 cases, 99 controls). Most studies used the 47kDa membrane lipoprotein gene as target (69%) or the porA gene (18%). Patients were more often male (69%) with a median age of 31.5 years, and the prevalence of HIV, when tested, was 38%. In a majority of specimens tested by syphilis stage, the pooled sensitivity was below 50% (Table). A high degree of heterogeneity was detected. Pooled sensitivities were significantly lower in studies conducted in Africa compared to those conducted in North America. Pooled sensitivities from West European countries were systematically higher than those from African countries.

**Conclusion:** The new Bio-Rad Dx CT/NG/MG Assay has been found to be highly performant and very easy to use. We also appreciated the quality of its associated sample collection systems and the simplicity and specificity of the Dx Real-Time System instrument and software. This kit is well suited for the routine simultaneous detection of Chlamydia trachomatis, Neisseria gonorrhoeae and Mycoplasma genitalium in urogenital specimens from asymptomatic and symptomatic patients.

**Methods:** For this prospective study, urogenital and anorectal samples were obtained from symptomatic and asymptomatic patients attending the Alfred Fournier Institute for a STI screening, a medical consultation or a biological check-up, from December 2009 to April 2010. For clinician-collected genital (urethral, endocervical, vaginal) and ano-rectal samples, a Bio-Rad flocked swab was collected in addition to the routine technique’s swab. Urine samples from each patient were dispatched between the different techniques. For each molecular test, DNA was extracted by manual extraction method then amplified according to each manufacturer’s instructions.

**Results:** A total of 1697 specimens from 955 patients (327 men and 628 women) were analyzed. The prevalence of CT, NG and MG was respectively 6.2%, 0.4% and 1.4% in women, and 11.6%, 10.1% and 3.4% in men. For CT, the relative sensitivity of the Dx CT/NG/MG Assay was 98% (50/51) versus Roche and 92.7% (51/55) versus Gen-Probe; the relative specificity was 100% (649/649) versus Roche and 99.9% (953/954) versus Gen-Probe. For NG, the relative sensitivity of the Dx CT/NG/MG Assay was 100% (30/30) versus culture and 90.9% (10/11) versus Gen-Probe; the relative specificity was 99.7% (675/677) versus culture and 100% (998/998) versus Gen-Probe. For MG, all positive samples obtained with the Dx CT/NG/MG Assay were sent to Bordeaux to be tested with an inhouse real-time PCR test: a concordance of 95.8% (22/23) was found.

**Conclusion:** The new Bio-Rad Dx CT/NG/MG Assay has been found to be highly performant and very easy to use. We also appreciated the quality of its associated sample collection systems and the simplicity and specificity of the Dx Real-Time System instrument and software. This kit is well suited for the routine simultaneous detection of Chlamydia trachomatis, Neisseria gonorrhoeae and Mycoplasma genitalium in urogenital specimens from asymptomatic and symptomatic patients.

### Table: Pooled sensitivities of TpPCR by stage and specimen, heterogeneity (For value of Cochran test, I²) and significance of heterogeneity.

| Specimens          | Pooled sensitivity | Pooled specificity | Cochran test | I² | P          |
|--------------------|--------------------|--------------------|--------------|----|------------|
| Blood              | 95.8% (95% CI: 93.8–97.8) | 97.9% (95% CI: 93.0–99.4) | 0.001 | 0% | <0.001    |
| Urine              | 96.0% (95% CI: 93.0–98.6) | 99.0% (95% CI: 97.5–99.9) | 0.001 | 0% | <0.001    |
| Oral secretion     | 93.6% (95% CI: 89.3–97.2) | 97.0% (95% CI: 93.0–99.9) | 0.001 | 0% | <0.001    |

**Objective:** To reduce costs and increase throughput, we developed a quantitative real-time duplex PCR (qPCR) for the simultaneous diagnosis of Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (Ng). The costs being reduced by duplexing, we tested clinical samples for both pathogens in all samples whenever either Ct or Ng was requested by clinicians to measure the added value of such a systematic duplex approach.

**Methods:** The porA region for Ng and the cryptic plasmid for Ct were targeted (Jaton et al., JCM, 2006). The duplex PCR was run on an automated platform (MagnaPure LC (Roche) for the extraction, Tecan liquid handling system for the 384 plate assembly, ABI 7900 (Applied Biosystems) for the TaqMan assay on 11470 clinical samples received in our laboratory (2006–2010). Among these specimens 78% were from women and 22% from men.

**Results:** For 65% of specimens (7408/11470), testing for both Ct and Ng were requested by the clinicians, whereas for 34% (3918/11470) and for 1% (144/11470) only Ct or Ng was requested, respectively. The rate of positivity was 5.8% (661/11470) for Ct and 1.1% (130/11470) for Ng. Twenty-two specimens were Ct+/Ng− (18% women corresponding to n = 4), 639 Ct+/Ng− (70% women, n = 428), 108 Ct−/Ng− (25% women, n = 27), 10701 Ct−/Ng− (79% women, n = 8499). The added value of the dual approach, i.e. a result positive when Ct or Ng was not requested by the clinician was 0.8% (5/661) of positive cases for Ct (3 women) and 9.2% (12/130) of positive cases for Ng (10 women). Thus, among the 144 samples for which Ct was tested but not requested, 3.4% (5/144) were positive whereas among the 3918 samples for which Ng was tested but not requested, 0.3% (12/3918) were positive. Interestingly, among the 31 samples positive for Ng from women, 10 (32%) were not requested by the clinicians.

**Conclusion:** This dual PCR allows high throughput real-time PCR and might be a very useful tool in large epidemiological studies of sexually transmitted infections. Moreover, given the nearly absence of
specific signs and the similar sexual transmission of both agents, it is very difficult to argue to test only one of those pathogens, especially for *C. trachomatis* infection which is commonly asymptomatic and for women regarding *N. gonorrhoeae*.

**P1687** Fully automated microfluidic PCR for the detection of *Chlamydia trachomatis* with the BD MAX™ instrument

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**Objectives:** To transfer our routinely used *Chlamydia trachomatis* (CT) assay to the BD MAX™ instrument (Becton Dickinson) and to validate it for various commonly used transport media and urine. The BD MAXTM is a fully integrated and automated device for the extraction and detection of nucleic acids from up to 24 specimens within about 2.5 hours. It supports fluorescence detection at 2 wavelengths, Cal Red for the process control and FAM for the target sequence. Specimens undergo extraction, purification, amplification and detection using the respective extraction strips and microfluidic cartridges with only two manual pipetting steps (adding specimen to sample prep tube and primer/probe solution to the T1 tube).

**Methods:** Microfluidic PCR in a final volume of 4ul was optimized (primer, probe and MgCl2 concentrations, annealing/extension temperature and time). After LOD determination, the optimized assay was challenged with urgenital specimens (186 Amplicor standard transport medium, STM, Roche; 122 e-Swabs, Copan; 122 native urines) which had previously been analyzed using our home-brew real-time PCR after easyMAG extraction. Total assay and hands-on times were compared for the two methods. In addition, specimens from two different commercial systems (72 Viper and 50 ProbeTec, both Becton Dickinson) in their respective transport systems were analyzed.

**Results:** The assay had an analytical sensitivity of 1 to 10 organisms per PCR and thus was about as sensitive as our routinely used procedure. Only 4/552 (0.7%) clinical specimens were inhibited, i.e. were negative for CT and had a cycle threshold (Ct) of the process control >36. After discrepant analysis, overall sensitivity and specificity were 93.7 and 99.7% with 94.7 and 100% for STM, 100 and 100% for e-swabs, 91.4 and 100% for urines, 100 and 100% for Viper, and 88 and 96% for ProbeTec. False negativity correlated with high Ct values in the reference assays and may have been influenced by the smaller sample volumes tested (100 versus 400ul for transport media and 500 versus 1000ul for urine). As compared to our routine test format, the BD MAXTM had a slightly longer total assay time but a considerably shorter hands-on time.

**Conclusion:** BD MAX™ is an easy to use and fully automated platform for the sensitive and specific detection of CT in various commercial transport media and urine. It can be used as a primary diagnostic tool or as a confirmation test.

**P1688** Vaginal swabs are the optimal sample for screening women for *chlamydial and gonorrheal infection using the Roche Cobas™ 4800 system

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**Objective:** To evaluate the performance of self-obtained and clinical collected vaginal swabs for use with the cobas® 4800 CT/NG assay.

**Methods:** The cobas® 4800 CT/NG assay is a DNA-based amplification assay for *C. trachomatis* (CT) and *N. gonorrhoeae* (NG) performed on a fully automated platform. The assay detects both the wild-type and Swedish-variant forms of CT and has dual NG targets. In a study evaluating the performance characteristics of this assay, women attending STD, family planning, and gynecology/obstetrics clinics, who had provided urine and endocervical swabs were randomized to either self-collection (SC) or clinician collection (CC) of vaginal samples. The infection status of the participant was determined using results of urine and endocervical samples tested with other nucleic-acid based tests (Gen-Probe AC2 and BD ProbeTec). A positive result was required from each of the comparator assays to classify a participant as infected.

**Results:** The overall sensitivity of vaginal swab specimens from 1,722 women was equivalent to other sample types with sensitivity of 94.3% and 100% for CT and NG, respectively. For comparison, the CT sensitivity for endocervical and urine samples was 88.7% and 90.6%, respectively. Interestingly, the sensitivity of SC was higher than that of CC for CT: 98.1% and 90.4%, respectively. For NG, the sensitivity was 100% for both methods of collection. The lowest sensitivity of vaginal swabs (86.4%, n = 22) was seen in clinician-obtained samples collected at Family Planning clinics. In these same clinics, SC swabs had CT sensitivity of 100% (n = 31). The overall specificity was 99.8% and 100% for CT and NG, respectively. The specificity for SC and OC was 99.7% and 99.9%, respectively.

**Conclusions:** Vaginal swabs using the cobas® 4800 CT/NG assay performed as well, or better than, endocervical and urine samples for women. These specimens are simple to collect and transport and may improve clinic flow by reducing the need for speculum-aided pelvic exams in asymptomatic women being screening for these infections. Women may self-sample more vigorously than clinicians resulting in higher sensitivity, thus self-collection of this sample type should be encouraged.

**P1689** Evaluation of Seeplex STD6 ACE detection kit for the diagnosis of six sexually transmitted pathogens

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**Objectives:** Traditionally, the diagnosis of bacterial sexually transmitted disease (STD) has been dependent on the isolation of the causative pathogens by culturing endocervical or urethral swab specimens on selective media. While such procedures typically provide excellent diagnostic accuracy, they are often time consuming and expensive. A multiplex polymerase chain reaction (PCR) assay was evaluated for detection of six STD causative pathogens based on the Seeplex STD detection system.

**Methods:** The Seeplex STD6 ACE Detection (Seegene Inc.) assay employed six pairs of DPO primers specifically targeted to unique genes of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, and *Trichomonas vaginalis*. A total of 347 specimens (155 of cervical swab and 192 of urine) collected for 2 months were tested, and results were compared to those obtained with a combined multiplex PCR.

**Results:** The accuracy was 100% between Multiplex PCR and monoplex PCR assay for both sensitivity and specificity. Furthermore, the presence of two pathogenic bacteria (*C. trachomatis*, and *N. gonorrhoeae*) was tested in 347 clinical samples by Seeplex STD6 ACE Detection and the ProbeTec ET CT/GC test (BD Inc.). The results of these two tests were 99.71% concordant for *C. trachomatis* and 100% concordant for *N. gonorrhoeae*.

**Conclusion:** Multiplex PCR assay using Seeplex STD6 ACE Detection proves to be a novel cost-effective and fast diagnostic tool with high sensitivity and specificity for the simultaneous detection of six STD pathogens.

**P1690** Usefulness of *Neisseria gonorrhoeae* systematic PCR detection on urogenital specimens with the Abbott real-time *Chlamydia trachomatis/Neisseria gonorrhoeae* assay

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**Objectives:** To evaluate the usefulness of *Neisseria gonorrhoeae* (NG) systematic PCR detection on all urogenital samples sent to diagnose a sexually transmitted infection (STI) including *Chlamydia trachomatis* (CT) screening.

**Methods:** From January 1, 2010 to December 16, 2010 an Abbott RealTime CT/NG assay was systematically performed on all urogenital specimens sent to diagnose a STI. Molecular detection of NG was compared with the results of Gram-stained smear and culture.

**Results:** 3989 CT/NG PCR were performed during this period (3311 women and 678 men). 137 specimens were positive for CT (93 women
Comparison of three commercial molecular assays for detection of Chlamydia trachomatis and Neisseria gonorrhoeae

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Objective: Several automated systems to detect Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG) are currently available. Here we determined and compared the performance of the Roche COBAS® AMPLICOR CT/NG Test (CA), the Siemens VERSANT® CT/ GC DNA 1.0 (kPCR) assay and the Abbott RealTime CT/NG assay (M2000).

Methods: Five hundred and three samples (143 urines and 364 swabs) were prospectively collected, and divided into three aliquots that were subsequently used in one of the three assays. Samples positive for Neisseria gonorrhoeae (NG) in the Roche CA assay were confirmed by a cppB/16S real-time PCR as this system is known to report NG false-positives. In case of discrepancies the result found by two of the three assays was taken as the final outcome.

Results: 503 samples were tested for CT in the CA, kPCR and M2000 resulting in 43 positive, 456 negative and 4 discrepant outcomes. Discrepancies observed included 1 sample negative in the CA- but positive in the kPCR and M2000-assay. In addition, three samples were negative in both the CA and M2000-assay but positive in the kPCR-assay. The Ct values of these latter 3 samples reported by the kPCR were above 35 and became negative after retesting.

In addition, 438 samples were tested for the presence of NG with all three assays, resulting in 6 positive and 431 negative outcomes. Furthermore, 1 discrepant result was observed (negative in the CA and M2000 assay but positive in the kPCR assay). However, after analysing the kPCR amplification plot this sample was interpreted as negative.

Conclusion: The three commercial assays have identical performance. However, positive Neisseria gonorrhoeae in the Roche COBAS® AMPLICOR CT/NG Test must be confirmed as this system reports NG false-positives. The VERSANT® CT/GC DNA 1.0 (kPCR) assay has the lowest hands-on time compared to the other two automated systems, therefore the kPCR system is very useful for high throughput molecular diagnostic testing.
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48 assays was performed to further investigate specimens providing discrepant results.

Results: A total of 708 clinical specimens (293 male urines and 415 swab specimens, of which 356 self-collected vaginal swabs, 45 swabs from cervix and 14 swabs from male urethra) were analyzed. The results were concordant in 98.5% of cases (697/708). Out of 708 samples, 50 provided positive results (17 men, 33 women). Three urine specimens and 8 vaginal swabs provided discrepant results. Out of 5 specimens providing positive results in the reference CT assay, 4 were false-negative in the cobas® 4800 CT test. Out of 6 positive results by the cobas® 4800 assay, five were false-positive. After discrepancy analysis, the prevalence of the CT infection was 7.7% (55/708). The specificity and sensitivity of the cobas® 4800 CT/NG test were 92.7% (urine specimens 94.1%, swab specimens 92.1%) and 99.2%, respectively. The 3 false-negative results in swabs could be explained by the procedure not consistent with the manufacturer’s instructions. Indeed, swabs were not inserted directly into the cobas® media vials.

Conclusion: The cobas® 4800 CT/NG test is suitable for high throughput identification of the C. trachomatis infection.

Evaluation of Versant® CT/GC DNA 1.0 assay for the detection of Chlamydia trachomatis and Neisseria gonorrhoeae in urine specimens in selected STD patients

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Objectives: Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (GC) are the two most common sexually transmitted bacterial infections in the developed countries. Since the introduction of nucleic acid amplification tests (NAATs) for CT/GC detection in genital tract specimens, this approach has become the most used diagnostic method. Moreover, urine has also been validated as an adequate specimen for diagnostic testing in many studies. Here we report on the evaluation of a newly installed system for CT/GC detection in urine specimens.

Methods: A total of 700 first void urine specimens were obtained from patients (330 males and 370 females) attending the STD Outpatients Clinic of the St. Orsola University Hospital in Bologna, Italy. Samples were tested by VERSANT® CT/GC DNA 1.0 Assay (Siemens Healthcare Diagnostics Inc., Tarrytown, USA), a multiplex Real-Time PCR assay for simultaneous CT/GC detection. A total of three specimens were collected from each patient, respectively: one urine sample for VERSANT® testing and two urethral or endocervical swabs for the detection of C. trachomatis and N. gonorrhoeae by culture.

Results: Out of the 700 specimens, 83 (11.9%) and 41 (5.9%) were found positive by VERSANT® for CT and GC, respectively. C. trachomatis and N. gonorrhoeae were isolated from 55 (7.9%) and 33 patients (4.7%), respectively. Sensitivity of VERSANT® compared to culture was 100.0%, since no patient was scored positive by culture and negative by VERSANT®. When a urine sample was scored positive by VERSANT®, but the corresponding swab for C. trachomatis culture was negative, an “in house” omp1 Nested PCR assay was performed on remnant VERSANT® extracted DNA. 23/28 samples with discordant results were confirmed positive by Nested PCR. VERSANT® specificity compared to N. gonorrhoeae culture was 98.8%, whereas its specificity compared to C. trachomatis culture was 95.7%. Specificity for CT rose to 99.2% when results obtained by Nested PCR on discordant specimens were considered.

The overall CT prevalence in male patients with symptoms of urethritis was 37.7%, whereas GC prevalence was 29.9%. In this group of patients, 5.2% of the subjects presented CT/GC co-infection. Regarding female patients presenting discharge or dysuria, we noticed a CT prevalence of 27.8% and no GC cases.

Conclusion: VERSANT® CT/GC DNA 1.0 Assay demonstrated to be a highly sensitive and specific technique for CT/GC detection in several groups of patients attending a STD Clinic.

Molecular detection of Chlamydia trachomatis and Neisseria gonorrhoeae in (self)collected vaginal swabs and rectal swabs with the Cobas 4800 system

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Objectives: Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG) are the most prevalent sexual transmitted diseases worldwide. In recent years molecular diagnostics for the standard sample types like cervical swabs and urines has been highly adapted in most laboratories. Currently more difficult sample types, with regard to inhibition, sensitivity and specificity, like rectal swabs and (self collected) vaginal swabs are received more often in the laboratories. This study describes the performance of the new Cobas 4800 for the detection of CT and NG in these difficult sample types.

Methods: A total of 1100 co-collected swabs were tested (900 vaginal; 200 rectal). Informed consent for co-collection was obtained from all patients. All swabs were tested routinely with the M2000 system (Abbott) and the co-collected samples were tested in the Cobas 4800 system (Roche) according to the manufacturers and tested blinded. Discrepant results were retested with an independent real time PCR method.

Results: From 900 vaginal swabs 10% (n = 90) were positive for CT and 0.5% (n = 4) for NG and in 200 rectal swabs 12% CT positives (n = 24) and 6% NG positives (n = 12) were identified. In the vaginal samples 9 discrepant results between the Cobas 4800 and M2000 system were identified. Five with borderline values and 4 with clear different results (1 NG, 3 CT). Two M2000 positive results (Ct 35.7, 32.3) were available for home brew analysis and could not be confirmed. In the rectal samples 8 discrepant results were found including 3 clear differences (2 NG; 1 CT). The CT discrepant sample was positive in the Cobas 4800 system (Cp 33.1) and negative in the M2000 system. For NG 2 samples were negative in the Cobas 4800 system but positive (Ct 23.8; 32.0) in the M2000 system. All 3 clear discrepant results were available for home brew analysis and confirmed the Cobas 4800 results. Conclusion: Both sample types showed a high concordance between the two systems (kappa 0.95 for CT; kappa 0.93 for NG). For detection of vaginal swabs only small differences were found both for CT and NG but these were equally spread between the two systems. There is no difference between self collected and clinician taken vaginal swabs. All clear rectal swab discrepancies confirmed the Cobas 4800 results. This remarkable difference will be further studied. In general, (self collected) vaginal swabs and rectal swabs show reliable results for routine detection of CT and NG.

Evaluation of the Cobas® 4800 CT/NG test using clinician and self-collected vaginal swabs, cervical specimens in PreservCyt Solution, and pharyngeal throat wash specimens

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Objective: To evaluate the limit of detection (LOD), inclusivity, exclusivity, and interfering substances of the cobas® 4800 CT/NG Test using vaginal, PreservCyt, and pharyngeal specimens.

Methods: Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG) cultures were diluted into pools of negative patient specimens at six concentrations to determine the LOD (lowest concentration giving a ⩾ 95% hit rate). A panel of 15 serovars of CT, plus the Swedish variant (mCVT), and 45 strains of NG was diluted into each sample matrix to determine inclusivity. To ensure specificity, a panel of 184 non-CT and non-NG organisms that may be found in the oral or urogenital region was tested at ⩾ 105 CFU or copies/mL. The organisms were diluted into CT/NG positive and negative samples. 10 CT/NG positive and negative samples were spiked with blood (up to 5%), leukocytes (up to 105 cells/mL), cervical mucus, and saliva at concentrations up to 10% to
Comparison of the VERSANT® CT/GC DNA assay and the Evaluation of the new VERSANT® kPCR CT/GC DNA 1.0 Combo2 assay had a significantly higher sensitivity than the VERSANT assay in detecting C. trachomatis in male urine.

Objectives: To evaluate the Siemens VERSANT® CT/GC DNA 1.0 (kPCR) assay for detection of Chlamydia trachomatis and Neisseria gonorrhoeae.

Methods: Aliquots of randomly selected fresh urine specimens were transferred into the Siemens VERSANT® Urine Transport Kit and the Gen-Probe APTIMA® Urine Specimen Collection Kit for Male and Female Urine Specimens. Samples were tested on the VERSANT® kPCR Molecular System with the kPCR assay and the TIGRIS® DTS® Automated Analyzer with the AC2 assay, respectively. Samples with discrepant results were subjected to repeat testing by both methods. Supplemental testing by use of the Gen-Probe single analyte APTIMA assays for individual testing for CT or GC (APTIMA® GC Assay, AGC) on the TIGRIS were performed to resolve discrepant results. Specimens confirmed positive by both methods or with discrepant results resolved by the supplemental testing with a single analyte APTIMA assay were defined as true positives.

Results: A total of 784 male urine specimens was examined of which 120 (15.6%) were positive in kPCR only. Twelve of 22 samples were positive in AC2 only. Ten of 12 'invalid' kPCR tests were repeatedly 'invalid', two repeat tests became positive. Nine of the 12 AC2 discrepant positives were confirmed positive by the repeat test with AC2 and the APTIMA® CT Assay (ACT). None of the 3 kPCR discrepant positives were confirmed by ACT. Thus, the sensitivity of the kPCR assay was 94.7% (124/131) compared to 100% (131/131) of the AC2 assay. The difference was statistically significant ($\chi^2 = 5.28, 0.05 > p > 0.01$). The specificity of the two assays was 99.5% (650/653) for kPCR and 99.5% (650/653) for AC2. Four male urine specimens contained N. gonorrhoeae (0.5%) of which one specimen additionally contained C. trachomatis. The latter was detected by both kPCR and AC2. One of the GC-positive samples was 'invalid' in the kPCR assay but confirmed positive by the AGC assay.

Conclusion: Both methods had a high specificity, but the APTIMA Combo 2 assay had a significantly higher sensitivity than the VERSANT assay in detecting C. trachomatis in male urine.

Evaluation of the Siemens VERSANT® CT/GC DNA 1.0 (kPCR) assay for detection of Chlamydia trachomatis and Neisseria gonorrhoeae

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Objectives: The Siemens VERSANT kPCR system is an automated system which combines extraction of nucleic acids from 96 samples with subsequent real time PCR. The VERSANT CT/GC DNA 1.0 (kPCR) assay detects Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (GC) in a multiplex real time PCR on this system. We compared this assay with the BD ProbeTec™ ET System (PT). In addition we blindly tested approximately 300 urine samples obtained at a different lab.

Methods: Three different sets of samples were tested in the kPCR: (1) PT pretreated samples (for each CT or GC positive sample a negative sample of the same run was also collected) (2) prospectively collected urine samples during routine CT/GC testing and (3) urine samples obtained in a blinded fashion by an external lab facility. The first and second set were tested by kPCR and PT and were used to refine the interpretation of the kPCR. GC positive PT samples were confirmed by real-time PCR targeting the Opa gene. The third set was tested by kPCR and Roche Cobas Amplicor (CA) with GC confirmation by Opa-PCR. Samples were judged with the newly derived rules of interpretations.

Results: Set (1): 308 PT samples were tested in kPCR for CT and GC. 130 were CT positive and 22 were GC positive in both tests. 1 discrepency in CT (positive in PT and negative in kPCR). 1 discrepency in GC, but after confirmation in agreement with kPCR. Set (2): 243 urine samples tested in PT and kPCR with 16 CT positives and 5 GC positives in both tests. In this set two samples were repeated in kPCR as the Ct value was above 35 cycles. Both samples became negative after retesting. With this interpretation rule PT and kPCR were in complete agreement. Set (3): 292 CT samples were tested in CA and kPCR. 27 positives, 260 negatives and surprisingly 5 discrepant results were obtained. After testing these 5 samples by the Dutch CT reference centre all 5 samples were in agreement with kPCR.

267 GC samples were tested in CA with GC confirmation and kPCR. 2 positive and 265 negative results were obtained in both tests.

Conclusion: If the above mentioned rules of interpretation were used the VERSANT CT/GC DNA 1.0 (kPCR) assay demonstrated to be a robust diagnostic tool which is easy to use. In addition no GC confirmation is required.

Evaluation of the new VERSAT® kPCR CT/GC DNA 1.0 assay for detecting Chlamydia trachomatis compared to the Aptima Combo 2 assay

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Objectives: The VERSAT® kPCR CT/GC DNA 1.0 assay is a automated qualitative multiplex assay for the detection of Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (GC) on swab and urine specimens. The purpose of this study was to investigate the analytical and performance characteristics of the fully automated VERSAT® kPCR CT/NG DNA 1.0 assay for detection of Chlamydia trachomatis in comparison with that of the commercially available Gen Probe APTIMA Combo2 assay.

Methods: The routine laboratory performances of the assay were evaluated on a total of 470 clinical specimens. 188 specimens were randomly selected. Then 188 positive specimens: 94 from male and 94 from women (47 urine samples and 47 urethral swabs for each group) were selected on the CT results obtained with the Roche COBAS® AMPLICOR CT/NG assay. These samples were simultaneously tested with VERSAT® kPCR CT/NG DNA 1.0 assay and the Gen-Probe APTIMA COMBO 2 assay performed on the Tigris DTS system. At last, to evaluate a potential cross-contamination during specimen processing, 94 positive and negative specimens (results obtained with the Roche
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COBAS® AMPLICOR CT/NG assay were analyzed in the VERSANT® kPCR instrument in an alternating order. To evaluate the limit of detection (LoD) the QCMD 2009 09B was used and serial dilutions were performed (concentration range between 0.44 and 3560 copies/ml for urine matrix, and 0.47 to 1900 copies/ml for swab matrix). Each concentration was tested twenty times and the LoD was determined by the logistical regression.

**Results:** Concerning random samples, the concordances between the 2 assays were 99.1% and 97.3% respectively for swab and urine specimens. With the positive women samples, concordances were 100%, and with the positive men samples, concordances were 97.9% and 100% respectively for swab and urine. No cross contamination was detected on the positive men samples, concordances were 97.9 and 100% respectively for swab and urine specimens. With the positive women samples, concordances were 100%, and with the positive men samples, concordances were 99.1% and 97.3% respectively for swab and urine specimens.

**Conclusion:** The results obtained from this prospective and retrospective study used the VERSANT® kPCR CT/GC DNA 1.0 assay and the APTIMA COMBO 2 Assay were comparable. The VERSANT® kPCR represents a convenient high-throughput and sensitive system for the routine clinical diagnosis of Chlamydia trachomatis infection. However, we propose to improve the assay by enlarging the grey zone in order to increase the specificity of Versant kPCR CT/GC DNA 1.0 assay.

**Chlamydia trachomatis and other bacteria as aetiological agents to pelvic inflammatory disease by 16S rRNA gene sequencing**

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**Objectives:** The most common causes of pelvic inflammatory disease (PID) are Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (Ng) in which both are easily detected by culture or PCR of genital samples. For other bacterial species it may be more difficult to use cultivation or PCR of genital samples to get a causative agent for PID. In Sweden, laparoscopy is considered as a gold standard for PID-diagnosis. In this study, we have used 16S rRNA gene sequencing from abdominal fluid to identify bacteria that could be associated with PID.

**Methods:** Women undergoing diagnostic laparoscopy due to abdominal pain at Skåne University Hospital in southern Sweden were included in the study. Abdominal fluid from 73 women aged 15−54 years was collected. Of these, 33 patients that were diagnosed as non-PID (endometriosis etc) served as a control group to the remaining 40 PID cases but more than half of the cases were possibly caused by other less established pathogens. No cases of Ng were detected which reflects the low incidence of gonorrhoea in Sweden. As 16S rRNA gene sequencing predominantly found relevant pathogens in PID-cases compared to DNA from normal skin flora of non-PID cases, it is concluded that it may be a complementary diagnostic tool. In cases of mixed infections, Ripsseq is a valuable tool to separate sequences and determine the bacterial species.

**Evaluation of the new Cobas 4800 CT/NG test for detecting Chlamydia trachomatis and Neisseria gonorrhoeae in urogenital swabs and urine specimens**

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**Objectives:** To evaluate the new automatic system cobas 4800 in urogenital swabs and urine specimens in comparison with COBAS AMPLICOR for detecting C. trachomatis (CT) and N. gonorrhoeae (NG) in urogenital swabs and urine specimens in both women and men. Our aim was to compare that urine specimen results to those obtained by urogenital swabs.

**Methods:** A total of 696 clinical specimens for CT and NG testing from both symptomatic and asymptomatic patients were used in this study. These samples comprised 480 urogenital swab specimens (276 endocervical and 212 urethral) and their corresponding self-collected urine specimens (a total of 208 urines: 156 women and 52 men) submitted from the Center of Sexually Transmitted Infections located in Seville, Spain. First, we compared the results of urogenital swabs processed with cobas 4800 CT/NG test (c4800) (Roche Diagnostics) versus those processed with COBAS AMPLICOR CT/NG test (CAM) (Roche Diagnostics). Second, we analysed the results obtained with 208 urogenital swabs specimens with their corresponding self-collected urine samples in c4800. Discordant results were analysed with MultiNA system (Shimadzu Biotech).

SPSS Statistic program v18 (IBM Iberica) were used for the statistical analysis.

**Results:** Data are shown in Table 1. Statistical data for sensitivity (%), specificity (%), positive predictive value (%), negative predictive value (%) and kappa value, respectively, were:

- 1. CAM vs c4800 for CT detection: 77.9, 100, 100, 96 and 0.86.
- 2. CAM vs c4800 for NG detection: 68.3, 100, 100, 97.2 and 0.8.
- 3. c4800 swab specimens vs urine specimens for CT detection: 92.9, 100, 100, 98.9 and 0.96.
- 4. c4800 swab specimens vs urine specimens for NG detection: 100, 100, 100, 100 and 1.

Discordant results were analysed with MultiNA system and all of them were negatives. These data suggest that results obtained by CAM were false-positive results. Besides, we studied that clinical data backed up the MultiNA and cobas 4800 results. For Ng, it is known that false-positive results for other non-pathogenic Neisserias are possible with CAM.

**Conclusions:**

1. We had an excellent agreement between CAM and c4800; so, we could use either in routine workflow.
2. Self-collected urine specimens are as well as urogenital swabs for diagnosing and screening for C. trachomatis and N. gonorrhoeae infections.
3. In this study, no positive cervical specimens for Ng were found, but the results obtained for men would allow the same conclusion.

|                | Cobas AMPLICOR (Swab samples) | Cobas 4800 (Urine samples) |
|----------------|--------------------------------|-----------------------------|
|                | CT    | NG   | CT    | NG   |
| **POS**        | 60    | 0    | 28    | 0    |
| **NEG**        | 17    | 411  | 13    | 447  |
|                | **POS** | 2    | **NEG** | 0    |
| **POS**        | 17    | 411  | 13    | 447  |
| **NEG**        | 0    | 0    | 0    | 0    |
| **POS**        | 17    | 411  | 13    | 447  |
| **NEG**        | 0    | 0    | 0    | 0    |

**Cervical**

|                | c4800 | CAM |
|----------------|-------|-----|
| **POS**        | 31    | 0   |
| **NEG**        | 8     | 227 |
| **POS**        | 31    | 0   |
| **NEG**        | 8     | 227 |

**Urethral**

|                | c4800 | CAM |
|----------------|-------|-----|
| **POS**        | 29    | 0   |
| **NEG**        | 9     | 174 |
| **POS**        | 29    | 0   |
| **NEG**        | 9     | 174 |
**Demonstrating the performance of a fully integrated, low-cost, ultra-rapid PCR device with true point-of-care applications**

M. Green, A. Larry, D. Shenton, D. Pearce (Trowbridge, UK)

**Objectives:** To develop a highly sensitive, ultra-rapid, multiplex PCR method with fully integrated DNA preparation and ambient-stable reagent presentation. To be used in conjunction with a novel electrochemical detection method to demonstrate low copy number amplification and detection in under 20 minutes, for a point-of-care (POC) diagnostic test for Chlamydia trachomatis (CT).

**Methods:** The method employs a custom developed microfluidic PCR card, utilising a thin-film laminate construction, to achieve rapid heat transfer, in conjunction with an ultra-rapid thermocycler. All reagents necessary to perform the extraction, amplification and detection are deposited into the card and air dried, at the point of manufacture. Novel, ambient-stable reagent formulations with an 18 month shelf-life have been developed. A sample is added to the card and DNA extracted from the sample. The resulting eluate reconstitutes the dried PCR reagents and a 40-cycle multiplex PCR is performed using rapid thermocycling in 17 minutes. Amplified target is detected using electrochemically labelled target specific probes and a double-stranded DNA specific exonuclease to release the electrochemical label. Released label is read by applying a voltage to a screen printed carbon electrode, and at a known oxidation potential the label is oxidised producing a measurable current. The unique rapid performance of this device has been demonstrated in terms of analytical sensitivity and reagent stability under ambient storage conditions. Multiplex capability is demonstrated in this test with the presence of the control DNA.

**Results:** Analytical sensitivity of the device was evaluated by testing dilutions of CT DNA in the presence of the control DNA. The results show detection of CT down to 50 copies when co-extracted, amplified in duplex and detected electrochemically, with the control DNA (see graph). Stability tests on the reagents, dried into the device, showed stability for 18 months, stored at ambient temperature (20–25°C). Reagent performance after 18 months storage was shown to be equivalent to performance at time 0.

**Conclusion:** The results show that this device can be used to perform ultra-rapid multiplex PCR with no user intervention after sample addition, allowing minimally trained staff to carry out the assay in under 20 minutes, meeting the need for a ‘true’ POC device. Ambient stability of the reagents negates the requirement for any specialised storage conditions.

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**Spa typing and multilocus variable-number tandem-repeat analysis of methicillin-resistant S. aureus in The Netherlands**

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**Objective:** To elucidate the spread and transmission routes of MRSA an epidemiological typing method is indispensable. Currently Spa typing is one of the methods most frequently applied and has been used successfully in the Netherlands. It is a standard international method.

Multilocus Variable-Number Tandem-Repeats Analysis (MLVA) as described by Schouls et al. is a PCR method based on fragment analysis of eight repeat loci. In this study we compare the results of both methods and their significance in epidemiological typing.

**Methods:** For this study we typed 1047 MRSA isolates sent to our lab for typing. All strains were typed by spa- and MLA typing.

An overall comparison was made between these two typing methods. The most frequent Spa types and MLVA types were investigated in depth i.e. related to their geographical dispersion with help of the geographical tool on the Dutch MRSA website: http://mrsa.rivm.nl

Conclusions and Comments: MLVA typing can sub-divide common spa types significantly and consequently provide a significant understanding of the spread strains. However, MLA typing did not breakdown the animal related spa types t011 and t108.

As with spa-typing, MLA by means of its simplicity is suitable for web based tools to submit data into data bases and to make queries. Subsequently we should aim to build national and international networks using MLA typing method to elucidate the transmission of MRSA.

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**Entamoeba dispar: genetic diversity of clinical isolates-based on four tRNA gene-linked short repeat loci**

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**Objectives:** E. dispers is, to some extent, capable of producing variable focal intestinal lesions in animals and of destroying epithelial cell monolayers in vitro as well as producing amoebic liver abscess in hamsters. However evidence is still insufficient to link E. dispers with human diseases. A recent search for new polymorphic sequences in E. histolytica unearthed a number of loci suitable for strain typing, and revealed a unique gene organization at the same time. These loci are all characterized by the presence of A + T-rich STRs that vary in both number and sequence between strains.

In this study, genetic polymorphism of four tRNA-like STR-containing loci including S-Q, D-A, A-L and R-R were analyzed in order to clarify further the genotypic differences among E. dispers isolates.

**Methods:** A total of 28 E. dispers from gastrointestinal disorder patients were characterized using PCR and sequences methods.

**Results:** Sequence analysis showed 9, 12, 7 and 8 different patterns based on variation of units in this repeat-containing region of R-R, D-A, A-L and S-Q loci, respectively.

**Conclusion:** The results demonstrate an extensive genetic variability among E. dispers clinical isolates. The repeat-containing regions of R-R,
**P1705** Molecular characterisation of high-pathogenicity *Yersinia enterocolitica* biosertype 1B/08 clinical isolates collected in Poland, 2009

K. Zacharczuk*, R. Gierczynski, J. Szych, N. Rokosz, W. Rastawicki (Warsaw, PL)

**Objectives:** *Yersinia enterocolitica*, is one of the major foodborne pathogen that can cause yersiniosis in human. Infections with *Y. enterocolitica* can cause illness ranging from self-limited gastroenteritis, mesenteric lymphadenitis to postinfectious complications such as arthritis and erythema nodosum. Among pathogenic *Y. enterocolitica* predominating types of isolates in Europe belonging to biosertype 4/O3. However, in 2003, the first cases of infections caused by high-pathogenicity *Y. enterocolitica* biosertype 1B/08, were reported in Germany and in Poland. In the following years we observed continuous increase of *Y. enterocolitica* 1B/08 isolations from human in Poland. The highest number of the isolations was in 2009, when 64 clinical isolates of this high-pathogenicity biosertype were collected in our country. We investigated genetic similarity of these isolates and the presence of the major virulence markers including high-pathogenicity islands YSA and HPI.

**Methods:** All tested isolates (n = 64) were assigned to biosotype according to the scheme of Wauters. The serotype was determined by a latex agglutination. Genetic relatedness of the isolates was assessed by pulsed-field gel electrophoresis (PFGE) with XbaI and NotI enzymes, that is the current gold standard for *Y. enterocolitica* genotyping. The presence of virulence genes (ail, ystA, irp1, yst1M, ysrS) was examined by PCR.

**Results:** All the investigated isolates of *Y. enterocolitica* belonged to biosertype 1B/08, and yielded PCR amplicons for virulence determinants: ail, ystA, and for signature genes: irp1, yst1M, ysrS which are associated with the major genomic-islands of the high-pathogenicity *Y. enterocolitica* biosertype 1B. The PFGE profiles were endonuclease specific and homogeneous for the all 64 clinical isolates.

**Conclusion:** The biosertype 1B/08 isolates of *Y. enterocolitica* collected in 2009 in Poland were found to belong to the high-pathogenicity lineage. PFGE analysis revealed that these isolates are highly related and together constitute a single strain sensu stricto. Our results strongly suggest a common source of the isolates.

**P1706** Interlaboratory comparison of DiversiLab® rep-PCR clustering against characterised Acinetobacter baumannii isolates

PG. Higgins*, A.M. Hujer, K.M. Hujer, R.A. Bonomo, H. Seifert (Cologne, DE; Cleveland, US)

**Objectives:** DiversiLab® is a powerful epidemiological typing tool that uses micro-fluidic chips to provide standardized, semi-automated rep-PCR fingerprints, potentially allowing users to archive, compare and share fingerprints. We sought to investigate the reproducibility of DiversiLab® rep-PCR fingerprints between two laboratories with the aim to determine if the fingerprints and clustering are laboratory-specific or portable.

**Methods:** 100 non-duplicate *A. baumannii* isolates were used in this study including representative isolates belonging to *A. baumannii* worldwide clonal lineages 1–8 previously identified using DiversiLab®. A cluster was defined as isolates sharing ≥95% similarity. DNA isolation and rep-PCR was performed separately in the two laboratories. PCR products were run on micro-fluidic chips and analysed in the Agilent 2100 Bioanalyzer. Rep-PCR patterns generated in laboratory A were compared to those from laboratory B. Samples were tested blind. We compared both strain vs. strain and cluster vs. cluster. The Pearson Correlation (PC) and Kullback-Leibler (KL) statistical methods were employed in the analysis.

**Results:** 100 repPCR patterns from laboratory A were compared to 100 patterns from laboratory B. The PC statistical method gave greater similarity than KL and was used for all further analysis. 12 isolates from laboratory A showed >98% similarity with the corresponding 12 from laboratory B and were considered identical. 64 isolates showed 95–97.9% similarity with their corresponding isolates. 23 isolates showed 90–94% similarity with the corresponding isolates, while 1 isolate showed 87.4% similarity and therefore appeared unrelated. In the majority of cases, differences between fingerprints were related to band intensity and not absence of bands. However, intra-laboratory clustering was conserved; isolates that clustered in laboratory A, also clustered in laboratory B. Therefore, clustering was conserved, but fingerprints showed more variability.

**Conclusion:** Our data suggest that individual rep-PCR libraries should be generated in different laboratories to serve as reference standards for regional analysis of outbreaks. Clustering of isolates was reproducible, demonstrating the robustness of rep-PCR. This comparison allows conclusions regarding clonality to be reached independent of the laboratory where the analysis is performed.

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**Table 1. Distribution of pulsortypes and sequence types among VRE isolated from blood.**

| Clone | (Number of Strain) | Variants | Sequence Types |
|------|--------------------|----------|---------------|
| Clone1 | A (12) | ST117 | |
| Clone2 | B (8) | ST280 | ST18 |
| Clone3 | C (2) | ST17 | |
| Clone4 | D (2) | ST17 | |
Multilocus sequence typing used as a tool to confirm the ability of susceptible Helicobacter pylori strains to gain resistance to clarithromycin during eradication therapy

S. Jeverica*, A. Ihan, B. Tepeš (Ljubljana, Rogaška Slatina, SI)

Primary resistance of H. pylori to clarithromycin and metronidazole is the most common reason for eradication failure, followed by poor compliance to therapy and mixed susceptible and resistant H. pylori strain infection. To distinguish between mixed infections and H. pylori switch to resistance phenotype during eradication therapy, we proceeded with multi locus sequence typing (MLST) of H. pylori strains isolated from stomachs of patients before and after eradication therapy.

The study took place from November 2008 to December 2009. We collected H. pylori isolates from gastric biopsies from 133 patients who were never treated for H. pylori before or after antibiotic therapy. Five patients had eradication failure with the first isolate susceptible and second isolate resistant to clarithromycin. To analyse genotypes of first and second H. pylori isolates, we compared H. pylori strain sequences of 7 housekeeping genes with MLST.

Five patients had clarithromycin-resistant H. pylori before eradication therapy and were H. pylori-resistant to clarithromycin after eradication therapy. The sensitive and resistant colonies of each of the H. pylori populations, taken from patients before/after antibiotic therapy, had identical sequence types (ST) obtained with MLST. None of the patients became resistant to clarithromycin during the therapy due to mixed infection with H. pylori. All 5 patients with eradication failure were colonised with the same H. pylori strain before and after therapy. The factors favouring H. pylori survival and switch to antibiotic-resistance during eradication therapy might probably enable milder environmental conditions for H. pylori survival during the therapy.

One of such factors is the ineffective destruction of mucosa-adhered H. pylori by immune cells during therapy which may be due to immune deficit like interferon-γ deficiency or locally induced immune deficit by biologically active H. pylori molecules like strain specific H. pylori lipopolysaccharides.

Evaluation of the performance of the Chlamydia trachomatis detection kit and Ct Genotyping RHA kit compared to the Cobas Amplicor CT/NG in SVS from females visiting a STI clinic

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Introduction: Improving diagnostic methods for the detection of Chlamydia trachomatis (CT), including genotyping, can contribute to control of CT by acquiring knowledge on epidemiology, transmission, sexual networks and pathogenicity. In the present study, we have compared the performance of the Chlamydia trachomatis detection and genotyping (Ct-DT) kit (Labo Bio-medical Products BV, Rijswijk, The Netherlands) with the COBAS Amplicor CT/NG (Roche Diagnostics Systems, Basel, Switzerland) in a well described female population colonised with a sexually transmitted infection (STI) clinic.

Methods: Self obtained vaginal swabs (SVS) were collected from females visiting a STI clinic. The presence of Chlamydia trachomatis DNA was determined by the COBAS Amplicor CT/NG. In agreement with the manufacturer, 200µl of processed COBAS Amplicor CT/NG medium was used for DNA isolation using the Qiaegen DNA mini kit (Qiagen GmbH, Hilden, Germany). For the Ct-DT kit, 10µl DNA was used. All CT positive samples were used for serovar typing. Discrepant samples were retested using COBAS TaqMan CT Test v2.0 (Roche Diagnostics Systems, Basel, Switzerland). A sample was considered CT positive (comparison standard) if both NAAT were positive or if one of these NAAT and the retest was positive.

Results: In all, 772 clients were included in the original study. COBAS medium was available from 71 CT positive clients and 179 CT negative samples were randomly selected. With the Ct-DT kit, 68 out of 71 CT positive samples (97%) tested positive and 1 borderline, leaving 2 discrepant results. Retesting of the latter two samples using the COBAS TaqMan assay resulted in two positive tests. All COBAS Amplicor CT negative samples were also negative with the Ct-DT kit. The sensitivity, specificity, positive and negative predictive value of the Ct-DT kit were 97%, 100%, 100% and 99%, respectively, if the borderline result is included in the positive results. Genotyping results are presented in Table 1. Serovars D/DA, E and F were most prevalent. The serovar distribution is comparable to previously published Dutch data.

Conclusion: Compared with COBAS Amplicor CT/NG, the Chlamydia trachomatis detection and Ct Genotyping RHA Kit combination is a sensitive and highly specific assay to detect Chlamydia trachomatis. Moreover, it is a more rapid and easy to perform method to detect the most commonly detected serotypes compared to PCR-RFLP typing.

Extended multilocus variable-number tandem-repeat analysis of Clostridium difficile

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Objectives: PCR Ribotyping is currently used in the UK for epidemiological investigations to track transmission and identify emerging variants of Clostridium difficile. Although PCR Ribotyping differentiates over 300 types, it is not always sufficiently discriminatory for epidemiological investigations. MLVA is a more highly discriminatory molecular subtyping method which can identify/confirm transmission events. The aim of this study was to identify further novel VNTR loci and evaluate their discriminatory power for prevalent C. difficile ribotypes.

Methods: Eight novel loci were identified, using Tandem Repeats Finder software on the genomes of C. difficile 630 and QCD-32g58, and evaluated alongside seven published loci against a panel of C. difficile. Isolates were selected from a collection of epidemiologically clustered and diverse C. difficile isolates from 10 different PCR ribotypes (001, 002, 012, 014, 015, 017, 020, 027, 078 and 106). The 15 loci were amplified by PCR using fluorescently labelled primers. Four PCR products, each labelled with a different fluorophore, were mixed together with HiDi formamide and LIZ600 size standard (Applied Biosystems). PCR product size was determined by multicoloured capillary electrophoresis using the 3130xl Genetic Analyzer (Applied Biosystems).

Results: When all 299 isolates were analysed using the eight novel作坊Birmingham VNTR loci, each novel locus showed tandem repeat number variability between ribotypes. Isolates were grouped into tight ribotype clusters, with the exception of isolates from two ribotypes, 014 and 020, which clustered together in one group. Using extended MLVA (eMLVA) all isolates clustered in concordance with ribotyping data but were also differentiated within ribotypes.

Conclusion: This study demonstrates that by extending MLVA for C. difficile to the 15 VNTR loci, common ribotypes can be distinguished in a way that mirrors the true phylogenetic structure of C. difficile providing an insight into genetic diversity of the C. difficile population. Clinical isolates also can be meaningfully sub-typed giving results relevant for outbreak investigation and infection control.
Allele-specific real-time PCR for cost-effective canonical SNP genotyping of Bacillus anthracis

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Objectives: We have previously shown the emergence of unique Bacillus anthracis MLVA genotypes in Bulgaria. To further characterize these strains, an Allele-specific Real-time PCR assay (ASRT-PCR) was developed and validated based on 12 standardized canonical SNPs (Van Ert 2007). According to these canSNPs all B. anthracis isolates are grouped into twelve global sub-lineages correlating with geographical and historical distribution of this agent.

Methods: Two forward allele speciﬁc primers with 3’ terminal SNP and a common reverse primer were designed for analysis of each canSNP. The allele-discriminating primes incorporated an additional destabilizing mismatch at the SNP penultimate site which has been reported to result in greater speciﬁcity (Wangkumhang 2007). The type of mismatch was adjusted according to the principle of Little et al. 2001. Primers were tested against available B. anthracis genomes in GenBank as well as with reference strains of various canSNP sub-lineages representative of all possible alleles. The assay was optimized in a closed-tube format as EVA Green Real-time PCR followed by melting curve analysis. ASRT-PCR was applied for genotyping of 51 isolates originating from different regions in Bulgaria previously characterized by MLVA8. Phylogenetic analysis was performed with MEGA4 software.

Results: ASRT-PCR resulted in an unambiguous allele discrimination in each of the twelve target canSNP and all of the tested reference strains were correctly classiﬁed into their respective sub-lineages. By applying a touch-down PCR protocol it was found that not only extensive optimization proved unnecessary but all SNPs could be analyzed with identical PCR conditions. Most of the Bulgarian isolates (50/51) including those represented by unusual MLVA8 genotypes, were classiﬁed in the A.Br.008/009 sub-lineage which is predominant in Europe, Middle East and Asia. Interestingly a single isolate was referred to the A.Br.001/002 sub-lineage which is rare and appears to have either originated and/or expanded signiﬁcantly in China (Simonson et al. 2009).

Conclusions: The ASRT-PCR developed and validated in this study is a robust and cost-effective alternative to the TaqMan™ MGB or hybridization probes for canSNP typing of B. anthracis. The A.Br.008/009 sub-lineage is predominant in Bulgaria but testing more isolates from the Balkan Peninsula where B. anthracis is endemic is necessary to establish the regional population structure.

New approaches to genotyping of clinically relevant bacteria: typing of Escherichia coli by single nucleotide polymorphism pyrosequencing

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We have developed a single nucleotide polymorphism (SNP) scheme based on multilocus sequence typing (MLST) for typing of E. coli clinical isolates. The SNPs are read by pyrosequencing and the sequences are converted into allelic patterns that can be analyzed using the same tools used for MLST studies. With this approach we have studied two collections of clinical isolates of E. coli obtained blood and urine cultures. The two collections show an epidemic structure, with four major complexes (including the international ST131 complex) accounting for nearly forty percent of the isolates. One of the major clones, related to the known CFT073 urapathogenic strain, was signiﬁcantly more abundant among urine isolates. The method is easy to perform, is cheaper than other methods currently in use, it provides an output that is easy to read and interpret, and most important it is a high throughput approach that will be useful for typing of large collections of clinical isolates.
Likewise, pairwise alignment analysis was performed between closely related isolates to identify genomic differences.

**Results:** Whole genome clustering segregated the 50 isolates and reference stnashes into 8 different MapTypes, each corresponding to a different serovar. Of the 34 Saintpaul isolates: 25 were outbreak and 9 non-outbreak. This was highly consistent with PFGE data with one exception, where Optical Mapping grouped a putative non-outbreak isolate with outbreaks. The outbreak isolates were highly similar to each other (<0.2% difference). The outbreaks as a group were 1.2% different from the non-outbreak isolates. Indels represented the major variation between outbreak and non-outbreak.

**Conclusions:** Optical Mapping segregated the isolates into their respective MapTypes, each corresponding to a different serotype. Outbreak and non-outbreak isolates were also differentiated. No significant differences were identified among the outbreaks, whereas numerous differences were observed between outbreak and non-outbreak as well. These differences could not be discerned with PFGE. The clonal nature of the outbreak isolates suggests a single event or common source for the outbreak.

**P1715 Multilocus sequence typing characterisation of Streptococcus dysgalactiae subsp. equisimilis isolates from Portugal**

M. Pinho*, M. Ramirez, J. Melo-Cristino on behalf of the Portuguese group for the study of streptococcal infections

**Objectives:** Streptococcus dysgalactiae subsp. equisimilis is the β-haemolytic Lancefield group C or G streptococcal species most commonly reported worldwide and is increasingly recognized as an important human pathogen. To gain further insights into the clonal structure of S. dysgalactiae subsp. equisimilis population, a recently described multilocus sequence typing (MLST) scheme was used. The current study also aimed to evaluate the congruency of the MLST results with those obtained by other typing methods used in S. dysgalactiae subsp. equisimilis epidemiology.

**Methods:** Thirty-six isolates (28 Lancefield group G and 8 group C) obtained from invasive and non-invasive infections were selected for analysis by MLST. These isolates belonged to a collection of 314 S. dysgalactiae subsp. equisimilis strains associated with human infection in Portugal previously characterized by pulsed-field gel electrophoresis (PFGE) and emm typing. The relationships between the sequence types (STs) as determined by MLST were determined with goeBURST (http://goeburst.phyloviz.net/). Clonal complexes were defined as STs that were linked through single locus variants.

**Results:** A total of 22 STs were found among the 36 isolates analyzed by MLST. They were grouped into 5 clonal complexes and 11 were singleton. The diversity of STs observed was in line with the heterogeneity of PFGE clones (n = 11) and emm types (n = 17) presented by the isolates analyzed. Invasive and non-invasive isolates were not completely separated by any of these techniques. Several emm types were found associated with more than one ST and three STs harboured more than one emm type. The heterogeneous distribution of emm types across the STs was also evident for PFGE clones indicating a low level of correspondence between the three typing methods. In contrast to the situation observed with PFGE and emm, goeBURST analysis showed that distinct STs and clonal complexes were associated with either the Lancefield group C or group G carbohydrates.

**Conclusion:** The MLST analysis of S. dysgalactiae subsp. equisimilis isolates supports the existence of several genetic lineages responsible for human infections in Portugal, in agreement with PFGE and emm typing results. The variability of the MLST groups in terms of PFGE, emm type and group carbohydrates also points to the genetically heterogeneous nature of this streptococcal species.

**P1716 Genetic lineages and plasmid profile of extended-spectrum β-lactamase producing Escherichia coli isolates of food and animal origin**

S. Somalo*, Y. Sáenz, V. Estepa, S. Martínez, L. Vinué, C. Torres (Logroño, ES)

**Objectives:** To determine the genetic lineages and the plasmid content in a collection of Extended-Spectrum-β-Lactamase (ESBL)-producing E. coli isolates recovered in previous studies from food and healthy dog faecal samples.

**Methods:** Fifty isolates were included, 30 of food origin and 20 of faecal samples of healthy dogs. They produced the following ESBLs (number of isolates food/animals): CTX-M-14a (13/5), CTX-M-14b (3/2), CTX-M-9 (4/0), CTX-M-1 (0/3), CTX-M-32 (1/2), and SHV-12 (9/8). Multilocus sequence typing was carried out by PCR and sequencing and plasmid profile was determined by PCR based replica plating. Phylogenetic groups of isolates were determined by PCR.

**Results:** A high diversity of sequence types (ST) was identified among the food (20 different ST) and animal isolates (13 different ST). Six of them were new (ST1911, ST1912, ST1913, ST1914, ST1968, and ST2027). Ten ST were detected in more than one isolate (ST665, ST359, ST224, ST155, ST117, ST10, ST57, ST23, ST1914, and ST1615). The ST665 and ST224 were identified among isolates of both food and animal origin. The ST10 complex (ST10Cplx) (including ST10, ST43, ST176, ST617) was detected in 6 isolates of food and animal origin. The ST23Cplx and ST350Cplx were identified in 4 isolates of animal origin and the ST155Cplx in 2 isolates of food origin. Phylogroups detected among ESBL-positive isolates were (number of isolates food/animal): A (8/8), B1 (13/5), B2 (1/1), D (4/4), and U (4/2). All isolates of the ST10Cplx were ascribed to the phylogroup A. The two isolates typed as ST23 were of phylogroup A and harboured SHV-12. The two isolates of phylogroup B2 produced CTX-M-9 and CTX-M-14b, and were typed as ST770 and ST372, respectively. The plasmid profile of isolates in relation to the ESBL were as follows (replicon type/number isolates): CTX-M-14a (FIB-FII/13; FI1/11; N/5; FC2/2; B/O/10; K/6; Y/1; X/1; P/1); CTX-M-14b (I1/13; FIB-FII/1; FI1/2; Y/1; HI2/1), CTX-M-9 (FIB-FII/2; HI2/1; II2/2; FC2/2; K2/1; A/C/1; B/0/1); SHV-12 (I1/17; FIB-FII/14; FI1/1; K/11; B/0/2; P2; FC2/2; N1/1; Y1/1; T1/1, CTX-M-11 (I3/1; FIB-FII/3; P1/1; K1/1) and CTX-M-32 (HI1/1; K2/2; FIB-FII/2; P2/2, K2/2).

**Conclusion:** A high diversity of sequence types was detected among ESBL-producing isolates of food and animal origin, being those of ST10Cplx the most frequently detected in both origins. Phylogroups A and B1, and FIB-FII and I1 replicon types are mostly found among these isolates.
former included 25 isolates from two epidemiologically linked clusters partially subtyped by PFGE using Apal but differentiated by recently published variable-number tandem-repeat fingerprinting (MLVF) and multilocus variable-number tandem-repeat analysis (MLVA) protocols (J. Clin. Microbiol. 2010. 48:3600–3607). Bacterial cultures were prepared according to a standardized protocol with overnight growth on Trypticase Soy Agar (BD, Franklin Lakes, NJ). Aliquots of washed diluted cultures were dried on fused silica slides and Raman spectra were generated (ca. 2 hrs/24 samples). Using on-board analysis software, similarity dendograms were produced using a squared Pearson correlation coefficient of 99.98 as the cut-off point.

Results: The SpectraCell system identified the EMRSA-15 and EMRSA-16 strain types although in rare instances isolates were grouped outside their respective major strain clusters. The two epidemiologically distinct EMRSA-15 groups were also identified with greater sensitivity and specificity than PFGE but comparable to MLVA and MLVF.

Conclusions: The power of typing and evaluation of the most frequent ribotypes in our country as well as detection of toxin genes by Real-time PCR.

Methods: During the period of November 2008 to March 2010, 65 stool samples were analyzed from patients with mild to severe enterocolitis and history of previous antibiotic therapy. The cases were registered from nine hospitals in Sofia and Plovdiv. Isolation and identification of C. difficile was performed by standard microbiological techniques and latex-co-agglutination test. Production of toxins A and B was analyzed by enzyme-immunoassay (EIA). Detection of toxin genes tcdA, tcdB, cdtA and cdtB (binary toxin) was performed by Eva Green Real-time PCR. PCR-r ribotyping coupled with fragment analysis on capillary gel electrophoresis was applied as a typing method.

Results: The overall C. difficile isolation rate was 23% (15/65). Production of toxin A and B was detected in 21, 5% stool samples by EIA. Three toxigenic variants have been distinguished by Eva Green Real-time PCR: 53.3% (8/15) toxin A+ B+; 20% A+ B+ and 26.7% A− B−. The binary-toxin genes cdtA/B was detected by PCR in one of the tested A+ B+ strains. Six ribotypes were distinguished among the 15 clinical strains. The most prevalent ribotypes were: 017 − 40% (6/15); 002 − 13%; 014/020 −13% and 012, 046, 078 were represented by 7% each. Four of the ribotype 017 isolates originated from a single hospital. Patterns were compared to reference ECDC C. difficile collection. Thirteen percent of C. difficile isolates were correlated to unknown PCR-ribotypes.

Conclusion: Infections of C. difficile are an emerging problem in Bulgaria. The incidence of C. difficile infections increased in the recent years and at least 5 cases led to serious outcome and death. The significant number of cases C. difficile diagnosed with outbreak ribotypes might represent a significant problem in the future. The results of the current study would improve the diagnostic and therapeutic preparedness of the Bulgarian hospitals when dealing with C. difficile infections.

Blastoecystis sp. subtype 4 is common in Blastocystis-positive patients presenting with acute diarrhoea

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Objective: Blastocystis is possibly the most common non-fungal eukaryotic organism found in the human intestinal tract. Extensive genetic diversity within the genus has been demonstrated, and the genus comprises at least 13 subtypes (STs), 9 of which have been found in humans. It is hypothesised that differences in clinical outcome of Blastocystis carriage is linked to differences in genetic makeup. The aim of study was to identify the prevalence of Blastocystis and distribution of Blastocystis subtypes in patients presenting with acute diarrhoea.

Methods: Faecal samples from 444 Danish patients presenting with acute diarrhoea were submitted to standard panels of microbiological analyses and moreover tested for Blastocystis by culture. Positive samples were subtyped by PCR and sequencing. Acute diarrhoea was defined as three or more loose or watery stools per day for less than two weeks. Analysis of subtype frequency distribution was performed using the one-way Chi-Square goodness of fit test and analysis of categorical data was performed using Fisher’s exact test (two-tailed).

Results: A total of 25 patients (5.6%) were positive for Blastocystis by culture and PCR, 19 (76%) of whom were positive for Blastocystis sp. ST4 (P < 0.001). ST2 was seen in 4 patients (16%); ST3 and ST1 were each seen in one patient. All ST4 and ST11 or more were females, a positive test was significantly associated with the female gender (P < 0.01); only one male in the group of patients aged 11 or more was positive for Blastocystis. A total of seven Blastocystis-positive patients were positive for pathogenic bacteria or virus, four of whom were positive for ST4.

Conclusion: Comparing the study population with patients suffering from chronic, HIV-related or travel-related diarrhoea, the overall prevalence of Blastocystis in patients with acute diarrhoea was relatively low, whereas the relative prevalence of ST4 was remarkably high. Hence, since the relative prevalence of ST4 in patients presenting with other types of diarrhoea in Denmark is low, the role of Blastocystis sp. ST4 in the etiology of acute diarrhoea should be investigated further.

Application of three different variable number tandem repeat typing schemes on Staphylococcus aureus within the east and west Midlands

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Objectives: Variable number tandem repeat (VNTR) typing can be performed on several loci to provide a strain specific numerical identifier useful in epidemiological studies such as monitoring transmission within a clinical setting. Currently in Staphylococcus aureus three key schemes have been investigated with varying levels of discrimination shown by each loci. The objectives of this study are to compare these schemes on clinical isolates from the East and West Midlands (UK) and identify the most discriminatory loci.

Methods: A collection of methicillin resistant S. aureus (MRSA) and methicillin sensitive S. aureus (MSSA) were obtained from clinical studies previously performed within the East and West Midlands. Isolates were selected on the basis of distinct PFGE and Staphylococal Interspersed Repeating Unit (SIRU) profiles. Prior to VNTR typing the restriction modification (RM) test was performed on the isolates to determine the clonal complex (CC). Isolates were selected based on CC to be typed by VNTR using the 21 loci from three published schemes. PCR amplification of the VNTR loci were subsequently performed and capillary gel electrophoresis using QIAxcel to determine repeat number.

Results: CC22 (EMRSA-15) is the most frequently observed healthcare associated strain within the Midlands region. From an initial screen of 80 MRSA and 66 MSSA isolates, 28 and 4 isolates respectively were determined to be CC22. The other epidemic strain seen within the Midlands is CC30 (EMRSA-16) and 4 of the MRSA plus 6 of the MSSA isolates were defined as CC30 For development of a calibration panel to compare the VNTR typing schemes only CC22 and CC30 isolates were included from the MRSA. Due to the higher level of variability in SIRU profiles observed within the MSSA, isolates from CC1 (6) CC8 (2) and CC45 (4) were also included. The number of repeating units at each locus was determined on this panel of isolates.

Conclusion: Initial work demonstrates that all three VNTR typing schemes can be used to produce strain specific identifiers important in epidemiological studies and influencing infection control in outbreaks.
The discriminatory power of each locus varies and will be investigated in greater depth in further work. The most discriminatory loci will be selected to be used in a robust typing assay examining the epidemiology of hospital associated S. aureus within the East and West Midlands.

**P1721** Evaluation of DiversiLab system performance for typing of multi-resistant bacterial pathogens

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**Objective:** To evaluate the typing performance of rep-PCR using the semi-automated DiversiLab (DL) system for typing a range of multi-drug resistant (MDR) pathogens commonly recovered in nosocomial infections.

**Methods:** Performances of DL – including reproducibility, typeability, discriminatory power, concordance with pulsed field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) – as well as its epidemiological concordance were evaluated using collection of 160 well-characterised MDR strains of vanA E. faecium (VREF) (n = 12), K. pneumoniae (n = 34), A. baumannii (n = 33), P. aeruginosa (n = 41) and E. coli (n = 40).

**Results:** DL showed excellent performances for typing VREF and K. pneumoniae collections, displaying 100% typeability and reproducibility, a discrimination index (DI) >95% and >99% concordance with PFGE and MLST. Good performance values were also observed for other species except that (i) DL presented DI <95% for A. baumannii and E. coli collections (93.9% and 92.6%, respectively) mainly due to clustering of isolates belonging to distinct PFGE/MLST types into the same DL type and (ii) DL showed poor concordance with PFGE (87.9%) and MLST (78.6%) regarding P. aeruginosa because isolates belonging to clone ST235 were classified into distinct DL types. Excellent (100%) epidemiological concordance was observed, as all outbreak-associated isolates clustered in identical DL types, distinct from unrelated isolates of the same species.

**Conclusions:** DL revealed to be a reliable typing tool for outbreak investigation but was generally less discriminant than PFGE analysis. Due to problems of concordance between the two methods, MLST type should not be infer from DL type for P. aeruginosa.

**P1722** Molecular epidemiology of human listeriosis in Andalucía, Spain

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**Objectives:** The aim of this study was to perform a retrospective study genotyping 154 isolates from human listeriosis cases occurred in the region of Andalusia (southern Spain) in the period 2005–2009.

**Methods:** Serotyping was performed for 1 and 4 somatic antigens using commercial Listeria antisera, and by multiplex-PCR serogrouping according to the method described by Doumith et al. (2004). PFGE was performed according to the PulseNet protocol with the Apal enzyme. The similarity of PFGE profiles was evaluated using the BioNumerics software. The multiplex PCR protocol described by Chen and Knabel (2007) was used for the identification of isolates belonging to L. monocytogenes ECI, ECH, and ECII epidemic clones.

**Results:** The 154 isolates were grouped into four serotypes: 4b [94 (61.3%) ] strains, 1/2b [30 (19.5%) ] strains, 1/2a [27 (18%) ] strains and 1/2c [3 (2%) ] strains. Sixty-two Apal distinct pulstypes were recognized among the 154 human isolates. Thirty-seven isolates (24%) showed unique Apal pulstypes, and the remaining 117 strains (76%) were assigned to 25 Apal clusters, 60% in clusters of more than two isolates. Sixty-nine (45%) of the strains are included in six PFGE types. Regarding the unique pulstypes, they accounted for 24% of the strains and 60% of the pulsestypes: 49% of serogroup 4b, 27% of serogroup 1/2b and 24% of serogroup 1/2a.

The EC markers were found in 62 (40.3%) L. monocytogenes isolates tested. The ECI marker was present in 43 (46.2%) 4b serotype isolates, ECII in 10 (10.7%) 4b serotype isolates, and ECIII in 9 (33.3%) 1/2a serotype isolates.

**Conclusions:** A large proportion of the human listeriosis cases under investigation could be grouped into molecular subtype clusters, and our cases may there be related to international food borne outbreaks, involving both the United States and European countries. In conclusion, this is the first large Spanish molecular epidemiology study of human listeriosis and the results obtained could be helpful in reinterpretting the epidemiology of L. monocytogenes in southern Spain.

**P1723** Comparison between automated rep-PCR and MALDI-TOF for molecular typing of AmpC-producing Proteus mirabilis

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**Objectives:** The clinical relevance of Proteus mirabilis infection is increasing as well as the resistance of this bacterium against antimicrobial treatments. We studied the clonal relationship among AmpC-producing P. mirabilis from nosocomial and community source in the Health Area of University Hospital Complex of Santiago de Compostela (Spain). Results obtained by automated rep-PCR and whole-cell matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) are compared.

**Methods:** Isolates were obtained from routine cultures at the University Hospital Complex of Santiago de Compostela from 2006 to 2009. Only one isolate per patient was considered for analysis. Species identification was performed by both, Vitek 2 System and MALDI-TOF MS. Antimicrobial susceptibility testing of isolated bacteria was assayed using Vitek 2 System. Isolates showing intermediate or total resistance to amoxicillin-clavulanic, cefotaxime or ceftazidime according to CLSI breakpoints were selected for screening of AmpC production by the double-disk synergy test with phenyl boronic acid, third generation cephalosporins and cloxacillin. Multiplex PCR for identifying family-specific AmpC [l]-lactamase genes were used.

Molecular-epidemiological analysis of the strains was performed by both, automated rep-PCR and MALDI-TOF MS AXIMA (SARAMIS).

**Results:** DiversiLab analysis results detected great variability among bacterial isolates. The AmpC-producing P. mirabilis isolates (35 in total) showed 29 different banding patterns. Isolates from community source clustered near in the dendrogram. The strains with lower similarity coefficient (<70%) were from community home patients. The comparison among spectra obtained by MALDI-TOF analysis also showed the proximity of the community strains in the dendrogram (similarity coefficient >70%). The indistinguishable/similar isolates according to DiversiLab criteria also were located nearby in the dendrogram constructed by SARAMIS software. However, the more divergent strains mismatch between both methods.

**Conclusions:** MALDI-TOF MS for species identification and automated rep-PCR (DiversiLab System) for clonal strain typing are sensitive and reproducible techniques which combined use may be a useful tool for fast and accurate molecular-epidemiological analysis of bacterial outbreaks. However, further studies should be designed to establish the correlation between genomic and proteomic methods.

**P1724** Epidemiological typing of Clostridium difficile isolates from China

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**Objectives:** Whilst Clostridium difficile infection (CDI) is well recognised in Europe and North America there are few reports from Asia, mainly from Korea. In China CDI has been reported only from Shanghai. We have surveyed a large teaching hospital in Changsha, Hunan, 550 miles from Shanghai for CDI and collected clinical/epidemiological data
and applied PCR ribotyping and multilocus variable-number tandem-repeat analysis (MLVA) subtyping.

**Methods:** Twenty-one isolates from April 2009 to February 2010 were obtained from 70 patients at Xiangya Hospital with symptoms of CDI. Twenty-one isolates from April 2009 to February 2010 were obtained from 70 patients at Xiangya Hospital with symptoms of CDI. Re-identification according to the new taxonomy and speciation of organisms. However, some bacteria, such as *Brachyspira*, *Brucella* and *Mycoplasma* species, are poorly represented by some commercial databases but are important human and animal pathogens. The aim of this study therefore was to determine if MALDI-ToF was a useful tool in the identification of *Brachyspira*, *Brucella* and *Mycoplasma* species to species level.

**Methods:** Isolates were obtained from collections held at the Veterinary Laboratories Agency (VLA), Weybridge, UK and at Kingston University, Kingston-upon-Thames, UK. Standard Bruker protocols were used for analysis of bacteria spotted directly to the target plate and also for ethanol/formic acid extracts. Samples were analysed using a Bruker Autoflex 2 MALDI-ToF machine. MSP (mass spectral projection) reference spectra and dendrograms were created using MALDI Biotyper software (Bruker Daltonics). The MSP reference spectra were then added to the Bruker standard database and used to identify known and unknown strains.

**Results:** The same *Brachyspira* and *Mycoplasma* (Figure 1) species clustered together, but were also suitably distinct other species to make identification to the species level possible for these two genera of bacteria using MALDI Biotyper software. Different *Brucella* species were not in distinct clusters and it was not possible to reliably identify species from each other by the standard algorithm. However, it was possible to distinguish them from other bacteria, based on the scoring given by the MALDI Biotyper software. In particular, some species of *Brucella* may be difficult to distinguish from *Ochrobactrum* species by standard methods, but the MALDI-Biotyper scores showed the *Brucella* species to be very distinct from species of this genus.

**Conclusions:** The results demonstrate that MALDI-ToF is able to speciate important *Brachyspira* and both human and veterinary *Mycoplasma* species and differentiate them from other bacteria. It was also be able to distinguish the important zoonotic pathogen *Brucella* species from closely related organisms. MSP spectra for these organisms are now being further validated and will possibly added to the main commercial Bruker database to facilitate speciation of these organisms.
Molecular characterisation of methicillin-resistant Staphylococcus pseudintermedius of canine origin from central Italy

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Objective: In veterinary medicine, methicillin resistant Staphylococci have emerged as a clinical challenge in the last few decades. From a clinical point of view, S. pseudintermedius(Sp) is probably the most significant species of the Staphylococcus Intermedius Group (SIG), being an important canine opportunistic pathogen, often isolated from dermatitis, otitis and other secondary infections in dogs. The aim of this study was to unravel the genetic relatedness of Methicillin Resistant Staphylococcus pseudintermedius(MRSP) strains from Italy by using some molecular typing method like Staphylococcal Cassette Chromosome (SCC)mec typing, dru typing and detection of leukocidin toxin (lukS).

Methods: A total of 30 MRSP isolates were identified by PCR using pse primer from dogs with different clinical symptoms in central Italy. SCCmec elements were characterized by multiplex PCR and lukS gene were detected by PCR. Genetic diversity was accessed by dru typing.

Results: All the strains identified previously as methicillin resistant SIG were reconfirmed as Sp by species specific PCR. 19 out of 30 MRSP showed dru type d9a (63.3%), 8 samples were d11a (26.6%) and 3 samples were d10h (10%) type. SCCmec typing revealed the presence of four types (II-III, III, IV, and V). SCCmec II-III was most frequently seen (16/30, 53.3%). SCCmec III was detected in 3 isolates (10%) and other 3 isolates showed SCCmec IV (10%). Five isolates were SCCmec type V (16.6%). Three were non type able by SCCmec typing. Overall, isolates with d9a and SCCmec II-III were predominating. All the isolates were positive to lukS gene and the sequencing of one amplified lukS gene PCR product revealed the homology with deposited sequence of lukS of MRSP in NCBI.

Conclusion: Overall, the results of the present study allowed us to conclude that associations exist between dru type d9a and SCCmec II-III, d11a and SCCmec V, d10h and SCCmec IV in contemporary clinical isolates of Sp from dogs in central Italy. The lukS gene expression seems to belong to all MRSP investigated here, not showing any specific relation to dru type, SCCmec type or site of infection. The SCCmec V was most predominant in US and Canada, but in this study this type is identified as close to European predominant type SCCmec II-III. In conclusion, the emergence of health associated strains like MRSP with SCCmec IV and V in dogs should be seriously considered to avoid the MRSP transmission from animal to humans and vice versa.

Horizontal spread of highly resistant Gram-negative rods – evaluation of the Diversilab® typing method

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Objectives: The worldwide prevalence of highly resistant Gram-negative rods (HR-GNR) is increasing. Typing methods are needed in case of an outbreak or to monitor the endemic situation. In this study we investigated the performance of the Diversilab® typing method in comparison with the AFLP typing method.

Methods: A collection consisting of 653 HR-GNR, that were obtained during a 6 months prospective survey in 18 Dutch hospitals, was typed by AFLP and Diversilab®. Results were compared directly to each other. Subsequently, the sensitivity and specificity of Diversilab® were calculated, using AFLP as the reference method. Furthermore, results were compared by means of epidemiological linkage and Cohen’s kappa for agreement was calculated.

Results: Diversilab® considered significantly more isolates (275) to belong to a cluster than AFLP (198). The sensitivity was 83.8%, and the specificity was 78.6%. When epidemiological linkage was included in the analysis, Diversilab® considered 9 isolates as secondary cases which were considered unique in AFLP. Only 2 secondary cases according to AFLP were missed by Diversilab®. This results in a Kappa for agreement of 0.983.

Conclusion: In daily practice a typing method has to be used in combination with epidemiological information. When this was done, Diversilab® showed to be a highly reliable method for the typing of HR-GNR. This in combination with the ease of use and the speed, makes Diversilab® an appropriate screening in routine clinical practice. When a cluster is suspected and the consequences of these findings are substantial, a confirmatory analysis should be performed.

Rapid typing of Listeria monocytogenes by high-resolution melting curve analysis of inlB

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Objectives: In listeriais outbreaks and for epidemiological investigations a fast and accurate protocol to subtype L. monocytogenes is essential for control and prevention of listeriosis. In outbreak situations the L. monocytogenes serotyping scheme, based on somatic (O) and flagellar (H) antigens, has limited value for tracking isolates. For rapid identification and typing of isolates in routine diagnostics, a PCR-based typing method targeting a single genetic region would be preferential in terms of cost, simplicity, turnaround time, and potential for standardization, since PFGE, MLST and MVLST still represent time-consuming and cost intensive approaches. The aim of the study was the evaluation of a rapid typing method based on polymorphisms of the internalin B gene.

Methods: One hundred seventy two clinical and 267 food derived L. monocytogenes isolates and 20 isolates from culture collections were typed by high resolution melting curve analysis of a specific locus of the internalin B (inlB) gene. All obtained melting curve profiles were verified by sequence analysis.

Results: All tested L. monocytogenes isolates yielded 15 specific melting curve profiles (figure). Sequence analysis revealed that these 15 melting curve profiles corresponded to 18 distinct inlB sequence types. All clinical isolates were assignable to all defined 18 STs, whereas two STs were absent in food isolates. The most frequent STs were ST-2, ST-9 and ST-1 among the clinical isolates and ST-7 and ST-9 and ST-1 among the food isolates. The high resolution melting curve profiles obtained correlated to the five phylogenetic groups I.1, I.2, II.1, II.2, and III (figure).

Conclusion: High resolution melting curve analysis constitutes an inexpensive assay, which represents an improvement in typing compared to classical serotyping or multiplex PCR typing protocols. This new method is a rapid and powerful screening tool for simultaneous preliminary typing of up to 384 samples in about two hours in advance to more demanding methods such as PFGE or MLST.
**P1730** Evaluation of a novel fluorescent amplified fragment length polymorphism assay and optical mapping to probe the genetic diversity of *Staphylococcus aureus*

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**Objectives:** To evaluate and compare non-biased genome sampling approaches for probing genetic diversity within *Staphylococcus aureus* and implementation as tools for dissecting major methicillin resistant clones.

**Methods:** Fluorescent amplified fragment length polymorphism (FAFLP) is an analytical methodology that allows random sampling of chromosomal DNA through double endonuclease digestion and selective amplification using restriction-site specific primers. Optical mapping, on the other hand, utilizes a single endonuclease for digestion of individual chromosomal DNA molecules as a gross alignment and orientation method. *S. aureus* isolates from six major multilocus sequence types (MLST) were sampled using both approaches in parallel. Genomic DNA was restricted using the endonuclease combination Csp6I and BglII for FAFLP and NoI for optical mapping. FAFLP products were analysed on an automated sequencer using GeneMapper and Bionumerics software. Optical maps were analysed against entire genome sequences using MapSolver. Derived FAFLP profiles and optical maps were analysed in conjunction with staphylococcal cassette chromosome mec (SCCmec) and MLST data.

**Results:** FAFLP extracted ~0.65% of the genome and generated a profile consisting of 50–85 fragments of 50–555bp. Optical mapping encompassed the whole genome, generating 200–230 fragments between 1100 and 94000bp. Unique sequences specific to isolates and clusters were identified from optical maps. At 95% similarity target, 13 and eight unique profiles were exhibited with FAFLP and optical maps respectively. Four highly similar clusters exhibiting differences of up to 14% with unique profiles were observed with the SCCmec type.

**Conclusions:** FAFLP identified genetic micro-heterogeneity between and within clusters as it sampled more loci relative to the genome area. Differential fragments identified between MRSA lineages by FAFLP can be used in diagnostic assay development. Optical mapping identified macro-variation within the whole genome and indicated genetic transfer events possibly caused by recombination or transposition. Sequenced genome analysis generated in-silico maps aiding genome comparisons and synteny assessment. Understanding and mapping the evolution of MRSA lineages requires such methods to probe the genetic variation on a micro and macro scale.

**P1732** Multilocus sequence typing of *Propionibacterium acnes* with diverse origin

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**Objectives:** *Propionibacterium acnes* is a Gram-positive, slow growing, aerotolerant anaerobic bacillus, predominantly found on the skin of adults. It is, however, considered an opportunistic pathogen and is mostly associated with acne vulgaris and rarely also with severe infections such as infective endocarditis, prosthetic joint infections and deep sternal wound infections following cardiothoracic surgery. It is however, not known if specific subtypes are more prone to cause invasive infection compared with commensals. In order to characterize *P. acnes* isolates, phylogenetic analysis has been performed based on sequencing of the housekeeping gene recA, a putative hemolysin gene (tly) and the transcarboxylase 12S gene (Tc12S). So far, based on those genes, four major groups are known. The aim of the present study was to obtain a well-defined multilocus sequence typing (MLST) protocol for *P. acnes*, in order to investigate the genetic heterogeneity of *P. acnes* isolates with diverse origin.

**Methods:** The MLST was based on internal fragments of nine housekeeping genes, lac, otx, fba, coa, zno, gms, pak, cob and cel. All the MLST genes as well as recA, tly and Tc12S were PCR amplified and sequenced in 29 different *P. acnes* isolates with diverse origin including all known subtypes, i.e. 1A, 1B, II and III. Allelic profiles based on concatenated sequences from each MLST gene were performed, where the most common sequence type (ST) was graded with the lowest figure. To identify different sequence variants phylogenetic analysis was subsequently performed.

**Results:** The MLST analysis identified 23 STs within the collection. Furthermore, the phylogenetic trees showed a superior capability to discriminate *P. acnes* isolates when based on the concatenated sequences compared with analysis based on the sequence of each gene individually and also compared with only using recA, tly and Tc12S.

**Conclusion:** The results support the hypothesis that *P. acnes* is more diversified than the four major groups known up till now. Our data suggest that presented MLST protocol is superior when investigating the heterogeneity of *P. acnes* isolates with diverse origin compared with using recA, tly and Tc12S.
Changes in Clostridium difficile type distribution: a national reference laboratory perspective

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Objectives: The aim of our national reference laboratory at THL is to support regional and local infection control teams to identify Clostridium difficile (CD) and monitor the effects of prevention and control measures. When the national surveillance and ribotyping service began in 2008, the emphasis and concern was around identifying ribotype 027. As diagnostic methodology and awareness has improved along with new commercial applications, several hospitals are now able to identify type 027 directly from clinical samples. The objective of this study is to analyse the type distribution of CD isolates sent for genotyping in Finland in 2008–2010.

Methods: All clinical laboratories have reported all CD findings (positive culture and/or toxin production) from stools to the National Infectious Disease Register since 2008. The laboratories have also been asked to send CD isolates from severe cases and persistent outbreaks to the reference laboratory at THL for genotyping. All sent toxigenic isolates have been ribotyped, and PFGE has been performed when necessary.

Results: In 2008–2010 only 13/20 hospital districts sent isolates for ribotyping. The number of isolates sent varied between 1–72/district/year. The total number of isolates sent for typing has decreased every year since 2008 covering now 5.5% of all reported CD findings. Also the incidence of CD associated infections in Finland has decreased since 2008. More sporadic ribotypes are identified. In 2008, 36% of the strains analyzed were of type 027, in 2009, 26%, and in 2010, 12%. The distribution of other ribotypes has been rather stable, type 001 being the most common followed by ribotypes 014, 020, 005, 011, 002, 023, 018, 003, 078, 056, and about 30 ribotypes that are identified sporadically.

Conclusion: Since we respond to the needs of regional infection control teams, our typing data represents persistent and virulent strains rather than the wholesome distribution of ribotypes in Finland. Utilization of the typing service, local activities and available methodology vary considerably between different regions. Special care must be taken when analyzing data received by new commercial applications with some limitations. Isolation of CD enabling more complete clonal, virulence and susceptibility analysis is still essential to monitor the evolutionary changes among the CD population behind the disease.

A population study on Chlamydia trachomatis in adolescents in a high-incidence area in north Norway reveals multiple new MLST genotypes and the new Swedish mutant (nvCT)

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Objectives: Our study aimed to; (1) assess the prevalence of C. trachomatis (CT) among adolescents in Finnmark county, (2) map CT genotypes in Finnmark and compare with variants in Tromsø and Trondheim, (3) investigate possible spread of the nvCT, and (4) examine associations between specific genotypes and clinical symptoms.

Methods: Finnmark borders Northwest Russia and Finland and is an extended county with sparse population in minor municipalities. This population based cross-sectional study inviting all high school students in five Finnmark towns included a web based questionnaire and urine samples (participation rate: 77.4%; n = 1476; females 725/males 751; mean age: 17.2 years). Parallel to this cohort providing 60 CT positive samples, positive clinical CT samples were collected from the routine laboratories in Tromsø (n = 80), Trondheim (n = 88), and Finnmark (n = 20) giving a total of 248 samples. High resolution multilocus sequence typing (MLST) targeting five genetic regions and ompA sequencing were used to characterize CT in the PCR positive urine samples. nvCT was verified using a PCR detecting the plasmid mutation. Results: CT prevalence in the Finnmark study was 5.6% among the sexually active (females: 7.1%, males: 3.9%). Complete MLST profiles were obtained for all samples with detection of 50 sequence types (ST), of which 62% (31/50) were novel. 12 new alleles in the MLST scheme were detected. Sequencing of ompA identified 11 unique genotypes. By combining MLST and ompA, 54 genotypes were identified. 57.3% (142/248) of the isolates had STs that were present in Finnmark, Tromsø, and Trondheim. But different STs predominated at different sites. New STs were present in all three areas. nvCT was present in 1.6% (4/248) of the samples. The common genovar E predominated in Tromsø and Trondheim (56%) whereas genovar G (36.2%) was most frequent in Finnmark. CT infected females with rare STs reported urogenital symptoms more frequently (72.2%) than those with common STs (52.1%). Due to small number of specimen (n = 41), the difference was not statistically significant.

Conclusion: A CT prevalence of 5.6% was as expected according to surveillance data with Finnmark reporting incidence rates twice the national average. 31 new STs and 12 new alleles in the MLST scheme were detected. The MLST system had a 4.5 higher discriminatory power compared to conventional ompA genotyping. Only four cases of nvCT indicated a limited spread in North and Middle Norway.

Molecular tools for the diagnosis of culture-negative infections

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Objective: Universal pathogen PCR by use of broad range 16S primers (16S-PCR), followed by sequencing, is a highly specific, technically demanding diagnostic tool in the routine laboratory. Although still being a niche indication, reserved mainly for severe or life threatening infections, acceptance of this method by clinicians is increasing. Despite uncertainties in interpretation of culture negative/PCR positive cases, 16S-PCR might provide the life threatening clue. However, contamination, either during pre-analytics or in the molecular laboratory, poses the risk of pointing in the wrong direction. By discussing well documented clinical cases both, the power and the limitations of 16S-PCR in daily routine work will be discussed.

Methods: 16S-PCR followed by cycle sequencing was done using the SepsiTest kit (Molzym, Germany) according to the manufacturer’s instructions. Amplicons were sequenced commercially (gate, Germany) and sequences were analysed using at least two different algorithms (NCBI BLAST, leBIBI or SepsiTest-Blast). Mixed sequences were analysed using a specific programme (RipSeq by iSentio, Norway). The most common specimen was blood (n = 23), followed by aortic or mitral valve tissue (18) and synovia/synovial fluid (n = 10). All specimens were obtained and analysed as part of routine diagnostic procedures.

| Result of s16S-rDNA (partial) sequencing | number |
|-----------------------------------------|--------|
| no amplification/sequencing             | 24     |
| weak positive, not sequenced            | 6      |

(M) mixed sequence
Results: Sixty-five samples were processed in 2010. Out of 41 positive PCR reactions, 35 could be successfully sequenced, representing 21 different taxa. Six sequences were mixed sequences. The most common sequence was of *Staphylococcus aureus*. Confirmation by concomitant culture was possible in 13 cases, the remainder being negative in (repeated) culture. While in some cases the result of molecular identification was regarded as etiologic significant, in other cases interpretation was equivocal (i.e. etiologic relevant pathogen vs. contaminant).

Conclusion: 16S-PCR followed by sequencing is a powerful method if culture stays negative. Technical challenges are weak positive samples and mixed sequences. Highly trained personnel, great experience and strict rules during pre-analytics are obligate prerequisites. A main problem is interpretation of the finding. While in some cases contamination might pose uncertainties, the result of molecular identification often provides the lifesaving clue.

Materials and Methods: FISH (Lucoces, Macon) analysis was conducted always using the bottle which became positive first by the BacT ALERT® 3D system (Bio Merieux) of 152 positive blood cultures from different patients. Dependent on the Gram stain either a Gram negative (Bac I) or a Gram positive panel (Bac II) were used. Pathogens included in both panels are regarded as being responsible for approximately 90% of blood stream infections. The time to result was 30 min and the hands-on time only 10 min.

Results: Fifty-eight Gram negatives and 94 Gram positives were identified by conventional methods. In comparison to the latter, the test agreement was 84.9% (129/152). In 14 out of 152 cases (9.2%) the microorganism identified by the reference method was not included in the respective panel by FISH. Of the remaining 138 cases, in 8 (5.8%, 5 of a Gram negative and 3 of a Gram positive pathogen) the result of the FISH test differed from that of the reference method being either false positive or false negative.

Conclusion: These data suggest that this novel FISH test accurately identifies blood stream pathogens in positive blood cultures and may considerably reduce the time to result.

A novel FISH test for rapid pathogen identification in positive blood cultures

A. Makristathis*, S. Riss, M. Hirschl (Vienna, AT)

Objectives: Conventional blood culture is regarded the gold standard for the detection of blood stream pathogens. In the present study, a novel commercially available fluorescence in situ hybridization (FISH) test has been validated allowing for rapid bacterial pathogen identification in positive blood cultures.

Material and Methods: FISH (Lucoces, Macon) analysis was conducted always using the bottle which became positive first by the BacT ALERT® 3D system (Bio Merieux) of 152 positive blood cultures from different patients. Dependent on the Gram stain either a Gram negative (Bac I) or a Gram positive panel (Bac II) were used.
Conclusions: Culture-independent analysis of bacteria by 16S RNA PCR and sequence typing in cardiac biopsies is an important diagnostic tool to identify etiology of endocarditis even years after antibiotic treatment. Thereby, DNA of viable and also of dead bacteria can be detected in a very sensitive way at the site of infection. Culture-independent analysis may also help to discriminate bacterial contaminations from true infections.

**P1739** 16S ribosomal RNA gene sequences from cardiac valve tissue biopsies convey the diagnosis of culture-negative endocarditis

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Objectives: Culture-negative endocarditis accounts for nearly half of all cases of infectious endocarditis. Prior or concurrent antibiotic treatment at the time of blood cultures taken accounts for 45–60% cases of this situation; the remainder are caused by slow-growing and fastidious organisms. We hypothesized that detection of the bacterial 16S ribosomal rRNA gene from peroperative cardiac valve tissue sample and subsequent sequencing and species identification was more sensitive than culture for the identification of the cause of endocarditis.

Methods: Infecting strain identity was achieved by culture and by using polymerase chain reaction (PCR) that targeted highly conserved regions of the 16S RNA gene to diagnose the cause of endocarditis in peroperative cardiac valve tissue biopsies from 179 patients in Norway. The identity of cultivated organisms were identified by conventional techniques and mass spectrometry, while the identity of noncultivated infecting agents was determined by sequencing 16S rRNA gene-specific PCR products and comparing them with known 16S rRNA gene sequences from a wide range of bacteria. Thereby, the sensitivity and performance of 16S rRNA gene-based identification and culture in detecting the cause of endocarditis was compared.

Results: The 16S rRNA gene PCR sequencing was by far more sensitive than culture and yielded a broad range of Gram-positive and Gram-negative species that were the likely cause of endocarditis. Interestingly, one case each of Tropheryma whipplei, Bartonella quintana, and Bartonella henselae were among the species identified. Thereby, the approach served to broaden the etiologic diagnosis of culture-negative endocarditis.

Conclusion: PCR that targets highly conserved regions of the 16S rRNA gene and subsequent sequence comparison enabled the identification of cultivated and noncultivated endocarditis agents from peroperative cardiac valve tissue biopsies. Direct detection of microbial agents by PCR has clearly served to broaden the etiologic diagnosis of endocarditis. Thereby, the diagnosis of the cause of endocarditis can be secured and also be a guide for optimal antibiotic therapy to be implemented.

**P1740** Immunomodulatory effects of voriconazole and caspofungin on human peripheral blood mononuclear cells stimulated with Candida albicans and Candida krusei

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Objectives: Candida infections are frequently associated with high morbidity and mortality in immunosuppressed patients. Although Candida albicans is the most frequently isolated species of candidiasis patients, the occurrence of infections caused by other Candida species is increasing. Cell-mediated immunity and humoral immunity are the principle protective immune response against fungal infections. Phagocytic cells are known to be important in clearing fungal infections. Antifungal agents such as voriconazole and caspofungin enter phagocytic cells and cause intracellular activities. The purpose of this study was to evaluate the immunomodulatory effects of voriconazole and caspofungin on human peripheral blood mononuclear cells (PBMC) stimulated with C. albicans and C. krusei.

Methods: Human PBMC isolation was done by Ficoll-hypaque density gradient centrifugation method. Cell proliferation was assayed by colorimetric method using MTT 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT). The cytokine levels in the human PBMC culture supernatants stimulated with C. albicans and C. krusei were determined by ELISA.

Results: The addition of voriconazole and caspofungin lead to proliferation of PBMC. In the presence of voriconazole and caspofungin, the levels of IL-2, IFN-γ, and IL-6 increased remarkably in PBMC stimulated by C. albicans and C. krusei. Otherwise, the combination of antifungal drugs and PBMC stimulated by Candida spp. did not increase the levels of TGF-β and IL-10.

Conclusion: Our results suggest that voriconazole and caspofungin have immunomodulatory effects on human PBMC stimulated with Candida spp. The interaction between antifungal drugs and PBMC stimulates Th1 type cytokine secretion. Cytokine stimulation from immune cells can assist in the elimination of fungal pathogens. So, immune modulators such as cytokines may be useful as therapeutic adjuvants to antifungal drugs for treatment of infections caused by Candida spp.

**P1741** Lipopolysaccharides O1 antigen and outer membrane proteins contribute to virulence and protective efficacy of O1 antisera in Klebsiella pneumoniae

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Background: To explore the mechanisms enabling Klebsiella pneumoniae to disseminate through the bloodstream, we characterized the roles of lipopolysaccharides (LPS) O antigens and outer-membrane proteins (OMPs) in the pathogenesis of K. pneumoniae causing community-acquired pyogenic liver abscess (PLA).

Methods: The O1-antigen seroepidemiology of K. pneumoniae clinical isolates was analyzed by magA-mutant hyperimmune mouse serum. The virulence of deletion mutants was compared with that of the wild-type strain by intraperitoneal and intragastric infections in mice. The in vivo protective capacity of O1 antiserum was tested by experimental K. pneumoniae infection.

Results: The O1 serotype was more prevalent in PLA strains, and the O1 serotype isolates had a higher frequency of serum resistance. Mutation of the O1 antigen changed serum resistance in K. pneumoniae. The O1-antigen deficient mutants became susceptible to complementation of these genes showed that only. The O1-antigen deficient mutants became susceptible to complementation of these genes showed that only.

Conclusions: Our findings indicate that LPS O1 antigen and LPS-associated OMPs are important virulence factors for PLA caused by K. pneumoniae. LPS O1 antiserum could be a useful vaccine candidate against infections by K. pneumoniae with loose capsule structures.

**P1742** Isolation of genetic loci associated with phagocytosis and virulence in Klebsiella pneumoniae using a Dictyostelium model

Y.J. Pan, T.L. Lin, C.R. Hsu, J. Wang* (Taipei, TW)

Phagocytosis resistance is an important virulence factor in Klebsiella pneumoniae. Dictyostelium has been used to study the interaction between phagocytes and bacteria because of its similarity to mammalian macrophages. In this study, we used a Dictyostelium model to investigate genes for resistance to phagocytosis by NTUH-K244, a strain of K. pneumoniae. Peptidoglycan-associated lipoprotein (Pdi) and murein lipoprotein (LppA) were essential for K. pneumoniae to proliferate and survive in blood. Immunization of mice against LPS O1 provided protection against infection with capsular type K2 strain, but not capsular type K1 strain.

Conclusions: Our findings indicate that LPS O1 antigen and LPS-associated OMPs are important virulence factors for PLA caused by K. pneumoniae. LPS O1 antigen could be a useful vaccine candidate against infections by K. pneumoniae with loose capsule structures.
complementation restored the phagocytosis resistance phenotype. These two mutants were also susceptible to phagocytosis by human neutrophils and revealed attenuated virulence in a mouse model, implying that they play important roles in the pathogenesis of K. pneumoniae. Furthermore, we demonstrated that clpP, which exists in an operon with clpX, was also involved in resistance to phagocytosis. The transcriptional profile of clpX was examined by microarray and revealed a 3-fold lower level of expression of capsular synthesis genes. Therefore, we have identified genes involved in resistance to phagocytosis in K. pneumoniae using Dictyostelium, and this model is useful to explore genes associated with resistance to phagocytosis in heavily-encapsulated bacteria.

**P1743** Effect of endoplasmic reticulum stress pathway on mycobacteria-induced cell death

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**Objectives:** To investigate the role of endoplasmic reticulum stress pathway in mycobacteria infected cells and to determine its role in intracellular survival in host cells.

**Methods:** A549 cells and Raw 264.7 cells were cultured in MEM supplemented with 10% FBS and antibiotics. The cells were stimulated with mycobacterial antigen ESAT-6 and were infected with M. tuberculosis H37Rv (Mtbc). Macrophages infected with mycobacteria were lysed for intracellular survival CFU counting. Quantitative RT-PCR and western blot analysis were used for determination of mRNA expression or protein induction after antigen stimulation or mycobacterial infection. Cell viability was assessed with a Cell Counting Kit-8 or FACS flow cytometry. Reactive oxygen species (ROS) production was measured by laser-scanning microscopy using DHE staining.

**Results:** Mycobacterial secreted antigen ESAT-6 stimulation induced apoptosis with ER stress response. We found XBP-1 mRNA splicing, GRP78, ATF4 and CHOP mRNA induction was increased by ESAT-6 antigen stimulation. Similar results were observed with Mtbc infected macrophages. ER stress-mediated host cell response during mycobacterial infection was induced XBP-1 mRNA splicing, GRP78 and CHOP expression. Caspase-12 and caspase-3 were activated by ESAT-6 or Mtbc infection in macrophages. We also found ASK1/JNK signaling pathway was involved in ESAT-6-mediated ER stress apoptosis. Cytosolic calcium released by ER stress response was increased by ESAT-6 stimulation. It was confirmed by confocal microscopy after pretreatment of calpain inhibitor PD150606. Interestingly, we found induced phosphorylated α-subunit of eukaryotic initiation factor 2 was related to CHOP expression during Mtbc infection. Furthermore, we found the results of CHOP expression were important for Mtbc survival in macrophages. The increased CHOP expression was not observed in heat-killed bacteria but in live Mtbc.

**Conclusion:** These results demonstrate a stronger induction of ER stress response chaperones by not only mycobacterial secreted antigen ESAT-6 but Mtbc infection. Mycobacterial antigen induced intracellular calcium concentration, which resulted in ROS accumulation, and therefore induces the onset of ER stress-induced apoptosis. Live Mtbc could control ER stress pathway to survive in macrophages. These observations lend support to the hypothesis that ER stress mediated apoptosis may play an important role in the pathogenesis of tuberculosis.

**P1744** The role of aberrant tcdC sequences in toxin production by Clostridium difficile

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**Objective:** In the current model of Clostridium difficile toxin regulation, TcdC is a negative regulator/repressor of toxin production. Epidemic strains of C. difficile frequently carry an aberrant tcdC gene, characterised by a frame-shift mutation. We tested the popular hypothesis that this is the reason for the hyper-toxin-production phenotype reported for epidemic strains.

**Methods:** We developed a new method for precise manipulation of the C. difficile chromosome; a major breakthrough for this genetically intractable organism. This enabled us to correct the tcdC frame-shift mutation in the epidemic C. difficile strain, R20291 (the notorious Stoke Mandeville, UK outbreak strain), as well as substitute the native tcdC gene for that of the non-epidemic strain, 630.

**Results:** Production of toxin A and toxin B by C. difficile R20291 remained unchanged, regardless of the tcdC allele present in the chromosome.

**Conclusion:** This work demonstrates that an aberrant tcdC sequence alone does not explain the hyper-toxin-production phenotype reported for epidemic strains of C. difficile. Furthermore, it indicates that our current model of C. difficile toxin regulation needs revision.

**P1745** Analysis of cellular senescence induced by lipopolysaccharide in pulmonary alveolar epithelial cells

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**Objectives:** There have been few studies on the relationship between senescence and acute infection, although immunosenescence has been recently introduced. In this work, it was examined the possibility of lipopolysaccharide (LPS) causing cellular senescence in lung alveolar epithelial cells. The morphologic and biochemical characteristics of senescence induced by LPS were analyzed. Then, it was clarified how this cellular senescence phenomenon is associated with oxidative stress effect induced by LPS and whether antioxidants could inhibit reduced cellular viability by oxidative stress effect of LPS.

**Methods:** Human-like type II lung epithelial cell (A549) is obtained from cell-bank. Cell viability was checked using cell counting kit-8. Cell morphology was examined for morphological changed at 2, 5 and 7 days post-LPS treatment by phase-contrast microscopy. The presence of apoptotic cells was assessed by determining caspase activation using a Caspase-Glo 3/7 luminescence assay kit. Detection of cellular lysosomal content was determined by staining with acridine orange. The biochemical senescence was checked by the presence of senescence-associated β-galactosidase (SA β-gal) activity. We evaluated the determination of LPS-generated hydrogen peroxide and the effect of the antioxidant GSH.

**Results:** A549 cells exposed to LPS were showed decreased viability and growth arrest in concentration-dependent manner. The pre-apoptotic concentration of LPS developed morphological senescence changes and elevated SA β-gal activity. Also, senescence associated lysosomal content was increased in pre-apoptotic LPS. Exposure of A549 cells to LPS resulted in the formation of hydrogen peroxide in proportion to concentration. The ability of LPS to induce cellular viability inhibition was prevented by the presence of adequate levels of GSH.

**Conclusion:** This study revealed that LPS could induce cellular senescence in lung alveolar epithelial cells, and these phenomena were closely associated with hydrogen peroxide production by LPS. Taken together, it is suggested that LPS-induced cellular senescence may play an important role in limiting the tissue repair response after sepsis.

**P1746** Virulence genes focG and agn43aCFT073 are related to mortality in Escherichia coli bacteraemia

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**Objectives:** E. coli is a leading cause of bloodstream infections. More than 30% of bacteraemia cases in community- and hospital-acquired infections are due to E. coli, and it is the major cause of mortality from these infections. The pathogenesis of E. coli bloodstream infections is largely unknown, why preventive measures are lacking. We studied 198 E. coli strains causing bacteraemia according to virulence factor (VF) genes and the relation of these to mortality.

**Methods:** January 2003–May 2005, 198 E. coli blood isolates were collected at Hvidovre Hospital from 197 bacteraemic patients with E. coli
Stenotrophomonas maltophilia

Expression of virulence factors in S. maltophilia

The occurrence of VF's among the E. coli isolates ranged from <1% (kpsMT III) to 98% (fimH) (Table 1). The median of the aggregate virulence score was 14 (range 1–22). Total 30-day mortality was 11%, 24% among HA episodes and 10% among CA episodes. Among the 29 VF genes, only two were significantly related to mortality, i.e. focG (F1C fimbria) and agn43a (biofilm related antigen 43, allele a CFT073). focG was present in isolates from 39% (9/23) of patients dying within 30 days of culture, as compared to 20% (35/175) of isolates from patients not dying (p = 0.003). agn43a CFT073 was present in 61% (14/23) of isolates from patients dying within 30 days, as compared to 31% (55/175) of isolates from patients not dying (p = 0.005).

Conclusion: Overall mortality of E. coli bacteraemia cases was low (11%), however the two VF genes focG and agn43a CFT073 were significantly related to mortality. Thus, F1C fimbriae and biofilm formation capacity appear to play a significant role in the pathogenesis of serious E. coli infections. More studies on selected strains harbouring focG and agn43a CFT073 are needed in order to examine the virulence potential of such strains in further detail and whether prospective detection of these genes in blood culture E. coli isolates could influence treatment of bacteraemia.

Table: Occurrence of virulence factor genes in E. coli blood isolates from cases of bacteraemia (n=185)

| Virulence factor genes | Occurrence (%) |
|------------------------|----------------|
| Adhesins               |                |
| sfa/epIC               | 5 (3)          |
| bmaE                   | 7 (4)          |
| htap                   | 135 (69)       |
| focG                   | 44 (22)        |
| gfaD                   | 2 (1)          |
| hha                    | 93 (49)        |
| pagKH                  | 142 (73)       |
| sfaC/focD              | 67 (36)        |
| Biofilm related         |                |
| agn43                  | 173 (60)       |
| agn43a                  | 93 (35)        |
| agn43b                  | 56 (29)        |
| agn43c                  | 60 (30)        |
| Iron uptake             |                |
| cbrA                    | 176 (79)       |
| fyuA                    | 162 (63)       |
| mott                   | 111 (50)       |
| kpsHT                  | 65 (35)        |
| kpsHA                  | 143 (75)       |
| Proteins                |                |
| hpa                     | 46 (22)        |
| kpMT II                 | 163 (62)       |
| kpMT III                | 170 (85)       |
| hsaT                    | 11 (5)         |
| Toxins                  |                |
| cdtB                    | 19 (5)         |
| cdtF                    | 56 (20)        |
| A4G                    | 67 (34)        |
| C5                    | 89 (45)        |
| Miscellaneous           |                |
| kpsA                    | 23 (12)        |
| malC                    | 125 (65)       |
| tige                   | 134 (68)       |

Stenotrophomonas maltophilia, belonging to the γ- subclass of proteobacteria, is found ubiquitously distributed in the environment and is gaining importance as a nosocomial pathogen.

In our previous work we analyzed genetic diversity of S. maltophilia by rep (repetitive extragenic palindromic)-PCR fingerprinting and gyrB gene sequencing, for a collection of 171 environmental and clinical strains. This revealed 11 genetic subgroups for S. maltophilia. A subset of 50 representative isolates for these groups was then used for further investigation of phenotypic properties. With respect to its role as opportunistic pathogen, potential virulence traits, as the production of extracellular proteases, haemolysins and siderophores were investigated. Furthermore, factors supporting colonization of a human host were examined by swimming and twitching motility and biofilm assays. Virulence was tested by co-culturing the bacteria with the amoeba Dictyostelium discoideum and Acanthamoeba castellanii as model organisms. After testing twenty different antibiotics on a small subset of strains, gentamicin, vancomycin, norfloxacin, tetracycline and co-trimoxazole, were chosen to determine MIC’s for the 50 S. maltophilia isolates.

Nearly all investigated isolates produced proteases and haemolysins and all of them produced siderophores. Motility assays revealed differences in swimming and twitching motility. Biofilm formation generally differed, but did not correspond to their genetic subgroups of the isolates. An exception is that all isolates from environmental group E2 showed only slight potential for biofilm formation. Virulence for amoebae was shown for about one third of the tested isolates and was in no relationship to clinical or environmental origin. All isolates were resistant to vancomycin and most to gentamicin. Most of them showed intermediate MIC’s for norfloxacin and tetracycline, and all isolates were susceptible to co-trimoxazole.

For motility assays, biofilm formation, virulence and antibiotic resistance generally no correlation to the previously defined genetic groups was found. In this context it was expected that housekeeping genes and rep-PCR fingerprints are not suitable markers to determine phenotypic properties of S. maltophilia.

Expression of virulence factors in Pseudomonas aeruginosa strains from nosocomial pneumonia and from non-infectious respiratory colonisation processes

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Objectives: Pseudomonas aeruginosa is a frequent agent of nosocomial pneumonia but is also found as colonizer of asymptomatic patients. Recently, strains from patients with pneumonia were shown to possess higher “serum-resistance” ability as compared to strains from asymptomatic colonized ICU patients. In this study we investigated P. aeruginosa strains collected from patients with pneumonia and from asymptomatic patients with regard to their lipolytic, haemolytic and elastolytic activity, all properties that are suggested to be virulence factors.

Bacteria and Methods: Bacteria: 171 P. aeruginosa strains were consecutively isolated from bronchoalveolar lavage fluids or transstracheal aspirates of ICU patients with monobacterial nosocomial pneumonia, and 49 strains were isolated from the respiratory tract of ICU patients without respiratory tract infections.

O-serotyping: All strains were serogrouped using O-antigen-specific sera for the 14 P. aeruginosa O serogroups. The haemolytic activity of the strains was tested on Columbia agar containing 5% sheep blood incubated over 72 h. Elastase and lipases: Lipolytic activity was tested on Tryptic-Soy-Agar supplemented with various fatty acid ester (1% Tween20—85); the expression of elastase was determined on 0,25% Elastin-Trishydrochloride agar.
The bacteria were cultivated on the various media for 3 days (lipase) or 7 days (elastase) at 37°C. Results: Higher frequency of the serogroups A (11.9% and 16.3%, respectively), B (14.3% and 21%), E (26.5% and 24.6%), and I (28.6% and 28%) was detected in both strain collections. Both strain collections did not differ significantly in their haemolytic elastolytic lipolytic, and activity (p > 0.05). Conclusions: The haemolytic, lipolytic and elastolytic activities are less likely to contribute to the putative higher pathogenic potential of P. aeruginosa pneumonia strains.

**P1749** Alginate exopolysaccharide acts as a ligand for P. aeruginosa invasion of lung epithelial cells through its interaction with p63

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Background: The interplay of P. aeruginosa with the airway epithelial cells is a crucial step in the pathogenesis of the respiratory infections caused by this microorganism. Both, bacterial and host factors modulate this interaction. In this work, we investigated the role of the alginate exopolysaccharide, a key virulence factor associated to severe respiratory infections, and the surfactant protein A (SP-A), a broad spectrum opsonin with membrane permeabilisation properties in this interaction.

Methods: Bacterial invasion capacity of human bronchoepithelial cells (16HBE13) and human type II pneumocytes (A549) monolayers was determined by standard invasion assays in presence of SP-A with the type strain PA01, an isogenic alginate hyperproducing mucA mutant (PAOMA), an isogenic alginate deficient algD mutant and 2 clinical isolates (PA22 and PA2C). The role of p63 in P. aeruginosa internalization by respiratory epithelial cells was studied using standard invasion assays in presence of a p63 blocking antibody or using p63 silenced cells by siRNA. Alginate binding assays were performed measuring purified P. aeruginosa FITC-labelled alginate union to A549 monolayers by fluorescence spectroscopy.

Results: Opsonisation of P. aeruginosa with SP-A did not affect the invasion of the airway epithelial cells by the microorganism. However, SP-A inhibited P. aeruginosa internalization by airway epithelial cells through direct interaction of the protein with the cells, suggesting that the microorganism attached to the cells through a specific receptor for SP-A such as p63. Specific anti p63 antibodies blocked the invasion of epithelial cells by alginate-producing P. aeruginosa strains. Furthermore, the invasive capacity of these strains was reduced in p63 silenced cells. Finally, the binding of purified alginate to p63 silenced cells or cells pretreated with p63 blocking antibodies was significantly reduced.

Conclusions: Altogether these results indicate that P. aeruginosa and SP-A share a common receptor, p63, on the surface of respiratory epithelial cells. Alginate exopolysaccharide appears to be involved in the interaction of the bacterium with this receptor and could play an important role during the process of infection by promoting bacterial internalization.

**P1750** In vivo gene expression and growth conditions of Pseudomonas aeruginosa in cystic fibrosis lungs

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Background: P. aeruginosa is the main pathogen responsible for progressive lung destruction in cystic fibrosis (CF) patients, in whom they persist despite aggressive antibiotic treatments. Biofilm and anaerobic growth conditions might be responsible for these treatment failures. We investigated by in vivo gene expression analysis whether P. aeruginosa grows anaerobically within the CF-lung.

Methods: We collected sequential sputa obtained after respiratory physiotherapy from 26 CF-patients over a 17 months period at the University Hospital Besançon. From each sputum we isolated one or two P. aeruginosa strains and extracted total DNA and RNA. Bacterial load and in vivo gene expression were determined by qRT-PCR on DNA and RNA preparations, respectively. In vitro anaerobic conditions were created with the Gene Bag system in an anaerobic jar. Genotypes of isolates were compared by RAPD.

Results: Sixty sputum samples and 68 isolates from 10 of the 26 patients were retained for final analysis. Each of the 10 patients carried one genotype. In vivo expression of nirS and norB, two anaerobically induced genes, was compared to their in vitro expression by the corresponding isolate under aerobic and anaerobic growth conditions. We found that in 9/10 patients nirS and norB expression was indicative of aerobic growth, whereas in one patient expression of these genes suggested anaerobic growth. In vivo expression of nirS and norB did not correlate with the patient's clinical stage (exacerbations vs chronic phase).

Conclusion: In sputum P. aeruginosa grows aerobically in 90% of CF patients. These isolates should therefore be susceptible in vivo to antimicrobials. This might explain the clinical improvement after antimicrobial treatments, however anaerobic growth conditions might prevail deeper in the lung.

**P1751** Comparative evaluation of enterotoxin 2 encoding gene expression levels in Shigella spp. and EIEC strains grown in the presence of eukaryotic HeLa cells and artificial culture media

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Objectives: Pathogenesis of Shigella spp. and enteroinvasive E. coli (EIEC) strains is dependent on their ability to express the invasive phenotype in the presence of different populations of eukaryotic cells, process involving complex interactions, whose results are leading to disruption, invasion and destruction of the intestinal barrier. The enterotoxin-2 (ShET-2) encoding gene (sen) is located on a inversion associated 140 Mda plasmid.

Purpose: To study the sen gene expression in 16 Shigella spp. and EIEC strains, in the presence of artificial culture media and respectively, in the presence of eukaryotic HeLa cells, in order to investigate the utility of this method for the further development of an alternative assay of the invasive potential to replace the in vivo guinea pig Sereny test.

Methods: 13 Shigella spp. strains and 3 EIEC strains isolated in Romania from children with dysentery and aqueous diarrhoea during 2005–2007 and positive for Sereny test were analyzed. The expression levels of sen gene in bacterial cells cultivated in the presence of eukaryotic HeLa cells were determined by SYBR Green Real-Time PCR. As control for relative quantification were used standard microbial culture obtained in liquid broth.

Results: sen gene was present in all selected strains. Cultivation on artificial media resulted in sen gene expression in only one strain, confirming that Shigella spp. and EIEC strains lose their invasion ability by conservation and passages on artificial culture media. In contrast, sen gene expression is induced in the presence of eukaryotic HeLa cell substrate in 15 of the 16 tested strains, the expression level being strain dependent.

Conclusions: These results demonstrate the potential utility of cell lines for the study of invasive potential of Shigella spp. and EIEC strains by the expression level analysis of bacterial genes involved in invasion, after the bacterial cultivation in cell cultures and the opportunity for standardization and optimization of an in vitro model for assessing the invasive potential.

**P1752** Virulence patterns of Pseudomonas aeruginosa nosocomial strains associated with different clinical infections

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Objectives: To purpose of this study was to perform the phenotypic and molecular characterization of the most important virulence factors in P. aeruginosa strains isolated from different clinical specimens, in order to establish an association between certain virulence patterns and the type of nosocomial P. aeruginosa infection.
Interaction between oral streptococci and two Prevolta species: an in vitro study
C.S. Singts*, A.C. Rodloff (Leipzig, DE)

Methods: The study was performed on 50 P. aeruginosa strains isolated from: respiratory samples (tracheal and bronchial secretions), dermal samples (wound secretions) and blood cultures. Six enzymatic virulence factors (lechitinase, lipase, gelatinase, amylase, caseinase, DNase, and hemolysins) were investigated by phenotypic assays based on cultivation of bacterial strains on culture media containing specific substrate, while eleven (elastase, alkaline protease, protease IV, rhamnolipid, haemolytic phospholipase C, non haemolytic phospholipase C, exotoxin A, exoenzyme S, exoenzyme T, exoenzyme U and ptyroendyme A) by PCR detection of the respective encoding genes.

Results: The results of phenotypic characterization of the analyzed P. aeruginosa strains showed that the patterns of soluble virulence factors varied among the strains isolated from different specimens, as well as in those with the same clinical origin. However, the strains isolated from wound secretions preferentially expressed DNase and hemolysins, but do not express gelatinase, whereas the strains isolated from tracheal secretions expressed the majority of soluble virulence factors tested by phenotypic methods, excepting gelatinase and hemolysins. The results of PCR assays showed that all P. aeruginosa strains isolated from all types of clinical specimens do possess genes encoding elastase, alkaline protease, rhamnolipid, exotoxin A and non haemolytic phospholipase C. Exoenzyme S was present in strains isolated from wound secretions and tracheal secretions, but was absent in those from tracheal secretions, while the gene encoding protease IV was present in a small number of strains isolated from wound secretions and tracheal secretions, and absent from those isolated from bronchial secretions.

Conclusion: The presence of genes encoding for haemolytic phospholipase C, pyroendyme A and exoenzymes S, T and U was associated and absent from those isolated from bronchial secretions. Our goal was to describe the behaviour of these two Prevolta species in the presence of two oral streptococci.

Objective: Earlier studies have shown that periodontitis is associated with a loss of colonization of S. sanguinis and an increased colonization of P. intermedia and P. nigrescens. Our goal was to describe the behaviour of these two Prevolta species in the presence of two oral streptococci.

Methods: The effect of clinical strains of S. sanguinis, S. anginosus was evaluated against reference strains of P. intermedia (DSM 20706) and P. nigrescens (DSM 13386) as well as clinical strains (one each). All clinical strains were identified using MALDI-TOF-MS. Suspicions of 0.5 McFarland from streptococci and Prevolta species (one each on Columbia blood agar (Oxoid, Basingstoke. UK) supplemented with 5% sheep blood, haemin (5 μg/L) (Sigma, Taufkirchen, Germany) and vit. K1 (Sigma). Colonies were counted after 5 days of anaerobic incubation. Each Streptococcus/Prevotella combination was repeated 5 times in parallel with a negative control (sterile broth above Prevotella).

Results: After 24 h of incubation a bactericidal effect of S. sanguinis (at least 3-log reduction of initial inoculum) was observed against both strains of P. intermedia while S. anginosus inhibited the P. intermedia DSM strain only. The same bactericidal effect but after 48 h was observed with S. anginosus against P. intermedia. No bactericidal effect was observed against P. nigrescens. The growth of P. nigrescens in the presence of the streptococcal strains remained either unchanged or slightly decreased.

Conclusions: Both S. sanguinis and S. anginosus have a significant bactericidal effect against P. intermedia. On the other hand, P. nigrescens

Prevalence of cytolytic distending toxin (cdtABC) virulence gene in Campylobacter spp. isolated from diarrhoeal and control cases in Peruvian children under 2 years of age
A. Lluque, J. Ruiz*, A. Prada, T. Ochoa (Lima, PE; Barcelona, ES)

Objective: The aim of this study was to describe the prevalence of cdtABC genes; associated with virulence of Campylobacter species by producing cytotoxin lethal for host enterocytes, in isolates from Peruvian children under 2 years of age, with and without diarrhea.

Methods: Fifty-three Campylobacter strains isolated from a cohort study in Lima, Peru were analyzed. Campylobacter species were identified by colony morphology, Gram’s staining and biochemical reactions. Species identification and the presence of cdtABC gene was performed by PCR with specific primers as described previously. A multiplex PCR using primers previously described was performed to differentiate between C. jejuni and C. coli.

Results: 27 (51%) strains were C. jejuni, and 26 (49%) C. coli. 29 (55%) were from diarrhoea cases, and 24 (45%) were from healthy control. The cdtABC gene was most frequently found in C. jejuni isolates 96% (26/27) (p < 0.001), the one that did not present this gene was from a control case. Only 8% (2/26) of C. coli presented this gene, the two strains belonged to diarrhoea case. 59% (17/29) strains that presented cdtABC gen were from diarrhea cases and 46% (11/24) were from control cases.

Conclusions: Campylobacter strains, specially C. jejuni carrying important toxin production and invasiveness genes are circulating in the population studied. This virulence factor was found not only in diarrhoea cases but also in clinical control cases of C. jejuni, for this reason a surveillance of this bacteria is necessary in both symptomatic and asymptomatic cases. Further studies are necessary in order to look for association among the presence of this gene and clinical features.
Microbial pathogenesis – miscellaneous

P1756 Mechanical ventilation up regulates Toll-like receptor 2 and activation of the lung by bacterial lipopeptide

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Objectives: To assess the impact of mechanical ventilation (MV) on the expression of Toll-like receptor 2 (TLR2) and responsiveness to Gram positive bacterial lipopeptide (BLP), in pulmonary epithelial cells and in ventilated rabbits.

Methods: In vitro experimental study and prospective, randomized animal study. Experiments were performed in two research laboratories from two distinct universities in France and Switzerland.

Subjects: Male adult rabbits.

Results: Cyclic stretch of human pulmonary epithelial cells lines increased both TLR2 mRNA and protein expression. Cells submitted to cyclic stretch also increased interleukin (IL)-6 and IL-8 secretion in response to the bacterial lipopeptide PAM3CSK4, a classical TLR2 ligand. A mild mechanical ventilation protocol induced a 60-fold increase TLR2 mRNA expression in lung tissue when compared with unventilated controls. The combination of mechanical ventilation and airway exposure to PAM3CSK4 acted synergistically in causing lung inflammation and injury. In addition, bacterial growth and pulmonary-to-systemic translocation were increased in rabbits subjected to MV as compared to spontaneously breathing animals in a model of Staphylococcus aureus pneumonia.

Conclusions: Mechanical ventilation increases lung expression of TLR2 and sensitizes the lung to bacterial TLR2 ligands. This may account for the propensity of mechanically ventilated patients to develop acute lung injury in the context of airborne bacterial colonization/infection.

P1757 Virulence of Salmonella enterica serovar Agona Pulse Field Type SAGOXB.0066, cause of the 2008 European outbreak – ex vivo studies

M.S. Martins*, M.P. McCusker, S. Fanning (Dublin, IE)

Objectives: To study the virulence of a Salmonella enterica serovar Agona PFGE-type SAGOXB.0066 strain, directly linked to a pan European outbreak in 2008.

Methods: Ex vivo studies were conducted with Caco-2 cells and human THP-1 macrophages. Salmonella Typhimurium SL1344 was used as a reference. The invasiveness and intracellular growth in Caco-2 cells was assessed using standard protocols. Survival assays were conducted in human macrophages for up to 7 days. Microscopy studies were performed on the macrophages. The supernatants of the cultures were recovered and kept for further analysis. The activation of macrophages was evaluated by the Griess test. An array of cytokines was used to discriminate antibiotic triggered background silent inflammatory bowel disease.

Results: Salmonella Agona SAGOXB.0066 showed an increased ability to adhere and invade Caco-2 cells when compared with Salmonella Typhimurium SL1344. This strain showed a 10-fold difference in adherence and a 20-fold difference in invasion. In the macrophage survival assays S. Agona showed a 10-fold increase. By extending the assay to 7 days S. Agona exhibited an ability to survive and replicate inside the macrophages, while the S. Typhimurium SL1344 infection was cleared. Microscopy studies showed distinct differences in cell differentiation induced by the strains. Macrophages infected with S. Agona showed a significant increase in the number and size of vesicles. Cell culture supernatants were tested for macrophage activation and cytokine production and showed significant differences in the responses induced between the two strains.

Conclusions: In this study, we tested the virulence of Salmonella Agona PFGE-type SAGOXB.0066. This strain exhibited enhanced attachment and invasion of Caco-2 cells. S. Agona showed enhanced survival in the THP-1 macrophages in comparison with SL1344. S. Agona was able to manipulate the macrophage apparatus, avoiding killing to survive even after 7 days post-infection. These findings may explain why this particular S. Agona pulse-field type has caused numerous outbreaks including the pan European outbreak that took place in 2008.

P1758 Bacterial lipoprotein acylation is dispensable for the pathophysiology of experimental group B streptococcal meningitis

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Objectives: Group B streptococcus (GBS) meningitis is particularly frequent in newborns. GBS bacterial lipoproteins (BLPs) are recognized by Toll-like receptor 2. The resulting inflammation initiates the pathophysiology of neuronal damage. We investigated the impact of BLPs acylation on inflammation and brain injury during experimental GBS meningitis.

Methods: GBS strains NEM316 and COH1 and their mutants defective in prolipoprotein diacylglyceryl transferase (delta-lgt) were used either live or ethanol-fixed to induce inflammatory reaction and brain damage. Ethanol-fixed bacteria were injected intracranially at 4×10^5 CFU per animal. Live bacteria were injected at 2×10^5 CFU (COH1) or 1×10^5 CFU (NEM316) and animals were treated 16 h later with ceftriaxone (100 mg/kg, s.c., bid). Cerebrospinal fluid (CSF) was sampled at 2, 4, 8, 12, 16 and 24 h after injection and analysed for myeloperoxidase (MPO), matrix metalloproteinase-9 (MMP-9) and cytokines. Brain damage in the cortex and the hippocampus was analysed by histomorphometry.

Results: (A) Ethanol-fixed bacteria: No differences could be found for CSF MPO and MMP-9 between wild type and corresponding isogenic mutant strains, while significant differences were observed for IL-1, IL-10 and IFN-γ. Comparison between NEM316 and COH1 in animals infected with COH1 revealed that MMP-9 and all cytokines except IL-10 were significantly higher in the COH1 group. Meningitis by ethanol-fixed GBS was characterized by low apoptosis in the hippocampus and no cortical damage. (B) Live bacteria: Productive infection was observed for COH1, while infection with 100x more NEM316 bacteria did not result in consistent bacterial growth. COH1 and its mutant revealed similar bacterial growth in the CSF, similar mortality curves and no differences in cytokine levels. Similar levels of hippocampal apoptosis and cortical damage were found in animals infected with COH1 or its mutant.

Conclusions: Differences in the initiation of inflammatory processes could be observed between strains when used after ethanol fixation. However, they did not translate into significant changes in the pathophysiology of meningitis by live GBS. This argues for LBP-acylation being dispensable for immune recognition during GBS meningitis. Hypervirulence of COH1 compared to NEM316 was confirmed in the present study by a stronger inflammatory CSF reaction and a increased ability to cause productive CSF infection in infant rats.

P1759 Characterisation of virulence factors and antimicrobial resistance in Escherichia coli strains causing pyelonephritis in children

J. Koren, K. Curuxa, M. Kmetou, L. Siegfried*, L. Kovač, H. Hupko (Bratislava, Kosice, SK)

Objectives: Urovirulence factors of E. coli play important role in urinary tract infections. This study was performed to investigate the following urovirulence factors in clinical isolates of E. coli causing admission to this study. She was diagnosed with ulcerative colitis during follow-up period and cured with colectomy. Histology in limited cases did revealed no more than nonspecific acute colitis.

Conclusions: Although this study confirms that AACH may have some relation either with K. oxytoca or C. difficile, unrecognised organisms or any other reason may be considered in patients who remain negative for both pathogens. Not seeing any flares in disease during the long term antibiotic free follow up in nearly all of the patients indicates that AACH is a distinctive entity. However, long term follow up may be necessary to discriminate antibiotic triggered background silent inflammatory bowel disease.

P1757 Characterisation of virulence factors and antimicrobial resistance in Escherichia coli strains causing pyelonephritis in children

J. Koren, K. Curuxa, M. Kmetou, L. Siegfried*, L. Kovač, H. Hupko (Bratislava, Kosice, SK)

Objectives: Urovirulence factors of E. coli play important role in urinary tract infections. This study was performed to investigate the following urovirulence factors in clinical isolates of E. coli causing...
Results: Out of 210 tested samples, 140 (66.7%) were found to be positive for *E. coli*. Five patients had 2 different *E. coli* strains. The tested VFs were found with the following frequency: afa in 105 strains (72.4%), pap in 99 (68.3%), sfa in 78 (53.8%), alfa-hly in 60 (41.4%), cnf1 in 55 (37.9%), and afa in 4 (2.7%) strains. Eight strains (5.5%) had no VF detected, 32 (22.1%) had one, 40 (27.6%) had two, 23 (15.8%) had three, 22 (15.2%) had four and 28 (19.3%) strains had five VFs. Sixty three (43.4%) isolates of *E. coli* strains were resistant to ampicillin and 16 (11.0%) isolates were not susceptible to trimethoprim/sulphamethoxazole; only 6 (4.1%) strains were resistant to ampicillin/sulbactam, 4 strains (2.7%) to cefuroxime, 3 isolates (2.1%) to ciprofloxacin and 1 isolate (0.7%) to gentamicin.

Conclusion: The results showed that *E. coli* is an important pathogenic agent of pyelonephritis in children, with aerobic bacteria as the most frequently detected VF.

Although 43.4% of strains were resistant to ampicillin and 11.0% to trimethoprim/sulphamethoxazole; only 6 (4.1%) strains were resistant to ampicillin/sulbactam, 4 strains (2.7%) to cefuroxime, 3 isolates (2.1%) to ciprofloxacin and 1 isolate (0.7%) to gentamicin.

Acknowledgment: This study was supported by project VEGA 1/0857/10 of Ministry of Education of the Slovak Republic.

**Pathogenic elements among staphylococci isolated from bloodstream and prosthetic devices-associated infections**

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Objective: Coagulase-negative staphylococci (CNS), especially *Staphylococcus epidermidis* and *S. haemolyticus*, are the leading causative agents of neonatal nosocomial sepsis. CNS are also a major cause of biofilm-mediated device-associated infections among hospitalized patients. The first step of staphylococcal infection is attachment to various surfaces followed by production of an extracellular polysaccharide intercellular adhesion (PIA), encoded by the ica operon, and ultimately, biofilm formation. Of clinical importance is also the possible production of TSST-1 and enterotoxins acting as superantigens, by CNS. In this study, CNS isolated from bacteraemias and device-associated infections in terms of methicillin resistance, biofilm formation, ica and toxin gene carriage, were compared.

Methods: A total of 348 CNS (163 from 69 bacteraemic infants and 185 from intravenous catheters, tracheal aspirations and other device-associated infections) were identified at species level by the API Staph System and Vitek 2 Advanced Expert System (bioMerieux, France). Susceptibility tests were performed by the disk diffusion method and Etest to antistaphylococcal agents. PBP2a production was tested by an agglutination test (bioMerieux). Biofilm formation was tested by Christensen’s method. Clonality was identified by PFGE analysis of SmaI and 1 isolate (0.7%) to gentamicin.

Conclusion: The results showed that *E. coli* is an important pathogenic agent of pyelonephritis in children, with aerobic bacteria as the most frequently detected VF.

Although 43.4% of strains were resistant to ampicillin and 11.0% to trimethoprim/sulphamethoxazole; only 6 (4.1%) strains were resistant to ampicillin/sulbactam, 4 strains (2.7%) to cefuroxime, 3 isolates (2.1%) to ciprofloxacin and 1 isolate (0.7%) to gentamicin.

Acknowledgment: This study was supported by project VEGA 1/0857/10 of Ministry of Education of the Slovak Republic.

**A role of triggering receptors expressed on myeloid cells and the myeloid differentiation factor 88 in Helicobacter pylori infection in children**

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Objectives: The host response to bacterial antigens is a key component of the pathogenesis of *Helicobacter pylori*-related diseases. Triggering receptors expressed on myeloid cells-1 (TREM-1) and TREM-2 are involved in the regulation of the immune response to lipopolysaccharide, and the myeloid differentiation factor 88 (Myd88) mediates Toll-like receptor signaling. The aim of this study was to investigate an association between TREM-1, TREM-2 and Myd88 expression and inflammatory response in the gastric mucosa in *H. pylori*-infected children.

Methods: Children referred for endoscopy with clinical symptoms indicating a pathology in the upper gastrointestinal tract were eligible for inclusion. *H. pylori* infection was confirmed by UBT and PCR of gastric biopsies. The expression of TREM-1, TREM-2 and Myd88 were measured from Andes, Sin Nombre, or Seoul virus infected cultures associated with hantavirus infection in humans.

Methods: VEGF receptor and VEGF protein and mRNA expression were measured from Andes, Sin Nombre, or Seoul virus infected cultures of human peripheral blood mononuclear cells (PBMC), THP1, and human pulmonary (HPEC/ST1.6) and dermal (HMEC-1) cells. We also documented changes in monocyte chemokine production by infected endothelial cell lines. Trans-endothelial electrical resistance assay was used to model vascular leak induction using co-cultures of infected monocytes and endothelial cells. Finally, we analyzed cellular junction protein expression profile changes that occurred as a result of this co-culture system.

Results: In support of previous findings, we report an induction of VEGF in both endothelial cell lines tested. We also provide evidence for increased expression of monocyte specific chemokines by infected endothelial cells. Furthermore, we report that cells from monocyte lineages up regulate VEGF after infection with hantaviruses and demonstrate co-culture of infected cell lines results in a synergistic lowering of TEER and alteration in expression of paracellular junction protein profiles.

Conclusions: The pathological mechanism responsible for inducing vascular leak in hantavirus infection remains poorly defined. Data from this work provide additional evidence to this disease have considerable contributions stemming from a deregulated immune response. Here we provide the first evidence of a direct interaction between immune effector cells with the infected endothelium that result in changes in vascular permeability during hantavirus infection.
Results: A total of 78 children were included in the study (40 *H. pylori*-infected and 38 – uninfected). Expression levels of TREM-1, TREM-2 and MyD88 were similar between *H. pylori*-positive and -negative groups both in the gastric epithelium and the lamina propria. Similarly, no differences were seen regarding TREM-1, TREM-2 and MyD88 levels and the cagA status or severity of gastric inflammation.

Conclusion: These results indicate, that in contrast to adults, TREM-1, TREM-2 and MyD88 do not play an important role in a local response to *H. pylori* in children.

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**P1763** Antibodies against a 20-kDa polysaccharide of *Staphylococcus epidermidis* inhibit adhesion and facilitate endocytosis to human macrophages

A. Spiliopoulou, F. Kolonitsiou, I. Spiliopoulou, N.K. Karamanos, D. Mack, E.D. Anastassiou* (Patras, GR; Swansea, UK)

Objectives: *S. epidermidis* is a leading cause of hospital-acquired and biomaterial-associated infection. 20-kDa PS is an extracellular polysaccharide of *S. epidermidis*, distinct from Polysaccharide Intercellular Adhesin (PIA). Immunization of rabbits with purified 20-kDa PS elicits production of antibodies that react specifically with 20-kDa PS and 20-kDa PS-producing *S. epidermidis* strains. Involvement of anti-20-kDa PS antibodies in bacterial adhesion to macrophages and endocytosis was studied.

Methods: *S. epidermidis* ATCC35983 (20-kDa PS (+), PIA (+), biofilm producing,icaADBC (+)) and clinical strain 1505 (20-kDa PS (+), PIA (+), biofilm negative, icaADBC (−)) and specific anti-20-kDa PS antiserum were used in the present study. Human monocytes collected from healthy volunteers were differentiated to macrophages. Bacteria were pre-incubated with PBS, pre-immune rabbit serum, and specific anti-20-kDa PS antiserum. Biotinylated bacteria were allowed to adhere to macrophages for 1 h and attached bacteria remaining after washing were detected by a NeutraAvidin-HRP TMB substrate commercial kit. Macrophages were incubated with bacteria for 1 h, extracellular bacteria were lysed with lysostaphin, cells were lysed with Triton and intracellular bacteria were counted by plating serial dilutions of lysates on agar plates.

Results: *S. epidermidis* ATCC35983 exhibits higher attachment as compared to the clinical strain. Preincubation of ATCC35983 with pre-immune serum does not alter adherence potential, whereas, incubation with anti-20-kDa PS serum diminishes attachment up to 89%. Preincubation of clinical strain with pre-immune serum and anti-20-kDa PS serum minimally inhibits adhesion to macrophages. In terms of anti-20-kDa PS involvement to endocytosis, preincubation of ATCC35983 with pre-immune serum does not alter endocytosis, whereas, preincubation with immune serum increases endocytosis by 10-fold. Preincubation of clinical strain with pre-immune or anti-20-kDa PS serum minimally enhances endocytosis (1.5-fold).

Conclusion: 20-kDa PS mediates attachment to human macrophages as specific anti-20-kDa PS antiserum inhibits adherence. Anti-20-kDa PS antiserum seems to exhibit opsonic properties as preincubation of ATCC35983 promotes endocytosis of bacteria in macrophages. 20-kDa PS could serve as target for opsonic antibody development.

**P1764** A new model to study microRNA modulation in response to *Mycobacterium tuberculosis* infection

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Objectives: MicroRNAs are critical regulators of the mammalian immune system through the fine-tuning of gene expression. *M. tuberculosis* (Mt) can persist alive and replicate into the host due to its ability to interfere with macrophages antimicrobial mechanisms. Up to date little is known on miRNAs role in tuberculosis pathogenesis. Aim of this study is to develop a cellular model to monitor the dynamic regulation of endogenous microRNAs in living cells during Mt infection.

Methods: Human macrophage cells (THP1) were transduced with a lentiviral vector, which expresses two transgens, green fluorescent protein and low-affinity nerve growth factor receptor (NGFR). By inserting a specific miRNA target sequence (miRT) into the 3-prime UTR of the GFP transcript, GFP expression is subject to miRNA-mediated regulation. NGFR is unaffected by miRNA activity and serves as a control. Transduced THP1 were infected with virulent Mt (H37Rv) or attenuated strains, *M. bovis* and BCG. At selected time points, GFP and NGFR expression was quantified by FACS analysis or by qRT-PCR. Prototypical studies were done with miR-155 and miR-146, reported to be key regulators of immune response in macrophages. MiR-223 is a hematopoietic stem cell-specific micro-RNA was used as internal reference.

Results: (a) THP1 cells were efficiently transduced at MOI 1 with no toxic side effects. (b) Consistently with findings in other experimental models, 100nM LPS caused specific raise in miR-146 and -155 levels, thus validating our viral load. Infection with H37Rv and BCG resulted in a time dependent increase of miR-146 levels, as indicated by progressive decrease in GFP fluorescence. Unexpectedly the levels of miR-155 did not change. MiR-223, which is involved only in cell differentiation, did not respond to infection. (c) Incubation of transduced THP1 with Mt cell lysates resulted in a strong time-dependent increase of miR-146 expression. This is consistent with previous studies reporting that lipooligosaccharidomannan (LAM) cause miR-146 increase upon binding of TLR2.

Conclusion: Here, we developed a novel and useful tool for real-time and fluorescence monitoring of miRNAs of interest in living cells. This model could represent a fast screening method to identify miRNAs that are differentially modulated by different Mt strains. This could be a fundamental step towards the comprehension of the pathogenesis of tuberculosis and the design of new drugs and vaccine strategy.

**P1765** Early correlates between viral load and host immune response in critical pandemic influenza disease at ICU admission

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Objectives: Evaluate the burden of viruses secreted by the respiratory tract in the infected patients.

- Identify early host response signatures characterizing those patients admitted to the ICU with the most severe form of the disease.

Methods: We recruited 23 patients attending the participants’ ICUs with primary viral pneumonia with negative respiratory and blood bacterial cultures at admission and 15 controls. Patients were divided into two groups, the MV group needed of invasive mechanical ventilation (n=15) and the NMV group composed of patients not needing of mechanical ventilation at any moment during hospitalization (n=8).

Viral diagnosis and viral load was performed on RNA from pharyngeal swabs by RT-PCR. Oseltamivir resistance was directly detected in the initial positive pharyngeal swab by RT-PCR and sequencing. Immune mediator levels in plasma were measured by using the multiplex BioRad 27-plex assay and ELISAs. For gene expression profiling, RNA was stabilized, purified, amplified, labeled, hybridized to Sentrix Human-v2 Expression BeadChip (Illumina) and scanned on Illumina BeadStation 500GX. Data was analyzed by Ingenuity Pathway Analysis 8.5 software.

Results: Viral load in pharyngeal swabs was 300 fold higher in the group of patients with the worst clinical condition at admission to the ICU. Comparison of cytokine profiles evidenced significant higher levels IL-8, MCP-1, IP-10, IL-12p70, IFN-γ, IL-6, VEGF, GM-CSF, IL-1α and IL-10 in the MV group. Correlation analysis demonstrated a positive associations between these mediators and viral load. Comparison of gene expression profiles between MV and NMV patients revealed 2449 genes differentially expressed between them (P <0.05, FDR <0.1). IPA
revealed that these genes participate in different host response-related signaling pathways.

**Conclusion:** At admission to the ICU, severe respiratory disease following pandemic influenza infection is characterized by an exuberant innate immunity program which correlates with uncontrolled viral replication in these patients. Understanding the role of this initial response to the virus in the genesis of the severe respiratory compromise observed in these patients could help to design better treatment strategies for this disease.

**P1764** Bacterial inoculum impact on the prognosis of patients with *Escherichia coli* bacteraemia

**L. Viñas Castillo* (Seville, ES)**

**Objectives:** 1. To confirm the association between the severe prognosis and the time to detection of growth (TDG) in *E. coli* bacteraemia. 2. To evaluate the influence of TDG by extended spectrum β-lactamases (ESBL) production.

**Methods:** Retrospective observational study. All patients with *E. coli* bacteraemia from 1 January to 31 December 2009 were identified. Data collected included prognosis factors known for *E. coli* bacteraemia (co-morbidities using the Charlson co-morbidity score, the presence of sepsis, severe sepsis and septic shock, and the Pitt score when the blood culture was obtained, supportive treatment and adequate empirical antimicrobial treatment), the TDG as an indirect measure of the bacterial inoculum, fatal clinical outcome (mortality in 48 hours and in 30 days) and other clinical and microbiological variables (source of infection, place of acquisition, ESBL production and the presence of polymicrobial bacteraemia). In a multivariate analysis were included the Charlson and Pitt score and the TDG as variables related to the global and early mortality.

**Results:** During the study period, 226 episodes in 212 patients with *E. coli* bacteraemia were analysed; of which twenty (9%) were polymicrobial bacteraemia. The median age was 63.3 years (range, 18.8–89.8). Median Charlson score was 2 (range, 0–10) and median Pitt score was 1 (range, 0–14). Thirty-one (14%) patients had severe sepsis and twenty-nine (13%) septic shock at presentation. The empirical antimicrobial therapy was adequate in 76% cases and its median delay was zero hours (range, 0–70). The most frequent source of infection was urinary tract (31%), followed by biliary tract (26%) and unknown (22%) infections. One hundred sixty-seven (74.5%) infections were nosocomial and health-care related infections. Thirty-nine (17%) patients died in 30 days and seventeen (7.5%) in 48 hours. The median TDG was 8.3 hours (range, 0.42–76.5). Thirty-one (15%) *E. coli* strains were ESBL producers. In a bivariate analysis, variables associated with a < 8th TDG were the presence of septic shock (p < 0.001) and the global mortality (p < 0.05) but not a ESBL production (p > 0.34). A multivariate analysis, showed that a < 8th TDG was related to global mortality (OR 2.69; 95% CI 1.09–6.62; p = 0.03).

**Conclusions:** 1. The time to detection of growth equal or less than 8 hours is an independent predictor fatal outcome in episodes of *E. coli* bacteraemia. 2. The TDG in *E. coli* bacteraemia is not modified by ESBL production.

**P1765** Role of surface structures of an atypical enteropathogenic *Escherichia coli* in attachment and induction of IL-8 production at early stages of intestinal cell infection in vitro

**S. Sampaio*, T.A. Gomes (São Paulo, BR)**

Atypical Enteropathogenic *Escherichia coli* (aEPEC) promote attaching and effacing (A/E) lesions in the intestinal epithelium, with intimate bacterial attachment to host cells mediated by intimin, an outer membrane adhesin. A/E lesions formation is dependent on the injection of effector proteins by a type 3 secretion system (T3SS). Recent studies have shown an important role of flagellar proteins in adherence and gut colonization in different bacterial pathogens.

**Objectives:** To evaluate the role of three surface structures of aEPEC strain 1711–4 (serotype O51:H40): flagellum, intimin and the T3SS in the adhesion and induction of IL-8 production at early stages of intestinal cell infection in vitro

**Methods:** Mutants deficient in FliC, the main flagellum protein (strain 17fliC-), EscN (the ATPase component of the T3SS) (strain 17escN-) and intimin (strain 17eae-), were constructed by insertion mutagenesis. Polarized monolayers of Caco-2 or T84 cells were infected separately with approximately 1.5 × 10⁵ colony forming units (CFU)/well of aEPEC 1711–4 or each isogenic mutant. Strains E. coli DH5α (non-pathogenic) and Salmonella Typhimurium (IL-8 inducer) were used as controls. Cell culture supernatants were collected two hours after infection and concentrations of IL-8 were measured by ELISA. Cells were lysed and the number of bacteria was determined.

**Results:** Two hours after infection of Caco-2 and T84 cells, the mean CFU/well of cell-associated aEPEC 1711–4 was at least 100 and 600 fold higher compared to the isogenic mutants (P < 0.001), respectively. In Caco-2 cells, only the 17fliC- mutant showed a significant reduction in the ability to induce IL-8 production, i.e., ~20 fold less than aEPEC 1711–4 (45 pg/ml) or the 17escN- and 17eae- mutants (35 and 38 pg/ml, respectively). Likewise, in T84 cells, only the 17fliC- mutant showed a significant reduction of IL-8 production (10 pg/ml) as compared to aEPEC 1711–4 (37 pg/ml) (growth rate: 24 h, P < 0.001). After 24 h of infection, there was no difference between IL-8 levels induced by aEPEC 1711–4 and its isogenic mutants in Caco-2 cells, whereas in T84 cells there was a twofold difference between IL-8 levels induced by aEPEC 1711–4 (240 pg/ml) and the 17fliC- mutant (150 pg/ml), in the same period.

**Conclusion:** Our data suggest that flagella, intimin, and the T3SS are necessary for efficient adhesion of aEPEC 1711–4 and FliC is the main IL-8 inducer at early stages after enterocytes infection in vitro.
Discussion: The duration of the lag phase was associated with median survival time whereas the maximal fungal biomass was related with survival. The combination of in vitro growth indices like the X0/Ymax ratio and in vivo virulence indices like SURSMST may be a useful tool for predicting virulence in Af.

May the brain parenchyma be influenced in cutaneous anthrax patients without central nervous system disorder? A magnetic resonance spectroscopy study

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Objectives: Cutaneous anthrax, caused by Bacillus anthracis, is the most common form of human anthrax. After gaining access of spores to tissues, the spores germinate into vegetative forms. The symptoms appear after producing of toxins by vegetative bacteria. The roles of anthrax toxins in the cellular pathology of infected hosts are mostly unknown. We aimed to evaluate whether the subtle metabolic cerebral changes are present in normal-appearing white matter on conventional magnetic resonance (MR) imaging, in patients with cutaneous anthrax, by using MR spectroscopy (MRS).

Methods: The group of patients with cutaneous anthrax had consisted of 5 males and 5 females not having systemic or neurological diseases, with ages between 16 and 63 (36.3±17.0) years. Thirteen healthy subjects with ages between 26 and 43 (34.1±5.2) years constituted the control group.

Brain magnetic resonance imaging (MRI) examination consisted of conventional imaging and single-voxel MRS. Magnetic resonance spectroscopy was performed by using a point-resolved spectroscopy sequence (PRESS; TR/TE: 2000/136, 128 averages). 20 mm × 20 mm × 20 mm voxels were placed in normal-appearing parietal white matter (NAPWM).

Conventional MRI and MRS findings were evaluated by three neuroradiologists, independently.

Results: Ten patients with cutaneous anthrax and 13 healthy control subjects, had no signs and symptoms in terms of involvement or diseases of central nervous system, were investigated with conventional MR imaging and single-voxel MRS. N-Acetyl aspartate (NAA)/creatine (Cr) and choline (Cho)/Cr ratios were calculated. There were no statistically significant differences between patients and controls in NAA/Cr ratios on the MRS findings (2.11±0.35 and 2.10±0.12, respectively, p=0.90).

However, Cho/Cr ratios were significantly increased in the patients compared to controls (1.18±0.12 and 0.99±0.11, respectively, p=0.001) (Figure).

Conclusion: MRS revealed metabolic changes in normal-appearing white matter of patients with cutaneous anthrax. Anthrax may cause subtle cerebral alterations, which may only be discernible with MRS. Increased Cho/Cr ratio possibly represents an initial phase of inflammation due to anthrax toxins in the central nervous system. Metabolic changes of cerebral parenchyma without neurological findings were showed in cutaneous anthrax. These changes may be related to systemic effects of anthrax toxins.

The cutaneous interface as a key event in Lyme borreliosis: study of different human pathotypes in a murine model

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Lyme disease, is an infectious disorder caused by a tick-transmitted bacteria: Borrelia burgdorferi. The skin constitutes an essential interface in this arthropod borne disease. Indeed, the primary manifestation is a cutaneous inflammation, the erythema migrans. Dissemination of spirochetes from the site of inoculation can lead to other manifestations typically involving the skin, heart, joints or central nervous system. Mechanisms responsible of this specific dissemination are not known. In this study we postulate that a specific cutaneous innate immune response could be involved in the bacterial organotropism. Part of skin innate immunity is constituted by the secretion of antimicrobial peptides (AMPs). Two families of AMPs are well-represented in the skin: the cathelicidin (CRAMP) and the defensin (mBD3 and mBD14). The role of these AMPs, of the TNF-α and of the chemokine MCP-1 has been assessed. We studied their potential role in the skin interface during the early development of Lyme borreliosis.

We challenged C3H/HeN mice with spirochetes from B burgdorferi sensu stricto strains initially isolated from human single skin lesions (EM), from disseminated skin lesions (multiple EM), from cerebrospinal fluid of a patient with neuroborreliosis. The Borrelia strain N40 isolated from an Ixodes tick was also tested as a reference strain. Mice were syringe inoculated and the skin innate immunity at the site of inoculation was studied for each strain. Gene expression was analyzed by quantitative RT-PCR. The different Borrelia pathotypes induce different gene expression profiles: N40 induces a high expression of cathelicidin, MR726 induces mBD-3 defensin. At 5 or 7 days post-infection, a strong induction of TNF-α and MCP-1 was noticed for all strains tested. MCP-1 is known to increase endothelial permeability, that could facilitate Borrelia dissemination. This study underlines the importance of the Borrelia strain choice, in the study of Borrelia pathogenesis.

Pseudomonas aeruginosa virulence potential: comparison between clinical isolates and pristine water isolates obtained from a hydrotherapy facility

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Objectives: Pseudomonas aeruginosa (PA) is a ubiquitous environmental bacterium capable of causing a variety of human infections. Several virulence factors (VF) have been detected in clinical PA, and these traits are also important for environmental bacteria, as previously reported. Thermae is a health care unit that uses natural mineral water (NMW) to treat several health conditions by inhalation and irrigation of different mucosal tissues. Thermae NMW cannot be disinfected and if PA appears in NMW it can spread through the water distribution system and arrive at the treatment equipments, contacting with the thermas users. The aim of this study was to understand the prevalence of VF in NMW PA and to compare them with PA clinical isolates.

Methods: PA collection was composed of 49 isolates obtained from a central hospital, 32 resistant to several antimicrobials and 16 susceptible, and 77 isolates obtained from a hydrotherapy facility, susceptible, in general, to antimicrobials. PCR of 7 different VF encoding genes was performed: 3 secreted VF genes (phenazine1, apr, lasB); 2 type III effector system genes (exoS, exoY) and genomic islands PAGI-1 and PAGI-2 genes (orf3 and c105, respectively).

Results: VF genes were more frequent in PA environmental isolates except for exoY and c105, present in a higher rate in clinical isolates. All VF genes were present in more than 60% of all environmental isolates, except for PAGI-2 gene (24%). Curiously, PAGI-1 gene was the more prevalent VF gene in the environmental collection, present in more than 90% of all these isolates. Regarding clinical isolates, VF genes were present in a lower rate, with 3 VF genes present in less than 50% isolates. ExoY was the more prevalent VF gene in clinical isolates (90%) and lasB the less frequent (41%). Comparing antimicrobial resistant clinical...
isolates to susceptible ones, all VF genes were detected in a higher rate in the former, except for apr. ExoY was present in all resistant isolates.

**Conclusion:** Environmental PA was obtained from a thermoe facility, where patients contact with pristine NMW to improve their health. Clinical PA isolates, obtained from human infections, presented lower rates of VF genes when compared to environmental ones. Although we cannot predict if the detected genes are functional, the remarkable conservation of genes encoding VF indicates that most isolates, regardless of source, possess the basic pathogenic mechanisms necessary to cause human infections.

**P1772** Chemokine secretion after stimulation with *Staphylococcus aureus* is increased in human neutrophil granulocytes from female donors compared to male controls

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**Objectives:** Male patients have a higher incidence of invasive infections with *Staphylococcus aureus* (*S. aureus*). The reason for this gender-specific difference, however, is unknown. The goal of our study was to investigate the role of chem- and cytokines secreted by neutrophil granulocytes (polymorphonuclear leucocytes, PMN) – the most important cells in the first line of defence against bacterial pathogens – in this setting.

**Methods:** We isolated PMNs from peripheral blood of age-matched healthy female and male human donors. After stimulation with opsonised *S. aureus* or lipopolysaccharide (LPS) the production of IL-8, MIP-1a, MIP-1b, IL-1ra, and TNF was measured using ELISA and Luminex® technology.

**Results:** PMNs of female donors incubated with *S. aureus* produced significantly more chemokines IL-8 and MIP-1b than male controls, whereas stimulation with LPS alone did not lead to significant differences. No significant differences in the production of TNF, IL-1ra, and MIP-1a were observed after stimulation with either *S. aureus* or LPS.

**Conclusions:** IL-8 and MIP-1b play an important role in activation and recruitment of PMNs. An increased production of these chemokines in the early stages of bacterial infections might contribute to the lower incidence of invasive bacterial infections in female patients.

**P1774** Dexamethasone reduces *Staphylococcus aureus*-induced production of inflammatory cytokines and matrix metalloproteases in primary human chondrocytes and synovial fibroblasts

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**Objectives:** *Staphylococcus aureus* (*S. aureus*) is the most frequent pathogen involved in acute bacterial arthritis. Joint infections due to *S. aureus* are associated with a high rate of sequelae and poor functional outcome. We hypothesize that concomitant glucocorticoid therapy may reduce joint destruction during staphylococcal arthritis.

**Methods:** Primary human chondrocytes, synovial fibroblasts and osteoblasts were infected with viable *S. aureus*. The production of the inflammatory cytokines IL-6 and IL-8 (CXCL8), the secretion of matrix metalloproteases (MMP-1, MMP-13), cell viability and intracellular bacteria with or without coinoculation with dexamethasone were studied for 5 days.

**Results:** Primary human chondrocytes produced significantly more IL-6 and IL-8 than fibroblasts and osteoblasts and high levels of matrix metalloproteases in response to *S. aureus*. Coincubation with dexamethasone led to a significant decrease in IL-6 and IL-8 production in all cell types. Additionally, dexamethasone led to a significant reduction in the *S. aureus* induced production of matrix metalloproteases by primary chondrocytes. No differences in cell viability and intracellular CFU were found between cells incubated with dexamethasone and controls.

**Conclusion:** The host immune system and tissue destructive substances contribute to permanent joint damage in *S. aureus* arthritis. The use of dexamethasone may modulate inflammation and tissue destruction and may eventually contribute to a better functional outcome after *S. aureus* arthritis.

**P1775** Relationship between *Bordetella pertussis* and chronic obstructive pulmonary disease

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**Objectives:** There is some evidence indicating the role of *Bordetella pertussis* as a trigger for pulmonary diseases. The aim of this study was to investigate the association of *B. pertussis* with chronic obstructive pulmonary disease (COPD).

**Methods:** In a case-control study, two groups including patients with COPD and age- and sex-matched control group were included. Serum samples were tested for *Bordetella* IgG and IgA by Enzyme Linked Immunosorbant Assay. A questionnaire including demographic characteristics, habitual history, and spirometry findings was completed for each patient. Data were analyzed using SPSS.

**Results:** Ninety patients with COPD and 90 subjects in the control group enrolled in the study. *Bordetella* IgG seropositivity was detected in 83 (92.2%) COPD patients and 46 (51.1%) controls (*P* = 0.001). There was no significant association between *Bordetella* IgA seropositivity and COPD. Of 90 patients with COPD 66 (51%) had mild, 31 (34.4%) moderate, and 13 (14.4%) severe disease. No significant association was found between the severity of COPD and the frequency of *Bordetella* IgG and IgA seropositivity.

**Conclusion:** The results suggest that there is an association between *B. pertussis* infection and COPD. Further studies should be planned to understand the potential pathogenic mechanisms that might underlie these associations.

**P1776** Altered protein secretion of *Chlamydia trachomatis* in persistently infected human endocervical epithelial cells

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**Objectives:** To observe altered protein secretion of *Chlamydia trachomatis* in persistently infected human endocervical epithelial cells.

**Methods:** We demonstrate that ampicillin exposure resulted in altered *C. trachomatis* secretory activity that is coupled with its adaptation to a persistent mode of growth using both primary human endocervical epithelial and HeLa cell infection models.

**Results:** We observed a decrease in secretable CPAF in the cytosol of epithelial cells with no evident reduction of CPAF product by *C. trachomatis*. In contrast, the expression of CopN and Tarp were down-regulated, suggesting *C. trachomatis* responds to ampicillin exposure by selectively altering the expression of secretable proteins. We also...
demonstrated that ampicillin exposure resulted in alterations to the outer membrane of C. trachomatis.

**Conclusion:** Together these results suggest that the regulation of both gene expression and the sub-cellular localization of secretable virulence proteins of C. trachomatis is involved in the adaptation of C. trachomatis to a persistent infection state in human genital epithelial cells.

**P1777** Screening of nuclear targeting proteins in *Acinetobacter baumannii* and their pathologic effects on host cells

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*Acinetobacter baumannii* is an important nosocomial pathogen that causes a variety of human infections, but pathogenic mechanisms of this microorganism are not fully understood. To investigate *A. baumannii* pathogenesis regarding the nuclear targeting of *A. baumannii* proteins and subsequent host cell pathology, we screened nuclear targeting proteins in *A. baumannii* and determined their cytotoxic activity in host cells. Thirty-seven functional or hypothetical proteins were predicted to carry the putative nuclear localization signal (NLS) sequences among 3,367 open reading frames of *A. baumannii* ATCC 17978. Of the 31 elements generated by the Gateway® recombinational cloning system, 14 *A. baumannii* proteins tagged with green fluorescent protein (GFP) targeted to the nuclei of host cells, whereas 17 GFP-tagged proteins localized in the cytoplasm. Among the 14 nuclear targeting proteins, S21, L20 and L32 ribosomal proteins and transposase were predicted to carry nuclear export signal (NES) sequences, but only transposase harbored the functional NES. After translocation to the nuclei of host cells, seven *A. baumannii* proteins induced host cell death. Five nuclear targeting proteins were found in *A. baumannii*-derived outer membrane vesicles (OMVs), suggesting that OMVs may deliver these proteins to host cells. Our data provide a novel insight into *A. baumannii* pathogenesis regarding nuclear targeting of bacterial proteins and extend our knowledge of bacterial pathogenesis.

**P1778** Effect of avian influenza A/H5N1 infection on human cellular microRNA profile – identification of gene regulatory pathways leading to adverse clinical outcome

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**Background:** Avian influenza remains as a serious threat to poultry and human health. Recently, a novel system of gene regulation in plant and mammalian cells has been revealed. This system is orchestrated by short RNA molecules (microRNAs) that are differentially expressed according to the physiological and pathological status of the cells concerned. Viruses can interact with the miRNA-mediated gene regulatory pathways. Therefore, the profile of host cell miRNAs may change as a consequence of avian influenza viral infection.

**Aims and Objectives:** This study aims at elucidating how avian influenza infection perturbs the human gene regulatory pathways leading to adverse pathological events, e.g. cytokine storm. The ultimate goal is to generate essential information for further studies to identify novel intervention targets to ameliorate the outcome of infection.

**Study design and Methods:** The human lung cell-derived H292 cell line were used to establish an in-vitro system for influenza infection. H5N1-infected cells were screened for the expression profile of miRNAs using a highly sensitivity, broad-catchhing approach, i.e. microarray. Similar data were obtained from H1N1-infected cells for comparative analysis to identify miRNAs that were differentially up/down-regulated following H5N1 infection.

**Results and Conclusions:** Based on the broad-catching miRNA microarray results, we found that dysregulation of miRNA expression were mainly observed in highly pathogenic avian influenza infection. A list of differentially expressed miRNAs was identified for subtypes H1N1 and H5N1, and the temporal pattern of expression was delineated.

Among the differentially regulated miRNA, it was found that hsa-miR-1246, hsa-miR-663 and hsa-miR-574-3p were highly up-regulated (>3-fold) in H5 infection compared to H1. Also, hsa-miR-100*, hsa-miR-21*, hsa-miR-141, hsa-miR-1274a and hsa-miR-1274b were found to be highly down-regulated (>3-fold) in H5 infection, particularly during the late post-infection phase compared to H1. It is worthwhile to further predict and characterize the function of these miRNAs by in-vitro and in-silico approaches.

**P1779** Dependence of group B streptococci adherence on serotypes and α-like protein genes

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**Objectives:** Adherence of Group B streptococci (GBS) to epithelial cells has been shown to be an important factor in the colonization of mucus membranes of human rectum and vagina. Therefore, the aim of the study was an assessment of the adhesion of chosen GBS strains to the human colon adenocarcinoma cell line (HT29) and human epidermoid vulvo-vaginal cells (A431) in relation to capsular polysaccharides of GBS and α-like protein genes.

**Methods:** The adherence of GBS to HT29 and A431 cell lines was tested. 26 strains of GBS from human sources belonging to Ia, Ib, II, III or V serotypes possessing different α-like protein genes such as alp2, alp3, bca, ε or rib and GBS standard strains in the conventional adherence assay were used. GBS strains were inoculated at 1×10⁶ CFU/ml/well (OD600 of 0.6−0.7) onto HT29 monolayers and A431 cells for 30 minutes. The infected cells were washed 3 times in PBS, fixed in formaldehyde and Gram-stained. The results were obtained semiquantitatively by counting bacterial cells in randomly chosen 20 fields. Each test was done in duplicate. This study was supported by grants no. N N401 042337 and N N401 042938.

**Results:**
1. Adherence of GBS strains to HT29 cell line was significantly higher than to A431 cell line.
2. For GBS serotype III and II a significant difference between adhesion to HT29 and A431 cell lines was shown.
3. Adhesion of GBS strains to HT29 was not dependent on serotype but on α-like protein genes. The most adhesive were the GBS strains containing rib and alp2 genes.
4. Adherence of GBS strains to A431 depended on both their serotype and α-like protein genes. Serotype III adhered to A431 cells the strongest, especially strains containing rib and alp2 genes.
5. GBS strains containing the rib gene adhered to HT29 and A431 cell lines more strongly than GBS strains possessing other α-like protein genes.

**Conclusions:** Adherence of GBS is not a species attribute, so it changes among strains and depends on GBS serotype and α-like surface proteins. Our results showed significantly higher adherence to HT29 cell line than to A431 and it is a confirmation that GBS is primarily a constituent the gastrointestinal tract and only secondarily colonises the vagina. Moreover, GBS adherence to vulvo-vaginal cells was closely related to serotypes and α-like protein genes in opposition to the adherence to HT-29 which showed a a relation only to rib and alp2 genes.

**P1780** The Soy protein of the *Porphyromonas gingivalis* secretion system functions as an independent virulence factor

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**Objectives:** *Porphyromonas gingivalis*, an asaccharolytic, Gram-negative anaerobic bacterium is recognized as an important pathogen of periodontitis. The bacterium secretes large amounts of cytotoxic proteases, gingipains, which are essential virulence factors. The mechanism of gingipain secretion is a subject of intense investigation and up to date several proteins have been identified as components of the outer membrane (OM) translocation machinery. Apart from functioning in the
gingipain secretion some proteins of the secretion apparatus may work as independent virulence factors. To verify this assumption we have compared ability of mutants deficient in individual proteins involved in secretion to form biofilm and adhere to professional phagocytes in the context of the outer membrane proteome.

Materials and Methods: Wild-type *P. gingivalis* W83 and isogenic mutants deficient of sop, porT, PG0022, PG0266, PG0543, and PG1403 genes essential for gingipains secretion were grown to an early stationary phase. Bacterial biofilm formation and adherence to the RAW 264.7 macrophages were estimated by crystal violet staining and FACS analysis, respectively. Gingipain activity was assayed in full culture and bacterial cell homogenates. The outer membrane fraction was separated, resolved by SDS-PAGE and subjected to peptide fingerprinting to identify proteins in the OM.

Results: In contrast to the parental strain, secretion system mutants show no gingipain activity in full cultures and in cell homogenates. All mutants adhered to the plastic surface far more efficiently than the wild type strain W83. Most interestingly, however, only the sop mutant vividly adhered to macrophages. To explain the mechanism of adherence, the proteome of the OM derived from wild-type, delta-sop and delta-PG0266 strains was compared. The proteome of both strains was found identical but different than that of the parental strain.

Conclusion: The lack of gingipain secretion strongly enhances the *P. gingivalis* binding to plastic surface. Conversely, gingipains are apparently dispensable for protecting the bacterium from adherence to macrophages. From the comparative analysis of mutant strains phenotype and the OM proteome, the Sov protein was identified as the virulence factor which prevents the direct contact between *P. gingivalis* and macrophages. From the comparative analysis of mutant strains was compared. The proteome of both strains was found identical but different than that of the parental strain.

P1781 Impact of different peritoneal dialysis fluids on bacterial and yeast growth of common pathogens

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Objectives: Peritoneal dialysis (PD) used in the treatment of patients with end-stage renal failure is often complicated by peritonitis. The major pathogens of PD associated peritonitis are staphylococci, followed by *P. aeruginosa*, *E. faecalis*, and *S. aureus*. The aim of this study was to evaluate the growth of the most common pathogens of PD associated peritonitis in PD fluids (PDFs) containing different glucose concentrations, icodextrin or amino acid.

Material and Methods: Growth kinetics of microorganisms were performed in the PDFs: Dianeeal PD4® (glucose 1.36%, 2.27%, 3.86%), Physioneal 40® (glucose 1.36%, 2.27%, 3.86%), Extraneal® (7.5% icodextrin), and Nutrineal PD4® (1.1% amino acid). The bacterial test isolates comprise *S. aureus* ATCC 25923, *S. epidermidis* ATCC 35983, *E. faecalis* ATCC 29212, *P. aeruginosa* ATCC 27853, and *C. albicans* ATCC 90029. Sabouraud bullion (SAB) and Mueller-Hinton Broth (CAMHB) were used as control broths. Ten milliliters of each PDF and of control broths containing an inoculum of approximately 106 CFU/ml were incubated for 24 h at 37 ºC, and for 24 ºC for yeasts. Samples were taken at 0, 2, 4, 6, 8 and 24 h the number of CFU/ml was determined.

Results: Influence on bacterial and yeast growth in terms of less pronounced growth was demonstrated for all eight PDFs when compared to the control broths. After 24 hours incubation a significant reduction in the number of viable bacteria (p < 0.05) was detected for each PDF compared to the initial inoculums. However, glucose-based PDFs supported less bacterial growth than non-glucose-based PDFs, particularly in staphylococci. After 4 hours, a significant reduction of the viable bacterial count of *S. aureus* and *S. epidermidis* (mean 0.82–1.44 log10 CFU/ml, p < 0.05) was detected in all glucose-containing PDFs regardless of the tested glucose concentration. When comparing the two different types of glucose-containing PDFs, growth of *S. aureus* was higher suppressed in Physioneal 40® within four hours than in Dianeeal PD4® (p < 0.05), whereas no difference in growth of *S. epidermidis* was detected. In *C. albicans*, suppressive effect on the fungal growth was detected after 24 hours in all PDFs.

Conclusion: In vitro study show that all tested PDFs attained inhibitory effect on the microorganism. This in vitro observation could be beneficial for prophylaxis and treatment of patients with recurrent PD-associated infections.

Pathophysiology of infectious diseases, in vivo

P1782 Can Salmonella cure cancer? Insights on a novel antitumoural mutant

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Objectives: *Salmonella enterica* serovar Typhimurium attenuated mutants have been tested in experimental treatments against different cancer lineages. It has been demonstrated by different groups that those mutants present a strong tropism for necrotic/hypoxic regions, colonizing areas that are inaccessible to conventional chemotherapeutic drugs. Our goal is to discuss the efficacy of a novel *Salmonella* Typhimurium attenuated mutant (under patent requirement) in the treatment of murine 3LL carcinoma and B16F10 melanoma.

Methods: The *S. enterica* Typhimurium mutant was constructed using the lambda red system. B16F10 melanoma and 3LL carcinoma cells were cultured in vitro and injected subcutaneously in the back of C57BL/6 female mice. Intratumoural injections of the mutant strain in different c.f.u. concentrations were administered when tumour volume reached approximately 50mm3. Results: When mice were administered with *S. enterica* titles equal or higher than 107 c.f.u. B16F10 tumour disappeared completely within 24 hours (3LL tumour became smaller and presented a necrotic aspect), but mice mortality was high probably due to a strong immunological response or tumour lysis syndrome, since oral and intravenous administration of the mutant strain in health mice did not cause any collateral effect. Administration of lower doses decreased tumour volume but did not eliminate the total tumour mass. Instead, within 4 days, part of the 3LL and B16F10 tumour masses sear and 80% of mice survive longer than the control group administered with PBS. Histological analyses of treated 3LL show hemorrhagic sites, polymorphonuclear cells infiltration and areas of cell death.

Conclusions: Our novel *S. enterica* mutant has potential antitumour efficacy but new treatment improvements must be done in order to obtain optimal results.

P1783 Induction of streptococcal experimental endocarditis by cumulative low-grade bacteraemia mimicking spontaneous bacteraemia in humans

T.R. Veicso, M. Amiguet, M. Giddey, J. Youillamos, V. Rousson, P. Moreillon, J.M. Entenza* (Lausanne, CH)

Objectives: Medico-surgical procedures in the oral cavity can provoke transient, high-grade bacteraemia. This is a risk factor for infective endocarditis, as supported by clinical studies and experimental endocarditis in animals. Yet, case control studies suggest that cumulative exposures to low-grade bacteraemia occurring during daily-life activities, such as tooth brushing, can also provoke IE. However, no experimental demonstration of this later possibility exists. Here we investigated the capacity of oral streptococci – one of the primary causes of infective endocarditis – to induce valve infection in a new model of experimental endocarditis induced by persistent, low-level bacteraemia.

Methods: Rats with sterile aortic vegetations were inoculated with 105c or 106cfu of strains *Streptococcus intermedius* or *Streptococcus gordonii*. Identical inoculum sizes were administered either by intravenous bolus (1 ml in 1 min) or by continuous intravenous infusion (0.0017 ml/min over 10h). Bacteremia levels were determined 1 min (bolus) or 2h (continuous infusion) after inoculation [expressed as
median (range) CFU/ml of blood]. Vegetation infection was assessed 24h later.

**Results:** Bacteria levels and rates of vegetation infection are shown in the table.

**Conclusion:** Persistent, low-grade streptococcal bacteremia represents a non-negligible, sometimes even similar risk of experimental endocarditis than transient high-level bacteremia. These experimental results support the hypothesis that streptococcal infective endocarditis can result from cumulating bouts of low-grade bacteremia provoked by daily-life events, rather than only after procedure-induced high-level bacteremia.

| Inoculum/Strain | Bacteria (CFU/ml) | Infected vegetation/total | Bacteria (CFU/ml) | Infected vegetation/total |
|-----------------|------------------|--------------------------|------------------|--------------------------|
| $10^6$ CFU S. intermedia | 10^3 | 58 (82%) | 4 (0-20) | 3/10 (30%) |
| S. gordonii | 10^3 | 88 (100%) | 1 (0-7) | 12/17 (71%) |
| $10^4$ CFU S. intermedia | 10^6 | 6/6 (100%) | 10 (1-24) | 7/10 (70%) |
| S. gordonii | 10^6 | 6/8 (100%) | 9 (1-13) | 6/8 (100%) |

**[P1786]** Role of *Candida albicans* and *Pseudomonas aeruginosa* direct interaction in the protective effect of *C. albicans* colonisation on *P. aeruginosa*-induced lung injury

**Objectives:** *Pseudomonas aeruginosa*, frequently responsible for ventilator associated pneumonia, can often be isolated in association with *Candida albicans*, mostly considered a colonizing agent in the respiratory tract. These pathogens can interact through their Quorum Sensing (QS) or adhesion molecules which may modulate each other's virulence. In a mouse model, prior airway colonization with *C. albicans* attenuated *P. aeruginosa*-induced lung injury. Our aim was to determine whether *C. albicans* was responsible for this protective effect through direct interactions, either by modulation of QS-dependent *P. aeruginosa* virulence or as a decoy target for *P. aeruginosa*.

**Methods:** Reference strains were *C. albicans* Ca SC 5314 (Ca) and *P. aeruginosa* PaO1. Ca ATCC 10231 (CaD) was used as a strain naturally deficient in the *C. albicans* QS molecule, farnesol. Germ-tube formation inhibition assay was used to confirm farnesol deficiency. In vitro with farnesol (1microM) on PaO1 QS-dependent virulence factors (pyocyanin, rhamnolipid, and elastase) were assessed by colorimetric assays. Ca EFG1−/− (CaDEfg1) was used as a non-filamentous strain. *C. albicans* was assessed as a target for *P. aeruginosa* by an in vitro co-culture killing assay. The in vivo effects of prior colonization by *C. albicans*, either farnesol-deficient or non-filamentous, were evaluated at 48h on *P. aeruginosa*-induced lung injury as assessed by alveo-capillar permeability to FITC-Albumin and on bacterial clearance assessed by *P. aeruginosa* burden.

**Results:** CaD strain was confirmed as farnesol-deficient since its supernatant did not prevent germ-tube formation (p < 0.001 vs. Ca). Farnesol in vitro significantly lowered *P. aeruginosa* QS-dependent virulence factor secretion (p = 0.0016 for pyocyanin, p = 0.0061 for rhamnolipid and p = 0.0043 for elastase). Ca filaments were confirmed as a target for *P. aeruginosa*, killed in an in vitro co-culture assay (p < 0.05 vs. non-filamentous strain), whereas CaDEfg1 survived. However, in vivo, prior airway colonization with either farnesol-deficient (CaD) or non-filamentous (CaDEfg1) strains did not decrease the protective effect of *Candida on P. aeruginosa*-induced lung injury (p = 0.005 for Ca and CaD, p < 0.05 for CaDEfg1) and bacterial clearance (p < 0.0001 for Ca and p = 0.05 for CaD and CaDEfg1).

**Conclusion:** Decreased severity of *P. aeruginosa*-induced lung injury occurring after *C. albicans* airway colonization is not due to farnesol nor to *C. albicans* as a decoy target.

**Objectives:** The aim of the study is to explore 3 originally synthesised biomaterials and bacterial colonisation risk and their impact on the surrounding tissue: material A – raw materials and products are crystalline, B – the substance is an amorphous, crystalline product; B+ – B etched in order to reduce the amorphous phase.

**Methods:** *Pseudomonas aeruginosa* and *S. epidermidis* were used for the preparation of suspensions in concentration of 100 and 1000 CFU/ml. Biomaterial samples were cultivated at 37 C for 2h to promote adhesion and then were implanted in intercapsular area of rabbits (for 2 and 4 weeks). The biomaterial was removed and, using plate count and sonification method the bacterial colonisation on the surface of the biomaterial was determined, however, we prepared preparations from the surrounding tissues, staining in haematoxylin-cosin and using immunohistochemistry methods thus determining TNF-A, B-defensin-2 and II-10.

**Results:** Biomaterial samples contaminated with *S. epidermidis* showed a low degree of colonisation. Two samples (A and B) after 2 weeks of exposure were sterile. *P. aeruginosa* showed a higher degree of colonisation with intensity from 0.21 CFU/ml (B biomaterial) up to 8.7 CFU/ml (B+ biomaterial). The most intense inflammatory reaction was observed around the *P. aeruginosa* contaminated biomaterials. Many TNF-A and II-10-containing inflammatory cells infiltrated tissues surrounding the aforementioned materials. Indicators of inflammation practically did not differ for the 2 and 4 week implants of biomaterials.

We observed some defensin-containing cells in tissue surrounding biomaterials after *P. aeruginosa* infection, while in other biomaterial contamination cases such cell amount were much more moderate.

**Conclusions:** The study showed that *P. aeruginosa* compared with *S. epidermidis* more intensively colonised biomaterials in the in vivo study. *P. aeruginosa* infection tends to cause depletion of B-defensin 2 production in tissues, and therefore may lower the host non-specific resistance. TNF-A and II-10 production expression is not affected by such a short (2 and 4 weeks) biomaterial implantation. Cytokine expression is characterised by pronounced tissue around biomaterials, which are contaminated with *P. aeruginosa*.

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**Molecular point-of-care tests**

**P1797** Clinical and economic impact of GBS intrapartum screening using molecular diagnosis

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(Paris, Nancy, FR)

**Objective:** Since January 2010, in our institution, the prevention of neonatal EOGBS infections is based on intrapartum screening using real time PCR. The objective of this study is to assess the cost of this strategy from the perspective of the French Health Insurance (Assurance Maladie) as well as the consequences in terms of number of infections, compared with prevention based on antenatal screening as used previously (2009).

**Materials and Methods:** An exhaustive data collection on EOGBS infections was performed. French Healthcare Reimbursement rates from 2009 were applied to data recorded in 2009 and data for the first 9 months of 2010. The cost of intrapartum screening by PCR could not be coded, and was evaluated on the basis of “Nonenclature Montpellier” reimbursement scheme.

**Results:** The incidence of EOGBS infections was 9.06‰ in 2009 (prevention based on antenatal screening by culture) and was 4.23‰ in 2009 (prevention based on antenatal screening by culture).
in 2010 (prevention based on intrapartum PCR screening) (p = 0.04).
Calculated on the basis of the 2009 activity, in 2010, if prevention had been based in its totality on antenatal screening, we would have expected 19 GBS infections with subsequent 90 neonatology hospitalization days and 33 days on ICU. In applying the 2009 rates, the cost for the health insurer would have been €90 311 in neonatology and €29 662 for antenatal screening by culture: a total cost of €119 973 (≈ cost A). In reality only 9 GBS infections were observed with 68 days of hospitalization in neonatology and 4 days in ICU. The actual cost of care for the French healthcare system was only €35 805 for neonatology hospitalization days. The cost for intrapartum screening by PCR would have been €113 705 if codified: the sum being €149 510 (≈ cost B) as total cost.

For the total of 2070 term deliveries and the 2130 live term births during the study period, there was a decrease in the number of hospitalization days of 24.44% in neonatology and 87.9% in ICU with an overall cost supplement estimated at €29 537 (≈ cost B minus cost A) if reimbursement codes were applied.

Conclusion: This study showed that intrapartum GBS screening by real-time PCR (GeneXpert Xpert GBS) resulted into a significant decrease in GBS infections and in the number of hospital days. The additional cost would theoretically be only €14.27 per delivery in order to avoid at least 10 infections annually in our institution. Additional multicenter studies should be performed to validate these preliminary results.

P1788 Quick and accurate diagnosis of toxigenic C. difficile combining Techlab C. diff Quik Chek Complete and GeneXpert C. difficile RT-PCR
P. Vandecandelaere*, R. Joseph, C. De Ridder (Ypres, BE)

Objective: We evaluated if selective addition of GeneXpert C. difficile PCR to the routine C.diff test algorithm could ameliorate the turnaround time for complete and correct results.

Methods: As standard procedure we perform a Techlab C.diff Quik Chek Complete assay (Techlab Cdiff) and a toxigenic culture (Cdiff) for all diarrheal stool samples.

Techlab Cdiff detects both glutamate dehydrogenase (GDH) and Clostridium difficile toxin A and B (ToxAB). Cdiff CDT is a panel of culture on Cdiff agar (BioMerieux) and a second tox A/B test (Immunocard Toxins A&B – Meridian) on colonies grown of stool samples that were toxin A/B negative directly on stool.

GeneXpert Xpert C. difficile RT-PCR (Cepheid) detects toxin B gene, the gene for binary toxin and the tcdC deletion. In the new test algorithm we perform a Cdiff PCR on stool samples with GDH pos and tox AB neg result (n = 76) and on stool samples with GDH neg and ToxAB pos result (n = 6). For GDH and ToxAB positive stools that were Cdiff negative we also performed a Cdiff PCR on the stool (n = 2).

Results: During the evaluation period 1136 stools could be included for evaluation and 84 PCR tests had to be performed. (7.4%). Overall 83 stools tested positive for toxin producing C. difficile (7.3%) and the results of 4 stools were inconclusive (Techlab Cdiff GDH pos and toxAB neg, Cdiff. PCR pos and CDT neg).

With the Cdiff QUIK CHEK 35 positive stools had a correct answer on day 1 (42.2% of the positive samples) and 82/1136 samples (7.2%) had a conclusive result (only GDH or toxAB test positive). By adding PCR we could give a conclusive result for those patients: 45/82 (54.9%) are PCR positive (41 results confirmed by CDT). These patients could be timely isolated to prevent nosocomial infections and the others were not unnecessarily isolated.

With the new test algorithm we can get a correct same-day result (positive and negative) for 99.3% of all stools (1128/1136) and for 96.4% of positive stools (80/83).

Conclusions: Selective addition of GeneXpert Cdiff PCR to Techlab C.diff Quik Chek Complete assay for diagnosis of CDAD allows us to give a same-day correct answer for C. difficile diagnosis for 99.3% of all stools and for 96.4% of the positive stools. GeneXpert Cdiff RT PCR has only to be performed on a minority of the stools (7.4%).
demonstrated with culture that the addition of throat and groin specimens increased the sensitivity of detection. The high price of rapid tests incites to pool the three specimens into one analysis. Preliminary data suggested that the sensitivity of PCR on throat specimen is lower than on the others. Pooling this specimen with others might influence the overall performance of the assay.

**Objective**: To compare performances of GeneXpert MRSA on throat specimens with those of nose & groin specimens.

**Methods**: Specimens were obtained using the eSwabs collection devices (Copan) from patients eligible for screening according to the hospital policy. Nose and groin samples were pooled in the same eSwab device (two nylon flocked swabs in one tube), whereas throat specimens were collected separately (one nylon flocked swab in one tube). Samples were eluted into 1 mL of liquid Amies medium. 150 microl. were used to perform the analysis with GenXpert MRSA, and the remaining was used for culture (enrichment broth followed by inoculation onto chromogenic M-select agar plates [Biorad]).

**Results**

From September 2010 to November 2010, 441 screening were performed and analyzed both by GenXpert MRSA and by culture. Among these, 377 (84%) were negative by both methods in both samples. Considering the results from culture as the gold standard, the sensitivity of GeneXpert MRSA was lower for the throat: 0.56 (95% CI, 0.40–0.72) than for the nose&groin: 0.84 (95% CI, 0.71–0.93); whereas the other performances (specificity, positive predictive value, and negative predictive value) were similar.

56 screenings were positive by GeneXpert MRSA (48 in nose&groin samples and 25 in throat samples). Among these, 8 (14%) were positive only in the throat.

**Conclusions**: Sampling of the throat contributed to a non negligible number of positive screenings (14%). However, the sensitivity of throat swabs was nearly significantly lower than the pooled nose and groin swabs. Although the reason for this finding is still unclear, we advise not to pool the three specimens since it could lower the sensitivity of MRSA detection with the GeneXpert assay.

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**Table 1a. Results of Xpert™ vanA and vanB PCR assays in comparison to enriched culture**

| Xpert™ vanA and vanB PCR assays | VRE culture on C-ID | VRE medium |
|---------------------------------|--------------------|------------|
| Positive                        | Negative           | Total      |
| VRE culture on C-ID             | VRE medium         |            |
| Positive                        | 38                 | 24         | 62         |
| Negative                        | 0                  | 188        | 188        |
| Total                           | 38                 | 212        | 250        |

**Table 1b. Results of Xpert™ vanA and vanB PCR assays in comparison to enriched culture**

| Xpert™ vanA and vanB PCR assays | VRE culture on C-ID | VRE medium |
|---------------------------------|--------------------|------------|
| Positive                        | Negative           | Total      |
| Positive                        | 6                  | 104        | 110        |
| Negative                        | 0                  | 160        | 160        |
| Total                           | 6                  | 264        | 270        |

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**P1791 Is VRE screening by Xpert™ vanA/vanB PCR superior to selective culture on chromID™ VRE indicator agar?**

H. Peltroche-Llacsaahuanga*, G. Haase (Aachen, DE)

Prevalence rates of VRE increased worldwide over the past 15yrs. Active control measures are being implemented in some hospitals e.g. detection of non-infected but gut-colonized patients that might serve as a source of the spread of VRE.

In this VRE surveillance study 270 rectal double swab samples recovered from patients at admission to the university hospital were tested by using the recently released Xpert™ vanA/vanB PCR assay (Cepheid, USA) in parallel to enriched culture on chromID™ VRE medium (C-ID; bioMérieux, FRG). Overnight enrichment in BHI with 3 g/L vancomycin preceded inoculation of the chromogenic agar, as described recently (Peltroche-Ll. et al.; 2009). Final species identification of microorganisms recovered from C-ID was achieved by mass spectrometry fingerprinting using MALDI-TOF (Microflex; Bruker Daltonics, FRG).

Presumed VRE were confirmed by vancomycin Etest. The study revealed highly disparate results for PCR and culture (Tab. 1a, b). The detected VRE rate was 21% comprising 54 vanA E. faecium, 4 vanA E. faecalis, 4 vanB E. faecium, and 2 vanB E. faecalis. Considering that the PCR assay is detecting genes that are not unique among VRE spp. (Ballard et al.; 2005), Consequently, the positive agreement of vanB PCR with culture of VRE is only 5%, whereas the vanA PCR revealed 70%. Of note, in case of 10 positive vanA PCRs, only Lactococillus spp., that are potentially able to acquire vancomycin resistance (Mater et al.; 2005), were cultured on C-ID.

Since Xpert PCR assays can be processed in less than 1h with minimal hands-on-time, results can be provided instantaneously enabling immediate respective patient management. Unfortunately, the costs for this PCR are about 6fold higher than for culture on C-ID agar. On the other hand culture needs at least 48h to reveal results.

However, as long as vanA/vanB PCR performed with specimens comprising complex intestinal microbiota is in such an extent unspecific for presence of VRE, the benefit for patient management is most questionable. Although rapid detection of VRE can be of help under certain circumstances, culture revealing the identification of the specific microorganism is still of advantage, particularly in case of VRE screening with potentially transferable resistance genes such as vanA/vanB.

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**Lab automation**

**P1792 Automating the bacteriology laboratory**

N. Bentley* (Cambridge, UK)

**Objectives**: Bacteriology laboratories historically have seen limited automation with much restricted to separate modules (e.g. blood cultures, urine microscopy) with scientific staff performing tasks associated with manual operations, e.g finding and carrying culture plates and loading incubators. Our laboratory has implemented total system automation using of Kiestra Lab Automation®. This has facilitated a paradigm shift in working practice enabling a focus on the patient rather than the process. The system has been in place for over a year and benefits are described.

**Methods**: The system covers 210 m² and comprises automatic plate selection, bar-coding, incubation and digital imaging, 8 bench workstations, a plate transport track, and management software. Plates are read as images on high resolution screens, individual colonies are digitally marked to direct future work by lower-grade staff. Incubation times are standardised, and quality management is facilitated by bar-coding, automated batch recording, and image storage. Working practices have been modernised to enable effective use of the system.

**Results**: Installation took 14 days with the extensive vocational training. Minor discrepancies observed during comparison of traditional and automated systems were resolved by image adjustments and increased staff experience. All sample types except urine utilise the system. After installation mean turn-around-times for MRSA screens fell from 30 to 22h, with daily analysis (mean 300 samples) reducing to 0.5h compared to 1.5h previously. A year on, the system enables further reduction in bench reading times, allows quick negative authorisations and automatic zone reading for antibiotic susceptibility. Assessments in the 3rd and 52nd weeks of operation show 7535 and 9848 plates were used for 3782 and 5186 samples, respectively; the mean time between failures rose from 6.2h to 7.5h.

**Conclusion**: A linked modular automation system allows experienced staff to focus on productive tasks and move away from traditional working practice. Allowing the laboratory to move away from “batch analysis” to patient centred bacteriology, facilitates 24/7 working, enables predictable turn-around-times and provides extensive quality metrics. Automation has enabled the laboratory to re-skill the workforce and reduce the headcount by 5 scientists while workload has increased by 4%. Furthermore, Kiestra will enable planned future laboratory consolidation to one site.
Experience with KIESTRA’s Total Lab Automation solution
to meet the challenge of universal MRSA screening for
Lister Hospital, a large UK district general hospital

G. Humphrey, C. Malone, H. Gough, FM. Awadel-Kariem* (Stevenage, UK)

Objectives: The Microbiology Department at the Lister Hospital introduced KIESTRA’s Total Lab Automation solution “It’s The Sample That Moves!”. This study was designed to assess the impact on productivity. Furthermore, the UK Department of Health has set all UK hospitals a target to screen all elective admission patients for MRSA carriage by March 2009, and all patients by March 2012. To meet these targets there is a need to increase capacity and to provide a rapid, cost effective screening method. The second objective of this study was to look at KIESTRA’s role, and the role of the Vision Toolbox in conjunction with KIESTRA, to address this need.

Methods: The Laboratory productivity index (LPI) was used to assess productivity before and after the implementation of KIESTRA in June 2008. To enable a major change in working practice, the first step was to ask the consultant staff to agree on a set of base line algorithms, thus defining what the departments product is. It also meant that the scientist could concentrate their professionalism on the small number of isolates that fell outside the predicted algorithms. In June 2009, the use of Vision Toolbox in conjunction with KIESTRA was examined to address the second objective.

Results: The Laboratory productivity index (LPI) has improved from 24.7 to 63.9, an increase by a factor of 2.6. This has been achieved by altering working practices so as to enable the automation to work at its most efficient level. This improvement was achieved by: a) halving the time taken to culture plates; b) developing a productive process where a team reads, picks, tests and carries out susceptibility tests in a seamless process; c) since 2006, absorbing 70% more work into the work stream with 21% less staff; d) implementing novel time-management processes to maximise productivity.

For the second objective, a validation trial of the Vision Toolbox against 324 positive samples and 240 negative ones resulted in accurate results with high sensitivity and specificity. It obviated the need for manual reading with added savings in speed and productivity.

Conclusions: The implementation of KIESTRA has resulted in measurable productivity improvements. Using Vision Toolbox in conjunction with KIESTRA has demonstrated significant promise in meeting the imminent challenge of universal MRSA screening.

Design of a new microbiology laboratory based on Lean Six Sigma principles

J.P. Brochet*, J.M. Cassorla, H. Palumbo (Le Haillan, Lyon, FR)

Objectives: In an already weakened economy, microbiology laboratories are under increased pressure to provide the highest quality patient test results. In this context, our laboratory had to consolidate the activity of 22 peripheral labs into one single platform. This challenge meant a new structure, had to be built its architecture and ensure integrated current and new automated solutions. In this competitive landscape, decreasing turn around time to deliver results to physicians is also a critical success factor to ensure better patient management. This is why we set up a project to apply Lean/Sigma principles to our microbiology lab.

Methods: Lean and Six Sigma are the two most powerful strategies for achieving operational and service excellence in any organization today. The project started by observing the laboratory in its current state – the physical layout and how samples were processed. The roadmap obtained outlined how the lab could improve processes to reduce waste and enhance efficiency, while simultaneously increasing results quality and reducing errors.

The Lean Lab assessment was been managed by a Lean Engineer and a Microbiology Application Specialist in two main phases. The first phase was performed in March 2008 to provide recommendations and the second in November 2010 to assess how the lab was successful in implementing them.

Results: The new microbiology laboratory was built with a surface of 177m² (former lab 60m²). The improvements enabled the management of 1385 specimen (an increase of +185% in terms of activity. Productivity was increased by 138% with 6.6 patients managed per working hour (productivity was previously 2.77 patients).

The time dedicated to quality management has been increased by more than eight times between March 2008 and November 2010 (1.5h to 12.5h). This enabled staff to focus on lab accreditation. Turn around time for urine specimens, which represents 67% of the total number of specimens, was decreased by 21% (providing a +2% increase of positive samples). These figures were achieved by integrating UF 1000i, PREVI™ Color Gram, PREVI™ Isola, VITEK 2 and automatic patient data capture.

Conclusion: The workflow of the entire Microbiology lab has been greatly improved through the newly optimized organization by matching workload/capacity (specimen processed/resources required). These improvements enabled the lab to prepare for the accreditation process and be able to handle an increased workload in the near future.

Efficiency of an automated streaking system for bacteriological diagnostics

J. Knobloch* (Lübeck, DE)

Objectives: The increasing economic pressure in microbiologic laboratories results in the need for increased automation. The automation of microbiological techniques can help to focus the personnel capacities on core duties. Therefore, the PREVI™ Isola automated plate streaking system was evaluated for its efficiency and quality in the processing of urine and stool specimen.

Methods: Manual streaking was compared to streaking using the PREVI™ Isola system for 500 urine and 250 stool specimen. 10μL of the urine sample as well as 10 μL of a 1:100 diluted urine was manually streaked on agar plates. For PREVI™ Isola streaking the original urine containers were placed in the system. For stool specimen the volume of suspension media (NaCl solution) was adapted to 1.5 mL to avoid strong dilution effects for watery stool specimen and about 0.2 g or 200 μL were resuspended and placed in the automated system. Agar plates were incubated for 18 to 24 h and the streaking results were compared. Additionally, the time needed for manual and automated streaking was measured for representative batches of the respective specimen.

Results: The quality of automated streaking was highly reproducible with a good separation of single standing colonies in a range of 10^6 to 10^7 cfu per mL. Streaking quality was equal in 78.0% for urine specimen and 75.6% for stool specimen. A superior result using the automated streaking procedure was observed in 16.0% for urine and 5.2% for stool specimen. Superior results for manual streaking were observed for 6.0% of urines and 19.2% of stool specimen. Superior results of the manual method were observed in stool specimen with a tenuous consistency. In watery stool specimen the sensitivity was reduced due to the dilution for few probes. The average hand-on-time was reduced from 84 s to 21 s per probe for urine specimen and from 91 s to 61 s for stool specimen, respectively.

Conclusions: Automated streaking decreased hand-on-times significantly for both urine and stool specimen. Standardization of the streaking procedure resulted in a higher streaking quality for urine specimen and an almost comparable streaking quality for stool specimen, indicating that automated streaking is able to reduce personnel cost together with an increased or at least equal quality. For stool specimen with tenuous consistency the manual streaking procedure should be used to avoid minor streaking quality.
**P1796** Lean Six Sigma methods as a core asset of the microbiology lab

J.M. Rosse*, T. Guesudet, C. Rieder-Monsch, J.M. Cassorla, H. Pahumio (Strasbourg, Montoison, Marcy-L’Etoile, FR)

**Objectives:** In an already weakened economy, clinical microbiology laboratories need to reduce cost, to improve productivity, to respond to accreditation requirement and to provide high-quality results. In this competitive landscape, decreasing turn around time to deliver results to physicians is also a critical success factor to ensure better patient management.

This is why we set up a project to apply Lean/Sigma principles to our microbiology lab.

**Methods:** Lean and Six Sigma are the two most powerful strategies for achieving operational and service excellence in any organization today. The project started by observing the laboratory in its current state – the physical layout and how the samples were processed. The roadmap we obtained outlined how the lab could improve processes to reduce waste and enhance efficiency, while simultaneously increasing quality result and reducing errors.

The Lean Lab assessment was managed by a Lean Engineer and a Microbiology Application Specialist in two main phases. The first phase was performed in November 2008 to provide recommendations and the second in June 2010 to assess how the lab was successful in implementing them.

**Results:** The identified improvements enabled the management of 1540 specimen by week (+24% of increase in terms of activity). Productivity was increased by 37% with 3.89 patients managed per working hour (former productivity was 2.84 patients). The time dedicated to quality management has been increased per three fold between the two steps (5h to 15h).

Turn around time for urine and genital specimens, representing 63% of the total number of specimens, was decreased by 10–11% (providing a 8% increase of positive samples for urine and 5% for genital swabs). These figures were achieved by integrating UF 1000i urine cell analyzer and PREVITM Isola automated plate streaker in our lab. The challenge was to manage an increased workload (+24%) inside the same microbiology laboratory that had only been extended by few square meters (30m²).

**Conclusion:** The workflow of our Microbiology lab has been greatly improved, 90% of the daily issues have been solved. Processes standardization and training program for skilled technicians were also two main outcomes. Being more focused on microbiology expertise helped to manage more analyses with added value and to save time.

**P1797** Is liquid microbiology by the WASP® reliable?

M.E. Bierma*, J.M. van Bree, M.J. van de Ven, T.C. Liebregts, N.L. Arents (Weldhoven, NL)

**Objectives:** The ESwab® and UriSwab® have been proven to be reliable specimen collection systems. Liquid specimen collection systems give the opportunity for automation in medical microbiology. In this study we evaluated the reliability of the Walk Away Specimen Processor (WASP®) (COPAN, Italy) by studying the endpoint: amount of growth and different species detected.

**Methods:** Randomly selected urine samples and ESwab samples received by our laboratory from clinical patients were processed manually and by the WASP. From urine samples 10 ml was inoculated on a bloodagar plate containing colistin and aztreonam and a chromogenic medium (CPS3 agar; bioMerieux; France) by both methods. After inoculation streaking was done using the same streakingpattern. The swabs of the ESwabs were manually plated on a subset of culturemedia and streaked in 4 quadrants. The WASP used 30 ml liquid medium of the ESwab to inoculate the plates and also streaked this in 4 quadrants. The subset of culture for the ESwabs depended on the origin of the specimen but was always the same for both the manual and the WASP method. After overnight incubation grown colonies were counted and identified according to conventional methods.

**Results:** In total 218 urine samples and 79 ESwab samples (19 vaginal swabs; 17 throat swabs; 14 wound swabs; 14 rectal swabs; 5 ear swabs; 4 fecal swabs; 2 cervical swabs; 2 nose swabs; 2 abscess swabs) were included in the study. Table 1 shows the methods compared to each other in terms of species grown and their amount of growth. Most discrepancies (33/47 (70%)) were encountered in urine samples.

**Conclusion:** The WASP is capable of fully automatically inoculating liquid specimen and produces more frequently a higher amount and/or additional species as compared to standard manual procedures.

|                | manual | WASP |
|----------------|--------|------|
| number of samples | 207    | 206  |
| spec with more growth by manual | WASP method (1) | 218 |
| spec with more growth by manual - WASP method | 30 - 79 |
| % spec with more growth and in an additional species by manual - WASP method | 1.3 |
| % value for difference in more growth and/or additional species (1) species grown in only one of the two methods excluded | 0.05% - 9.11% |

**P1798** Automation in routine bacteriology – experience with the WASP inoculation robot

O. Nolte*, H. Haag, M. Kommerell, S. Brunner-Zillikens (Constance, DE)

**Objective:** Our laboratory is a privately owned laboratory with an average of ~100 specimens for bacteriologic examinations, daily. Workflow, cost effectiveness and daily sample size are highly different from academic or tertiary hospital centres. To optimize workflow and save hands-on-time, we introduced an automated inoculation device. Being the first laboratory in Germany, we got the WASP (Walk Away Specimen Processor, Copan, Italy) installed in Sept. 2010. After three month of operation we were able to rate the benefit of automation in our laboratory.

**Methods:** Using the WASP, urines (in Sarstedt Monovettes), stool and swabs were inoculated and streaked automatically. The majority of specimens, however, are not yet delivered as eSwabs but as conventional swabs or specimen containers. These were transferred to empty tubes, prefilled with physiologic saline, upon reception. All materials were streaked by WASP according to specified protocols already established in our routine diagnostics.

**Results:** LIS-connected WASP was ready for accepting routine samples for method verification immediately after installation. Transfer of our specific streaking protocols to the WASP was laborious (manual typing) but easy. Although eSwabs have been proven to be better than standard swabs in a couple of independent studies, senders are not fully organised yet to use eSwabs. Therefore, still more than 85% of specimens (with the exception of urines) have to be transferred to pre-filled tubes prior to inoculation by WASP. All specimen-types were accepted and automated streaking was in general much better than manual streaking (i.e. clearly distinguishable single colonies [4 quadrant streaking pattern] even from stool, virtually no need for repeated inoculation). Following method verification, WASP optimized workflow in the order of 1 FTE.

**Conclusion:** Although our laboratory is comparatively small in terms of daily sample size, WASP reduced manual workflow in the expected order (time savings due to automated streaking, easier processing of colonies, elimination of repeated streaking) and eliminated potential sources of error (printed vs. manual label on each plate). Our example shows that even smaller sized laboratories may effectively use and benefit from automation in the bacteriology lab. For optimal use of instruments like WASP, liquid-based microbiology needs to be more widely implemented.
Use of a sputum dipper and sputum liquifying solution to process sputum specimens prior to inoculation with the Copan WASP instrument

**P1790**

**Objectives:** Sputum and trach aspirate (SP/TA) specimens are not very amenable to automated planting instruments due to the viscous nature of the specimens, which can also make it difficult to obtain reproducible results with manual planting methods. We have previously shown that, using a new pre-packaged mucolytic agent, Sputum Liquifying Solution (SLS) (Copan), SP/TA specimens can be prepared (liquefied) for processing on the Copan WASP instrument. When 1 ml of SP/TA specimen was combined with 1 ml of SLS and plated with a 30 ul loop on the WASP, culture results were comparable to manually plated SP/TA specimens. To better facilitate the transfer of SP/TA specimens to tubes of BHI, Copan has developed a new device that, using a new pre-packaged mucolytic agent, Sputum Liquifying Solution (SD). This study evaluated the utility of the SD for use with SLS.

**Methods:** In a pilot study, we determined that the SD picks up about 400 ul of SP/TA specimens, suggesting that using the SD with 0.5 ml of SLS should produce comparable cultures to what we obtained using 1 ml of SP/TA specimen combined with 1 ml of SLS (maintaining ~1:1 ratio of specimen to SLS). In this study we compared the results of SP/TA specimens processed in 2 different ways with all specimens plated with the WASP to BAP, Choc, and Mac plates. One set of plates was inoculated from a 1-ml SLS tube to which 1-ml of purulent specimen was added using a 1-ml syringe. The second set of plates was inoculated from a 0.5-ml SLS tube to which SP/TA was added with an SD. The WASP utilized a 30ul loop with the same streaking pattern for all specimens.

**Results:** Results were sorted into 4 groups: no growth (NG), pharyngeal flora (PF), yeast, and potential pathogens (PP). Each result was semi-quantitated as no growth, few, moderate or many colonies. From a total of 168 specimens tested, there were 218 results. The same qualitative result was obtained for 216/218 results (11 NG, 118 PF, 16 yeast; and 71 PP). Of the 216 results in qualitative agreement, 202 had the same quantitation. 2 results from 2 specimens had moderate B strep x.

**Conclusions:** The Copan Sputum Dipper is a very effective transfer device which, when used with SLS, facilitates processing of SP/TA specimens with the WASP, producing accurate, reproducible test results. SD/SLS processing is an acceptable alternative to manual planting and provides the benefits of automation.

WASP, a Walk Away Specimen Processor enables accuracy and cost benefits managing multiple specimens on a single culture plate

**P1800**

**Objectives:** Microbiology laboratories are burden with high costs to process numerous samples for the identification of colonized patients with methicillin-resistant *Staphylococcus aureus* (MRSA) or vancomycin-resistant enterococci (VRE) and extended spectrum β lactamase bacteria (ESBL) to prevent nosocomial infections. Automation in microbiology is essential for handling the many samples and new strategies are necessary for reducing chромogenic plates and staff time costs. Copan, in the last 2 years, introduced a fully automated Walk Away Specimen Processor (WASP) for bacteriology analysis. The objectives of this study are to validate the unique WASP feature to inoculate, streak and label 2 different samples on the same agar plate 1 without cross-contamination 2) without labeling or specimen transposition errors and to 3) calculate the cost saving.

**Study method:** Simulated 1.0 McF ESwar positive (ESP) samples prepared with *E. coli*, *H. influenzae* MRSA, MSSA and VRE and ESwar negative (ESN) samples were used. A 10ul inoculation loop and inverted “Christmas tree” streaking pattern were used in the WASP protocol. First, 500 of each sample type were loaded on the WASP to have an ESP and an ESN on the same plate. After, all ESN and ESP samples were randomly loaded to simulate real life processing conditions and the Two Specimens per Plate Protocol was selected. All plates were incubated at 35°C for 24hrs; labeling errors and cross-contamination were monitored. Materials cost saving was calculated.

**Results:** In the first 500 plates inoculated with ESN and ESP samples all labels were placed on the correct side of the plate and all the positive cultures corresponded to the ESP sample label and all negative cultures corresponded to the ESN sample label on the plate. No cross-contamination was found in all the 500 plates inoculated with ESN and in the ¹ plate inoculated with ESP samples; no cross-contamination was found in the 500 randomized samples inoculated on the same plate. 1000 plates were used to culture 2000 samples, at a cost of 1.5€ per plate resulting in 1500€ savings in plates only, the actual staff savings depends on each institution.

**Conclusions:** It was demonstrated that the WASP can reliably and accurately inoculate 2 samples on the same plate without errors and provide a 50% cost saving for materials as well as reduced staff time. The ability of inoculating 2 samples on the same plate can be implemented for screening MRSA, VRE or ESBL colonized patients.

**P1801**

**Objectives:** Quantification of bacterial cultures is necessary to be able interpret culture results. Clinical relevance of these cultures is often based on the amount of growth. Therefore, first inoculation of clinical samples needs to be standardized and reproducible. Furthermore, the yield of single colonies in mixed cultures is essential to perform further testing for identification and AST. A novel inoculation method was introduced by Kiestra Lab Automation (the Netherlands). Inoculation with the automated InoqulA is based on bacterial spreading through a magnetic bead instead of an inoculation loop or similar device. The objective of this study was to determine the reproducibility and quality of this novel principle.

**Methods:** Three known bacterial species (*E. coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*) were used in different combinations and different dilutions (table 1). These 9 samples were inoculated in 15 fold on CPS3 chromogenic agars (bioMerieux) with the InoqulA (10 microl/plate). Subsequently, the 9 different samples were inoculated in 6 fold on CPS3 chromogenic agars with the InoqulA and manually. By using Vision Technology Software the percentage of growth on an agar plate was determined. The bacterial species which were used all have a distinctive appearance on the CPS3 agar (*E. coli* is pink, *K. pneumoniae* is green and *S. aureus* is whitish). Because of this it is easy to determine whether single colonies are present.

**Results:** The first 3 (1−3) samples (0.5 McFarland) all showed 95% confluent growth and single colonies (sc) after 25mm of spreading, the second 3 (4−6) samples showed 75% confluent growth and sc after 15.8 mm and the last 3 (7−9) samples showed 50% confluent growth and sc after 18.6 mm. The range of distribution (RD) was less than 10% in the InoqulA, whereas the RD in the manually inoculated samples...
was 50%. Distinct single colonies were observed less in the manually inoculated samples compared to the automatically inoculated samples.

**Conclusion:** The InoqulA is able to perform reproducible, high quality first inoculation results compared to manually inoculation. Multiple inoculations with the InoqulA of the same samples showed less than 10% variation. Se were observed in all samples, but less in the manually inoculated samples. Based on the percentage of confluent growth and the distance to the appearance of the amount of growth can be deduced.

**P1802 Evaluation of the UriSwab® as a tool to diagnose urinary tract infections**

N.L. Arents*, U. Bayir, M. Förster, A.R. Jansz (Veldhoven, NL)

**Objectives:** Getting patients to collect a reliable urine specimen is a challenge. Filling the test tube without contaminating the inside with bacteria or the outside with urine is a well-known problem. Recently the UriSwab® (COPAN, Italia; US) became available and is claimed to be more practical to use and thus may increase the reliability of the sample. While urinating, two sponges on an applicator are inserted in the stream and absorb exactly 1200 mcL of urine. The sponges retain the fluid during transit preventing the sample from leakage and bacterial overgrowth. In this study we investigated the reliability of the US considering the species grown, their amount of growth and the presence of squamous and bladder cells.

**Methods:** In the first part of the study urine samples were collected by US and sterile container from clinical patients during a single miction episode. The container and the US (after centrifugation) were both processed immediately by inoculating a tri-plate containing bloodagar, McConkey agar and Streptococci selective agar with 10 mcL of urine. After 24 hours storage at room temperature (representing transport time) both collection systems were again processed in the aforementioned way. Growth colonies were identified by standard methods. In the second part of the study, employees of our laboratory were asked to collect a urine sample by US and sterile container directly at awakening before they washed themselves. Both samples were processed by Gram staining and the presence of squamous and bladder cells was scored by standardized methods (ranging from absent to +4). A difference of at least 2+ was considered relevant.

**Results:** Table 1 shows the result for the first study. The second study showed the absence of squamous cells in both samples in 59 cases, identical presence of squamous cells in 38 cases, at least 2+ or more squamous cells in the container sample in 3 cases and at least 2+ or more squamous cells in the UriSwab sample in 0 cases. Bladder cells were not observed in any sample.

**Conclusion:** The number of species, their amount of growth and the presence of squamous cells did not differ significantly between the UriSwab and container method independent of the time of processing. The UriSwab® (COPAN, Italia; US) became available and is claimed to be more practical to use and thus may increase the reliability of the sample.

| Table 1: UriSwab samples compared to samples collected in a sterile container |
|------------------------------------------------|
| study 1 | sample processed after 0 hours 24 hours number of samples | 47 47 | 25 25 | 15 15 | more growth on UriSwab / container sample | 1.5 [1] 1.5 [1] | more growth on UriSwab / container sample | 0.0 0.0 | p value for difference | 0.2 1 |

**P1803 The utilisation of the bacterial mode on UF 1000i for the screening of urine samples submitted to a microbiology laboratory for culture**

J. Jarankova*, J. Protivcinsky (Brno, CZ)

**Objectives:** The UF1000i urine analyser (Sysmex) is routinely utilized for microscopic examination of the urine sediment in clinical biochemistry laboratories. Our aim was to evaluate a suitability of UF 1000i bacterial mode for a pre-culture urine screening in a clinical microbiology practice and to assess the system reliability during a fast diagnostics of urinary tract infection (UTI).

**Methods:** In our study, 1162 fresh urine samples were collected. These samples were simultaneously run on the UF 1000i and inoculated onto blood agar and MacConkey agar plates and incubated at 37°C from 18 to 24 hours. Results were then compared with respect to the UTI flag presence generated by the analyzer. To more precisely interpret the results, diagnosis, clinical findings of UTI, antibiotic administration, changes in blood count, concentration of C-reactive protein (CRP), and the presence of the other originator of the UTI such as *Ureaplasma urealyticum, Mycoplasma hominis* were considered.

**Results:** Of all samples, with the cut-off values of UTI set to 10 white blood cells/µl and 100 bacteria/µl, 91.3% were correctly evaluated by the UF1000i, falsely positive samples were found in 8.2% while the total percentage of falsely negative samples was 0.5%. After implementation of the UF 1000i into our workflow, the number of true positive samples increased by 10% as compared to the simple culture results. It was found that as many as 53% of all samples submitted to the microbiology laboratory were unnecessarily examined since UTI was ruled out by using the UF 1000i. The system sensitivity and specificity were 97% and 95% respectively. The UF 1000i positive predictive value of the UTI was 82.6% while negative predictive value was 99%.

**Conclusion:** The UF 1000i has proved to be a suitable tool for pre-culture urine screening in a clinical microbiology laboratory practise. All results, gained by the UF 1000i, possess a high negative predictive value whereby a presence of UTI can be easily excluded and thus, a reduction in hospitalization of patients, rational antibiotic administration can be applied leading to the faster diagnostics and a financial benefit for health care providers.

**P1804 Automated microscope and flow cytometer to screen bacteriuria: a comparison**

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**Objectives:** There is strong recommendation to use automated methods as a screening tool for detect negative urine samples of patients, except children, pregnant woman and immune-compromised. We evaluated two automated screening test for urinary tract infections (UTI). Material and methods. A total of 500 urine specimens from outpatients were selected for the study. All urine samples were analyzed, in parallel, with the Sysmex UF1000i (BioMérieux), Iris Diagnostics iQ200 (Izasa) and bacterial culture, the latter considered the gold standard. Before testing urine samples each day, positive and negative controls were running in the automated systems according to the manufacturer's instruction. The cut-off values used were 140 bacteria/microlitter (UF1000i) and 2,800 small particles/microlitter (iQ200).

**Results:** Sensitivity (S), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) found are listed in the table. We found major discrepancies (positive samples detect as negative) in 14 cases with iQ200 and 6 cases with UF 1000i.

| Table 2: Comparison of UF1000i and iQ200 systems |
|------------------------------------------------|
| | UF1000i | iQ200 |
| S | 85.3% | 84.9% |
| Sp | 76.0% | 65.2% |
| PPV | 65.9% | 59% |
| NPV | 30.3% | 11.8% |

**Conclusions:** The two methods were acceptable to use as screening test. We found a higher S and NPV with UF1000i. Although labour and
time are saved with the screening methods, like iQ200 and UF1000i, we
need clinical judgment (to combine different indicators of infection) and
interpretation. National and European guidelines are needed.

**P1805** Comparison between UF-1000i (Sysmex) flow cytometer and
bacterial culture in rapid diagnosis of urinary tract infection

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Objectives: In order to assess the analytical performances of UF-1000i
cytometer (Sysmex) in rapid diagnosis of urinary tract infections (UTIs),
we conducted a study to select a suitable cut-off value for bacteria and
leucocytes.

Methods: A total of 948 consecutive urine samples were tested in
parallel by UF-1000i and routine culture (gold standard for the diagnosis
of UTIs). Urine samples were from hospital and general practice patients
and were representative of all age groups. CFU quantification was
performed on TSA-5%SB using standard 1 mL loops and incubating
plates overnight at 37°C in air. In UF1000i system, cells in the urine
were stained with fluorescent dyes for membranes and/or DNA. The combined
analysis of scattered light and fluorescence allowed the identification and
quantification of particles as leucocytes, bacteria and yeast-like cells.

Results: Cultures were considered positive with \( \geq 10^3 \) CFU/mL. UF-
1000i results were considered positive at cut-off values of \( \geq 70BACT/\mu L \)
and of \( \geq 100WBC/\mu L \). On this basis of the 948 urine samples, 38% of
the cytometer results were true positive. 47.5% true negative, 12.0% false
positive and 2.95% false negative. 12 false negative results were
from potentially neutropenic patients (from haematology/oncology, renal
transplant wards). Excluding this group of patients, whose urine samples
are routinely cultured, cytometer false negative results lowered to 1.8%.
The system performed with a sensitivity of 95.5%, a specificity of 79.9%,
a NPV of 96.4%, a PPV of 75.9%.

Conclusions: UF-1000i (Sysmex) was useful in ruling out UTIs, with
an acceptable amount of false negative results when cut-off values for
WBC and bacteria are properly settled. False positive results depend
on cytometric detection of viable and dead bacteria. Rapid counting of
leucocytes and bacteria improved turnaround time of negative results
and laboratory workload.

**P1806** Screening for urinary tract infection in children: evaluation
of diagnostic performance and optimal cut-off for Sysmex
UF1000i flow cytometer

R. De Roso*, S. Grasso, M. Acolio, M. Modolo, P. Stano, A. Camporese
(Pordenone, IT)

Objectives: The diagnosis of urinary tract infection (UTI) in pediatric
patients is challenging because the clinical presentation is often not
specific and the gold standard test, urine culture, does not supply
same-day results. A lot of studies in adult population evaluated the
performance of flow cytometer for detection of bacteriuria and now this
instrumentation is admitted in the routine of many laboratories, providing
results in a few minutes. We aimed to evaluate analytical performance
and optimal cut-off value of Sysmex UF 1000i for bacteria counting by
comparing culture, to guide early diagnosis and treatment of UTI in a
pediatric population.

Methods: Sysmex UF1000i (Sysmex Co. Japan), a fluorescence flow
cytometer intended for urinalysis purpose, has a specific analytical
channel for bacteria counting. 350 samples were obtained from children
up of 10 years (212 female, 138 male; 124 in- and 226 out-patients)
from clean catch midstream urine or, from 94 infant younger than one
year, by a sterile collection bag or urethral catheterization. Samples were
examined by Sysmex UF1000i and cultured onto CLED and
CNA agar plates (Kimia, Padua, Italy) by means of 10 mL loop. The
results of UF1000i analysis for each sample were compared with urine
culture results, considered positive as \( \geq 10^3 \) CFU/mL, and the instrumental performance evaluated using receiver
operating characteristic (ROC) analysis.

Results: 202 samples were negative at bacterial culture (57.7%) and 148
were positive (42.3%). Different cut-off values for instrumental bacteria
count were considered. At a bacteria count \( \geq 10^3 \) UF1000i results were:
true positives 146 (41.7%); true negatives 104 (29.7%); false negatives 2
(1.3% of all positive samples); false positives 97 (27.7%). In this setting
we obtained a sensitivity of 98.6%, a specificity of 51.7%, with a area
under the curve = 0.956, a negative predictive value of 98.1% and a
positive predictive value of 60%.

Conclusion: The obtained results indicate that Sysmex UF1000i is a
valid method for ruling out samples do not need to be cultured. In our
experience cut off points for bacteria count in children up to 10 years
differed remarkably from those of adult population, so that the threshold
for significant counts should be selected on age basis. Moreover at the
above mentioned point of bacter count, leucocytes count didn’t improve
the screening of culture positive samples.

**P1807** Evaluation of automated microbiology identification
systems: comparison of the Sensititre Aris® and Vitek® 2
when testing Gram-positive and -negative pathogens

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(Marshfield, US)

Objectives: To evaluate the accuracy of Gram-positive and –negative
bacterial identifications using commercial automated systems includ-
ing the Sensititre ARIS® (Trek Diagnostic Systems) and Vitek® 2
(bioMérieux) compared with existing identification algorithms.
Automated systems have been shown to have variable failure rates
requiring periodic re-evaluation of contemporary isolates to assure
continued performance. Correct pathogen identifications are mandatory
for diagnostic, prognostic and therapeutic purposes.

Methods: Clinically significant challenge pathogens (404 total) from
patients attending a large regional medical centre were collected
prospectively in 2010 and included staphylococci (108), enterococci (40),
streptococci (13), Enterobacteriaceae (136), Pseudomonas aeruginosa
(59) and other non-fermentative bacilli (48). Isolates were tested
according to the manufacturers’ recommendations using Sensititre®,
GPID and GNID, and Vitek® 2 GP and GN identification panels;
results were compared with existing laboratory biochemical algorithms,
including use of the Phoenix® PID and NID panels. Consensus was
defined as all 3 systems providing matching results. Any system result
discrepant from the other 2 was considered erroneous, and alternative
testing methods were used for adjudication.

Results: This evaluation generated a total of 1,212 identifications
between the current laboratory testing algorithm and the automated
systems. Overall, 95.3% and 88.9% of Vitek 2 identifications were
correct at genus and species levels, respectively, for this challenge
set compared with 91.3% and 81.4% for Sensititre. Both systems
provided acceptable \( \geq 90% \) species-level identifications for
\( E. coli \), Klebsiella, group B streptococci and Enterococcus spp. but performed
less well for other Enterobacteriaceae, other non-fermentative bacilli
and other staphylococcal species. Sensititre was inferior in identifying
P aeruginosa (79.7% at the species level) compared with Vitek 2 (100%).
Results for the two assay systems are in the Table.

| Isolate Species/Groups | Sensititre% Agreement | Vitek2% Agreement |
|------------------------|-----------------------|-------------------|
|                        | Genus| Species | Genus| Species |
| E. coli                | 97.9 | 97.9   | 100  | 100     |
| Klebsiella spp.        | 90.0 | 90.0   | 100  | 100     |
| Other Enterobacteriaceae | 90  | 73.3   | 88.9 | 82.6    |
| P. aeruginosa          | 96.6 | 79.7   | 100  | 100     |
| Other NonFermentative GNB | 81.2 | 64.9   | 81.3 | 62.5    |
| Staphylococcus aureus   | 86.1 | 86.1   | 100  | 100     |
| Other Staph species     | 96.1 | 96.1   | 100  | 100     |
| Streptococcus. Group B | 100  | 100    | 100  | 100     |
| Enterococcus spp.       | 100  | 100    | 100  | 100     |

Conclusions: Both the Sensititre® and Vitek® 2 systems produced a high
level of accurate identification at the genus level (91.3% and
95.3%, respectively) but less well at the species level (81.4% and
88.9%). In particular certain Enterobacteriaceae, non-fermentative bacilli
and staphylococci other than \( S. aureus \) were found to be problematic.
Limitations with each system were apparent and require consideration with implementation.

**P1808** Efficiency of an automated Gram-staining system for bacteriological diagnostics

J. Knobloch* (Lübeck, DE)

**Objectives:** Automation of time consuming manual procedures in bacteriological laboratories can help to focus the personnel capacities on core duties. Therefore, the PREVI™ Color automated Gram-staining system was evaluated for its efficiency and quality in processing of slides inoculated with a wide variety of clinical specimen as well as pure bacterial cultures.

**Methods:** Manual Gram-staining was compared to automated staining using the PREVI™ Color system for 700 slides from different specimen (BAL n = 50, genital swabs n = 98, sputum n = 100, Stool n = 100, urine n = 151, wound swabs n = 151, and pure bacterial cultures n = 50). For the manual staining slides were heat fixed and stained by the classical Gram-staining procedure. For automated staining fixation of the slides as well as staining was performed within the PREVI™ Color system. The staining method was blinded prior microscopy. Additionally, the hand-on-time needed for manual and automated staining was measured for representative batches of slides.

**Results:** The quality of automated Gram-staining was interpreted by microscopy of slides blinded for the staining method. The overall staining quality was equal in 71.3%. A superior result using the automated system was observed in 21.1% and for manual staining 7.6%. For complex specimen (genital swabs, sputum, stool, and wound swabs) significant differences were observed with only 59.2% equal results and superior results in 29.8% for automated staining and 11.0% for manual staining. Minor differences were observed in specimen with low complexity (BAL, urines, and pure bacterial cultures) with 87.4% equal results and superior results in 9.6% and 3.0% for automated and manual staining, respectively. The average hand-on-time was reduced by 34%.

**Conclusions:** Automated Gram-staining decreased hand-on-times significantly. Standardization of the staining procedure resulted mainly in higher or equal staining quality for all specimen, indicating that the introduction of automated Gram-staining is able to reduce personnel cost together with an increased or at least equal quality of staining results.

**P1809** Evaluation of the Vitek2 ANC card for the identification of clinical isolates of anaerobic bacteria

E.H. Lee*, J. Degener, G.W. Welling, A. Velo (Groningen, NL)

**Objectives:** The aim of this study is to compare the accuracy of the VITEK 2 ANC card (bioMérieux, Marcy l’Etoile, France) with 16S rRNA gene sequencing for identification of anaerobic species.

**Methods:** An evaluation of the VITEK 2 ANC card was performed with 301 anaerobic isolates, including 100 species that were not contained in the database. Each strain was identified by 16S rRNA gene sequencing, which was considered to be the reference method. Inocula were made from subcultures grown for 24 to 48h on BBA agar. Cells were suspended in 0.45% NaCl, pH 5.7, and adjusted to a McFarland number 2.7 to 3.3 using a calibrated VITEK® 2 DENSICHEK (bioMérieux, Marcy l’Etoile, France).

**Results:** Of the 301 isolates, 79.4% (239/301) and 60.1% (181/301) were correctly identified to the genus and species level respectively. Of the 201 isolates species which are included in the database, correct genus and species identification were obtained for 95.6% (192/201) and 90.0% (181/201). For the 100 isolates unclaimed at the species level, 47.0% (47/100) gave correct identification to the genus level and 16.0% (16/100) were accurately designated as not identified. Strains which were difficult to characterize to the species level using current phenotype methods, were misidentified by VITEK 2 ANC card. For example, Peptoniphilus harei and Peptoniphilus asaccharolyticus, Fusobacterium nucleatum and Fusobacterium nucleofemor, Prevotella nigrescens and Prevotella intermedia cannot be easily distinguished from each other.

**Conclusions:** The VITEK 2 ANC card allows rapid identification of anaerobic bacteria within 6h. This system is an acceptable method for identification of those species which are included in the database.

**Mobile genetic elements**

**P1810** The small resistance plasmid p9123 is widespread among enteric pathogens and enhances their fitness

C.S. Wong, L.M. Hall, V1. Emme* (Bristol, London, UK)

**Objectives:** We previously demonstrated the small sulphonamide and streptomycin resistance plasmid p9123 enhances the fitness of Escherichia coli K12 JM109 by approximately 5% per generation, even in the absence of an evolutionary history. Here, we investigated the fitness impact of the plasmid on other strains of enterobacteriaceae, with particular emphasis on enteric pathogens.

**Methods:** BLAST searches were used to identify homologues of p123 to establish the distribution of the plasmid. p9123 was introduced into selected enteropatogenic bacterial strains by electroporation. Sulphonamide and streptomycin susceptibility was determined by Etest. Fitness was estimated by determining the growth rates of strains with and without p123 in nutrient broth. Statistical analysis was carried out with the student’s t-test.

**Results:** An interrogation of Genbank revealed 12 occurrences of p9123 or closely related plasmids. Of these, nine were among isolates of enteric pathogens such as Shigella spp., Salmonella enterica sv. Choleraesuis and enterotoxogenic E. coli (ETEC). Some variants encode additional resistance determinants such as tet(A) or ampC. Acquisition of p9123 by the bacterial strains studied elevated their sulphonamide MICs from 4–48 mg/L to >1024 mg/L whereas their streptomycin MICS increased from 3–12 mg/L to 32–128 mg/L except in the case of Shigella flexneri M90T which is intrinsically highly resistant to streptomycin due to rpsL mutation. Fitness results are shown in the table. Acquisition of p9123 significantly improved the fitness of S. flexneri M90T, whereas in EPEC E2343/69 and S. Typhimurium LT2 the plasmid had a neutral effect on fitness.

**Conclusion:** The resistance plasmid p9123 has no detrimental effect on the fitness of a variety of pathogenic enterobacteriaceae and confers a fitness benefit in S. flexneri as previously observed in non-pathogenic E. coli. The widespread distribution of the plasmid among enterobacteriaceae may hence be due to its fitness neutral and/or enhancing properties. The spread of a beneficial plasmid among pathogens is of concern as it may potentially enhance the spread of the pathogenic strains themselves.

**P1811** Complete sequences of two 'untypable' plasmids carrying blaCTX-M-1 group genes

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**Objectives:** A local survey of genes conferring resistance to extended-spectrum β-lactamases identified conjugal plasmids that could not be typed by the commonly-used PCR-based replicon typing scheme carrying blacTX-M-15 (pJIE143; from Escherichia coli) or blacTX-M-62 (pJIE137; from Klebsiella pneumoniae). blacTX-M-15 appeared to be associated with ISEc1 alone in pJIE143. pJIE137 was found to carry a class 1 integron with several insertions separated from ISEc1- blaCTX-M-62 by a segment that is not closely related to known plasmids.

The aim of this study was to completely sequence pJIE143 and pJIE137.
Methods: Transconjugants carrying pJEI43 or pJEI37 were subjected to S1 nuclease/pulse-field gel electrophoresis to confirm that only one plasmid species was present and for size estimation. Plasmid DNA was extracted from transconjugants, treated with Plasmid Safe DNasease to remove chromosomal contamination and amplified using GenomiPhi. Purified DNA was tagged with molecular barcodes and pooled with other plasmids for emulsion PCR and Roche 454 Titanium pyrosequencing. Data for each plasmid were separated bioinformatically, sequences were partially assembled using the Newbler software provided and PCR was used to complete assembly.

Results: pJEI43 produced a single >34 kb contig with 180x coverage that could be assembled as a circular molecule with the usual 3 kb ISEcp1-blaCTX-M-15 transposition unit inserted in the plasmid backbone, flanked by direct repeats. The whole backbone sequence is ~99% identical to pBS512_33 from Shigella boydii, which carries no known resistance genes and has a put-type replicon, also to pCR from Citrobacter rodentium. pJEI37 produced a 37 kb contig (128x coverage) that corresponded to most of the plasmid backbone and six shorter contigs (0.5–9 kb, >50x coverage) that corresponded to parts of the previously mapped resistance regions containing several repeats. All seven contigs were assembled into a single 58 kb circular molecule and parts of the pJEI37 backbone were found to be related (~70% identical) to IncN plasmids

Conclusions: pJEI43 illustrates that ISEcp1-blaCTX-M-15 is not always associated with Tn2 and may be found on plasmids that are quite different from the IncF and IncI groups commonly carrying this gene. pJEI37 also represents a novel plasmid type with independent insertions of two resistance regions.

Plasmid-mediated antimicrobial resistance: characterising extended-spectrum β-lactamases in Escherichia coli isolated from cattle

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Objectives: The aim of this study was to characterize the clonality of blaCTX-M bearing Escherichia coli strains from the United Kingdom, harbouring blaCTX-M and other antimicrobial resistance genes with respect to their relative plasticity and ease of dissemination.

Methods: Bovine E. coli isolates (n = 52) collected between the period of March and October 2007, found to posses the blaCTX-M gene were examined. Resistance phenotypes were determined using disk diffusion assay. Isolates were also analysed by pulse field gel electrophoresis, conjugation, plasmid profiling, miniaturized DNA microarray analysis and PCR based replicon typing (PBRT) and IncI plasmid MLST (pMLST).

Results: All strains were found to be resistant to cefotaxime (CTX) and harboured large plasmids (50–100 kb) of varying macro-restriction profiles (25–100% similarity) and PFGE patterns. The isolates encoded either CTX-M group 1 (blaCTX-M-1, -15 and -32) or CTX-M group 9 (blaCTX-M-14 and -14b) gene, with three isolates encoding both. The isolates harboured multiple plasmids of varying incompatibility (Inc) groups including: IncI, IncFIB and IncI. Forty-seven of the fifty-two strains transferred plasmids conferring cefotaxime resistance to recipient E. coli K12 strains with relative frequency of transfer between 1.25 x 10^{-5} and 9.23 x 10^{-3}. Thirty-nine of the resultant transconjugants harboured a single IncF, IncN or IncI plasmid. These plasmids were found to be resistant to multiple antimicrobial agents. IncN strains were further characterised using pMLST and found to be the same as types from human isolates of E. coli and Shigella sonnei.

Conclusion: These data confirm the variable nature of bovine CTX-M ESBL E. coli based on PFGE profiles, although only a limited number of CTX-M sequence types were seen. Interestingly, 20 isolates encoded the blaCTX-M-15 gene, the most prevalent β-lactamase worldwide, although none of these were the ST131 clone. CTX-M encoding plasmids were found to be largely on IncN and IncI plasmid groups with the ability to transfer to a recipient E. coli strain. The presence of these genes on transferable plasmids expands its available niche and increases the probability of transfer of multi-resistant plasmids harbouring blaCTX-M genes, between bacteria, including zoonotic, commensal and pathogenic bacteria, with potential consequences for both public and animal health.

Objective: To characterize the genetic environment of aac(6′)-Ib-cr and qnrS1 genes and the plasmid content in Enterobacteriaceae

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Methods: Ten Escherichia coli, five Klebsiella pneumoniae, and two Enterobacter aerogenes isolates were recovered from clinical and environmental samples. Antibiotic susceptibility patterns were determined using agar dilution methods. Several resistance genes were analyzed by PCR and sequencing: 1) blaTEM, blaBLA-M, blaOXA, blaSHV, qnrA, qnrB, qnrS, qepA, aac(6′)-Ib-cr and qoxAB; 2) presence of type 1 and 2 integrons; and 3) mutations in gyrA and parC genes. Molecular typing and phylogenetic group were determined by MLST and PCR or PCR-RFLP. Number and plasmid type were analyzed by PFGE-S1 and PCR-based replicon-typing (PBRT). Characterization of addiction systems were carried out by PCR. The genetic environment of quinolone resistance genes were studied by PCR-mapping or cloning and subsequent sequencing.

Results: The aac(6′)-Ib-cr gene was detected in all tested isolates and blaCTX-M-15 gene in all but one E. coli. Three K. pneumoniae isolates presented as well qnrS1 and one E. coli and all K. pneumoniae strains harboured oxqAB gene. Most of the isolates showed high level quinolone resistance due to mutations in gyrA and parC genes. All K. pneumoniae isolates belonged to the KpI phylogenetic group and to sequence-types ST341 and ST433. E. coli isolates belonged to B1, B2 and D phylogenetic groups and to the sequence-types ST224, ST468 and ST131. All E. coli and E. aerogenes and one K. pneumoniae strain presented type 1 or 2 integrons. Several plasmid types were detected: IncC, IncFIA, IncFIB, IncN, IncR and colE. The following addiction systems were identified: pemK1, hok-sok, ccdAB, sfrBC and vagC/D. Different genetic environments of aac(6′)-Ib-cr gene were detected among studied isolates: 1) located in the variable region of a type 1 integron; 2) located in a Tn1721 transposon; 3) related to aac(3)-II gene; and 4) related to mrx gene. The genetic environment of qnrS1 gene was the same in all the positive strains, presenting an IS26 and a truncated ISEcl2 upstream of the gene and an hypotetical protein and a resolvase downstream.

Conclusions: The aac(6′)-Ib-cr gene can be located in different genetic environments, associated or not with integrons and the bacteria that carry this gene contain four different plasmid types and a high diversity of addiction systems.

Novel gene cassettes and integrons in environmental antibiotic-resistant bacteria

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Objectives: To evaluate the occurrence of integrons and diversity of gene cassettes arrays among Enterobacteriaceae and Aeromonadaceae isolated from urban wastewaters.

Methods: Screening of integrase genes was performed in 697 isolates by dot blot hybridization; integrase-positive strains were further characterized in terms of phylogenetic affiliation to species level and antimicrobial resistance profile. The genetic location of integrons was determined and strains containing plasmid-borne integrons were selected for mating experiments. Integron's variable regions were characterized by sequencing analysis.

Results: The prevalence of intI genes was 3.73%. One third of intI genes were located in plasmids and could be transferred at frequencies of 10–5 and 10–6 transconjugants/recipient cell. Twelve different integrons (4 representing novel arrays) were detected and included 3 new gene
Mobile genetic elements

cassettes: a novel aadA variant (aadA17), a gene putatively involved in cell signalling and biofilm formation (skyA) and an open reading frame encoding a putative protein of unknown function (orfER1.20). In addition, a novel insertion sequence (ISAS12) was found. Approximately 80% of strains, most of them belonging to Aeromonas, were resistant to at least 3 antibiotics of different classes.

Conclusions: The high prevalence of multiresistant isolates highlights the urgent need to employ effective means of effluent disinfection to avoid the dissemination of antibiotic-resistant bacteria. Moreover, the presence of novel integron structures in treated effluents suggests that domestic wastewater environments may favour the formation of novel combinations of gene cassettes. Results obtained also emphasize the importance of Aeromonas as potential vectors of dissemination of integrons and antibiotic resistance genes.

Presence of mobile genetic elements carrying virulence and antibiotic resistance genes in non-pathogenic Vibrio strains

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Objectives: Non pathogenic Vibrio species can be occasionally responsible for infectious diseases in humans. Horizontal gene transfer and the acquisition of foreign DNA is a fundamental process in the evolution of most bacterial species. Many genes can be mobilized in extra-chromosomal elements thus having the possibility of mix or recombine in the aquatic environment.

In this study we have screened a collection of North Adriatic marine Vibrio strains for the detection and analysis of mobile genetic elements such as the V. cholerae pathogenicity island (VPI-2) and class 1 integrons. Methods: A total of 152 Vibrio spp. strains were isolated from water, plankton, sediment and fish samples obtained in the area of the northern Adriatic sea during the period 2006-2009. The identification of the environmental strains at the species level was performed by standard biochemical methods. DNA extracted from vibrios with a standard protocol was subjected to PCR. The obtained PCR products were analyzed by sequencing.

Results: In order to investigate the transfer of VPI-2 from V. cholerae to non-pathogenic Vibrio species we have screened the environmental strain collection with different pairs of primers designed on some pathogenicity island gene clusters (5′,3′,TMRF,nacC,nanH,FAGHE). A total of 18 strains carried at least one of the genes. Four strains (NPV3,NPV6,NPV7,NPV18) presented both the 5′ and 3′ insertion sites of the pathogenicity island VPI-2 into the chromosome and the nanH gene. The strain NPV18 also carried the nagC gene. The TMRF and FAGHE clusters have not been detected in any of the four strains. Moreover, we found some other strains carrying only partial fragments of the island. A total of 13 environmental non-pathogenic Vibrio strains, all isolated from fish farms, show the presence of the integrase gene from class1 integrons. Strains were screened for the presence of the integrase gene from class1 integrons. Strains were screened for constant class1 integron using primers designed in the 5′ and 3′ conserved segments (in-F/in-B); eight strains carried the constant class1 integron while 5 strains seem to have only the integrase site.

Conclusions: This study showed possibility for a non pathogenic Vibrio strain of acquiring integrons containing AR cassettes and an entire or partial pathogenicity island containing virulence factors. We suggest that non pathogenic vibrios might represent a marine reservoir of virulence and AR genes and of genetic elements of medical interest and for this reason this could constitute a risk for human health.

The Repository of Antibiotic-resistance Cassettes – an online database of gene cassettes found in mobile resistance integrons and a web-based application for annotation of DNA sequences containing these cassettes

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Objectives: Gene cassettes carried by mobile resistance integrons (MRI; generally classes 1–3) frequently contribute to transmissible antibiotic resistance (including multi-resistance) in Gram-negative bacteria. Gene cassette nomenclature is complicated and currently largely unregulated, so that many cassettes are incorrectly named in publications and incorrectly or incompletely annotated in sequences in GenBank. We previously used an automated annotation system (called Attacca) to identify over 130 different (>98% identical) cassettes carrying antibiotic resistance genes in sequences of MRI in GenBank. Our aim was to make a database containing these cassettes and our cassette annotation system available to the research community as a web-based application.

Methods: Our previously published method uses a database of known gene cassettes, automated BLASTn searches and computational grammars to identify gene cassettes in arrays in MRI in all sequences in GenBank. We have made our cassette database accessible online and the Attacca system has been adapted to a web-based application that allows annotation of individual sequences submitted by researchers. The annotation system was also modified to use amino acid sequences to distinguish between different β-lactamase variants encoded by closely related cassettes.

Results: The Repository of Antibiotic-resistance Cassettes (RAC) website (http://www2.chi.unsw.edu.au/rac/) is offered as a free service that allows researchers to browse our database of known gene cassettes, which includes notes about different cassettes and links to exemplar sequences. After registration, requiring only an e-mail address, users can submit DNA sequences (e.g. obtained by PCR amplification of cassette arrays or from sequencing of complete plasmids) for accurate annotation of gene cassettes. Gaps in annotations are flagged for manual review and any novel cassettes identified will be assigned appropriate names. Initially the details and sequences of these cassettes will be available to the submitter only. Following publication elsewhere, or given permission from the submitter, they will be added to the public database and may then appear in annotations of sequences submitted by all users.

Conclusions: RAC is an on-line application that allows browsing of a comprehensive database of antibiotic resistance gene cassettes, accurate annotation of gene cassettes in DNA sequences and appropriate naming and incorporation of new cassettes.

Mobile genetic elements: a comparative study of their presence in bacteria from inanimate surfaces in Hospital Infante D. Pedro, Aveiro, central Portugal, in 2005 and 2008

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Objective: Due to the high antibiotic pressure, multidrug resistant bacteria are abundant within the hospital environment. This study aimed to compare the presence of mobile genetic elements in Gram-negative bacteria collected from inanimate surfaces within Hospital Infante D. Pedro, Aveiro, in 2005 and 2008.

Methods: Samples from different inanimate surfaces were collected, during one month period in 2005 and the same period in 2008. Sterile swabs were rubbed in the surfaces and then placed in rich medium (TSB), and incubated overnight at 37°C. Serial dilutions were plated in MacConkey agar. Phenotypically different colonies were selected and their clonal relationship was evaluated by rep-PCR. Identification to the species level was determined by 16S amplification. Screening for class 1 integrons, insertion sequence common regions (ISCRI) and sul3 gene was performed by PCR, using appropriate primers.

Results: In 2005, 85 phenotypically different bacteria were isolated, from which 45 did not show clonality and were further studied; from these, 33% isolates were positive to intI1 gene. ISCR1 was detected in 33% of the integron positive isolates. In 2008, 227 phenotypically different bacteria were isolated; 153 did not show clonality and were further studied; from these 46% possess class 1 integron, 60% of which possess ISCR1. In some isolates of 2008 the variable region could not be amplified although the intI1 gene was present. In these cases the presence of sul3 gene was investigated, revealing its presence in 60% of the isolates. Escherichia coli, Klebsiella spp., Pseudomonas spp. and Proteus mirabilis were the most frequently found microorganisms.

Conclusion: This study reveals a high prevalence of mobile genetic elements, namely in intI1 and sul3 genes, and ISCR1 element in bacteria
from inanimate surfaces. These elements are responsible by horizontal gene transfer among bacteria and may therefore be implicated in the multidrug resistance profile observed in bacterial species. It is likely that these bacteria can find their way into debilitated hospitalized patients.

New species and unusual pathogens: usefulness of molecular tools for bacterial identification

A quantitative assessment of *Borrelia* and *Bartonella* DNA and tick-borne encephalitis virus RNA in *Ixodes persulcatus* Schulze ticks collected in Chelyabinsk region of Russia

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*Borrelia* and *Bartonella* DNA and tick-borne encephalitis virus (TBEV) RNA were detected in *Ixodes persulcatus* Schulze ticks collected in May 2009 in Chelyabinsk region, Ural, Russia by means of PCR with subsequent electrophoresis or real-time PCR with fluorescent *TagMan* probes. *Borrelia burgdorferi* sensu lato DNA was found in 36.6±5.0% ticks analyzed. Phylogenetic analysis of the PCR product nucleotide sequences showed 2 species *Borrelia afaxelli* and *Borrelia garinii*. Threshold cycles Ct=23.4–46.3 corresponded to approximately 1–105 genome equivalents in reaction mixtures or nearly 101–106 DNA copies per an infected tick. Among 76.5% of studied ticks low bacterial loads with Ct>35 were observed. *Bartonella* DNA was revealed in 38.9±5.2% *I. persulcatus*. Based on phylogenetic analysis of nucleotide sequences of the Bartonella-specific PCR-products *Bartonella quintana* and *Bartonella henselae* were found. Threshold cycles varied from Ct=25.7 till 39.5 with evident prevalence of samples with Ct<30. It might suggest higher amounts of *Bartonella* DNA in taiga ticks compared to *Borrelia* DNA. The tick-borne encephalitis virus RNA of Siberian genetic subtype was detected in 16.9±4.3% of *I. persulcatus* using reverse transcription with subsequent real time PCR with *TagMan* probe. Based on threshold cycles Ct=21.3–48.1 and taking into account RNA isolation and reverse transcription efficiency the viral loads could be estimated as 104–107 genome-equivalents per a tick. The TBEV quantities exceeded the tick-borne bacteria amounts. Experimentally observed frequencies of mixed infections with *Borrelia* and *Bartonella* in 13.0% of ticks fitted well to theoretical estimations based on independent distribution of the tick-borne bacteria among vectors. Bacterial loads for individual and mixed infections have similar spans but Ct>39 were more typical for mixed infections.

Combined DVC-FISH method for in situ detection of viable *Arcobacter butzleri* cells

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Objectives: Arcobacters can enter into a viable but non-culturable (VBNC) state, which is a problem for the detection by traditional cultural methods of this emerging human pathogen. Moreover a standardized reference method of detection has not so far been proposed. In this study we have developed a technique for the specific enumeration and rapid discrimination of viable and non viable cells, combining direct viable count (DVC) procedure with fluorescent in situ hybridization (FISH) method.

Methods: The reference strain *Arcobacter butzleri* DSM 8739 was used to develop and set up the technique. DVC method was modified and adapted to *A. butzleri* analysis by testing different times of incubation and concentrations of DNA-gyrase inhibitors. The tested antibiotics were ciprofloxacin, novobiocin and nalidixic acid. We combined incubation times of 3, 6, 7, 8, 9 and 24 hours with concentrations of 0.3, 0.4, 0.5, 0.6 and 0.8 µg/mL. FISH was performed by using the probe ARC94, with the sequence 5'TGGCACCATTAGCTGCAG CA3', targeting 16S rRNA. The detected bacteria were counted by direct plate counting, fluorescence microscopy and by the viability kit LIVE/DEAD® BacLight. These data let us select the best time of incubation and antibiotic concentration without compromising the cell viability. Furthermore, the theoretical detection level of the method was tested in phosphate-buffered saline (PBS) method.

Results: The optimal conditions for the DVC-FISH method were the incubation with 0.3 µg/mL of ciprofloxacin during 6–7 hours. We observed cell degradation or decrease of the number of bacterial cells with times of incubation higher than 9 hours and/or concentrations higher than 0.5 µg/mL. With this DVC-FISH combination we obtained more than 2 times the original size of *A. butzleri* allowing the detection of elongated viable cells. The sensitivity established for the DVC-FISH method in phosphate-buffered saline was 10³ UFC/ml.

Conclusion: Our results show that the DVC-FISH combination is an effective, rapid and culture-independent useful method to detect and identify viable and non viable *Arcobacter* cells. Therefore, DVC-FISH method could be applied for detection of these bacteria in different clinical samples as well as for the analysis of possible sources of transmission.
MALDI-TOF

P1822 Faster microbial identification with MALDI-TOF mass spectrometry and digital imaging: a pilot study

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Objectives: In this pilot study, we assessed the potential acceleration of identification with Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) as compared to conventional methods (CM) and investigated whether identification time can be further minimized by combining MALDI-TOF MS with the "It's The Sample That Moves®" system (ITSTM®, KIESTRA Lab Automation, The Netherlands), a fully automated incubation system which produces digital images of agar plates using different photographic settings to detect early growth. Finally, to assess the potential clinical impact of accelerating identification but not susceptibility testing, we evaluated how availability of microbial identification results could influence prescribed antibiotic treatment.

Methods: Prospective study time and results by CM of 219 consecutive positive blood culture isolates was assessed. Retrospectively, MALDI-TOF MS identification was performed using a Microflex mass spectrometer (Bruker Daltonik, Bremen, Germany) and the Bruker Biotyper database 3.0. Identification time and accuracy was measured. Discrepancies were resolved by 16S rRNA sequencing. Minimal incubation time to detect growth with digital imaging by ITSTM® and subsequently perform MALDI-TOF MS identification was determined for different microbial species. Adjustments in antibiotic treatment regimen, based on availability of pathogen analysis results, were appraised retrospectively from the Electronic Patient Record system.

Results: Pathogen identification by MALDI-TOF MS succeeded in 95.7% of all isolates. MALDI-TOF MS and CM were concordant in 94.1% of all isolates. The combination with ITSTM® resulted in a mean time gain of 30.6 hrs compared to CM. The incubation time in ITSTM®, needed for detection of microbial growth and successful subsequent MALDI-TOF MS analysis, was lowest in Gram negative rods and highest in yeasts. All microbial groups showed significantly longer incubation times in lower concentration (1×10^4 CFU/ml, 1×10^2 CFU/ml). Early microbial identification without susceptibility results led to an adjustment of the antibiotic regimen in 12% of all patients.

Conclusion: With the combination of ITSTM® and MALDI-TOF MS, microbial identification could be significantly accelerated as compared to CM. Workflow could be optimized, costs reduced, pathogen identification accelerated and potentially lifesaving switches in antibiotic regimen could occur earlier.

P1823 Tapping the potential of intact cell mass spectrometry with a combined data analytical approach

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Objectives: Identification of microorganisms with matrix assisted laser desorption ionization time of flight (MALDI-TOF) intact-cell mass spectrometry (ICMS) has proven to be a valuable alternative to time consuming biochemical identification approaches. However, due to the complexity and noisiness of MALDI-TOF spectra, the taxonomic resolution of the method is often not fully exploited by commercial tools, which often rely on a single data analytical approach.

Methods: In the present work, we propose a workflow which uses the spectral diversity of a commercial database (SARAMIS) to narrow down the search field at a certain taxonomic level, followed by a refined classification by supervised modeling. As supervised learning algorithm, we have chosen a shrinkage discriminant analysis approach, which takes colinearity of the data into account and provides a scoring system for biomarker ranking. This ranking can be used to tailor specific biomarker subsets, which optimize discrimination between subgroups, allowing a weighting of misclassification. The suitability of the approach was verified based on a dataset containing the mass spectra of three Yersinia species Y. enterocolitica, Y. pseudotuberculosis and Y. pestis. Thereby, we laid the emphasis on the discrimination between the highly related species Yersinia pseudotuberculosis and Yersinia pestis.

Results: All three species were correctly identified at the genus level by the commercial database. Whereas Y enterocolitica was correctly identified at the species level, discrimination between the highly related Y. pseudotuberculosis and Y. pestis strains was ambiguous. With the use of the supervised modeling approach, we were able to accurately discriminate all the species even when grown under different culture conditions.

Conclusion: The proposed workflow, which combines the diversity of a commercial database with the discriminative power and feature selection capabilities of supervised classification has the potential to ameliorate the taxonomic resolution of intact-cell mass spectrometry.

P1824 Methods for the immediate identification of bacteria in charcoal-containing BacT/ALERT® blood cultures by MALDI-TOF mass spectrometry

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Objectives: Direct analysis of positive blood culture broth using MALDI-TOF can potentially identify the organism within one hour of the bottle flagging positive. However, isolating microorganisms from charcoal-containing blood culture broth in sufficient purity for MALDI-TOF analysis is challenging using known methods, due to the size distribution of charcoal particles and the selective adherence of certain species. This study was designed to evaluate the performance of two new methods developed for processing positive charcoal-containing BacT/ALERT® blood culture broth for immediate identification by MALDI-TOF MS.

Methods: BacT/ALERT® FA bottles were inoculated with 40–400 CFU and 10 mL human blood, and incubated in a BacT/ALERT® 3D Microbial Detection System. Samples of positive broth were treated by one of two methods depending on their Gram morphology. For broth containing Gram-positive cocci in clusters, a sample was sonicated to detach adsorbed bacteria, treated with a proprietary flocculating agent and purified over a density cushion. For non-staphylococci, a sample of broth was subjected to differential centrifugation and purified over a density cushion. Bacteria enriched by these methods were treated with formic acid-acetonitrile and the extracts applied to a MALDI target plate. Samples were then overlaid with HCCA matrix and analyzed with an Axima Assay system (Shimadzu Corp.) and SARAMIS® database (bioMérieux).

Results: Staphylococci processing method: 17 of 18 FA broth samples containing individual strains of staphylococci gave correct ID by MALDI-TOF (8/8 Staphylococcus aureus and 9/10 Staphylococcus epidermidis).

Non-Staphylococci processing method: 19 of 20 FA broth samples containing individual strains gave correct ID by MALDI-TOF (4/4 Escherichia coli, 2/2 Klebsiella pneumoniae, 2/2 Enterobacter aerogenes, 2/2 Pseudomonas aeruginosa, 2/2 Enterococcus faecalis, 2/2 Enterococcus faecium, 3/4 Streptococcus pneumoniae/mitis and 2/2 Streptococcus pyogenes).

Conclusion: Both new methods provided acceptable performance (94.7% correct) for the identification of bacteria directly from charcoal-containing blood culture broth using MALDI-TOF mass spectrometry.

P1825 Improved method for the immediate identification of pathogens in BacT/ALERT® standard aerobic blood cultures by MALDI-TOF mass spectrometry

J.M. Hyman, J.D. Walsh* (Durham, US)

Objectives: The ability to characterize bloodstream infections within the first hour after a positive blood culture result would boost the clinical relevance of the diagnostic information provided. MALDI-TOF mass spectrometry (MS) has emerged in recent years as a fast and reliable method for the identification of microorganisms. This study was designed to evaluate the performance of a novel method for processing
positive BacT/ALERT® Standard Aerobic (SA) blood culture broth for immediate identification by MALDI-TOF MS.

**Methods:**
BacT/ALERT® SA bottles were inoculated with 40–400 CPU and 10 mL human blood, then incubated in a BacT/ALERT® 3D Microbial Detection System. A sample of positive broth was briefly treated with proprietary lysis buffer and passed through a 0.45 micron membrane filter. Following several washes, microorganisms were recovered by scraping the filter surface and smearing the cells directly onto a MALDI target plate. Samples were analyzed with an Axima® Assurance system (Shimadzu Corp.) and SARAMIS™ database (bioMérieux).

**Results:**
A total of 46 samples of positive BacT/ALERT® SA broth were tested, each containing a separate strain, representing eleven species commonly associated with positive blood cultures (Staphylococcus aureus, Staphylococcus epidermidis, Candida albicans, Escherichia coli, Klebsiella pneumoniae, Enterobacter aerogenes, Pseudomonas aeruginosa, Enterococcus faecalis, Enterococcus faecium, Streptococcus pyogenes and Streptococcus pneumoniae/mitis group). The overall accuracy of this rapid method was 97.8% to the species level (45/46 strains). One of eight S. epidermidis strains tested was not identified.

**Conclusion:**
This study demonstrates the effectiveness of a novel lysis filtration (LF) method to process positive blood culture broth for analysis by MALDI-TOF. The patent-pending selective lysis buffer used in this method quickly and effectively dissolves blood cells while leaving microorganisms intact. The LF method has the advantage of requiring less manual manipulation and no centrifugal processing, and produces a clean concentrated paste of microorganisms in 10–12 minutes that is optimal for MS analysis.

**P1828 Use of MALDI-TOF technology for the rapid microbiological diagnosis from blood samples**

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**Objective:**
To evaluate how MALDI-TOF technology impacts the time to microbiological diagnosis from blood samples and the concordance with standard identification methodology.

**Methods:**
155 positive blood cultures have been analysed in parallel using MS MALDI-TOF Autoflex III (Bruker) and a variety of standard procedures (Wider, Vitek II, Api, or manual identification). Time to identification and concordance with standard methods was investigated. Kappa correlation coefficient was estimated to evaluate the correlation between both diagnostic approaches.

**Results:**
Use of MALDI-TOF reduced a median of 14 hours (range 12–18 hours) time to species identification from positive blood samples. Identified microorganisms comprised 27.1% Gram negative [E. coli (23), P. aeruginosa (9), A. bioﬁlli (1), E. aerogenes (1), K. pneumoniae (2), K. oxytoca (2), A. hydrophila (1), B. fragilis (1), E. cloacae (1) and A. baumannii (1)] and 72.9% Gram positive microorganisms [S. aureus (13), S. epidermidis (35), S. hominis (25), S. capitis (2), S. warneri (2), S. haemolyticus (9), S. simulans (3), S. cirudans (5), S. pneumoniae (1), E. faecalis (6), E. faecium (1), P. acnes (1), L. monocytogenes (1), M. butace (4), Bacillus circulans (2), Clostridium perfringens (2), and Corynebacterium sp (1)]. Globally, concordance was 95.8% (k=0.928) for species identification, and 98.7% for genus identification (k=0.977).

For Gram negative rods, concordance was 95.2% (species, k=0.946), and 100% (genus), while for Gram positive microorganisms concordance was 82.3% (k=0.627), and 98.2% (k=0.931) for species and genus identification, respectively. Gram positive discordant identifications [Maldi/standard ID] were as follows: [St. viridans (6)/St. oralis (1), St. salivarius (1), St. australis (1), St. canis (1), St. pneumoniae]; [Corynebacterium spp/P. penitens]; [S. epidermidis/R. mucilaginosus]; [S. haemolyticus (1)/S. hominis (1)]; S. haemolyticus (11)/S. epidermidis (9) S. warneri (1) S. simulans (1)]. Gram negative discordant results [Maldi/standard ID] were infrequent: [P. aeruginosa/P. stutzeri]; [Aeromonas hydrophila/A. veronii].

**Conclusion:**
The use of MALDI-TOF for the identification of microorganisms from positive blood cultures reduces considerably the time to results. MALDI-TOF identification has shown an excellent correlation with standard identification procedures for Gram negative (genus and species level) and Gram positive microorganisms (genus level).

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**Objective:**
Matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a new approach for rapid identification of microorganisms. It is well recommended as a tool having the ability to replace or support conventional methods in the routine settings. Aim of this study was to compare two commercially available MALDI-TOF MS instruments in the routine diagnostic laboratory at the Institute of Hygiene, Microbiology and Environmental Medicine, Medical University of Graz.

**Materials:** In 2010, 1016 microorganisms, 911 bacteria and 105 yeasts were tested in parallel with two different MALDI-TOF MS Systems. The MALDI target plates of both MALDI-TOF MS instruments provided from Bruker Daltonics and Shimadzu Corporation were inoculated with the smear method using HCCA Matrix according to manufacturer’s recommendations. The generated mass spectra were analysed automatically from the company’s databases Bruker Daltonics and AnagnosTec GmbH, respectively. Using the given scores the results were sectioned at species, genus or family level. For Identification of isolates without a result or no concordant results 16S rRNA gene sequencing for bacteria or ITS sequencing for yeasts were applied.

**Results:** Of the 1016 isolates on species level the Shimadzu instrument identified 78.7%, the Bruker instrument 77.3%. The genus level was identified in 6.2% on the Shimadzu instrument and 16.5% on the Bruker instrument, respectively. The overall agreement of the instruments was 61.3% on species level and 20.3% on genus level. On species level the 911 bacterial isolates were slightly better identified with the Bruker instrument (82%) than with the Shimadzu instrument (77.1%), whereas the 105 yeasts were better identified from the Shimadzu instrument (93.3%) than from the Bruker instrument (36.2%).

**Conclusion:** In summary, both instruments, from Bruker and Shimadzu were able to provide rapid and accurate results and are easily to implement in a routine diagnostic laboratory.

A. Irazo Tatay*, J. Frasquet Artés, G. Fagundez Machaiin, M. Gobernado Serrano (Valencia, ES)

**Objective:**
Assay mass spectrometry for identification of various filamentous fungi isolated and identified in samples from two groups of patients, one with fungi skin diseases and the other with hematology and oncology underlying diseases.

**Methods:** Samples were grown in fungi culture usual mediums. Fungi were identified using our routine techniques of the laboratory, which consist in the observation of their differential macroscopic growth, and microscopic differences in their structures such as conidia and hyphae among others. These isolates subsequently were analyzed by mass spectrometry. For that purpose a previous extraction protocol, using formic acid and acetonitrile, was made. The extract was analyzed in the microflex mass spectrometer (Bruker Daltonik GmbH, Bremen). Spectra obtained were compared with the program MALDI biotype 2.0 43.8.

**Results:**
Mass spectrometry achieved correct identification of the following filamentous fungi:
Fungi of patients with hematology and oncology underlying diseases: Hypomictecum: Aspergillus fumigatus (1), A. flavus (1), A. terreus (1), A. thermomutatus (1). Dermatocaceae: Alternaria alternata (3).
Fungi of patients with fungus skin diseases: Dermatophyton, Trichophyton tonsurans (1), T. rubrum (1), Scopulariopsis brevicaulis* (1), Microsporum gypseum (1).

There was no reliable identification in the case of: Scedosporium apiospermum (1), S. prolificans (1), Trichophyton interdigitale (1), T. verrucosum (1).

In these cases we had score values below 1.7 and the organisms were proposed by MALDI biotyper were bacterium.

Of the 15 filamentous fungi tested were correctly identified 11 (73.3%). The range of score we obtain for correct identifications was between 1.465 for A. terreus and 2.005 for A. thermomutatus.

Conclusion: Mass spectrometry should improve to be a fungal identification reference method. In the list of 10 micro-organism proposals, following the correct one there was no same or similar genus fungi which could cause confusion or doubt in the identification. In some cases we observe what we have called “score jump”, that is a great difference of score between the true identification and the following failed identification in the ranking, and also not being related taxonomically (fungi-bacteria). We believe score jump gives greater value to the result, because is not just about the organism most similar to, but it also sets a big distance with the following, which means there are not more possible similarities than the real.

**P1829 Replacement of classical non-automated methods by MALDI-TOF mass spectrometry for routine identification of bacteria and yeasts**

G. Coppers*, K. Martens, G. De Sutter, J. Heyligen, E. Oris (Genk, BE)

**Objectives:** To evaluate replacement of classical non-automated methods by MALDI-TOF MS (MDT) for identification of Bacteria and Yeasts from clinical samples in our routine laboratory. Components of this evaluation are accuracy, reproducibility, turnaround time (TAT) and cost.

**Methods:** Accuracy: 850 clinical isolates collected during a 11 month period are identified by MDT in parallel with the routine classical identification (CI). In brief CI consists of Gram staining, growth and susceptibility features, coagulase, dnanase, oxidase, motility, non-automated biochemical and agglutination tests, and/or API* bioMérieux.

MDT is performed by a Microflex® LT Bruker spectrometer with FlexControl v 3.0 and MALDI Biotyper v 2.0 software with Reference Library 3.0. In addition 50 ATCC reference strains are identified by MDT.

Reproducibility of MDT is assessed with 4 ATCC strains in 10 inter-run and 4 intra-run identifications for each strain. Acceptance criteria for MDT are a score value of >1.9 for species and 1.7–1.9 for genus identification.

Measurement of TAT is done from specimen delivery to identification result reporting.

In order to establish the price for both methods, costs of reagents, labor, equipment purchase and lease are calculated.

**Results:** Of the 900 identified strains with MDT, 96% are concordant with CI (table 1), by which Cumitech 31A criteria are met to accept the new identification method.

Intra-run and inter-run reproducibility of MDT assessed with 4 ATCC strains is 100% (qualitative). Reproducibility of score values (quantitative) is comparable for all strains: E. faecalis 29212 (mean: 2.390); range: 2.236–2.501; SD: 0.060); E. coli S922 (mean: 2.395; range: 2.304–2.494; SD: 0.047); S. aureus 25923 (mean 2.359; range 2.188–2.459; SD: 0.057); P. aeruginosa 27853 (mean 2.369; range: 2.260–2.485; SD: 0.052).

Mean TAT for MDT identification is 24 hour, which means a gain of 24 hours in comparison to CI.

The mean cost of one routine CI (without API*) in our laboratory setting is 2.93 euro; the mean cost of one MDT identification is 3.16 euro.

**Conclusion:** For routine identification of Bacteria and Yeast in our laboratory, CI can be replaced by MDT with at least a comparable test performance and an improvement in TAT of 24 hours at a similar cost.

**P1830 Improvement of identification of anaerobes by MALDI-TOF mass spectrometry analysis**

M. Marschall*, U. Schumacher, I. Autenrieth (Tübingen, DE)

Matrix Assisted Laser Desorption/Ionisation (MALDI-TOF-MS) is increasingly used for identification of bacterial isolates. MALDI-TOF-MS identification is based on the comparison of a spectrum of mass signals of an isolate with a variety of spectra stored in a database. In contrast to other identification systems, the MALDI-TOF-MS database allows amendments of additional species regarding to individual requirements. Here we describe the implementation of MALDI-TOF-MS analyses (MTA) for identification of anaerobic species.

In July 2009 MTA was established as the first line identification method for anaerobic bacteria. Validation of the MTA-results was performed by the following protocol: each anaerobic isolate was analyzed by MTA in parallel to standard identification procedures. In case of discrepant results or if no identification could be obtained with MTA, additional identification assays and gene sequence analysis have been performed. When 15 of 15 strains of a given species had been identified correctly by MTA, the MTA-result of this species was accepted as validated and standard identification was aborted. Spectra of sequenced species, not represented in the database were added leading to continuous expansion of the database. Until today we successfully validated MTA based identifications of 27 anaerobic species, representing 80% of the anaerobic clinical isolates.

One of the main advantages of the MTA is that spectra of new species result in “No Identification” but not to misidentification, triggering attention to unusual strains. Using this protocol we were able to isolate surprisingly often rare identified species including Bacteroides dorei, Bacteroides xylanisolvens, Porphyromonas hominis, Porphyromonas timonensis, Eggerthella lenta, Robiniella peoriensis, and Synergistes species. Due to the short time needed for MTA of anaerobe cultures especially the identification of delicate species (eg Porphyromonas species, Peptostreptococcus anaerobius) has increased significantly up to 200%.

MALDI-TOF-MS is a promising new tool for a rapid, accurate and cost-effective identification of anaerobic species.

**P1831 Identification of clinically relevant anaerobic bacteria using two different MALDI-TOF mass spectrometry methods**

M. Knoester*, A. Veloo, J. Degener, E. Kuijper (Leiden, Groningen, NL)

**Objectives:** Identification of anaerobic bacteria in routine diagnostics is difficult and time consuming. Recently, matrix-assisted laser desorption and ionisation-time-of-flight mass spectrometry (MALDI-TOF MS) has been introduced as a rapid and reliable identification method for routine application in diagnostic laboratories. The objective of this study was to compare two commercially available MALDI-TOF MS systems for identification of clinically relevant anaerobic bacteria with 16S rRNA gene sequencing as gold standard.

**Methods:** A selection of 79 clinical isolates, representing 19 different genera, were tested and compared with identification obtained by 16S rRNA gene sequencing. The strains were tested in the Bruker system with a Microflex mass spectrometer (Bruker Daltonik, Bremen, Germany) using FlexControl software (version 3.0). The Biotyper
Excellent identification of coagulase-negative staphylococci using MALDI-TOF despite a rough procedure

J. Ahman, A.-K. Gustafsson, M. Bergman Jhungström, L. Serrander, M. Sundqvist*(Växjö, Karlskrona, Linköping, SE)

Objectives: The identification of coagulase-negative staphylococci (CoNS) to species level is traditionally labour intensive and sometimes expensive. Previous studies using MALDI-TOF (MS) have shown promising results using optimised conditions. We tested the performance of the Bruker Microflex LT system to identify CoNS without an extraction procedure and with 20h transport of the matrix overlaid sample between two laboratories.

Methods: A total of 176 CoNS were collected from several laboratories in Europe and the USA. They were previously species identified using various phenotypic and genotypic methods to Staphylococcus (68), S. capitis (40), S. hominis (28), S. haemolyticus (16), S. cohnii (8), S. warneri (7), S. lugdunensis (6) and S. simulans (5). All isolates were cultured on blood agar over night, put on the target plate and overlaid with matrix. The target plate was stored in a dark environment in room temperature and transported to another laboratory resulting in a delay of 20 hours from application of the sample to analysis. The identification of isolates was performed on a Microflex LT instrument (Bruker Daltonics, Germany) and massspectra were automatically analysed in the MALDI Biotyper 2.0 Software (Bruker Daltonics, Germany). Discrepant results were further analysed with 16S rRNA sequencing.

Results: A score value of >2.000 was at first run reported for 77% of the isolates. After retesting the isolates with first score value <2.000 the final median score value was 2.085 (range 1.705–2.376). The MS ID was in concordance with the previous species ID in 155/176 isolates. The 21 isolates showing different ID were analysed using 16S rRNA sequencing, confirming the species suggested by MS in 20/21 isolates. In one S. hominis isolate (verified by 16S rRNA) the 1st MS report was S. epidermidis (score 1.938) and when retested S. hominis (score 1.705). Two isolates previously reported as S. cohnii and S. epidermidis were identified by MS as S. aureus, which was also verified by 16S rRNA analysis.

Conclusions: We confirmed previous findings that the Microflex LT with the Biotyper software 2.0 is accurate in the identification of clinical isolates of CoNS. We show that the performance was very good despite a rough handling of the sample, i.e. without an extraction procedure together with a 20h delay of analysis from sample preparation.
**Results:** Among these 40 CF *Burkholderia* strains, 20 of them isolated from one CF patient were identified as *Burkholderia gladioli* by MALDI-TOF MS analysis and the other 20 as *Burkholderia multicaulis* isolated on three different CF patients. Analysis of each raw spectra obtained by the BioTyper software (Bruker Daltonics) allows us to create a phylogenetic tree between the different strains and to highlight epidemic spread within CF populations and colonization of a patient by different *Burkholderia* species.

**Conclusion:** In conclusion, MALDI-TOF proves very useful to type the *Bcc* complex. In order to validate results obtained by MALDI-TOF spectra analysis, triple-focus sequence typing (recA, gltB, gyrB) will be used and should allow tracing the global spread of Bcc bacteria.

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**Methods:** Ten cfiA-positive *B. fragilis* isolates from a previous study as well as 13 cfiA-positive meropenem non-susceptible isolates from routine cultures were compared to 125 cfiA-negative isolates of the same survey. cfiA gene detection was performed by PCR with specific primers. Strains were cultured anaerobically on Fastidious Anaerobic Agar® (LABM, Bury, UK) with 5% horse blood at 35°C. 4 cfiA− (1−4) and 4 cfiA+ (5−8) isolates were spotted on the target plate after ethanol/ formic acid extraction. One colony was smeared directly on the target plate. The spots were over laid by alfa-cyano-4-hydroxyoximinic acid matrix. Spectra were obtained with Microflex LT mass spectrometer and analyzed with Biotyper 2.0 software (Bruker Daltonics GmbH, Bremen). The relatedness between spectra was determined using the composite correlation index (CCI) tool of Bruker Biotyper. CCI values around 1 represent a high relationship between spectra.

**Results:** Visual inspection of the mass spectra revealed several peak differences between both groups. A CCI matrix was created using 48 raw spectra of isolates 1−8 after extraction. Mean of CCI values between respectively cfiA− (1−4) and cfiA− (5−8) isolates was 0.98 and 0.93 and was higher than the mean (0.63) of CCI values (t-test; p < 0.001) if CfiA− isolates were compared with cfiA+ isolates. Spectra obtained after direct transfer were matched into the CCI matrix. The CCI matching results classified unequivocally all strains in the expected group. In the dendrogram the spectra were clustered in a cfiA+ and a cfiA− group without overlap.

**Conclusion:** Our data suggest it is possible to differentiate cfiA-positive from cfiA-negative isolates and so predict carbapenem resistance in *B. fragilis* strains with MALDI-TOF MS. This discrimination is not based on the absence or occurrence of specific peaks. The protein profiles of these two genotypically distinct groups differ at a decade of m/z values. Since the occurrence of two separate genetic divisions of *B. fragilis* is not clustered geographically, the rapid detection of carbapenem resistance can probably be applied universally. It would be interesting to add and mark cfiA positive isolates to the MALDI-TOF MS databases used for bacterial identification, as a surrogate marker for detection of carbapenem resistance.
Identification of Acinetobacter calcoaceticus—Acinetobacter baumannii complex strains by MALDI-TOF mass spectrometry
P. Espinal*, I. Roca, J. Vila (Barcelona, ES)

Objective: Members of the Acinetobacter calcoaceticus—Acinetobacter baumannii (ACB) complex (A. calcoaceticus, A. baumannii, 13 TU) are phenotypically closely related making their identification very difficult for routine diagnostic laboratories. Although ARDRA (amplified rDNA restriction analysis) and PCR-based methods have shown good correlation with DNA-DNA hybridization results, they are both laborious and time consuming. The aim of this study was to identify members of the ABC complex using matrix-assisted laser desorption/ionization time of flight (MALDI-TOF MS).

Methods: The study included a set of external reference strains of described genomic species and a set of known clinical genomospecies from our collection: 1 each of A. junii, A. haemolyticus, A. baoguoi, A. radioresistens, A. calcoaceticus; 20 A. baumannii; 19 genomospecies; 3; and 20 genomospecies 13TU. All strains were verified by ARDRA, 16S rDNA restriction analysis (ITS), recA typing, presence/absence of blaOXA-51, and MALDI-TOF MS. Bioinformatic tools were applied for sequence analysis and MALDI-TOF spectra from were processed using the BioTyper Software (Bruker Daltonik).

Results: Overall, both reference and clinical strains were correctly identified by ARDRA, ITS and recA sequencing. The blaOXA-51 gene was only found in A. baumannii strains. MALDI-TOF MS correctly identified all the genomic strains except A. baumannii with a low score. However, MALDI analysis provided specific spectra of peaks for all representatives of every given genomospecies, allowing direct identification of ABC complex strains when specific profiles were compared.

Conclusion: We have shown that each genomic species has a unique MALDI-TOF MS pattern that can be used as a fast, simple and reliable alternative to identify members of the ABC complex. However, current databases need to be expanded to allow for the automated identification of A. baumannii 13TU. The rapid and accurate identification of clinically significant strains will provide better therapeutic alternatives against nosocomial/community-acquired resistant ABC complex strains.

Identification of non-tuberculous mycobacteria by MALDI-TOF mass spectrometry fingerprinting
A.J. Buss*, M. Timke, M. Kostrewa (Groningen, NL; Bremen, DE)

Objectives: Next to the clinical important Mycobacterium tuberculosis complex (MTC) Nontuberculosis mycobacteria (NTM) are pathogen especially in immunocompromised and senior people. Identification of NTM species is important for diagnosis and optimal treatment. The aim of this study is a comparison of genus probe and MALDI based identification results and to investigate the discriminatory power of MALDI-TOF molecular profiling, even for long-time stored samples.

Methods: 19 Mycobacterium species from a laboratory collection were characterized conventionally using PCR followed by reverse line blot hybridization of the amplified products to an in-house or commercial (Innolipa, Innogenetics) DNA probe assay. Biomass was harvested and analyzed using the MALDI Biotyper. Mycobacterium samples were heated at 95°C for 30 min, washed twice with 500 μl of water. The pellet was extracted with 70% formic acid and pure acetonitrile in equal amounts. The supernatant was measured in a Microflex™ (Bruker Daltonics, Germany) mass spectrometer and spectra analyzed using MALDI Biotyper 2.0 software. Furthermore, identification results of Mycobacterium spp. biomass stored for 3 to 4 years on pyruvate-enriched Loewenstein-Jensen medium at 4°C and of freshly cultured ones were compared for 11 samples. Samples with divergent identification results between reverse line blot hybridization and MALDI-TOF and “no reliable identification” MALDI Biotyper samples were sequenced and will be used for database complementation after finalization of analysis.

Results: Mycobacterium strains were identified as M. avium, M. fortuitum, M. gordonae, M. kansasi, M. malmoense and M. terrae. Some strains were assigned to different species by MALDI Biotyper than by the conventional identification methods, some as a different species and few mycobacteria were not identified using the MALDI Biotyper. Adding new species to the database enable their secure identification in future times. Dendrograms were calculated with acquired and database spectra. Diverging identification results were examined by additional methods and discussed.

Conclusion: It has been demonstrated that MALDI-TOF MS is a rapid and reliable method for discrimination and identification of NTM species.
spectra were measured for the clinical isolates whereas multiple spectra were obtained from the reference strains and added to the original spectrum database. The Bruker pre-defined score intervals was applied, were obtained from the reference strains and added to the original spectrum database. The Bruker pre-defined score intervals were applied, were obtained from the reference strains and added to the original spectrum database. The Bruker pre-defined score intervals were applied.

**Results:** MALDI-TOF mass spectra were successfully obtained from 35/46 clinical isolates and 17/20 reference strains. Spectra from clinical isolates were evaluated against the modified spectrum database yielding 31/35 with correct best match (81.6%) based on molecular identification. In 18 cases (51.4%) the log-score value was $\geq 2.0$ indicating reliable species identification. In five cases, all with a log-score below 1.7 an incorrect best match was suggested three of which was incorrect at the genus level. Overall, the MALDI-TOF identification performed best for *T. interdigitale*, *A. benhamiae*, *T. erinacei*, *T. tonsurans*, and *T. schoenleinii* (15/19 correct with log-score $\geq 2$), while only 2/10 clinical *Microsporum* isolates and 0/3 *E. floccosum* isolates yielded high quality spectra and correct identification.

**Conclusion:** Successful protein extraction is a significant criterion for successful MALDI-TOF MS analyses, and despite optimisation of sample preparation and supplementation of the database with 20 reference isolates several clinical isolates failed to yield high quality spectra. This may be related to the multifaceted structure and robust nature of dermatophytes rather than a general complication when analysing fungi with MALDI-TOF MS.

**[P1842] Direct identification of microorganisms from the bioMérieux Bact/Alert blood culture system by MALDI-TOF is possible J.D. Haigh*, D. Ball, M. Eydmann, M. Millar, M. Wilks (London, UK)**

**Objectives:** Several studies have reported poor results when trying to identify microorganisms directly from the bioMérieux Bact/ALERT blood culture system using MALDI-TOF. The aim of this study was to evaluate two new methods for direct identification of microorganisms from this system.

**Methods:** Aliquots were removed from any positive bottle and processed by two different methods. 1. Enrichment by centrifugation over a density cushion, and 2. Bruker MALDI Sepsityper™ method. In both cases, the resultant pellets were extracted with formic acid and acetonitrile, applied onto a steel MALDI target and overlaid with a-cyano-4-hydroxy cinnamic acid matrix. Analysis was performed with the Bruker Microflex LT MS running Bruker MALDI Biotyper software (V2.0). Results were compared to the identification results obtained after subculture in the conventional manner.

**Results:** Thus far, 181 positive blood cultures have been processed by both methods (see table). Overall the two methods gave similar results with 74% correct identifications obtained with the Bruker Sepsityper™ method and 75% for the enrichment method. Gram negative bacilli (GNB) could be identified directly, 5/6 (83%) of GNB which could not be identified by the enrichment method and 7/7 (100%) of those which could not be identified by the Sepsityper™ method, were derived from charcoal containing bottles. There were 10 mixed cultures which, neither method was able to identify successfully, these were excluded from the analysis.

**Results of direct ID by MALDI from positive blood cultures**

| Organism Type  | Enrichment method | Sepsityper™ method |
|---------------|-------------------|--------------------|
| Number (%) of correct IDs | Number (%) of correct IDs | |
| **Staphylococcus** | 52 (76) | 49 (71) |
| **Gram negative bacilli** | 62 (91) | 61 (90) |
| **Streptococcus** | 13 (65) | 14 (65) |
| **Gram positive bacilli** | 2 (15) | 5 (38) |
| **Others** | 0 | 0 |
**P1843** Streptococcus identification using proteomic, metabolic and molecular biologic approaches

M. Risch, T. Leibundgut, U. Nylegger*, D. Radjenovic, L. Risch (Liebefeld, CH)

**Objectives:** The taxonomy of Streptococci currently comprises 17 genera of catalase-negative Gram-positive cocci split off the genus Streptococcus since 16S rRNA gene sequencing patterns deviated from identifications based solely on haemolysis reaction, colony size, biochemical properties and presence of Lancefield antigens. 16S rRNA assays are considered as 'gold standard' but they suffer from non-applicability in daily routine. MALDI TOF fails in producing significant matches of *S. pneumoniae* with genome databases.

**Methods and Results:** We here collect 75 clinical routine samples revealing the agar plate identity of *S. mitis* and further delineate their specificity using MALDI TOF (Bruker) and Vitek (BioMérieux), completed by 16S rRNA (800 bp) in selected disparate results. Complete genus and species agreement: 25 (33%); disagreement: 50 (66%), of which 14 cases (28% of disagreeing cases) concerned genus disagreement, the remainder only species disagreement. Species identification prior to release of the results was made in 13 instances using conventional optochin test, colony appearance, Gram staining and latex-enhanced agglutination assays Pastorex (Bio-Rad, Marnes, France). In 8/15 cases MALDI erroneously revealed *S. pneumoniae* with samples clearly being another species, the most frequent disagreement being *S. pneumoniae*/*S. mitis*/oralis. With two samples, further distinction potential was brought by 16S rRNA-Gene PCR – in one case: *S. anginosus* (MALDI was right, Vitek result: *S. sanguinis*) and in the other: *S. pneumoniae* (MALDI was right, Vitek was loaded with metabolically weak sample – artefact).

**Conclusion:** Our results indicate that MALDI TOF has a reduced specificity in correctly identifying Streptococci. Often, isolates remain misidentified as *S. pneumoniae*, most belonging to *S. mitis*.

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**Biofilm: diagnostic approaches to therapeutic measures**

**P1845** Evaluation of colonisation of pacemakers and defibrillators from strains that produce biofilm and their study with atomic force microscopy

S. Arampatzï*, G. Giannoglou, E. Protonotariou, S. Logothetidis, S. Paraksaevaidis, V. Vasilikos, I. Styliadou, E. Diza (Thessaloniki, GR)

**Objectives:** Part of the pathogenesis of infections in patients with cardiac implants is the ability of microorganisms to create biofilms, which are resistant to the immune responses and antimicrobial agents. Recently, Atomic Force Microscopy (AFM) is being used for studying phenomena such as the irreversible cell adhesion and biofilm formation. The aim of this study was the investigation of colonization of implantable cardiac pacemakers and defibrillators in hospitalised patients, the detection of possible biofilm creation by these strains, and their study with AFM.

**Methods:** The study included 26 patients that were hospitalized in Cardiologic Clinic from which, 25 brought pacemaker and one defibrillator. Reason for admission was replacement of the pacemaker system. Tissue specimen from the fibrous capsule surrounding the pacemaker and swab from coating of fibrous capsule was obtained from each patient. Specimens were cultured following routine methods. Bacterial identification and antimicrobial susceptibility was performed with VITEK2-automated system (bioMérieux, France). The Tube method was used for the detection of slime-producing ability and in vitro biofilm formation was quantified by the polystyrene adherence assay. All isolates with high and moderate slime production were studied with AFM taping mode technique on a surface of Highly Oriented Pyrolytic Graphite (HOPG). The surface roughness was estimated by the measurement of root-mean-square roughness (Rrms) and peak-to-peak distance (P2P) parameters.

**Results:** (1) Pacemakers and defibrillators showed high rates of colonization (74%), mainly by *Staphylococcus hominis* and *Staphylococcus epidermidis*. (2) 50% of the strains isolated (mainly *S. hominis*,...
**Biofilm: diagnostic approaches to therapeutic measures**

*S. epidermidis* were positive for slime production with the tube method, while the quantitative method showed that 15% of the strains were high-slime producers (*S. hominis, Rhizobium radiobacter*) and 7% were moderate producers (*S. hominis, Leuconostoc* spp.). (3) The study of surface roughness on HOPG with AFM showed that high and moderate slime producers presented different roughness (Rrms and P2P) compared with slime non-producers.

**Conclusions:** (1) Implantable cardiac pacemaker systems are highly colonized by strains of normal skin microflora 50% of which may produce slime. (2) AFM is a powerful technique that can be used for studying the matrix of microorganisms that create biofilm.

**Evaluation of biofilm formation on coronary angiography catheters and their study with atomic force microscopy**

*S. Arampatzis*, G. Giannoglou, E. Protonotarios, S. Logothetidis, Y. Chatzissisis, S. Hatzimiltiadis, I. Styliadis, E. Diiz (Thessaloniki, GR)

**Objectives:** Catheter related infections (CRI) are a significant cause of morbidity and mortality in hospitalised patients. The pathogenesis of most CRI is complex and multifactorial and has been linked with the capacity of microorganisms to form sessile communities, known as biofilm. AFM can be used for imaging biological samples and in the investigation of phenomena such as the irreversible cell adhesion which is the initial stage of biofilm formation. Aim of this study was the investigation of colonization of coronary angiography catheters by different strains, in patients hospitalised in a Cardiologic Clinic, the detection of possible creation of biofilms by these strains and their study with AFM.

**Methods:** From a total of 26 hospitalised patients in a Cardiologic Clinic for coronary angiography, a catheter section about 20 cm was taken after its removal. Duration of catheter retention time was 8.8 ± 3.4 hours. No patient showed local or generalized infection during hospitalization. Specimens were cultured following routine methods. Detection of possible creation of biofilms by these strains and their study with AFM.

**Results:** (1) Coronary angiography catheters showed high rates of colonization (73%), mainly by *Staphylococcus epidermidis* and *Staphylococcus hominis*. (2) 33% of the strains isolated (mainly *S. hominis, S. epidermidis*), were positive for slime production while the quantitative method didn’t show strains that were high-slime producers and only 20% were moderate producers (*S. hominis, S. epidermidis*)

**Conclusions:** (1) Coronary angiography catheters are highly colonized by strains of normal skin microflora, 33% of which may produce slime. (2) AFM is a technique that can be used for the detection of biofilm matrix.

**Visualisation of microbial biofilm on explanted central venous catheters by two-photon laser scanning microscopy**

F. Tessaro*, I. Caola, M. Lorenzato, F. Piccoci, G. Nollo, P. Cucigl (Trento, IT)

**Objectives:** To visualize microbial biofilm by two-photon laser scanning microscopy (TPLSM) on central venous catheters (CVC) removed from patients.

**Methods:** In TPLSM an infrared laser is used to deliver two infrared photons to excite visible fluorescence from a fluorochrome. Because infrared photons are typically absorbed by biological systems less than visible light, this technique allows deeper penetration and optical sectioning, reducing cell phototoxicity and dye photobleaching.

We used a two-photon laser scanning system mounted on an upright microscope. The light source was a Ti:Sa fs-laser tunable in the 690–1040nm range.

Four fluorochromes were preliminarily tested on microbial biofilm grown in vitro: Fluorescein-5(6)-isothiocyanate (FITC), 5-Carboxyfluorescein (FCARB), Fluorescein Acid Yellow (FLAY), Rhodamine 6G (RH6G). Glass cover slips were inserted in a screw-cap tube with 3 ml of bacterial suspension and incubated in a oscillating waterbath at 37°C. After 3 days, colonized cover slips were extracted, washed in HEPES, fixed in 2.5% glutaraldehyde in HEPES, and stained with a fluorescent dye (1 mM in water). Two-photon excitation was carried out at wavelengths from 800 to 1000 nm in 20 nm steps and fluorescence intensity was obtained by image analysis. Two photon auto-fluorescence of non-stained polyurethane CVC was similarly quantified.

**Results:** FITC, FCARB, FLAY, RH6G showed a decrease of relative fluorescence intensity to less than 50% for excitation wavelength respectively higher than 960, 940, 960 and 840 nm. CVC auto-fluorescence was reduced after 860 nm.

**Conclusion:** TPLSM allowed high magnification of bacterial biofilm on CVC removed from patients. Optical sectioning of thick (20–30 micrometers) biological residuals and the detection of bacteria close to the catheter surface was obtained.

**The effects of ferulic and gallic acids on motility and biofilm control of pathogenic bacteria**

A. Borges*, M.J. Sauavedra, M. Simões (Vila Real, Porto, PT)

**Introduction:** Biofilms are multicellular communities and represent the prevalent mode of microbial life in nature, industrial processes, and health. It is estimated that biofilms contribute to more than 80% of all infections in humans. A particular characteristic is their extreme resistance to antimicrobial treatments. Moreover, the emergence of resistant bacteria to conventional antibiotics clearly shows that new biofilm control strategies are required. Most of the antibiotics available today come from natural origin, especially from microbial sources. Recent findings indicate that some natural phenolic compounds commonly found in plants have anti-biofouling potential.

**Objectives:** The aim of this study was to evaluate the activity of two phenolic acids ([gallic acid (GA) and ferulic acid (FA)]) against bacterial...
motility and biofilm formation by Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Listeria monocytogenes.

Methods: The anti-biofouling activities of GA and FA were tested against the bacteria using a microtiter-plate assay for biofilm mass quantification (crystal violet staining) and viability activity assessment (alamar blue staining). The effects of the phenolics were also tested on bacterial swimming, swarming and twitching motilities.

Results: The phenolics tested showed a higher potential to reduce the mass of biofilms formed by the Gram-negative bacteria comparatively to those Gram-positive. In terms of viability, FA and GA promoted reductions higher than 70% for all the biofilms tested. The application of FA and GA promoted a significant decrease in swimming, swimming and twitching motilities. GA caused total inhibition of swimming (L. monocytogenes), swimming (L. monocytogenes, E. coli and S. aureus) and twitching (L. monocytogenes, E. coli and S. aureus) motility. FA caused total inhibition of swimming (L. monocytogenes and S. aureus), swimming (L. monocytogenes, E. coli and S. aureus) and twitching (L. monocytogenes, E. coli and S. aureus) motility. These results are important because motility inhibition can account for biofilm control.

Conclusion: FA and GA demonstrated potential to inhibit bacterial motility and to control biofilms of pathogenic bacteria. Further studies will be developed in order to assess the effects of their combination in biofilm control.

P1850 Isolation of genes involved in biofilm formation of a Klebsiella pneumoniae strain causing pyogenic liver abscess

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Community-acquired pyogenic liver abscess (PLA) complicated with meningitis and endophthalmitis caused by Klebsiella pneumoniae is an emerging infectious disease. To investigate the mechanisms and effects of biofilm formation in virulence of K. pneumoniae causing PLA, we used microtiter plate assay to determine the levels of biofilm formed by K. pneumoniae mutants from a transposon mutant library of a K. pneumoniae PLA-associated strain. A total of 4 biofilm-increased mutants and 23 biofilm-decreased mutants were identified. One of the 4 biofilm-increased mutants was sugl insertion mutant. Further characterization showed that mucoviscosity, expression of capsular polysaccharide (cps) gene and production of CPS caused in sugl mutant strain. One of the 23 biofilm-decreased mutants was treC insertion mutant. Deletion of treC not only impaired biofilm formation from early stage but also reduced the production of CPS. In addition, biofilm cells had higher expression levels of treC. In vitro and in vivo competition assays revealed that the treC mutant strain was attenuated in competitiveness with regard to biofilm formation and colonization during infection in mice. treC affects K. pneumoniae colonization through biofilm regulation. Biofilm formation may play a role in K. pneumoniae PLA pathogenesis.

P1851 Molecular genotyping, adhesion and biofilm formation in methicillin-resistant Staphylococcus aureus clinical strains

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Objectives: In the present study we evaluated the correlation between methicillin resistance and biofilm formation in S. aureus clinical isolates.

Methods: A total of 36 methicillin-resistant (MRSA) and 73 methicillin-susceptible (MSSA) S. aureus clinical strains from different source of isolation were genotyped by Pulse-Field Gel Electrophoresis (PFGE). Resistance to methicillin was confirmed by the detection of the mecA gene. Adhesion to and biofilm formation on polystyrene were evaluated by the microtiter plate assay in aerobic, microaerophilic (5% CO2), and anaerobic atmospheres.

Results: Twenty-one different PFGE types were identified. A total of 8 and 16 PFGE types were found among MSSA and MRSA, respectively, while 3 were shared by both groups. Among PFGE types shared by more than 5 isolates, PFGE types 1 and 15 were identified only in MRSA, while PFGE types 2, 3, 7, 10 and 14 consisted only of MSSA. Among PFGE types shared by both groups, MRSA isolates predominated in PFGE type 4, while MSSA were more prevalent in PFGE types 5 and 6.

Biofilm level formed in anaerobiosis was significantly lower than that observed under aerobic and microaerophilic conditions (p < 0.001), either comparing MRSA and MSSA or within MRSA strains. Overall, MRSA did not differ from MSSA with regard to adhesion and biofilm levels, regardless of atmosphere tested. However, significant differences in biofilm formation in aerobiosis was observed among specific PFGE groups including MRSA strains. In particular, MRSA strains belonging to PFGE type 4, comprising also MSSA isolates, formed significantly more biofilm than that observed in MRSA isolates belonging to other PFGE types and to MSSA strains of the same group. No difference in adhesion levels was observed among pulsortypes.

Conclusion: Specific PFGE types represented by MSSA or MRSA were found, thus suggesting that certain MRSA and MSSA isolates segregate in different genetic lineages. Overall, MSSA and MRSA strains do not differ in adhesion and biofilm formation. Nevertheless, a clonal group of MRSA, defined by PFGE 4, exhibited a significant increased ability in forming biofilm with respect to other PFGE groups comprising MRSA as well as to all MSSA isolates.
Comparison of various antimicrobial agents as catheter lock solutions in an in vitro model of coagulase-negative staphylococcal biofilm

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Objectives: Coagulase-negative staphylococci (CoNS) are the main causative agents of catheter-related bacteraemia (CRB). The antibiotic lock technique (ALT) has been used to treat catheter colonization; however, the optimum choice of antimicrobial agents and their corresponding concentrations and exposure times have not been determined. In addition biofilm regrowth after ALT has not been evaluated either, so our objective was to evaluate the efficacy of different ALT solutions using an in vitro model of central venous catheter (CVC) infection.

Methods: The following lock solutions were evaluated: daptoycin (DAP) 5 mg/mL (reconstituted with lactated Ringer’s [LR]), teicoplanin (TEC) 5 mg/mL, both alone and combined with gentamicin (GM) 2.5 mg/mL, levofloxacin 2.5 mg/mL, clarythromycin (CLA) 5 mg/mL and ethanol 20%. PBS was used as control. Experiments were performed on full intracran Safety® 14G catheters (Braun Medical, Spain) inoculated with two clinical strains of methicillin resistant CoNS. AL solutions were exchanged every 24 h for 72 h. After 72 h, catheters were reincubated another 24 h with fresh media. Catheters were drained, flushed and sonicated at 0,4,8,24,48,72 and 96 h to assess CFU/mL. Scanning electron microscopy was performed to evaluate persistence of biofilm at 0,72 and 96 h.

Results: All antibiotic combinations resulted in significant reductions (p < 0.05) of log(10) cfu/mL at 72 h for both organisms compared with controls. DAP-LR resulted in significant reductions of log(10) for both organism versus TEC (p = 0.001). Only DAP was able to reduce logCFU below the limit of detection at 72 h, however neither DAP nor TEC prevent regrowth after 24 h of ALT removal. SEM studies showed persistence of biofilm at 72 and 96 h. DAP combinations were not better that DAP alone, although they were significantly better than TEC combinations. None of them were able to prevent regrowth at 24 h after ALT removal. TEC+GM resulted in improved efficacy compared to TEC alone at 72 h. Ethanol alone reduce logCFU below the limit of detection but did not prevent regrowth. DAP-Ethanol and TEC-ethanol eradicate biofilm at 72 h, but only DAP-ethanol prevent regrowth at 24 h after ALT.

Conclusion: Our CVC model demonstrated that ALT with 5 mg/mL of DAP-LR showed consistent better activity than ALT with 5 mg/mL of TEC, however both were unable to eradicate biofilm after removal of ALT. DAP-Ethanol eradicates biofilm and prevent regrowth so it should be explored in clinical trials.

Application of Escherichia coli biofilm supported on clinoptilolite for biosorption of cadmium from aqueous solution

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Objectives: Biofilms are microcosms consisting of microorganisms distributed throughout a matrix of fibrous and highly hydrated extracellular polymeric substances (EPS). They play an important role in natural aquatic environments. Since heavy metals pollution represents a serious problem for human health and for life in general and bacterial biofilms to be able to concentrate metal species from dilute aqueous solutions and to accumulate them within their cell structure the aim of this study was to investigate the ability of a biofilm of Escherichia coli, an effective agent for metal adsorption, supported on clinoptilolite for the removal of cadmium from aqueous solutions.

Methods: Adsorption experiments were carried out in a laboratory-scale batch model with clinoptilolite and clinoptilolite covered with a bacterial biofilm. Escherichia coli were isolated from hospital environment. The effect of initial heavy metal concentrations, pH, and agitation time on the removal efficiency was studied. Finally, experimental results were analysed using four isotherm equations.

Results: It was found that the clinoptilolite exhibits lower adsorption to cadmium than clinoptilolite covered by biofilm and adsorption of the cadmium is influenced by several parameters such as cadmium initial concentration, biosorption time and solution pH. The results showed uptake values of 6.8 mg/g and 9.6 mg/g in the batch model with clinoptilolite covered by biofilm, respectively, for initial concentrations of 10 and 100 mg/L. It was also observed that as the initial cadmium concentration increases, the uptake increases too, but the removal percentage decreases. Maximum absorption efficiency was achieved at pH value of 6. Among the models tested, namely the Langmuir, Freundlich, and Sips isotherms, the biosorption equilibrium for cadmium was best described by the Sips model.

Conclusion: It is concluded that the presence of biofilm increased the uptake efficiency of clinoptilolite and the biofilm tested is very promising for the removal of cadmium from aqueous solution.

Phenolic antioxidants that target the staphylococcal membrane may have potential as biofilm-eradicating agents

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Objectives: Bacterial biofilms are refractory to the action of many antibacterial agents currently in clinical use. Since approximately 80% of bacterial infections in humans involve a biofilm component, this represents a significant contributing factor to antibiotic treatment failure. To address this problem, it is important to discover and develop novel antibacterial agents which possess anti-biofilm activity. Here we report that a number of phenolic antioxidants show potent activity against staphylococcal biofilms in vitro, suggesting that these compounds, or derivatives thereof, may represent candidates for the treatment of biofilm infections.

Methods: Susceptibility testing was performed by broth microdilution according to British Society for Antimicrobial Chemotherapy (BSAC) guidelines. The Calgary biofilm device was used to establish Staphylococcus aureus and Staphylococcus epidermidis biofilm MICs (bMICs) and minimum biofilm eradication concentrations (MBECS). The effects of antioxidants on the membrane of S. aureus SH1000 were assessed using the BacLight™ assay and by monitoring K+ release via atomic absorption spectroscopy.

Results: Of 15 phenolic antioxidants, 14 displayed antibacterial activity against planktonic cultures (MIC 0.25−64 mg/L) of S. aureus SH1000, and the prolific biofilm-forming strains S. aureus UAMS-1 and S. epi- dermidis RP62A. Eight of these compounds eradicated biofilms formed by all three test organisms, with bakuchiol and totarol demonstrating the greatest potency (MIC 4 mg/L, bMIC ≤<8 mg/L, MBEC ≤<16 mg/L and MIC ≤<4 mg/L, bMIC ≤<4 mg/L, MBEC ≤<16 mg/L respectively). By contrast, none of 16 established antibacterial agents tested were able to eradicate staphylococcal biofilms (MBEC ≤<256 mg/L). All compounds displaying anti-biofilm effects acted on the membrane, causing leakage of intracellular K+ and/or >60% loss of membrane integrity.

Conclusion: In contrast to established antibacterial agents, several phenolic antioxidants exhibited good anti-biofilm activity, eradicating staphylococcal biofilms. All antioxidants that were able to eliminate biofilms displayed membrane-disrupting activity, suggesting that activity against the membrane is necessary to sterilise staphylococcal biofilms. However, several established antibacterial agents and phenolic antioxidants which disrupt the membrane failed to exhibit activity against biofilms, indicating that membrane-damaging activity alone is not sufficient for biofilm eradication.

Multidrug-resistant Acinetobacter baumannii and Pseudomonas aeruginosa biofilm formation and the destruction activity of macrolide antibiotics

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Objectives: Acinetobacter baumannii and Pseudomonas aeruginosa are the most common cause of nosocomial and biofilm related infections. Recently, Hospital infection by these strains acquired multidrug
resistance (MDR) has been problem. The aim of the present study is to evaluate the effect of mature biofilm destruction activity of macroide antibiotics because macroides indicate inhibitory effect of biofilm formation.

Methods: The total four clinical isolates of both *A. baumannii* and *P. aeruginosa*, including for two each MDR strains were used in this study. These strains were collected from 4 Japanese hospitals. Antibiotic susceptibilities were determined using the micro dilution method. To form biofilm, 10 micro-L of each bacterial suspension of McFarland No.1 were added to 190 micro-L of TSB with 1% glucose in 96-well plates and incubated at 37°C for 24hr under anaerobic conditions. Mature biofilms washed twice with milli-Q water and added 200 micro-L of 1, 10, or 100 mg/L of clarithromycin or azithromycin. After 24hr or 72hr of each macroide exposure, biofilms were washed and stained by 1% crystal violet. Absorbance at 595nm was used as a quantitative measurement of the biofilm.

Results: *A. baumannii* had less quantity of biofilms formation than *P. aeruginosa*. The biofilm destruction effect by the exposure of 1 and 10 mg/L macroides 24hr was not shown. However, the amounts of biofilms which drug-susceptible *A. baumannii* and *P. aeruginosa* produced decreased to half by 72hr exposure with 100 mg/L of macroides. A difference of the biofilm destruction effect was not found between clarithromycin and azithromycin.

Conclusion: When it was treated clarithromycin or azithromycin at 100 mg/L more than 72hrs, the biofilm destruction effect was confirmed.

**P1858** Improved antibiotic activity against *Pseudomonas aeruginosa* biofilms using a novel surface active lamellar body mimetic LMS-611

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Objectives: The opportunistic pathogen *Pseudomonas aeruginosa* grows within the cystic fibrosis (CF) lung as a biofilm, which hinders antibiotic activity due to the physical nature of the biofilm. The aim of this study was to assess the activity of a panel of antibiotics in the presence of a novel surface active lamellar body mimetic LMS-611 to determine whether it can potentiate antimicrobial activity against *P. aeruginosa* biofilms.

Methods: *P. aeruginosa* (PA01, PA14, H183 and two clinical CF strains) biofilms were grown on a Nunc Immunosorp peg plate model for 48 hr on a rocking platform. Biofilms were tested in a checkerboard array using LMS-611 in combination with piperacillin, aztreonam, meropenem, gentamycin, tobramycin, erythromycin, and ciprofloxacin. The effect of pretreating biofilms with LMS-611 at different time points (5 min, 1, 8 and 24 h) prior to challenging with piperacillin and gentamycin was also investigated. Scanning electron microscopy was also performed on treated biofilms.

Results: LMS-611 demonstrated no antimicrobial activity on its own. In combination studies it was shown to be most efficacious at 5% (v/v), significantly reducing the sessile MIC’s of piperacillin (4−16 fold), gentamycin (4−32 fold) and ciprofloxacin (1−16 fold). Potentiation of the antibiotics was also demonstrated with aztreonam (2−4 fold), meropenem (1−4 fold) and tobramycin (1−16 fold). No effect was observed for erythromycin. It was also shown that short pretreatments (5 min and 1 h) with LMS-611 did not reduce sessile MIC’s, but after 8 h a two-fold reduction was observed for piperacillin and gentamycin. Scanning electron microscopy demonstrated that the biofilm was scant and dispersed following LMS-611 combinational treatment.

Conclusion: As the clinician’s ability to treat *P. aeruginosa* within the CF environment diminishes, novel agents that potentiate existing antimicrobial compounds are an attractive option. This study has shown that LMS-611, a surface active agent with biophysical properties, can reduce the sessile MIC’s of key CF antibiotics. Our data indicates that disruption alters the biophysical properties of biofilms, which may improve access of the antibiotics into the bacterial cells.
**Methods**: E. faecalis isolates were from infections of prostatectomy and knee joints. Minimal biofilm eradication concentration (MBEC) for ampicillin, vancomycin, linezolid, ciprofloxacin, and rifampicin alone and in combinations was determined. E. faecalis biofilms in microtiter wells or on beads of bone cement were treated with the same antibiotics for various times followed by plating to quantify surviving bacteria.

**Results**: The E. faecalis isolates displayed MBEC's for the various antibiotics in the 64–512 mg/l range. When combined with 8 mg/l rifampicin, MBEC for ciprofloxacin and linezolid dropped to 16–32 mg/l for the four isolates tested. In biofilms formed on plastic surfaces significant bacterial killing (>2–3 log decrease in colony forming units (cfu)) was seen after an 8 hrs exposure to antibiotics. Thus we performed time-kill experiments on biofilms of four isolates formed on plastic surfaces and on bone cement beads under these conditions. For all isolates, the combination of ciprofloxacin and rifampicin, followed by linezolid and rifampicin, was most potent in reducing the number of cfu. E. faecalis resistance to rifampicin developed in our system when biofilms were subjected to this antibiotic alone. We observed no resistance to rifampicin when bacteria were subjected to a combination of ciprofloxacin and rifampicin.

**Conclusions**: Our data show that combinations of ciprofloxacin or linezolid with rifampicin have good effect on E. faecalis biofilms in vitro. The use of such combinations should be considered for testing in humans with for example joint prosthesis infections with E. faecalis.

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**Methods**: In this study we used Escherichia coli, Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter dissolvens, Staphylococcus aureus and Enterococcus faecalis which were isolated from Foley catheters previously. We determined MICs of each isolate in presence of BisEDT and BisPDT by microdilution technique and biofilm formation in presence of BTs was measured quantitatively by microtiter plate and crystal violet methods.

**Result**: MICs and biofilm inhibition percentage amounts of each bacterium in presence of biocides are shown in table 1. Results showed that BTs at MIC and sub-MIC concentrations inhibited biofilm production significantly (P < 0.05).

**Conclusion**: The unique antibiofilm properties of BTs may be useful to prevent or treat catheter associated urinary tract infections (CAUTI) due to bacteria that can attach and produce biofilm on catheter surfaces. It seems that coating catheters with BTs can inhibit biofilm formation on their surfaces significantly and prevent CAUTI in patients.
Coral-associated bacteria as a novel source for antibiofilm and quorum quenching agents

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Objectives: Resistance to the drugs is more common today with most of the bacterial pathogens, which limits the use of antibacterial agents that directly implies pressure on the pathogens in turn resulting in development of resistance. Hence an alternate target viz. quorum sensing (QS) system of bacteria has been targeted. QS inhibition also called quorum quenching is a promising method to combat multidrug resistant pathogens since it inhibits the production of biofilm & other virulence factors of the bacterial pathogens without affecting the normal growth, thus allowing no chance for the pathogen to gain resistance. Coral ecosystem being an unexplored reserve for microorganisms, are recently reported to have Actinomycetes as potential producers of bioactive compounds. The likeliness of getting novel bioactive compounds from these coral ecosystems is more prominent, since many such microorganisms of this ecosystem are novel. Hence, coral associated (CA) bacteria were screened for antibiofilm and quorum quenching agents.

Methods: Bactericidal activity was tested against Staphylococcus aureus ATCC 11632, meticillin resistant Staphylococcus aureus ATCC 33591, S. aureus clinical isolates, Pseudomonas aeruginosa ATCC 10145 and Serratia marcescens, since an ideal quorum quencher should not have any antibiotic activity. The agents with no bactericidal activity were subjected to a set of assays both quantitative and qualitative for revealing their antibiofilm and quorum quenching activity. Biofilm quantification using crystal violet stained quantification method and its architecture in treated as well as controls were visualized under CLSM.

Results: Out of 41 coral associated actinomycetes screened, 5 isolates have shown antibiofilm and 13 isolates have shown quorum quenching activity against both Gram positive and Gram negative pathogens. Isolate CA-3 has shown complete inhibition of biofilm formation by S. aureus and its drug resistant counterparts. CA-21, 23, 27, 34 and 41 were shown to inhibit the virulence factors like swarming, swimming, pigment production, biofilm formation and extracellular enzymes production that are controlled by the QS system of P. aeruginosa and S. marcescens significantly.

Conclusion: Coral ecosystem thus proves to be an unexplored reserve for novel anti-infectives. Purification and characterization of these agents will reveal a novel bioactive compound that is effective against multidrug resistant pathogens.

Fluconazole susceptibility, oxidative stress response and production of extracellular hydrolases in Candida glabrata clinical isolates

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Objectives: Candida glabrata is an opportunistic human pathogen causing both mucosal and bloodstream infections. The understanding of the factors contributing to virulence of this second most common Candida pathogen after C. albicans is essential for a more efficient control of infections caused by this fungus. The aim of this study was to determine the susceptibility of the C. glabrata clinical isolates to fluconazole and agents inducing oxidative stress as well as to assess their factors of virulence.

Methods: Susceptibilities of isolates to fluconazole were assayed by the broth microdilution method in 96 well plates according to the proposed CLSI (formerly NCCLS). The zone inhibition assay on YPGE medium was used for determination of susceptibilities of isolates to 7-chlorotetrazolo[1,2-c][1,2,4]triazine (CTBT). Minimal inhibitory concentrations to menadion and H2O2 were determined by spot assay. Cell surface hydrophobicity was measured by the water-octane two-phase assay. Biofilm formation on untreated polystyrene 96-well plate was quantified using the 2,3-bis(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide (XTT) reduction assay or crystal violet (CV) staining followed by measuring of absorbance using a microplate reader.

The protease production was detected on medium containing bovine serum albumin. The phospholipase production was detected on medium containing sterile egg yolk.

Results: Among 38 C. glabrata clinical isolates 28.9% were resistant to fluconazole. All isolates were susceptible to hydrogen peroxide, diamide and 7-chlorotetrazolo[1,2-c][1,2,4]triazine (CTBT) inducing an increased formation superoxide and other reactive oxygen species in fungal cells. The mean relative cell surface hydrophobicity (CSH) of isolates was 21.9. All isolates showed biofilm formation. A high biofilm formation was observed among 60.5% of isolates. The 76.3% and 84.2% of isolates displayed varying degrees of extracellular proteinase and phospholipase activity, respectively. The fluconazole resistant isolates displayed a significantly higher phospholipase production compared with fluconazole sensitive ones.

Conclusion: The results demonstrate a differential distribution of factors contributing to virulence of C. glabrata clinical isolates and point to the association of their resistance to fluconazole with phospholipase production.

A novel glycolipid biosurfactant from marine Serratia marcescens disrupts pathogenic Candida albicans BH and Pseudomonas aeruginosa PAO1 biofilms

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Objectives: To study the anti-biofilm activity of novel glycolipid biosurfactant isolated from marine Serratia marcescens.

Methods: The marine strain was isolated from the surface of Symphylia sp., a coral obtained from the seashores of Mandapam, Bay of Bengal, India. Morphological, biochemical and 16s rRNA analysis were performed for identification of the bacterial isolate. The culture was identified as Serratia marcescens. Cell free supernatants of Serratia marcescens grown in liquid medium were tested for antimicrobial activity against the pathogenic Candida albicans BH and Pseudomonas aeruginosa PAO1. Biofilm activity of S. marcescens was analyzed by drop collapse, blood hemolysis and surface tension reduction methods. The antimicrobial biosurfactant was purified by solvent extraction and sillicic acid column chromatography and characterized by TLC, FTIR and GC-MS. Minimum inhibitory concentration (MIC) against the planktonic cells of C. albicans BH and P. aeruginosa PAO1 was determined using standard broth microdilution assay. Modified biofilm disruption and anti-adhesion activity of biosurfactant against C. albicans and P. aeruginosa was studied in microtiter plates and on glass surfaces. Confocal laser scanning microscopic analysis along with IMARIS imaging technique was used to study the extent of biofilm disruption by the biosurfactant.

Results: The marine bacterium, identified as S. marcescens displayed strong antimicrobial activity against Candida albicans BH and Pseudomonas aeruginosa PAO1. Surface tension of the growth medium decreased from 52.0 to 29.6 mN/m after 24h of growth. Characterization of the biosurfactant showed presence of glycolipid as evident from TLC, FTIR and fatty acid analysis. The novel glycolipid biosurfactant contained glucose and palmitic acid moieties. The MIC against C. albicans BH and P. aeruginosa PAO1 was >30 µg/ml. Biosurfactant was inhibited and pre-established biofilms of C. albicans and P. aeruginosa were dispersed at sub-MIC concentrations of the biosurfactant. Confocal laser scanning microscopic analysis showed effective removal of preformed biofilms of P. aeruginosa PAO1 and the fungal, C. albicans biofilms. The study shows antimicrobial biosurfactant obtained from S. marcescens effective in controlling fungal and bacterial biofilms.

Conclusion: The results may provide a clue for the development of novel natural strategy to control detrimental fungal and bacterial biofilms.
Molecular virology

**P1865** RT-PCR microarray detection of viral respiratory pathogens in acute exacerbations of COPD in France

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**Objectives:** Most of COPD acute exacerbations (AE-COPD) are associated with bacterial or viral respiratory tract infections. The relative importance of different respiratory pathogens and relevant microbiological data in AE-COPD remains unclear. We assessed the epidemiology of viral respiratory infections in AE-COPD using RT-PCR microarray detection methods in Champagne-Ardenne (Northern France).

**Methods:** 51 stage 2–3 COPD patients were included in a prospective 12 months follow-up study and followed monthly. At each AE-COPD and one month later, clinical score (dyspnea, sputum volume and purulence), respiratory functional tests, blood samples and induced sputum samples were collected. Sputum samples were analyzed by a RT PCR microarray detection system detecting 17 different viral respiratory pathogens and by classical viral culture assays.

**Results:** 53 documented AE-COPD occurred in 26 patients, whereas 25 patients did not exhibit any AE-COPD. Viral respiratory pathogens were detected in 23 (43%) AE-COPD. The diagnostic yield of viral identification by PCR was 6.7 times higher than conventional viral culture. Human metapneumovirus (hMPV) and human rhinovirus (HRV) infection were detected in respectively 15% and 21% of AE-COPD, whereas the other viruses identified were influenza A (6%), influenza B (2%), parainfluenza III (2%) and respiratory syncytial virus (2%). Demographic characteristics, including COPD severity, were not associated with the risk of viral infection in AE-COPD. The clinical severity of AE-COPD was not associated with respiratory viral pathogens detection (P > 0.15 for all). Viral infection appeared to have no effect on subsequent readmissions or mortality rate over a study period of 1 year.

**Conclusion:** Our study demonstrates that hMPV and HRV infections are important pathogens in AE-COPD and may be considered as triggering factor for AE-COPD.

**P1866** Laboratory investigation of the Greek measles 2010 outbreak

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**Objectives:** In the prospect of measles virus elimination by 2010 a new epidemic is currently ongoing in Greece and other European countries. The purpose of this study is the laboratory investigation of the current Greek measles outbreak.

**Methods:** Epidemiological information was collected from 24 serologically tested measles patients during the 2010 measles outbreak in Greece. Measles virus RNA, isolated from the clinical samples using the Qiagen viral RNA mini kit, was used in RT-PCR to amplify the nucleoprotein 450bp region, which was then sequenced and studied in terms of nucleotide variation and phylogeny.

**Results:** 37.5% of the cases belonged to the Roma population of Bulgarian nationality, 29% belonged to the Greek non-minority population, 25% belonged to the Greek Roma population and 1 had Albanian nationality. Of those cases, 21% were less than 1, 33% were 1–14, 25% were 15–29 and 21% were older than 30 years old. All of the detected viruses were of the D4 genotype, which is currently circulating in Greece among the above mentioned population groups. Phylogenetic analysis revealed that the examined strains belonged to the 4th subgroup of the D4 measles virus strains.

**Conclusion:** The same measles virus D4 group is also circulating in other EU countries. The first cases and clusters in Greece were among persons of Bulgarian nationality, probably related to the measles outbreak in Bulgaria which started in April 2009. The Greek 2010 outbreak, which counts 126 patients until now, is epidemiologically linked to the Bulgarian outbreak. The high proportion of Greek nationals, mainly from Roma communities, underlines that despite the high national immunisation coverage with measles-mumps-rubella vaccine, pockets of unvaccinated populations still exist. It is essential to continue the epidemiological surveillance of measles in Greece to monitor the transmission pattern of the virus and the effectiveness of measles immunisation, which eventually will lead to the elimination of the virus in our country.

**P1867** Respiratory viruses involved in acute respiratory infections in a Greek paediatric population during the winter period of the years 2005–2008

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**Objectives:** Viruses are the major cause of pediatric acute respiratory infections (ARI) and yet many cases of illness remain uncharacterized. Our aim was to reveal the distribution of several respiratory viruses in children diagnosed as having an influenza-like illness (ILI) or other respiratory tract infections, over the winter period of the years 2005–2008.

**Methods:** Molecular assays including conventional and real time PCR protocols were employed to screen respiratory specimens for the identification of common or recently identified respiratory viruses. Specimens were collected by the clinicians or health care workers of the National influenza sentinel system organized by the Hellenic Centre for Disease Control and Prevention and of several pediatric clinics.

**Results:** Rhinopharyngeal or throat swabs from 3307 patients aged 0–18 yo, including 1856 males and 1451 females, were analyzed. A diagnosis of ILI was made in 1272 of the above total cases of ARI. Viral presence was confirmed in 2268 (68.6%) samples and included 979 respiratory syncytial viruses (hRSV), 660 influenza viruses (hINF), 537 rhinoviruses...
In-house real-time PCRs are more sensitive than large multiplex (MX) assays. The RespiFinder performed better than the xTAG RVP, especially for HCoV where a low sensitivity was obtained with the xTAG RVP.

**Results:** 366, 347, and 290 respiratory viruses were detected by the real-time in-house PCRs, the RespiFinder TwoStep and the xTAG RVP tests, respectively. Double infections were detected in 14, 6, and 2 samples by in-house PCR, RespiFinder TwoStep kit and xTAG RVP, respectively. Sensitivity and specificity of the commercial assays are shown in the table: INF was detected significantly less frequently by both RespiFinder and xTAG RVP compared to the in-house PCRs: P=0.01 and P=0.001 respectively; HCoV was detected less often by xTAG RVP only: P=0.001 compared to in-house PCRs. All other sensitivities were not significantly different. In general, samples found negative by the commercial assays tended to have a low viral load (based on Ct-value).

**Conclusions:** In-house PCRs were more sensitive than both commercial MX assays. The RespiFinder performed better than the xTAG RVP, especially for HCoV where a low sensitivity was obtained with the xTAG RVP.

**Background:** Sensitive and specific molecular detection is an important part of pathogen identification and epidemiological surveillance during pandemics and epidemics. Commercial collection and transport media, commonly referred as VTM, that maintain viability of organisms in specimens increase both infectious disease risk and RNA/DNA degradation by nucleases, oxidation and hydrolysis. A specifically designed molecular transport medium (MTM) for nucleic acid testing (NAT) has been utilized to preserve labile influenza RNA and nucleic acids from other pathogens. Specific Aim: We report the use of a MTM to inactive/kill high titers of influenza A/Vietnam/1203/2004 (H5N1) and pandemic A/Mexico/4108/2009 (H1N1) virus. Additionally, long-term preservation of released viral RNA for 62 days at ambient temperature and 37°C was evaluated by real-time RT-PCR.

**Methods:** For viral killing studies, collection swabs were loaded with 0.1 mL H5N1 (1.5×10^7 TCID50/mL) or viral storage buffer (negative controls), placed into MTM and held for 10, 30, or 60 minutes at ambient temperature. The samples were cultured using MDCK cells for 96 hours and visually examined for CPE with total TCID50 determined. Preservation of H5N1 and H1N1 virus was analyzed using real-time RT-PCR at 0, 1, 2, 5, 7, 14, 30, and 60 days post-inoculation. Additional RT-PCR amplification of large segments was performed to analyze integrity of preserved influenza H1N1 RNA. For this experiment, aliquots were placed into MTM, incubated at 37°C for 2 weeks and tested by RT-PCR.

**Results:** High pathogenic H5N1 influenza virus placed into MTM resulted in no detectable, viable virus and was equivalent to negative controls. RNA from H5N1 and H1N1 viruses preserved in MTM for up to 62 days at ambient temperature were detectable by real-time RT-PCR analysis with only minimal degradation of target signal. Additionally, influenza virus RNA was preserved for at least 2 weeks in MTM at 37°C using real-time PCR detection.

**Conclusion:** MTM rapidly kills microbes by lysing lipid membranes, and destroying proteins and enzymes (including nucleases). Further, MTM stabilizes and preserves the released nucleic acids from viruses and collected microbes. The ability to safely collect and ship clinical specimens at ambient temperature and detect viral RNA with NAT weeks later could be a valuable asset for tracking and surveillance of pandemic influenza (H5N1, H1N1/09) viruses or other emerging pathogens.
Objective: To compare the reliability of these methods to detect respiratory viral infection, and to know the viability of stored samples at 4°C more than 1 week.

Individuals, Samples and Methods: 151 respiratory specimens (67 pharyngeal swabs, 39 nasal washes, 27 nasopharyngeal swabs, 11 nasal swabs, 4 bronchial aspirates, and 3 broncoalveolar washes) belonging to different individuals were collected in viral transport media. 29 patients with lower respiratory infection (LR), 106 with upper respiratory infection (URI), and 16 with unknown symptoms. All samples were processed for Hamilton method according manufacture’s instructions, and two different “in-house” multiplex nested RT-PCRs (one against influenza virus A and B (IA, IB), respiratory syncytial virus (RSV), and rhinovirus, and the other one against parainfluenzavirus, metapneumovirus and coronavirus) were performed. The other two genome purification methods (Magmax and Roche) were developed in those samples stored at 4°C during one week at least.

Results: At least one virus was recovered in 61 (40.4%) samples (44 rhinovirus, 6 IB, 3 parainfluenzavirus, 4 RSV, 1 IA, 1 metapneumovirus, 2 mix infections) purified by Hamilton method. One week later, only 29 samples (19.2%) stored at 4°C were positive (p < 0.0001). This percentage was similar to the one observed performing Magmax method (19.8%, 30 samples), and, at the same time, to the one observed using Magnapure method (17.2%, 26 samples). The concordances were: 86.7% between Hamilton-Magnapure, 84.1% between Hamilton-Magmax, and 84.8% between Magnapure-Magmax. According clinical symptoms, a virus was detected in 16 (55%) patients of the LRIs, and in 41 (38%) of the URIs. Once the samples were processed and PCR performed, the percentage decreased significantly to 27% (8 patients) and 17% (18 patients) after the following week.

Conclusions: Delays of more than one week in the genome purification affects sensitivity in samples stored at 4°C. The three methods assayed have the same sensitivity, although the concordance was not optimal. The sensitivity decrease was not related to the respiratory symptoms.

Human rhinovirus species occurrence among adults with respiratory tract infection and asymptomatic individuals during two consecutive winter seasons

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Objectives: Human rhinoviruses (HRV) are classified into three species: HRV-A, HRV-B and HRV-C and are the leading cause of respiratory tract infections in humans. The disease outcomes associated with different HRV species and genotypes are poorly established. Several studies have documented high incidence and more severe illness caused by HRV-A and HRV-C viruses, however most of them have focused mainly on hospitalized pediatric patients during one season. The objective of this study was to investigate the incidence and circulation patterns of HRV species among adults with respiratory tract infection and asymptomatic individuals during two winter seasons.

Methods: Nasopharyngeal swabs provided by 11 primary care networks across 6 European countries, as part of the GRACE study (Genomics to combat Resistance against Antibiotics in Community-acquired LRTI in Europe) were included in this study. A total of 314 samples collected during the winter seasons 2007/2008 and 2008/2009 and positive for HRV as determined by real-time RT-PCR were selected for further analysis. The specimens were obtained from 290 adults with respiratory illness during the first (V1 = 253) or/and second visit (V2 = 44) to the general practitioner and 17 (V0) matched controls. A semi-nested RT-PCR targeting the VP1 capsid gene of all three HRV species was developed with degenerate primers designed in conserved motifs in VP3, VP1 and 2A HRV genes. HRV typing was performed by sequence analysis of VP1 gene.

Results: 162 strains were characterized including, 112 (69%) HRV-A, 20 (12%) HRV-B, 26 HRV-C (16%), and 4 (3%) HEV isolates. Among asymptomatic individuals HRV-A was detected in six cases, HRV-C in three and one subject was positive for HEV. HRV species distributions in the separate communities during the two seasons are presented in Figure 1. HRV-A infections were dominating in all 11 communities during both seasons, with the exceptions of Rotenburg/DE 2007/2008 and Bialystok/PL 2008/2007 with equal prevalence of HRV-A and HRV-C, and Utrecht/NL 2008/2009 with no HRV-A identified.

Conclusion: HRV-A were the most frequently detected rhinoviruses among adult outpatients and asymptomatic individuals during 2007/2008 and 2008/2009 winter seasons.
underscore the importance of pre-analytical step (nucleic acid extraction) to obtain reliable results in HPV DNA detection.

**P1873 Human papillomavirus typing in a cohort of 200 women in Greece**

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Objectives: Prevention programs for cervical intraepithelial neoplasia, which are mostly based on cytological examination have achieved a 22% reduction in incidence and mortality of invasive cervical cancer but still with limitations due to low sensitivity and the false negative rates reported by numerous studies. Human papillomavirus (HPV) detection and typing seems to be an important test enhancing diagnosis and follow-up of patients. The aim of this study was to evaluate the prevalence of HPV high- and low-risk human papilloma virus in a cohort of women by using molecular detection and typing.

Materials and Methods: Two hundred women aged from 19 to 59 years have been tested for the presence of HPV in cervical samples, by using the Linear Array method (ROCHE molecular diagnostics). The method includes the detection of 13 high-risk and 24 low-risk HPV types. The study was carried out during six months. When colposcopy was positive and the test negative, cross checking was performed by home-made PCR and RFLPs.

Results: Out of 200 cervical samples, 115 have been positive for HPV. High-risk HPV types (HR-HPV) were detected in 28 women, Low-risk HPV types (LR-HPV) in 39 and High-Low risk in 48 women samples. Among the high-risk HPV group, 14 shared DNA sequences of the 16 and 2 shared the 18 subtypes. Typing of the low-risk subtypes detected in 9 samples the G6, in 3 samples the G55, in 5 the G50A and in one sample the G11 respectively. Co-infection with two strains was detected in 29 samples, with three genotypes in 17 samples and with four genotypes in 26 samples. Three samples with colposcopy HSIL findings but negative for high-risk strains were re-tested by PCR. One was positive and two had different low-risk HPV types.

Conclusions: This was a pilot study accomplished in our laboratory. By using the linear array method we detected high- and low-risk HPV types in women. The most prevalent HPV types were the low-risk ones independently of colposcopy findings. Interestingly, in most of the cases, high- and low-risk types have been detected in the same sample. This typing profile seems to be the most prevalent. We propose here a new protocol taking in consideration both clinical and colposcopy findings in order to elucidate in a next step whether co-infection with two or more types is clinically important. Also, we aim to co-evaluate clinical, cytological and colposcopy findings with the results of Linear Array Method, performed in our laboratory, in the diagnosis of HSIL.

**P1874 Implementation validation of the Qiagen Artus HCV RT-PCR assay on the Rotorgene 6000 platform**

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Bonheiden (BE)

Objectives: To validate the Artus HCV RT-PCR kit (Qiagen), the first commercially available (CE-IVD) one-step HCV real-time PCR kit on the Rotorgene platform (Qiagen), for diagnosis and follow-up of HCV infections.

Methods: All validation experiments started with an extraction of 400 µl of EDTA plasma using the Qiagen MinElute Virus Spin Kit. Extraction was followed by a one-step RT-PCR on the Rotorgene platform according to the manufacturers guidelines. The assay was checked for analytical sensitivity, specificity, accuracy, precision and linearity following international publications on molecular validation methods (e.g. Rabenau, 2007).

Results:

1. Analytical sensitivity: To determine the limit of detection (LOD with a 95% hit rate) of the HCV kit, the Acrometrix Optiguan HCV RNA quantification panel (genotype 1) was used. Twenty samples with a concentration of 50 IU/ml were found positive and this with a mean Ct of 37.8, meeting our clinical validation criterion of LOD <50 IU/ml (based on literature). In Belgium, HCV genotypes 1 and 3 are the most common types. The Artus kit is developed to detect all HCV genotypes (1–6). Positivity was confirmed with genotypes 1a, 1b, 3, 4 and 5.

2. Specificity: According to previous validation guidelines, specificity was sufficiently demonstrated by the manufacturer: the HCV assay was tested on a broad range of viruses and bacteria and no cross-reactivity was found with any of these organisms.

3. Accuracy: The 2009 QCMD panel for quantification of HCV was used to check the accuracy of the Artus HCV kit. All samples showed a very good correlation with the QCMD results and were situated within our validation criterion of SD <0.5 log of the expected results (log IU/ml).

4. Precision: Two samples (10.000 IU/ml and 1000 IU/ml), were extracted in triplicate on 3 different days. The standard deviation (SD) was 0.4 for both samples, meeting our validation criterion of SD < 1 (Ct).

5. Linearity: Using the Optiquant HCV RNA quantification panel, the linearity of the HCV assay was assessed by testing 5 different concentrations in triplicate (range 400–1000 000 IU/ml). The HCV assay fulfilled our validation criteria (SD < 0.5 per triplo and 0.99 < R² < 1) (see figure). Values within this range will be reported in a quantitative way.

Conclusion: The Artus HCV RT-PCR assay met all our validation criteria and was implemented in the daily routine both as a qualitative (LOD 50 IU/ml) and as a quantitative assay (range 400–1 000 000 IU/ml).

**P1875 Comparison of BD ProbeTec™ HSV1 assays, ELVIS viral culture system, and TaqMan real-time HSV PCR assays in herpetic simplex virus detection of external anogenital lesions**

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Objectives: To evaluate the BD ProbeTec™ HSV1 Q assays, ELVIS viral culture system, and TaqMan real-time HSV PCR assays in herpetic simplex virus detection of external anogenital lesions.

Methods: Two hundred and four subjects with external anogenital lesions were enrolled from 9 geographically diverse clinical centers between September 29, 2009 and December 14, 2009. Two swabs were collected from each subject: the 1st swab was placed in BD™ Universal Viral Transport (UVT) medium and the 2nd was placed in a Q² Swab Diluent tube (data not presented). Each UVT swab specimen was tested with the TaqMan real-time PCR assays and ELVIS viral culture. In addition, a portion of each UVT specimen was transferred into a Q² Swab Diluent tube and tested with the BD HSV Q Assays. The PCR assays provided the viral quantification for each specimen in terms of...
copies/mL and all three methodologies provided HSV typing results for specimens.

**Results:** Among 204 ELVIS culture results, three HSV culture positive specimens were excluded from analysis due to the failure of typing analysis. One PCR specimen was excluded from analysis due to noncompliant specimen storage. The BD HSV Q Assays results were analyzed against the 201 ELVIS culture results and the 203 PCR results; see table.

The detection frequency of both HSV1 and HSV2 were similar between PCR and SDA at any viral titer tested. Both assays detected viral loads less than 10^3 copies/mL. In comparison with the other two methods, the ELVIS culture detection rate decreased at viral loads less than 10^4 copies/mL, unlike the ELVIS test system. Both the BD HSV Q Assays and the TaqMan PCR assays are highly sensitive and specific in detecting HSV infections in external anogenital lesions.

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**Conclusion:** The percent agreement between the BD HSV Q Assays and the well characterized TaqMan real-time HSV PCR is excellent. Both assays can detect positive specimens when the viral load is less than 10^4 copies/mL, unlike the ELVIS test system. Both the BD HSV Q Assays and the TaqMan PCR assays are highly sensitive and specific in detecting HSV infections in external anogenital lesions.

**P1877** Evaluation of AdvanSure HPV screening real-time PCR, Abbott real-time high-risk HPV test, and Hybrid Capture 2 HPV DNA test for the detection of human papillomavirus

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**Objectives:** Various commercial molecular assays for the detection of human papillomaviruses (HPV) are currently available. We compared the AdvanSure HPV Screening real-time PCR test (AdvanSure PCR) (LG Life Science, Korea) with RealTime High Risk HPV test (Abbott PCR) (Abbott Molecular, USA) and Hybrid Capture 2 HPV DNA Test (hc2) (Diagen Corporation, USA) for the detection of HPV.

**Methods:** The study population comprised 170 women who had been referred to the hc2 test from July to September 2010. The 170 samples obtained from cervical swabs were tested with AdvanSure PCR, Abbott PCR and hc2 tests, and 30 samples which showed any discrepancy in three assays were tested with Inno-Lipa HPV Genotyping (Innogenetics, Belgium). And the AdvanSure PCR and Abbott PCR tests were 47 compared for the detection of high-risk type 16 and/or 18. The hc2 test detects high-risk groups of HPV types (HR) (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and low-risk groups of HPV types (LR) (6, 11, 42, 43, and 44). The Abbott PCR detects LR type 66 including above HR types and can differentiate HPV type 16 and/or 18 from non-HPV type 16/18. The AdvanSure PCR assay detects above types detected by hc2 test plus HR (53, 70, 73, 26, 67, 69, and 72) and LR (34, 40, 54, 74, 3, 10, 27, 32, 55, 61, 62, 71, 81, and 84) and detects type 16 or 18 using additional probe.

**Results:** The clinical sensitivities of AdvanSure PCR, Abbott PCR, and hc2 tests for cervical intraepithelial neoplasia grade 2 or worse were 83.3%, 83.3%, and 77.8%, respectively, and the clinical specificities of those tests were 57.2%, 61.2%, and 50.0%, respectively. Comparing with Inno-Lipa HPV Genotyping test, the analytical sensitivities of AdvanSure PCR, Abbott PCR, and hc2 tests were 89.9%, 89.2%, and 97.7%, respectively, and the analytical specificities of those tests were 100%, 100%, and 71.8%, respectively. For the detection of type 16 and/or 18, the sensitivity and specificity of AdvanSure PCR test were 100% and 100%, respectively, and those of Abbott PCR test were 96.2% and 100%, respectively. And the kappa coefficient between AdvanSure PCR and Abbott PCR tests to detect type 16 and/or 18 was 0.9769, showing almost perfect agreement.

**Conclusions:** The AdvanSure HPV Screening real-time PCR test for the detection of HPV is comparable to Abbott RealTime high risk HPV test and Hybrid Capture 2 HPV DNA Test and can concurrently differentiate the HPV type 16 and/or 18 from other non-HPV type 16/18.
by both techniques. All samples were retrospectively tested using rt-PCR QIACMV after automatic DNA extraction with m2000sp. Rtp-PCR positive results were defined by \( \geq 52 \) copies/mL.

**Results:** Plasma samples were positive in 29.2%, 34% and 40.7% by AG, Cobas PCR and rtp-PCR, respectively. Thirty-nine samples (7.5%) were only positive by rtp-PCR, of these 9 were first and 15 last samples in episodes. Concordance between PCR assays was 83.7% (k = 0.63), Pearson 0.98 (p < 0.001) and rtp-PCR vs AG & Cobas PCR was 80.6% (k = 0.66). ROC curve analysis was applied using the existing treatment criteria based on AG and CobasPCR for triggering CMV preemptive therapy and a viral load of >173 copies/mL by rtp-PCR was established as the optimal value \((S = 65\%\ E = 73\%\ AUC = 0.728)\).

Episodes were detected in 71 (93%), 58 (76%) and 67 (88%) by AG, CobasPCR and rtp-PCR, respectively. Nine episodes (11.8%) were only detected by AG (all PCRn negative) and 5 (6.6%) only by PCR assays. For episode detection the first positive technique was rtp-PCR 51 (67%), AG 50 (65.8%) and Cobb PCR 37 (48%). The range for rtp-PCR was 52–3.9 \( \times 10^{5} \) copies/mL vs 60–1.0 \( \times 10^{6} \) copies/mL for Cobas PCR. Bland Altman Analyses CobasPCR vs rtp-PCR difference is 0.76 log.

**Conclusion:** Rtp PCR QIACMV detects earlier and more episodes than the COBAS® AMPLICOR PCR CMV. Rtp-PCR quantitative results were lower than end-point PCR (0.76 log). The threshold of 173 copies/mL is the optimal rtp-PCR CMV-DNA value for triggering preemptive treatment in allo-SCT. Results from this study suggest that this rtp-PCR assay is a useful tool for the clinical management of CMV in allo SCT patients.

**Evaluation of the extractor of nucleic acids incorporated in the Versant kPCR molecular system to use with the Trugene HIV-1 genotyping kit in plasma samples with viral loads <1000 copies/ml**

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**Objective:** Early detection of mutations associated with resistance (MR) to antiretrovirals (AR) is recommended to anticipate and prevent the therapeutic failure in HIV-infected patients receiving ARs. Many systems used to detect these MR are based in the DNA sequencing of the protease (PR) and the reverse transcriptase (RT) genes. One relevant limitation of these assays is their reduced reliability (related with problems of amplification and/or sequencing) for samples with low viral loads (<1000 copies/mL), due to the reduced amount of RNA presented in the samples analysed. The aim of this study was to evaluate the reliability of the semiautomated extractor of nucleic acids (NA) included in the VERSANT kPCR system (Siemens) in combination with the TRUGENE HIV-1 Genotyping Kit (Siemens) in samples with plasma viral loads <1000 copies/ml.

**Methods:** Forty eight plasma samples from different patients with viral loads ranging from 87 to 795 copies/mL, as determined with the VERSANT HIV-1 RNA 1.0 Assay (kPCR; Siemens), were studied. Extraction of RNA was performed with the VERSANT kPCR extractor module. DNA Sequencing of the RT and PR genes was performed by the TRUGENE HIV-1 Genotyping Kit and the OpenGene DNA Sequencing System (Siemens). The variables analysed were i) to get or not amplification and sequencing and ii) to detect major MR to AR in samples with plasma viral loads <1000 copies/ml.

**Result:** Thirty-eight (79.2%) samples were successfully sequenced (PR and RT genes). Thirty-three (86.8%) sequences belonged to subtype B. MR to PR inhibitors (MR-PI) was detected in 3 samples (0.9% of the PR sequences), whereas MR to nucleoside RT inhibitors (MR-NRTIs) and non-nucleoside RT inhibitors (MR-NNRRTIs) were detected in 11 (28.9%) and 14 (36.8%) of the RT sequences, respectively. The MR-PIs detected in the PR gene were L90M (n = 1), I54V + V82A (n = 1), and D30N + L33F + V82A + N85D (n = 1). In the RT gene, the most frequent MR-NRTI were V118I (n = 7), M184V/I (n = 4), T215Y/F (n = 4), and M41L (n = 3), whereas the MR-NNRRTI were V90I (n = 3), K103N/S (n = 2), G190A (n = 2), E138A (n = 2) and V108I (n = 2).

**Conclusion:** The extractor module of the VERSANT kPCR system is a highly reliable and convenient device to use with the TRUGENE HIV-1 genotyping kit for samples with plasma viral loads <1000 copies/mL. An elevated percentage of major mutations were detected with this system, particularly in the RT gene, supporting evidence of its utility in this type of samples.

**Quantitative real-time PCR with automated viral DNA extraction for diagnosis and monitoring of cytomegalovirus in allogeneic bone marrow transplant recipients**

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**Objectives:** Evaluation of the performance of a Real Time PCR (RT-PCR) assay on whole blood samples or cell free plasma over a Cytomegalovirus (CMV) antigenemia assay in diagnosis and monitoring of CMV infection in bone marrow transplant (BMT) recipients.

**Methods:** 896 samples from 37 allogeneic BMT recipients hospitalised at Patras’ University Hospital during a four-year-period (02/2006–03/2010) were included in this study. Viral load was detected either by RT-PCR on whole blood and cell free plasma, or CMV antigenemia assay. Viral DNA was automatically extracted. RT-PCR was performed using a commercially available kit that exploits the major groove binder technology. CMV lower matrix protein pp65 was detected by indirect immunofluorescence microscopy in \( 2 \times 10^{6} \) leukocytes/mL, per sample. High risk for CMV disease patients were identified according to the CDC guidelines,1, depending on their viral load.

**Results:** Antigenemia assay was shown to be less sensitive in detecting viral load (38.5%) than RT-PCR on whole blood (82.38%) or cell free plasma samples (71.23%). All methods showed the same specificity (96.45%–97.79%). Pairwise comparison of Roc Curves futher confirmed that the best method for CMV detection is RT-PCR on whole blood \((p < 0.001)\). Within the 4-year-period, 30 out of 37 BMT recipients (81%) showed a first incidence of CMV activation, as identified by RT-PCR on whole blood. Twenty out of 30 (66.7%) were positive by all three techniques (Table 1). CMV re-activation was detected in 22 out of the 30 (73.3%) by RT-PCR on whole blood, whereas, only 17 out of 22 (56.7%) were positive by all three techniques (Table 1). Further statistical analysis confirmed that RT-PCR on whole blood samples is more sensitive for CMV activation \((p = 0.0196)\) than RT-PCR on cell free plasma and CMV antigenemia assay. Finally, RT-PCR on whole blood detects re-activation of CMV disease (231 days) earlier than RT-PCR on cell free plasma (238 days), \((p = 0.05)\), and CMV antigenemia assay (281 days), \((p = 0.0273)\).

**Conclusion:** Real-time PCR based on automatic viral DNA extraction from whole blood samples is a quantitative, highly reproducible, more sensitive and equally specific method for diagnosis of CMV activation, as well as, monitoring of antiviral treatment in bone marrow transplant recipients, as compared to either real-time PCR on cell free plasma or the established CMV antigenemia assay.

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**Table 1. Summary statistics table**

|          | CMV activation | CMV re-activation |
|----------|---------------|-------------------|
|          | 
| Mean days before plasma | 108 (95% CI: 93–123) | 107 (95% CI: 91–123) |
| Median | 107 | 107 |
| 25th | 105 | 105 |
| 75th | 111 | 111 |
| Max | 123 | 123 |

Reference(s)

[1] MMWR Recomm Rep. 2000 Oct; 49(RR-10): 1–125, CE1–7.
**P1881** Could conjunctival swabs be useful to diagnose herpetic keratitis?
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**Objectives:** Herpes simple virus (HSV) is a common cause of corneal disease which ranges from superficial infection to chronic inflammatory herpetic stromal keratitis. The aim of this study was to assess if conjunctival swabs samples were equivalent to corneal scrapings to diagnose herpetic keratitis.

**Methods:** From June 2008 to May 2009, a total of 74 samples (37 conjunctival swabs and 37 corneal scrapings) collected from 37 patients with clinical keratitis were studied. Samples were obtained from each patient at the same time. Both types of samples were analyzed by real-time PCR using EasyMag (BioMerieux) as DNA extractor; real-time PCR was performed in a Light Cycler (Roche) by using TaqMan probes. Moreover, all samples were inoculated on A-549 and MRC-5 cell lines and evaluated for cytopathic effect (CPE) development for 7 days. When a CPE was observed, the viruses were typed by immunofluorescence (Microtrack).

**Results:** Real-time PCR was more sensitive than cell culture in both type of samples. 15/37 (40.5%) corneal scraping samples were PCR positive versus 9/37 (24.3%) conjunctival swabs. Cell culture allowed to detect HSV in 11/37 (29.7%) corneal scraping specimens and in 7/37 (18.9%) conjunctival swabs. Sensitivity, specificity, positive and negative predictive values and efficiency for cell culture performed on conjunctival swabs were 77.77%, 100%, 93.33% and 94.59%, respectively. For corneal samples these values were 73.33%, 100%, 84.61% and 89.18%, respectively. The agreement between both type of samples was 89.17% for real-time PCR assay and 83.78% (31/37) for cell culture detection.

**Conclusion:** These results showed that conjunctival swabs allowed to detect HSV in 60% of patients with PCR positive corneal scraping specimens, that are considered the optimal samples for herpetic keratitis detection.

**P1882** Molecular-genetic heterogeneity of tick-borne encephalitis virus in Transbaikal region
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**Objectives:** Tick-borne encephalitis (TBE) morbidity at Transbaikal Region is 3,4 per 100,000 population and characterized by higher lethality (from 22.5% in 2004 to 2.7% in 2009) in comparison with lower, than in adjacent territories, morbidity indicators, high percent of focal TBE forms (35.7%) and prevalence of rural population among the patients (62.0%). Regional population of TBE virus is insufficiently studied.

**Materials and Methods:** 37 strains of TBE virus isolated in 1995–2010 including 18 patients with focal TBE forms with lethal outcome, 11 from Ixodes persulcatus ticks, 2 from Dermacentor silvarum and 6 from wild rodents were studied.

Genotyping of strains was performed in real time by hybridization-fluorescent method with labelled genotype-specific fluorescent probes, restriction fragment length polymorphism (RFLP) assay, sequencing of protein E gene fragment 211 bp containing a marker amino acid in 206 position, sequencing of full-size gene of protein E.

**Main results:** Among isolates from died humans four strains were determined as a Siberian genotype of TBE virus, 13 – Far Eastern genotype, one strain was genotyped as a mixed isolate containing genome sites of Siberian and Far Eastern genotypes. Also, representatives of Siberian (4) and Far Eastern (1) genotypes, “886-like” (2) isolates and mixed-variants (4) were revealed among strains isolated from I persulcatus. Strains isolated from D silvarum were genotypes as TBE virus of the Far Eastern genotype. Strains from wild mammals represented four Siberian and two Far Eastern genotypes.

**Conclusions:** The findings indicate uniqueness and genetic heterogeneity of TBE virus circulating at the focal territories of Transbaikal region presented by various genotypes (Siberian, Far Eastern), “886-like” variants differed from all known genotypes of TBE virus at the genome level and mixed-variants containing sites of Far Eastern and Siberian genotype genomes. The role of different genotypes and variants in formation of regional epidemiological and clinical manifestations of TBE and the population immunostructures need further studying.

**P1883** Investigation of potential relationship between BKV with kidney and bladder cancers
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**Objectives:** Despite some reports published on the relationship between BK virus (BKV) and cancer, role of BKV in human cancers has not been completely understood. Furthermore, there are conflicting data on this subject. Objective of the present study was to determine the presence of BKV DNA in formalin-fixed paraffin-embedded kidney and bladder tissues and to investigate the BKV mRNA levels in these tissues.

**Materials and Methods:** 90 cancer tissues (40 bladder and 50 kidney) and 70 control tissues (25 bladder and 45 kidney) were included in this study. For detection of the BKV DNA, the nested PCR was performed from the tissues. Furthermore, real time RT-PCR was carried out for investigation of the BKV mRNA levels.

**Results:** Results of the nested PCR indicated that 23 (14.3%) of 160 samples were positive for BKV DNA. There was, statistically, a significant relationship between the cancer and presence of BKV DNA (p < 0.05). In the real-time PCR, BKV VP1 mRNA was detected in 69.5% of the BKV DNA positive samples. The levels of BKV mRNA were significantly higher in the cancer samples than non-cancer samples (P < 0.05).

**Conclusion:** These results suggest that BKV may be associated with development of bladder and kidney cancers. However, more extensive studies are required to elucidate the role of BKV in pathogenesis of cancer.

**Molecular diagnostics**

**P1884** A new method to inactivate genes on large conjugal plasmids for investigation of their biological function
J.L. Cottell*, M.A. Webber, L.J. Piddock (Birmingham, UK)

**Objectives:** The study of large conjugal plasmids is of increasing importance due to their role in the dissemination of antibiotic resistance (eg. Extended spectrum ß-lactamases) and virulence genes. As a result, hundreds of complete plasmid DNA sequences have now been elucidated. However, a lack of basic laboratory molecular methods to specifically perturb functional genes on plasmids has meant little work has investigated the biological properties of these plasmid borne genes. The aim of this project was to devise a molecular method to rapidly inactivate specific genes on plasmids, and to apply this method to the inactivation of genes on a large epidemic plasmid that encodes blaCTX-M-14.

**Methods:** A PCR amplimer encoding either the aph kanamycin resistance gene or an aph-green fluorescent protein gene (aph-gfpmut2) with homologous ends to the target gene for inactivation was generated. This PCR amplimer and the plasmid of interest were both transformed into an E. coli strain carrying a chromosomally encoded lambda red recombinase. Successful recombination between the plasmid and PCR amplimer resulted in the removal of some of the DNA from the gene of interest and insertion of aph or aph-gfp. Selection of colonies on kanamycin containing agar allowed identification of plasmids where homologous recombination into the selected gene had successfully occurred. This plasmid was transformed into E. coli DH5α and Salmonella Typhimurium SL1344 and the effect on antibiotic susceptibility, growth rates, conjugation frequencies and virulence was measured.

**Results:** Five plasmid genes were successfully inactivated by insertion of the aph and aph-gfp genes using this novel targeted recombination method.
method. Insertional activation of blacTX-M-14 with aph and aph-gfpmut2 reduced the MIC of cefotaxime for host bacteria from 32 μg/ml to 0.03 μg/ml. However, in this plasmid, inactivation of genes or insertion of gfp had no effect on bacterial host growth, virulence or conjugation frequency of the plasmid.

**Conclusion:** This new method for rapid targeted inactivation of plasmid genes will allow investigation of the function of selected genes and their role in the biology of large conjugative plasmids. The easy insertion of a gfp gene within selected regions in the plasmid allows potential for study of the role of specific plasmid genes in the dissemination and epidemiology of transferrable elements.

**P1885** Evaluation of a novel non-enzymatic method for enrichment of pathogen DNA from whole blood
A.J. Loonen, B. van Meerbergen, S. Neerken, R. Penterman, I. Dobbelzaer, K. Schmidt, P. van de Wiel, A.J. van den Brule* (Eindhoven, NL)

**Objectives:** Bloodstream infections are a major clinical problem. Blood culture is still the golden standard for identification of pathogens, however, this method is time-consuming. Rapid and sensitive identification of the causative pathogen is essential to select the most effective treatment.

In this study, a novel non-enzymatic method was evaluated for isolation of bacterial and fungal DNA from whole blood samples: Red and white blood cells are selectively lysed and the leukocyte DNA is simultaneously degraded, while pathogens remain intact. The performance of this novel method was compared to the reference method to date, i.e. MolYsis Complete5 kit (MolYzym) based on enzymatic processes.

**Methods:** A 10-fold dilution series containing methicillin-resistant Staphylococcus aureus, Candida albicans, and Pseudomonas aeruginosa was spiked into 1 and 5 ml whole blood. The purified pathogen DNA was quantified by qPCR for several assays, while the remaining leukocyte DNA was quantified using an RNaseP detection kit (Applied Biosystems).

**Results:** The new method and the MolYsis Complete5 kit revealed comparable Ct values in qPCR for all species tested with the expected linear relation between concentration and Ct value. 1–10 CFU spiked into 1 ml whole blood was detectable with all methods. Increase of the input volume to 5 ml resulted in a 5 times higher sensitivity. RNaseP qPCR results for eluates from 1 and 5 ml blood demonstrate effective removal of leukocyte DNA by both methods which enables detection of clinically relevant concentrations of pathogens from several milliliters of blood. The pathogen DNA can be isolated within 30 minutes for 1 ml and within 40 minutes for 5 ml blood, respectively, whereas the MolYsis Complete5 kit takes 2–3 hours.

**Conclusions:** This novel method allows sensitive and fast DNA enrichment from bacteria and fungi from whole blood. Starting from 5 ml blood, the lowest detectable concentration was 1 CFU/ml for the different pathogens and similar Ct values were obtained for both methods. However, at low pathogen concentrations increased detection rates were observed for the new method. Furthermore, this non-enzymatic method is less labour intensive and faster than the MolYsis Complete5 kit. It can be carried out as manual procedure on the bench, but is at present also integrated into a closed disposable cartridge for full automation.

**P1886** Development of a rapid, fully automated method for selective isolation of bacterial and fungal DNA from large volumes of whole blood
S. Neerken*, R. Penterman, B. van Meerbergen, I. Dobbelzaer, K. Schmidt, P. van de Wiel (Eindhoven, NL)

**Objectives:** Rapid identification of bacteria and fungi causing bloodstream infections is crucial to increase the survival chances of sepsis patients. Several attempts are made to replace traditional culture by faster molecular tests. However, the sensitivity of standard molecular techniques is limited by the fact that at maximum 0.2–0.5 ml blood can be used, because larger amounts of leukocyte DNA will inhibit the PCR. Our objective is to provide a rapid, fully automated isolation method that increases the sensitivity by enabling the large sample volume.

**Methods:** We developed a method for selective isolation of pathogen DNA from whole blood samples based on a non-enzymatic procedure. The red and white blood cells are lysed, while keeping the pathogens intact, simultaneously the leukocyte DNA is degraded and subsequently removed by filtration. Pathogens captured on the filter are chemically lysed. Finally, the pathogen DNA is collected for downstream processing with standard molecular techniques. The isolation procedure has been completely automated by integration into a disposable cartridge actuated by a table-top instrument.

**Results:** Different species of Gram-negative and Gram-positive bacteria and fungi (P. aeruginosa, S. aureus, C. albicans) were spiked into 1 and 5 ml human blood and bacterial and fungal DNA was isolated in the cartridge; total processing time before PCR was 30 and 40 min for 1 and 5 ml blood, respectively. Pathogen recovery and removal of inhibitors were tested by comparing Ct values of spiked blood samples with reference samples, the latter containing same amounts of bacteria spiked into buffer, that were lysed directly. Similar Ct values were obtained, demonstrating yields close to 100% and absence of PCR inhibition by leukocyte DNA. PCR for a standard human gene demonstrated a reduction of leukocyte DNA content by more than a factor 25. Increasing the sample volume from 1 to 5 ml revealed on average a gain of 2 Cts and higher hit rates at borderline concentrations (1–10 CFU/ml), demonstrating a direct relation between sensitivity and sample volume.

**Conclusion:** Our method enables fully automated specific isolation of pathogen DNA from up to 5 ml blood within 40 min with high yield and purity. Using 5 ml blood, pathogens spiked in concentrations as low as 1 CFU/ml were detectable. In combination with standard molecular procedures this allows the rapid identification of pathogens in blood at clinically required sensitivity levels.

**P1887** Comparison of six differentiation methods for identification of coagulase-negative staphylococci
A.J. Loonen*, A.R. Janz, J.N. Bergland, P. Wolffs, A.J. van den Brule (Eindhoven, Maastricht, NL)

**Objectives:** Coagulase-negative staphylococci (CNS) are a common cause of nosocomial infections and therefore pose a great threat to patients with an immunodeficiency. Rapid and reliable determination of CNS is therefore clinically relevant.

In this study, we compared the conventional Kloos and Schleifer test, the biochemical ID 32 Staph (bioMérieux), automated biochemical identification system Vitek2 (bioMérieux), partial 16S ribosomal DNA sequencing (MicroSEQ, Applied Biosystems), partial TUF DNA sequencing, and the proteomics approach matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS) to find the best method for accurate identification of CNS strains.

**Methods:** Approximately 150 strains, representing 19 CNS species, were included in this study. All CNS isolates were characterized by Vitek2 and when necessary partial 16S rDNA gene sequencing was performed. Because this method does not give 100% correct identification, due to either incomplete database or discrimination problems, all methods were used to analyze the included CNS strains. Data of all performed methods were compared to obtain a reference name, as a “gold standard”.

**Results:** 16S rDNA sequencing, TUF sequencing and MALDI-TOF seemed to have a correct identification rate of 70.6%, 93% and 99.3% respectively. For the Kloos & Schleifer test, ID 32 Staph and Vitek2 the correct identification was 80.4%, 86%, and 92.3% consecutively. No identification could be given for 2.1%, 19.6% and 0.7% of the strains when ID 32 Staph, Kloos and Schleifer or MALDI-TOF-MS was used respectively. Incorrect identification was given for 29.4%, 7%, 7.7%, and 11.9% of the strains when 16S sequencing, TUF sequencing, Vitek2 and ID 32 Staph was used respectively.

**Conclusions:** MALDI-TOF-MS showed the best results for rapid CNS differentiation (correct identity in 99.3%). In addition, this technique is fast, cheap and suitable for high-throughput. The current gold standard in many laboratories, a.o. our facility, is Vitek2 and partial 16S rDNA sequencing. Therefore, in our opinion, the gold standard should be replaced by MALDI-TOF-MS and TUF sequencing.
Objective: To evaluate the safety of PrimeStore® Molecular Transport Medium (PS MTM) in the diagnosis of TB and compatibility for use with two commercial TB diagnostics assays.

Methods: To evaluate safety the following 3 studies were performed with controls. 1) A 0.15ml and 0.5ml inocula of a known MDR strain was placed into PS MTM for 2 and 10 minutes then vortexed and cultured in the MGIT® liquid based system. 2) A known smear positive sputum specimen (>10 AFB/hpf) was placed into PS MTM for 1min and 5 mins followed by Zielh-Neelsen staining for morphological observation of cell wall integrity. 3) A reference H37rv strain with a concentration of 105−6 was used to perform a time kill assay. An inoculum of 0.5ml of the strain was placed in the PS MTM for 5s, 10s, 20s, 40s, 80s and 160s and thereafter subcultured onto 7H11 agar for each time exposure. To evaluate compatibility of the PS MTM with the two commercial assays (LightCycler Mycobacterium Detection kit, Roche®, South Africa and Genotype MTBDRplus, Hain LifeScience®, Germany) for TB diagnosis the following was done. Fifteen smear positive and 15 smear negative digested and decontaminated sputum specimens were processed with the Hain LifeScience and Roche assays and 0.5 ml of the remaining sediment was then inoculated into PS MTM and stored overnight. The 2 assays were then repeated from the stored PS MTM.

Results: In the safety study using MGIT liquid culture no growth was observed after 42 days incubation with both inocula in PS MTM whereas the control unexposed to PS MTM was positive for growth after 9 days. In the staining study, no AFB was observed after the 2 short exposure times. For the time kill study, no growth was observed after 42 days incubation at any of the time points, even at the 5s exposure to the PS MTM whereas colony forming units were observable after 7 days on the control plates. The compatibility studies for the Hain assay showed the same results with and without exposure to the PS MTM (smear positives were only tested). The Roche assay had the same results for the smear positive specimens however an additional smear negative specimen was detected after exposure to the PS MTM as compared to the control.

Conclusion: The PS MTM was safe in that it sterilized M. tuberculosis in a very short time period. For clinical specimens the medium was compatible with the 2 commercial assays and improved the diagnostic yield. Hence this medium allows for safe and rapid point of care diagnosis of TB.

Moleculardiagnostics

[Image 1 of 2]

P1889 Evaluation of PrimeStore® molecular transport medium for use in the diagnosis of TB

N.A. Ismail*, A. Dreyer, S.V. Omar, L.T. Daum, G. Fischer, P.B. Fourie, A.A. Hoosen ( Pretoria, ZA; Bethesda, US)

Objective: To evaluate the safety of PrimeStore® Molecular Transport Medium (PS MTM) in the diagnosis of TB and compatibility for use with two commercial TB diagnostics assays.

Methods: To evaluate safety the following 3 studies were performed with controls. 1) A 0.15ml and 0.5ml inocula of a known MDR strain was placed into PS MTM for 2 and 10 minutes then vortexed and cultured in the MGIT® liquid based system. 2) A known smear positive sputum specimen (>10 AFB/hpf) was placed into PS MTM for 1min and 5 mins followed by Zielh-Neelsen staining for morphological observation of cell wall integrity. 3) A reference H37rv strain with a concentration of 105−6 was used to perform a time kill assay. An inoculum of 0.5ml of the strain was placed in the PS MTM for 5s, 10s, 20s, 40s, 80s and 160s and thereafter subcultured onto 7H11 agar for each time exposure. To evaluate compatibility of the PS MTM with the two commercial assays (LightCycler Mycobacterium Detection kit, Roche®, South Africa and Genotype MTBDRplus, Hain LifeScience®, Germany) for TB diagnosis the following was done. Fifteen smear positive and 15 smear negative digested and decontaminated sputum specimens were processed with the Hain LifeScience and Roche assays and 0.5 ml of the remaining sediment was then inoculated into PS MTM and stored overnight. The 2 assays were then repeated from the stored PS MTM.

Results: In the safety study using MGIT liquid culture no growth was observed after 42 days incubation with both inocula in PS MTM whereas the control unexposed to PS MTM was positive for growth after 9 days. In the staining study, no AFB was observed after the 2 short exposure times. For the time kill study, no growth was observed after 42 days incubation at any of the time points, even at the 5s exposure to the PS MTM whereas colony forming units were observable after 7 days on the control plates. The compatibility studies for the Hain assay showed the same results with and without exposure to the PS MTM (smear positives were only tested). The Roche assay had the same results for the smear positive specimens however an additional smear negative specimen was detected after exposure to the PS MTM as compared to the control.

Conclusion: The PS MTM was safe in that it sterilized M. tuberculosis in a very short time period. For clinical specimens the medium was compatible with the 2 commercial assays and improved the diagnostic yield. Hence this medium allows for safe and rapid point of care diagnosis of TB.

P1889 Liquor protein profiling for the diagnostics of acute bacterial meningitis using MALDI-TOF mass spectrometry and ClinProt technology

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Objectives: Meningitis is a very serious, life threatening illness causing different complications in many cases. The diagnosis of meningitis is extremely important on the early stage of illness; however it may be delayed because some symptoms may initially be assumed to be related to another less serious condition. Meanwhile, acute meningitis, especially acute bacterial (purulent) meningitis, can progress very rapidly and result in death if it is not recognized and treated quickly. In our work, the ClinProt technology followed by MALDI-ToF mass-spectrometry (MS) analysis was applied to detect a combination of potential peptide markers in human cerebrospinal fluid (CSF) suitable for diagnostics of acute bacterial meningitis.

Methods: 104 CSF samples (35 from patients with acute bacterial meningitis, 38 from serous meningitis, and 31 from healthy controls) were collected. Of these, 20 samples of each group formed the main selections, while the rest ones – the additional (validation) groups. CSF samples were fractionated using weak cation exchange magnetic beads (MB-WCX kit, Bruker Daltonic, Germany) on a sample preparation robot following the manufacturer’s protocol. Eluates were applied onto a MTP 384 target plate polished steel; after drying at air a solution of 2,5-dihydroxybenzoic acid and α-cyano-4-hydroxycinnamic acid in a mixture of methanol/acetonitrile/water 5:4:1 was applied onto the sample. Mass spectra were collected by an Ultraflex MALDI-TOF MS and were analyzed using ClinProTools 2.1 software (both from Bruker Daltonics). Mathematical models for classification of mass spectra obtained after sample fractionation were generated using a genetic algorithm (GA) and a Supervised Neural Network (SNN).

Results: Three diagnostic models were generated to classify the bacterial/serous, bacterial/control and serous/control groups. Specificity of the models generated by both GA and SNN algorithms were 100% both for bacterial/serous and bacterial/control groups, while the sensitivity was no less than 80% in all cases. Only quite low values for both sensitivity (no more than 41%) and specificity (no more than 76%) were obtained for serous/control group differentiation.

Conclusion: The potential of CSF protein profiling for diagnosis of acute bacterial meningitis could be demonstrated. The approach was not appropriate to differentiate correctly the “serous meningitis” samples from the CSF of control group.

P1890 The impact of thermocycler variability on IVD CE kit validation

M. Span* (Landgraaf, NL)

Objectives: Thermocyclers are currently perceived by as black box systems that are assumed to perform as specified by the manufacturer. When combined with validated diagnostic CE IVD kits it is assumed that validation of the assay, as required by the ISO 17025:2005 and ISO 15189:2007 norm is adequately covered.

The objective of this study is to determine what the thermal performance is of a large population of thermocyclers, how big the variability is within this population and if this does have any impact on CE IVD kit validation.

Methods: 10.454 thermocyclers, both normal and real-time with both standard and fast blocks, have been calibrated using the 16 sensor dynamic MTAS system (CYCLERtest, NL). All thermocyclers were measured with an identical temperature protocol, being 30°C 60s, 95°C 180s, 30°C 60s, 90°C 180s, 50°C 180s, 70°C 180s, 60°C 180s and 30°C 60s to allow comparability. The parameters which have been measured are accuracy, uniformity, heating rate, cooling rate, overshoot, undershoot and plateau time.

Results: The thermocycler calibrations show that there is a large spread in both the accuracy and uniformity between different brands (figure 1, dots of different colors) and between different models of the same brand (figure 1, dots of the same color). When the calibration data are compared on individual serial number level the spreads in both accuracy and uniformity are even bigger.

Conclusion: When comparing the accuracy and uniformity between and within thermocyclers substantial differences can be observed. Practically this leads to situations that (q)PCRs will give reliable results on a particular thermocycler, but not on another thermocycler, even of the same brand and model.
Typically accredited labs consider CE IVD kits as sufficiently validated to give reliable results on the thermocycler on which it has been validated. However, CE IVD kit manufacturers typically validate their kits on <5–10 individual thermocyclers, assuming they all function alike. In the perspective of the large variability measured these small sample populations are statistically not representative for the total populations of thermocyclers. Therefore it can be concluded that a validated CE IVD kit in combination with a recommended thermocycler is no guarantee for reliable results and that diagnostic labs currently run the risk to generate false negative results or lower positive with all clinical consequences connected, due to underestimated thermocycler variability.

**Evaluation of automated DNA extraction devices for sepsis diagnostics**

S. Laakso*, M. Mäki (Helsinki, FI)

**Objective:** Aim of this study was to compare two automated. In Vitro diagnostic labeled DNA extraction devices. The performance of NorDiag Arrow (Nordiag) was compared to that of NucliSENS® easyMag® (bioMérieux) using positive blood culture samples. Both devices utilize magnetic particle based extraction technology, Arrow can process 1 to 12 samples simultaneously, whereas easyMag® is a higher throughput device capable of processing 1 to 24 samples. We analyzed the DNA extracts with the PCR and microarray-based Prove-it™ Sepsis assay (Mobidiag), which is designed to identify 60 Gram-negative and Gram-positive bacterial species from positive blood cultures. In this study, yields of DNA extracts were also studied using pure bacterial culture samples.

**Methods:** A set of 91 positive blood cultures from patients with suspected sepsis were randomly collected in HUSLAB, Finland. Blood culture bottles of BacT/ALERT 3D (bioMérieux) were incubated until flagged as positive. 250 μl of blood culture was used with the Arrow VIRAL NA kit and Arrow, and the elution volume was 100 μl. For easyMag® 100 μl of blood culture was used, and the elution volume was 55 μl. Prove-it™ Sepsis analysis was performed according to the manufacturer’s instructions and the obtained results were compared to those of conventional blood culture. DNA yields were studied using various amounts of E. coli pure cultures and specific qPCR.

**Results:** Prove-it™ Sepsis assay reported bacterial findings from 77 blood culture samples. 14 samples were reported as negative, containing bacteria not belonging to the pathogen panel of the assay. No difference was observed between the performance of Arrow or easyMag® with regard to the result reporting of Prove-it™ Sepsis. Of note was that Streptococcus dysgalactiae subsp. equisimilis was identified from two samples by Prove-it™ Sepsis, but the conventional method reported S. pyogenes findings. The DNA sequencing confirmed S. dysgalactiae subsp. equisimilis findings. Also, the difference was observed when the DNA yields of these two devices were compared.

**Conclusions:** Use of automated and standardized sample preparation methods together with rapid molecular assays can speed up the diagnostics of septic patients. Both tested DNA extraction devices were shown to be feasible for blood culture samples and the Prove-it™ Sepsis assay, providing the identification of pathogen in four hours.

**Molecular versus conventional diagnosis of intestinal parasitoses**

A. Calderaro*, C. Gorrini, S. Montecchini, G. Piccolo, C. Chezzi (Parma, IT)

**Objectives:** A field comparison in the period 2003–2010 between conventional and molecular methods for the diagnosis of infection by Entamoeba histolytica, E. dispar, Giardia intestinalis, Dientamoeba fragilis and Cryptosporidium spp. is reported analysing faecal samples from patients with gastro-intestinal signs and symptoms related to a clinical suspicion of intestinal parasitoses.

**Methods:** Two real-time PCR assays for the differentiation of E. histolytica and E. dispar and for the detection of D. fragilis, respectively, in faecal specimens were evaluated in comparison with microscopy and culture. Two real-time PCR assays for the detection of G. intestinalis and Cryptosporidium spp., respectively, were evaluated in comparison with the combination of microscopy, an immunocromatographic assay and an immunofluorescence assay.

**Results:** Conventional assays had a sensitivity of 88.4% for giardiasis, 43.5% for dientamoebiasis, 92.3% for cryptosporidiosis, 41.7% for amoebiasis and a diagnostic specificity of 100% in all cases, whereas molecular assays had 100% sensitivity and specificity in all cases. G. intestinalis. On a total of 422 patients, 112 cases of giardiasis were diagnosed by real-time PCR; only 99 cases were detected by microscopy and/or antigen detection traducing in 13 missed diagnosis. D. fragilis. On a total of 527 patients, 108 cases of dientamoebiasis were diagnosed by real-time PCR; only 47 cases were detected by microscopy and/or cultivation traducing in 61 missed diagnosis. Cryptosporidium spp. On a total of 552 patients, 13 cases of cryptosporidiosis were diagnosed by real-time PCR; only 12 cases were detected by microscopy and/or antigen detection traducing in 1 missed diagnosis. E. histolytica and E. dispers. On a total of 1,108 patients, 12 cases of amoebiasis and 79 cases of E. dispers infection were diagnosed by real-time PCR. Only 64 cases of E. histolytica/E. dispers infection were detected by microscopy and/or cultivation (S. histolytica and 59 E. dispers as determined by real-time PCR) traducing in a missed diagnosis of 7 cases of amoebiasis (6 of whom extra-intestinal).

**Conclusion:** All the real-time PCR assays proved to be in our hand a useful tool for the diagnosis of infections by protozoa showing a higher sensitivity than conventional assays. Therefore, if available and accessible, they would be the most accurate tool for the diagnosis of intestinal parasitoses.

**Use of PCR on urine samples for the diagnosis of toxoplasmosis after allogeneic stem cell transplantation**

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**Objective:** Incidence of invasive toxoplasmosis among T. gondii seropositive allogeneic stem cell transplantation patients is around 4% and the estimated mortality rate is 60%-90%. Rapid diagnosis and immediate onset of appropriate treatment are crucial for avoiding lethal outcome. We investigated the performance of PCR on urine samples for monitoring disseminated toxoplasmosis in 2 allogeneic stem cell transplantation patients.

**Methods:** Analysis using the repetitive 529bp DNA fragment of T. gondii (Genbank No:AF487550) through real-time PCR, was performed in parallel on blood and urine and according to clinical signs on CSF and bronchoalveolar lavage (BAL). Two patients underwent an allogeneic stem cell transplantation for acute lymphoblastic leukaemia. Both were positive for toxoplasmosis serology whereas donors were positive (for patient 1) or negative (for patient 2). After they presented clinical and radiological manifestation of pulmonary infection they were given a course of antibiotics and were systematically monitored by PCR performed on blood, urine, BAL and CSF in one case (patient 2).

**Results:** In patient 1, PCR in blood and urine were positive and induced the onset of antiparasitic treatment. PCR in urine remained positive after PCR in blood and turned negative few days later. After a 5 years follow up the patient evolution remained favourable. In patient 2, PCR in urine remained positive despite antiparasitic treatment and negativation of PCR in blood. The patient died from fungal co infection and cerebral manifestation of her leukaemia.

**Conclusion:** Recipients of stem cell transplantation, when positive for toxoplasma serology, need to be systematically monitored for toxoplasmosis as this potentially lethal disease can have a favourable outcome when treated early. Generally, diagnosis relies on PCR on
Two multiplexed nucleic acid tests for the detection of viral and bacterial pathogens in cerebrospinal fluid

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Objectives: The diagnosis of central nervous system (CNS) infections presents a challenge for clinicians, especially given the broad range of bacterial and viral aetiologies. Delayed pathogen identification and treatment may lead to life-threatening conditions and can be associated with high morbidity and mortality. Consequently, accurate and timely identification of the infectious agents is essential for patient management.

Here we present analytical study results of two qualitative, multiplex PCR tests being developed for the detection of clinically important pathogens targeting the CNS. Each panel detects and discriminates pathogens in a single well. Panel A focuses on viral and bacterial pathogens and Panel B on bacterial pathogens as well as the mecA antibiotic resistance gene.

Methods: Analytical reactivity and cross-reactivity were evaluated for each panel using deidentified left-over human cerebral spinal fluid (CSF) spiked with serial dilutions of individual viral or bacterial cultures and an internal control (to control for extraction efficiency and PCR inhibition). Extracted nucleic acids were amplified by either one-step reverse transcription (RT)-PCR (Panel A) or PCR (Panel B). Amplified products were sorted on a bead-array incorporating the xTAG® Universal Array coupled to magnetic microspheres. Detection was carried out on the Luminex MAGPIX® instrument.

Results: Both panels assessed were able to detect relevant levels of nucleic acids in CSF. Panel A identified viruses at around 100 copies/mL and Panel B detected bacteria at 3 CFU/mL. Each multiplexed panel showed good reactivity with the targeted pathogens and no cross reactivity across probe analytes. The time-to-result from extraction was less than 4 hours for each panel.

Conclusion: This study demonstrates that multiplexed detection of viral and bacterial pathogens in CSF is readily achievable in an assay system incorporating the xTAG® Universal Array and magnetic microspheres. Findings also support the suitability of the MAGPIX® instrument for this class of pathogen detection assay. The ability to simultaneously detect multiple viral and bacterial targets reduces the need for large CSF fluid volumes. The short turnaround time of each assay, run either alone or in tandem, is a key feature of this multiplexed system. Further development is likely to confirm that an assay system utilizing this approach will be a valuable tool for an aetiological diagnosis of CNS infections.

Development of a rapid method for Proteus spp. detection in urine by peptide nucleic acid fluorescence in situ hybridisation (PNA)

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Objective: The need to avoid empirical treatment of patients, and the fact that urine samples are among the most numerous of specimen types sent for microbiology studies, have prompted many researchers to explore methods to limit the time and expense of urine culture processing. The aim of this work was to develop a new peptide nucleic acid fluorescence in situ hybridization (PNA FISH) method for the rapid detection of Proteus spp., a genus related to the emergence of complicated urinary tract infections, especially for immuno-compromised patients.

Methods: The PNA probe was designed, optimized, tested on representative strains of the genus and other related strains, and, finally, a PNA FISH method was developed for application in urine samples.

Results: The PNA FISH method was optimized, and laboratory testing on representative strains from the Proteus genus and several related bacterial species, showed experimental specificity and sensitivity both of 100% (sensitivity, 95% CI, 81.5–100 and specificity, 95% CI, 91.4–100). Then, the PNA FISH method was adapted to the detection of Proteus in urine. Artificial urine samples were contaminated with decreasing pathogen concentrations and the PNA FISH method was able to detect, in approximately 2 hours, as low as 1 x 10^4 CFU/mL, a concentration considered indicative of infection for catheter associated urinary tract infections (CAUTI's).

Conclusions: PNA FISH is a very sensitive, specific and rapid method for Proteus detection in urine and it could be a reliable alternative to the currently used culture-based techniques as it may avoid the need for empirical antibiotic treatment.
from respiratory and urogenital samples. In this study, three verification parameters of the automated mid throughput QSSP DSP Pathogen Complex system have been investigated: (i) stability of eluates, (ii) repeatability, (iii) pretreatment for viscous samples and lysis of Gram-positive bacteria.

Methods:

i. Urine samples spiked with HIV and CMV were extracted using the QSSP Complex200 protocol. Eluates were incubated at 37°C, 5°C, and −20°C for 0–24, 0–31 d and 0–6 months, respectively. Eluates were analyzed using the artus® CMV RG PCR and the artus HI Virus-1 RG RT-PCR Kits. Both kits are CE-IvD marked for detection of the respective viruses from plasma; during development of the QSSP DSP Pathogen Complex system these assays were used for stability study with eluates generated from urine.

ii. Repeatability of the QSSP DSP Pathogen Complex system was evaluated with urine samples spiked with Chlamydia trachomatis. Three independent 96-sample runs were processed on different days using QSSP Pathogen Complex protocols with 200, 400 and 800 µl sample input (Complex200, Complex400, and Complex800). Eluates were analyzed using the artus C. trachomatis TM PCR Kit.

iii. Sputum spiked with Mycobacterium tuberculosis were liquefied with DTT. Liquefied samples were treated with lysozyme and M. tuberculosis DNA was extracted using the Complex200 protocol as well as the QiAamp® DNA Mini Kit as reference. Eluates were analyzed with the artus M. tuberculosis RG PCR Kit.

Results:

i. Eluate stability was shown for 24 hours at 37°C, 31 days at 5°C, and 6 month at −20°C for both viruses.

ii. Inter-batch variation based on CV-values ranged from 1.43–1.76%CV (Complex200), 0.64–0.98%CV (Complex400) and 1.67–2.24%CV (Complex800). The inter-run variations based on the CT-values were 0.84 (Complex200), 1.38 (Complex400) and 2.68%CV (Complex800).

iii. Extraction with the Complex200 protocol was as efficient as with the QiAamp DNA Mini Kit. Use of different lots of lysozyme had no influence on extraction of M. tuberculosis DNA.

Conclusion: Data shown are part of the verification of the QiAsymphony DSP Pathogen Complex system. The results show that the QSSP DSP Pathogen Complex system serves as reliable tool for extraction of viral RNA & DNA and bacterial DNA from urogenital and respiratory samples.

P1899 Performance of Gen-Probe’s Aptima Combo 2® Assay on the Panther™ system

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Objectives: The objective was to evaluate the analytical performance of the Gen-Probe APTIMA COMBO 2 (AC2) Assay on the PANTHER System, a new, fully automated molecular diagnostic analyzer that is CE marked, but not yet cleared by the FDA in the U.S.

Methods: Sensitivity and specificity were assessed by percent agreement to TIGRIS® DTS® Systems with individual negative urine specimens spiked with various concentrations of Chlamydia trachomatis (CT) and/or Neisseria gonorrhoeae (GC). A panel of 120 CT positives, 120 GC positives, 120 dual positives and 120 negatives were tested on three TIGRIS DTS Systems and three PANTHER Systems using the AC2 Assay.

Precision was evaluated using panels spiked with CT and/or GC rRNA into Swab Transport Media (STM). The panels were run in multiple replicates on three PANTHER Systems over a period of 24 days.

Analytical sensitivity was tested with three sample matrices. These were STM, urine processed with Urine Transport Medium (UTM), and PreservCyt Liquid Pap solution diluted with STM. Matrices were spiked with CT and GC rRNA to create panels at very low target concentrations which were tested on three PANTHER Systems.

Results: Positive agreement between the PANTHER Systems and the TIGRIS DTS Systems was 100%, with a lower 95% confidence interval of 99.5%. Negative percent agreement between the PANTHER Systems and the TIGRIS DTS Systems was 99.9%, with a lower 95% confidence interval of 99.5%. Total precision (%CV) for the AC2 Assay at the analytical sensitivity claim for CT and GC respectively was 6.8% and 3.3% for STM panels, 11.5% and 5.6% for urine panels and 6.9% and 4% for PreservCyt Liquid Pap solution panels.

Panels made at the analytical sensitivity claim for the TIGRIS DTS Systems gave 100% positive results on the PANTHER Systems, with a lower 95% confidence interval of 96%. The analytical sensitivity for the AC2 Assay on the PANTHER Systems was 5 fg/assay (2.5 IFU/mL) for CT and 250 fg/assay (125 CFU/mL) for GC, which is equivalent to the TIGRIS DTS Systems.

Conclusion: The fully automated PANTHER System from Gen-Probe provides precise results comparable to the TIGRIS DTS System when running the AC2 Assay.

P1898 Non-culture diagnosis of neonatal sepsis caused by Streptococcus agalactiae

A. De Zoya*, A. Vickers, K. Edwards, S. Gharbia, A. Underwood, A. Bedfors Russell, P. Heath, E. Galiza, I. Storey, A. Efstratiou (London, Birmingham, UK)

Objectives: Streptococcus agalactiae (Lancefield Group B streptococci (GBS) is a leading cause of sepsis, pneumonia and meningitis in neonates. The incidence of neonatal sepsis in the world ranges from 2–4 cases per thousand live births and early onset GBS infection occurs in approximately 2.6/1000 live births. Diagnosis of GBS infection in the newborn by conventional culture methods is slow and unreliable. Several PCR methods for the detection of GBS from maternal swabs have been developed. In this study we report the use of a real time PCR assay for detection of GBS in blood, CSF and tissue samples of neonates.

Methods: Oligonucleotide primers targeting the cylB gene were used to amplify a 264-bp fragment with the Roche Fast Start DNA Master Hybridisation Probes Mix. Hybridisation probes targeting an internal region of this product were used to confirm the presence of this product.

An internal process control (IPC) in the form of a linearised plasmid containing bacteriophage lambda DNA together with the cylB primer binding sites was also included. A total number of 462 blood culture negative clinical samples were analysed; including 112 clinical samples from 29 babies who had suffered sudden unexpected death in infancy (SUDI); 110 samples (35 EDTA bloods and 75 CSFs) from neonates with probable GBS sepsis or meningitis submitted to the WHO Streptococcus and Diphtheria Reference Unit (WHO SDRU) between 2006 and 2010 and 240 EDTA blood samples from neonates undergoing septic screens submitted from two hospitals in the UK for a separate study in 2009.

Results: Amongst the 112 samples analysed from 29 SUDI babies, eight were positive by PCR and amongst the 110 neonates with probable sepsis or meningitis, 17 were positive by PCR (16 CSF samples and 1 EDTA blood) and amongst the 240 EDTA blood samples from neonates undergoing septic screens, five were positive.

Conclusion: The RT PCR assay is superior to culture methods for detection of GBS from clinical samples. The method is rapid, sensitive, specific and reproducible. The use of a co-amplified IPC in the same reaction tube with the same primers as the target increases throughput and reduces cost. This is an invaluable tool in the rapid diagnosis of neonatal sepsis.
**[P1900] Performance of Gen-Probe’s Aptima® Assays for Chlamydia trachomatis and Neisseria gonorrhoeae on the Panther™ system**

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**Objectives:** The objective was to evaluate the analytical performance of the Gen-Probe APTIMA Chlamydia trachomatis (ACT) and Neisseria gonorrhoeae (AGC) Assays on the PANTHER System, a new, fully automated molecular diagnostic analyzer that is CE marked, but not yet cleared by the FDA in the U.S.

**Methods:** Sensitivity and specificity were assessed by % agreement to TIGRIS® DTS® Systems with individual negative urine specimens spiked with various concentrations of Chlamydia trachomatis (CT) or Neisseria gonorrhoeae (GC). A panel of 120 CT positives, 120 GC positives and 120 negatives were tested on three TIGRIS DTS Systems and three PANTHER Systems using Gen-Probe's ACT and AGC Assays. Precision was evaluated using panels spiked with CT or GC rRNA into Swab Transport Medium (STM). The panels were run in multiple replicates on three PANTHER Systems over a period of 24 days. Analytical sensitivity was tested with three sample matrices. These were STM, urine processed with Urine Transport Medium (UTM), and PreservCyt Liquid Pap solution diluted with STM. Matrices were spiked with CT and GC rRNA to create panels at very low target concentrations which were tested on three PANTHER Systems.

**Results:** Positive agreement between the PANTHER Systems and the TIGRIS DTS Systems was 100%, with a lower 95% confidence interval of 98.9% for the ACT and AGC Assays. Negative percent agreement between the PANTHER Systems and the TIGRIS DTS Systems was 100%, with a lower 95% confidence interval of 98.8% for the ACT and AGC Assays. Total precision (%CV) for the ACT Assay at the analytical sensitivity claim was 5.1% for STM panels, 3.5% for urine panels and 4.8% for PreservCyt Liquid Pap solution panels. Total precision (%CV) for the AGC Assay at the analytical sensitivity was 14.7% for STM panels, 5.1% for urine panels and 15.5% for PreservCyt Liquid Pap solution panels. Panels made at the analytical sensitivity claim for the TIGRIS DTS Systems gave 100% positive results on the PANTHER Systems, with a lower 95% confidence interval of 96%. The analytical sensitivity for the PANTHER Systems was 5 fg/assay (125 CFU/mL) for the ACT Assay and 250 fg/assay (125 CFU/mL) for the AGC Assay, which is equivalent to the TIGRIS DTS Systems.

**Conclusion:** The fully automated PANTHER System from Gen-Probe provides precise results comparable to the TIGRIS DTS System when running the ACT and AGC Assays.

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**[P1901] A novel, automated DNA extraction method from cervical SurePath® specimens**

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**Objectives:** The digene HC2 High-Risk HPV DNA Test® (HC2 test) is validated for use in Europe with cervical specimens collected in SurePath liquid based cytology media. We developed a new automated protocol (based on the QIAxasympm AXpH chemistry) to purify DNA from cervical SurePath® specimens.

In our initial R&D study presented here we investigated the performance of this protocol using (i) individual SurePath specimens and (ii) cell culture samples in SurePath medium spiked with potentially interfering substances for subsequent downstream use with the HC2 test.

**Methods:**

1. **Individual SurePath specimens:** To compare manual and automated sample preparation methods, DNA was isolated on the QIAxasympm AXpH using the newly developed AXpH SurePath protocol, which included a Proteinase K digest with extended lysis and as a reference, the established manual sample conversion method. The QIAxasympm eluates and the corresponding manually converted pellets were tested with HC2. Testing was conducted using 144 residual, de-identified, clinical SurePath samples. Cervical SurePath post-gradient specimens, retained after cytology screening, were used for this study.

   - **Interfering substances:** The impact of potentially interfering substances on the AXpH chemistry were tested by adding varying amounts of blood, lubricating jelly, contraceptive jelly, spermicidal gel, douche, feminine spray, and antifungal cream to low positive cell culture samples in SurePath Medium.

   - **Results:**

     i. **Individual SurePath specimens:** The newly developed QIAxasympm AXpH SurePath protocol resulted in 96% total agreement with the manual conversion method (138 of the 144 results were concordant, 6 were discordant).

     ii. **Interfering substances:** Of the 7 potentially interfering substances, no significant impact on the performance of the SurePath AXpH protocol has been observed. Mean RLU/co values of all low positive samples stayed above the cut-off (>1).

   - **Conclusion:** The newly developed QIAxasympm AXpH SurePath protocol demonstrates the feasibility of DNA extraction from cervical SurePath samples for use in hybrid capture based downstream applications. The performance of the automated DNA extraction method (1 strain not compromised by the presence of the tested potentially inhibitory substances at the tested concentrations). The QIAxasympm applications presented here are for research use only. Not for use in diagnostic procedures.

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**[P1902] Variability of ompC and ompF porin genes in multi-resistant clinical isolates of E. coli**

B. Ruiz del Castillo*, E. Roman, G. Moncaluán, L. Martínez-Martínez (Santander, ES)

**Objectives:** ompF and ompC code for the two major porins of E. coli (Ec). Multiple reports on structure, regulation and function (including their role in low level antibiotic resistance) of both porins in Ec-K12 are available, but there is limited information when considering Ec isolates from clinical sources. The aim of this study was to analyze the genetic variability of both ompC and ompF from multiresistant Ec strains.

**Methods:** Clonally unrelated multiresistant Ec (as defined by Rep-PCR) isolated from different patients, producing extended-spectrum β-lactamases (EcESBL, n = 46) or lacking these enzymes (EcNonESBL, n = 68) were studied. The complete ompC and ompF genes were amplified using specific primers, and their sequences were compared with those corresponding to Ec-K12 and to 24 other Ec strains deposited in Genbank. The conformational changes due to mutations found in our strains were studied by comparison with their corresponding crystal structures (OmpC: PDB ID code 2JIN and OmpF: PDB ID code 2OMP).

**Results:** All isolates included in this study expressed a complete copy ompC. A full-length ompC two isolates presented a truncated ompF gene with a stop codon at positions 127 and 122, respectively, that presumably does not codify for a functional OmpF. Homology analysis showed that the different Ec isolates deposited in Genbank presented 16 variations of ompC and 7 variations ompF. Regarding the clinical isolates of this study there are 46 EcESBL strains with 27 different ompC alleles and 15 ompF different alleles. The 68 EcNonESBL strains presented 16 different OmpS and 11 OmpFs. Among the changes affecting the pore structure of the porins, the more relevant were as follows: In OmpC, K317E (1 strain), R37E (1 strain), A156V/S157R (1 strain) and S157D (1 strain) mutations modify the inner constriction of the pore affecting channel selection; In OmpF, the V337R and Q339P changes (1 strain), located near the periplasmic end of the pore affect pore structure and ion selectivity of the pore.

**Conclusions:** We have observed some variability in the sequences of both ompF and ompC genes from multiresistant clinical isolates of E. coli, in comparison with the corresponding genes in the K12 strain. There was more variability in ompF than in ompC. Some of the observed changes affect pore size and ion selectivity, which may be of importance for antimicrobial resistance modulation.
**P1903** Cloning, soluble expression and immunoreactivity of HIV-1 CRF35_AD p24 protein in fusion with HP-thioredoxin from an Iranian clinical isolate

M. Farajollahi*, Z. Sharifi, F. Gorgipor (Tehran, IR)

There are two distinguished types of HIV virus, HIV-1 and HIV-2, that the first one causes the most prevalent and also lethal cases of the disease. As the p24 antigen is the most immunogenic protein of the virus and appears in blood in the early stages of the disease, antibodies directed to this antigen could be an important marker for early diagnosis of disease in low income settings.

The purpose of this study was to isolate coding sequence of the p24 protein from the sera of clinical samples gathered from Iranian patients, cloning and soluble expression of the p24 coding sequence of the gag gene in E.coli, and also assessing the immunoreactivity of the recombinant protein.

In this study the complete coding sequence of the Gag polyprotein was isolated using the nested RT-PCR from the serum of an infected individual and cloned in PTZ57R (T) vector and the resulting vector named PTZ-gag53-IR and sequenced. Coding sequence of p24 protein was isolated by PCR from the PTZ-gag53-IR vector and was inserted into pET102/D-TOP vector using TOP10 directional cloning strategy, and then expressed in E.Coli in fusion with thioredoxin. Immunoreactivity of the expressed protein was tested using immunoblotting and ELISA using blood samples of confirmed seropositive patients and healthy controls.

Sequencing results showed that the cloned sequence belongs to CRF35_AD subtype of HIV-1 virus which is highly prevalent in Iran and Afghanistan. Western blot and protein assay data confirmed that pET102D-TOP system effectively expressed recombinant p24 protein. Antibody assay using recombinant p24 protein from this isolate showed 100% sensitivity and 100% specificity although more samples need to be tested. This can be attributed to the native conformation of the antigen produced by this method and possible similarity of the protein sequence to those of infecting viruses in the tested population.

**P1904** Campylobacter jejuni cmeA/B transcription in poultry and human

M. Dzieciol*, M. Wagner, I. Hein (Vienna, AT)

**Objectives:** Campylobacter infections are amongst the leading causes of foodborne bacterial gastroenteritis in the developed world. The multidrug efflux pump CmeABC described in Campylobacter jejuni has been shown to be important for bile resistance and for successful colonization of chicken intestines and seems to be involved in resistance to fluoroquinolone and macrolide. The aim of the study was to develop a real-time reverse transcriptase (RT)-PCR method to study transcript levels of the multidrug efflux pump CmeABC in Campylobacter jejuni and to apply it to compare bile salt dependent cmeABC transcription in poultry and human C. jejuni strains.

**Methods and Results:** Ten poultry and eight human C. jejuni strains were cultured microaerobically at 42°C in Mueller-Hinton broth with or without the addition of the bile salts cholate (CA) and taurocholate (TCA). Normalization of real-time RT-PCR data to CFU revealed inconsistent transcription of 16S rRNA and low level transcription of cmeA and cmeB, which increased slightly but significantly upon addition of bile salts. Human C. jejuni strains had lower cmeA and cmeB transcription levels but could be induced in a similar manner as poultry strains.

**Conclusion:** Colony count normalized real-time RT-PCR was useful for determination of the cmeA and cmeB transcription on a cellular basis. The real-time RT-PCR method described is a valuable tool for future studies regarding the activity of the cmeABC operon, provides the opportunity to gather information about the transcript levels on a cellular basis and could easily be adapted to investigate other targets.

**P1905** The impact of rapid identification with PNA FISH® of selected bacteria and yeasts from blood cultures on antibiotic treatment and clinical advice: a cross-sectional study of 123 episodes of bacteraemia in 118 patients

S.H. Hartzen*, H. Colding (Hillerød, Copenhagen, DK)

**Objectives and Background:** Every laboratory is currently faced with a tight budget and has to make priorities concerning which diagnostic tests to use in order to maximize benefits from resource spending. Therefore, whenever a new diagnostic tool is assessed we have to ask the question: are benefits larger than the costs? In this study we tried to investigate whether the results of the PNA FISH® hybridization of positive blood cultures actually had any impact on 1) the antibiotic treatment of the patients and 2) on the clinical advice given to the clinicians concerning further diagnostic procedures and the origin of the infection.

**Methods:** PNA FISH®– short for peptide nucleic acid fluorescence in situ hybridization (AdvanDx, Boston, MA, USA) – uses fluorescent-labeled PNA probes in a 90 min. fluorescence in situ hybridization. Hybridization was performed according to the manufacturer’s manual. The following PNA FISH® probes were used: S. aureus, coagulase-negative staphylococci (CNS), E. faecalis, other enterococci, E. coli (EC), K. pneumoniae, P. aeruginosa, C. albicans, and C. glabrata.

**Design:** Cross-sectional study of 123 episodes of bacteraemia in 118 patients observed in our laboratory during a two months’ period in the autumn of 2010 where PNA FISH® technology was used.

**Results:** The most frequent isolated bacteria from the blood cultures were CNS (34/123) and EC (23/123). PNA FISH® had been performed in 123 out of 142 episodes of bacteraemia in 134 patients during this 2 months’ period. PNA FISH® had not been performed in 19 episodes either because the Gram stain made the test irrelevant or because the blood culture had become positive just before closure of the laboratory, in which case only Gram stain had been performed. The PNA FISH® result influenced the antibiotic treatment of the patient in 26 episodes (21%) and the clinical advice given to clinicians in 24 episodes (20%).

**Conclusion:** In our study of the influence of rapid identification using PNA hybridization we found that the PNA FISH® result only influenced antibiotic treatment and clinical advice in about 20% of the bacteraemia episodes. This result might be explained by such factors as good coverage of the empiric antibiotic treatment chosen, high a priori suspicion of the focus of the infection based on the clinical examination and finally on the distribution of microorganisms.

**P1906** Characterisation of probiotic and antioxidative properties of Nisin A producer L. lactis LL27 strain

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**Objectives:** The aim of this study was to evaluate the probiotic and antioxidative properties of Nisin A producer L. lactis LL27 strain. To determine the probiotic properties of LL27 strain; bile salt, pepsin, pancreatin and acid tolerance were examined. Moreover the ability of L. lactis to inhibit the adhesion of Escherichia coli ETEC and Salmonella Typhimurium SL344 to Caco-2 cells were identified.

**Methods:** L. lactis LL27 strain was grown in GM17 broth media for 18 h at 30 °C and cells were harvested by centrifugation. Cell pellets were washed twice and resuspended in PBS. For acid tolerance experiment, cells were inoculated in pH adjusted PBS and incubated at 30 °C for 4 h. The bacterial cells were inoculated in PBS adjusted to pH 2 and pH 3 containing 3% pepsin, PBS adjusted to pH 8 containing 1% pancreatin and GM17 broth containing 0.3% (w/v) bile salt. Samples were collected at 0h, 1.5 h and 3 h of incubation for the pepsin treatment, and at 0h and 4h of incubation for pancreatin and bile salt resistance assays. PBS at pH 7.2 was used as control. Caco-2 cells were routinely cultured in DMEM. Harvested overnight LL27, E. coli ETEC and S. Typhimurium SL344 cells were diluted in PBS (pH 7.2) to achieve a population to 108 cfu/mL. For adhesion inhibition assay, Caco-2 cells challenged with LL27 before tested pathogens.
Results: *L. lactis* LL27 exhibited high levels of resistance to low pH, bile salts, pepsin and pancreatin, which are conditions characteristic of the gastrointestinal tract environment. Moreover, *L. lactis* LL27 was found to adhere to Caco-2 cells at a rate of 13.1±2.3%. Adhesion inhibition assays showed that adhesion of *Escherichia coli* ETEC and *Salmonella Typhimurium* SL1344 to Caco-2 cells was reduced to 73.6% and 29.5%, respectively, when the cells were previously challenged with *L. lactis* LL27. The hydrophobicity, DPPH radical-scavenging activity and iron-chelating ability of the tested LL27 strain were found to be 83.48%, 75% and 29.5%, respectively. Scanning electron microscopy reveal the LL27 strain to be located inside Caco-2 cells.

Conclusion: The nisin A producer *L. lactis* subsp. *lactis* LL27 strain of food origin possesses the ability to inhibit adhesion of pathogenic microorganisms to Caco-2 cells and also was found to remain at a level of 67.4% (viable count) after 4 hours incubation at pH 3, which indicates that this strain may survive in gastrointestinal tract conditions and can be used as a probiotic strain.

**P1907** CagA status and *VacA* subtypes of *Helicobacter pylori* in relation to gastrointestinal complications in Iranian population

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Objectives: Gastric cancer is the most severe manifestation of chronic *H. pylori* infection. HP-induced carcinogenesis is mediated mostly through two potent polymorphic toxins; *vacA* and *cagA*. The aim of this study was to determine associations between different *cagA/vacA* genotypes and functions with gastrointestinal complications and evaluate whether there is correlation among genotyping, function and gastric malignancy in Iranian patients.

Methods: Collectively, 88 Hp-infected patients were evaluated for the presence and severity of gastric inflammation and atrophy according to OLGAG staining protocol. PCR genotyping of *vacA* s and m1 alleles plus *cagA* 3′ region was performed. Concentrated Culture Filtrates (CCF) of all of the studied strains were collected through liquid culture. HeLa cell line was incubated with CCF up to 24 hours. These strains were also co-cultured with AGS cells for 24 to 48 hours prior to analysis of the hummingbird phenotype. The number of vacuolated cells and the percentage of cells with hummingbird characteristics were determined by inverted light microscopy. Statistical analysis of data was performed using Kruskal-Wallis, Mann-Whitney U test and Bivariate correlations.

Results: Our results demonstrated that Hp strains with *vacA* s1m1 genotype and possess two or more EPIYA-C in their *cagA* 3′ region in comparison with other genotypes were significantly associated with gastric atrophy and inflammation among Iranian population (P < 0.05). The number of vacuolated cells incubated with s1/m1 CCF was significantly greater than that of s1m2 strains (P < 0.001). The hummingbird phenotype formation was significantly prominent in AGS cells incubated with strains having two or more EPIYA-C than all other divergent subtypes (P < 0.05). Functional assays revealed a correlation between hummingbird and vacuolation phenotypes in patients with gastric atrophy (P = 0.01, K = 0.537), intestinal metaplasia (P = 0.02, K = 0.608) and two or more premalignant lesions (P = 0.007, K = 0.894).

Conclusion: This analysis further recommends the application of *cagA* and *vacA* genotyping in population screening approaches, identifying high risk infections with functionally virulent strains, which may lead to the development of gastric premalignant lesions and ultimately gastric cancer.

**P1909** Integrated DNA extraction and purification on an automated microfluidic lab-on-chip from bacterial pathogens causing community-acquired lower respiratory tract infections

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Objective: Community-acquired lower respiratory tract infections (CA-LRTI) can be caused by bacteria or viruses, however, clinical symptoms cannot differentiate these etiologies. The overarching aim of the TheraEDGE project is to develop a point-of-care test (POCT) that will ensure a definitive etiological diagnosis of CA-LRTI within 30 minutes. In this study, we tested the feasibility of automated DNA extraction and purification on an in-house developed lab-on-chip (LOC) prototype utilizing the most common bacterial etiologic agents of CA-LRTI.
Methods: The disposable polymeric LOC comprises multiple reservoirs for buffer storage, two valves for fluidic interactions and a membrane for DNA purification (Figure). 50 μl of 1E±08–09 colony forming units/ml (cfu/ml) of reference strain cultures of Gram-positive (Staphylococcus aureus ATCC 25923 and Streptococcus pneumoniae ATCC 49619) and Gram-negative (Haemophilus influenzae ATCC 10211) bacteria were added to the LOC in the presence of a swab. Broth cultures were pre-treated with a constant and automated flow of enzymatic buffer from its reservoir with 3 minutes incubation at room temperature. Next, the pre-treated culture was treated with a guanidium-based lysis buffer followed by automated addition of ethanol to the lysate. Bacterial lysate was purified by passing through a DNA-binding membrane and DNA was eluted on-chip after a wash step. Experiments were done in quintuplicate on the LOC, and manually replicated in triplicate at the macroscale. DNA was quantified fluorometrically (Qubit dsDNA HS, Invitrogen).

Results: S. aureus yielded significantly higher amounts of DNA (average of 5 experiments: 1.0 μg/ml, 95% CI: 0.7–1.3) when undergoing LOC lysis in comparison to macroscopic DNA extractions (average of 3 experiments: 0.4 μg/ml, 95% CI: 0.3–0.5) (P=0.03, Mann Whitney U test). Yields from S. pneumoniae were also higher when lysed on LOC (average: 0.7 μg/ml, 95% CI: 0.6–0.9) in comparison to macroscale (average: 0.6 μg/ml, 95% CI: 0.2–0.9) but the difference was not significant (P=0.2). Similarly, DNA yields from H. influenzae were also higher on the LOC (average: 0.5 μg/ml, 0.4–0.6) compared to macroscale (average: 0.3 μg/ml, 95% CI: 0.2–0.4) but the difference was again non-significant (P=0.05).

Conclusion: We obtained higher or similar concentrations of purified bacterial DNA on an automated LOC device compared to manual macroscale experiments, which is promising for its application on a POCT.

Figure. Configuration of the lab-on-chip utilized for bacterial DNA extraction and purification

1. Enzymatic pretreatment buffer reservoir
2. Lysis buffer reservoir
3. Enzymolysis reservoir
4. Wash buffer reservoir
5. Elution buffer reservoir
6. DNA purification membrane
7. Elution chamber
8. Waste chamber
9. Valves to establish fluidic interactions

P1910 Reduction of workload of microbial gastroenteritis diagnostics by molecular pre-screening

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Introduction: Fecal samples from patients with diarrhea form a substantial part of the workload in diagnostic microbiology laboratories. The diagnosis of the bacterial and parasitic pathogens in gastrointestinal infections is routinely performed by a combination of culture, microscopy and antigen detection techniques. To reduce the workload of this combined approach, we emphasized that molecular methods can be used to screen the stool samples for positivity. PCR negative stool samples can be discarded from further analysis, leading to a reduction of workload and costs.

Objective: To determine the reduction of workload of gastrointestinal diagnostics at the parasitology and bacteriology labs using a PCR screening method.

Methods: A total of 267 stool samples from patients with gastroenteritis were subjected to PCR screening. From these samples, 89 were send in for parasitology, 97 were send in for bacteriology and 81 were send in for both. A multiplex PCR was used for the gastroenteritis-associated pathogens: Campylobacter (CA), Salmonella (SA), Shigella (SH), Giardia lamblia (GI), Dientamoeba fragilis (DF) Cryptosporidium (CR), and Entamoeba histolytica (Eh). DNA extractions of the samples were carried out on an EasyMag® DNA-extractor (bioMérieux) and three multiplex PCRs per sample were performed on a LightCycler® 480II (Roche Diagnostics).

Results: The prevalence of GI, CR, Eh, SA, and SH by PCR were low: 1.76%, 0.6%, 0.1% and 0.6% respectively. From a total of 170 samples send for parasitology, 33 samples (19.4%) were detected by DF-PCR and microscopy/ELISA, 18 (8.2%) were detected by DF-PCR only resulting in a prevalence of DF by PCR of 27.6%. From the samples send for bacteriology (n=178), 12 (6.7%) were positive by CA-PCR and culture techniques, whereas 22 (12.4%) were positive by CA-PCR only. The discrepancy between PCR and culture techniques for Campylobacter was due to very low bacterial loads (P<0.0001), in contrast to the discrepancy between PCR and microscopy/ELISA for Dientamoeba fragilis (p=0.106).

Conclusion: Without specific anamnestic data, pre-screening of 267 stool samples by PCR resulted in a reduction to 29.4% positive samples only that are eligible for confirmation by microscopy/ELISA for parasitology and a reduction to 19.7% positive samples only that have to be cultured e.g. for antibiotic susceptibility.

P1911 Evaluation of the PNA FISH® technology for yeast identification directly from positive blood cultures. An Italian experience

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Objectives: Yeasts are responsible for the majority of fungemias. Non albicans Candida spp. are frequently difficult to treat because of their sensitivity patterns. The PNA (peptide nucleic acid) FISH (fluorescence in situ hybridization) technology is designed to discriminate among medically important yeasts in blood samples, allowing clinicians to begin a proper empirical therapy.

Aim of the study was to evaluate the performance of PNA-FISH to directly identify yeasts from blood cultures.

Methods: Centers: 14 Microbiology labs of Italian Public Hospitals were included in a network coordinated by the Medical Mycology Committee – Associazione Microbiologi Clinici Italiani.

Samples: 72 blood cultures positive for yeasts at direct Gram stain; 4 blood negative cultures as control.

Technology: Yeast Traffic Light PNA FISH® AdvanDx, Woburn, USA able to discriminate C. albicans/C. parapsilosis, C. tropicalis and C. krusei/C. glabrata.

Reading: Lecture done in double by two different microbiologists and supervision by a third one.

Control: In case of discrepancy, complete reprocessing of the samples.

Results: The agreement between the traditional and the FISH techniques was 94.7% (72/76: 4 neg and 68 pos cultures). Complete agreement was observed for 33 Candida albicans/parapsilosis (C. albicans, 27; C. parapsilosis, 6); 17 Candida glabrata/kruzei (C. glabrata, 16; C. kruzei, 1); 7 Candida tropicalis; 6 C. albicans + C. glabrata. The test was negative in 5 cases of yeasts recognized by Gram staining but not-included in the FISH pattern (C. lusitaniae 2; C. guilliermondii 1; C. neoformans 1; G. capitatum 1).

Discrepancies occurred in 3 samples apparently positive only for C. albicans but mixed (C. albicans + C. parapsilosis, 2; C. albicans + C. glabrata, 1). One case of a mixed culture (C. tropicalis + C. glabrata) at the traditional identification resulted caused by C. glabrata only.

Finally, sensitivity of the FISH technique evaluated for 5 Candida species was 98.6%, and specificity 100%.

Conclusion: Distinguishing which yeast is causing fungemia, and whether the infection is due to multiple species, is important for the selection of antifungal therapy, particularly if results can be quickly transmitted to clinicians. The PNA FISH® testing is a very useful
Comparison of culture with two different qPCR assays for detection of rectovaginal carriage of *Streptococcus agalactiae* N. El Alaia*, I. Tency, G. Claesen, H. Verstraeten, P. Deschaght, E. Decat, G. Santiago, P. Coels, M. Temmerman, M. Vanechouette (Ghent, BE)

**Objectives:** Comparison of culture with two different qPCR assays for detection of Group B streptococci in pregnant women.

**Methods:** For a total of 100 pregnant women at 35–37 weeks of gestation, one rectovaginal ESswab was collected. ESswab was inoculated into Lim broth, incubated for 24 hours and plated onto chromID™ Strepto B agar (ChromAgar). DNA was extracted with the bioMérieux easyMAG platform, either directly from the rectovaginal ESswab or from the Lim broth enrichment culture. Two different qPCR formats were compared, i.e. the hydrolysis probe format (Taqman, Roche), targeting the sip gene and the hybridization probe format (Hybprobe, Roche), targeting the cfb gene.

**Results:** Both qPCR techniques identified 33% of the women as GBS positive. Only one culture-positive sample was qPCR-negative. qPCR, directly on the sample, already significantly increased the number of women found to be GBS positive (27%), compared to culture (22%). Moreover, the sensitivity of qPCR after Lim broth enrichment (33%) was again significantly higher than qPCR after DNA extraction directly from the rectovaginal swabs (27%).

**Conclusions:** When detecting GBS from rectovaginal swabs, the application of qPCR, irrespective of the target (sip or cfb) has increased the number of GBS positive women in comparison with culture.

Rapid identification of yeasts by PNA-FISH in patients with candidaemia

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**Objectives:** To analyse the performance of PNA FISH in detection of *Candida* spp. directly from positive blood culture bottles in a prospective clinical study.

**Methods:** Peptide nucleic acid probe fluorescence in situ hybridization (PNA-FISH) was performed directly from blood culture bottles positive for yeast growth documented by Gram staining. The Yeast Traffic Light probes for *C. albicans/C. parapsilosis*, *C. tropicalis*, and *C. glabrata/C. krusei* were used in the study. Results were compared to identification by conventional methods including the VITEK 2 system.

**Results:** A total of 53 patients with positive blood cultures were tested prospectively. Only one blood culture per patient was included in the study. Using conventional methods 33 *C. albicans*, 11 *C. glabrata*, 2 *C. tropicalis*, 2 *C. parapsilosis* and one each of *C. krusei*, *C. norvegensis*, *C. dubliniensis*, *C. lusitaniae*, and Saccharomyces cerevisiae were detected. The PNA FISH *Candida* assay showed 100% sensitivity and specificity for the *Candida* species included in Yeast Traffic Light kit. For two *C. albicans* tested in the beginning of the study, initial assay results were negative. However, repeated staining of these samples showed that both were positive for the *C. albicans/C. parapsilosis* probes indicating that lack of experience in sample preparation was the underlying factor for the negative result. Four *Candida* species (*C. norvegensis*, *C. dubliniensis*, *C. lusitaniae*, and Saccharomyces cerevisiae) not included in the Yeast Traffic Light probes were negative as expected. PNA-FISH was 24–48 h faster in identification of *Candida* spp. compared to conventional methods. With the help of PNA-FISH 12/53 (22.64%) (11 *C. glabrata* and 1 *C. krusei*) cases could have the possibility to receive early effective treatment 24–48 h earlier with an echinocandin instead of fluconazole.

**Conclusion:** The present study shows that the Yeast Traffic Light PNA FISH assay can provide rapid and reliable identification of five clinically relevant *Candida* species from positive blood cultures. The significantly faster identification of *Candida* spp. by PNA-FISH can be used as a guidance for early, effective antifungal therapy.

Clinical consequences of using PNA-FISH

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**Objectives:** The clinical guidance from microbiologists following blood cultures (BC) with Gram positive cocci in clusters depends on patient history and the pathogen retrieved. If the patient is severely ill or growth in all flasks occurs, initially, *Staphylococcus aureus* and not coagulase negative staphylococci (CoNS) is presumed which affects the clinical guidance. PNA-FISH (peptide nucleic acid fluorescence in situ hybridization) identifies pathogens within 90 min. The clinical consequences of early species detection were studied.

**Methods:** In November 2009, PNA-FISH was implemented for routine examination of Gram positive cocci in clusters directly from positive BC. Records of BC containing Gram positive cocci in clusters from August–October 2009 and 2010 were reviewed, and the guidance given was recorded.

**Results:** We identified 348 BC with Gram positive cocci in clusters (77 *S. aureus* and 248 CoNS). PNA-FISH identified the pathogen of 116 BC; 19 *S. aureus* and 97 CoNS. *S. aureus:* Of 19 PNA-FISH identified BC, 6 (32%) patients had antimicrobial therapy changed from no appropriate empirical coverage to specific narrow spectrum treatment; dicloxacillin. In addition, for 11 (58%) patients the broad spectrum treatment could be changed to specific *S. aureus* treatment at least one day earlier. All patients were eligible for pursuing possible *S. aureus* foci one day ahead of usual practice, e.g. performing echocardiography. Of 58 non-PNA-FISH identified BC, appropriate empirical treatment could have been given to 8 (14%) patients. Also, 36 (62%) patients could have had small spectrum *S. aureus* treatment one day earlier.

CoNS: Of 97 PNA-FISH identified BC, 73 (75%) had clinical consequences by stopping treatment of contaminants, but also by identifying possible catheter-related foci and initiating glycopeptide treatment and/or removal of the catheter one day ahead of usual practice. Of 151 non-PNA-FISH identified BC, 27% could have benefitted from PNA-FISH. Two patients could have avoided being readmitted on the presumption of *S. aureus* being grown.

**Conclusions:** In BC of 77 patients with *S. aureus* bacteremia and 248 patients with CoNS, PNA-FISH was able to improve guidance from clinical microbiologist to clinical doctors in 80% and 46%, respectively. The benefits were early diagnosis, early appropriate treatment, faster guidance to identify the infectious focus and avoiding unnecessary readmissions. Obviously, these improvements in patients care also hold economic benefits.

Integration of affordable molecular detection of β-lactamase genes with the CDS disc diffusion method of antibiotic susceptibility testing

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**Objectives:** To demonstrate integration of affordable molecular detection of different classes of β-lactamase genes with the CDS disc diffusion antimicrobial sensitivity test.

**Methods:** The methods employed are our frontline CDS test method followed by a series of multiplex and single plex real-time PCR tests on either the SmartCycler II or LC480 real-time PCR machines (both available within our Department). The PCR methods employed can be juggled to suit the typical β-lactamase resistance genes seen locally and can include ESBL's TEM, SHV, PER, VEB, GES, metallo-β-lactamases (IMP, VIM, SPM in multiplex format), NDMD, AMI, SIM, GIM, ampC enzymes (Perez Perez & Hansen 2002), oxy genes, CTX-M-1, CTX-M-2, CTX-M-9, CTX-M all groups except CTX-M-1 genes (can be multiplex format) and other combinations of gene targets. Variations in assays (and therefore the target genes) can be made with a simple change of probe label to suit the instruments available. This can be aided by use of real-time assay design software (Biosearch Technologies). A simple
Full automation of nucleic acid extraction with the dual
STARlet (Hamilton)-MagNAPure 96 (Roche) system: a preliminary experience

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Objective: To increase the diagnostic efficiency of our molecular diagnostic platform, where bacteria, viruses, parasites, fungi are detected by real-time PCR (RT-PCR) in clinical samples, the extraction step was fully automated with the STARlet liquid handling (Hamilton) followed by a MagNAPure 96 (Roche) instrument which can extract 96 samples in 1h. In order to assure correct and consistent extraction of DNA or ARN of a variety of pathogens, this new extraction robot was validated in our laboratory using clinical specimens.

Methods: Performance of the automated procedure was assessed by means of i) efficacy of the automation, i.e from the sample tube to DNA elution ii) absence of cross-contamination tested with 4 blood samples with high viral load (BKV, VZV, HSV and PBI9 (109–1012 copies/mL); and iii) the extraction efficiency compared with the MagNAPure LC (Roche) for DNA, and with EasyMag (BioMérieux) for RNA. We tested 24 EDTA blood positive for CMV (n = 18), EBV (n = 3), B19 (n = 2) and T. gondii (n = 1); 13 CSF positive for N. meningitidis (n = 2), S. pneumoniae (n = 2), H. influenzae (n = 1), M. tuberculosis (n = 2), EBV (n = 1), H5N1 (n = 1), JC (n = 1), VZV (n = 1), enterovirus (n = 1), T. gondii (n = 1); 22 respiratory samples positive for ARN viruses and 11 positive for bacteria (Mtbc (n = 8), M. pneumoniae (n = 1), P. pertussis (n = 1); 6 urogenital specimens positive for C. trachomatis (n = 3) or N. gonorrhoeae (n = 3); finally 14 difficult to extract Gram positive isolates and 1 C. albicans were tested. After extraction, nucleic acids were amplified with their specific RT-PCR using the ABI 7900 (Applied Biosystems).

Results: (i) Automation was complete. (ii) No contamination resulted from high copy number specimens. (iii) for 60% of specimens (54/91), the agreement between the new and the previous extraction procedure (difference of copies/ml) was <0.5 log, and for 90% of specimens (78/91) <1 log. Discrepancies were found at the limit of detection due to stochastic distribution.

Conclusions: A fully automated DNA/RNA extraction system was highly effective for different types of specimens and various pathogens. Although the determination of the diagnostic sensitivity and specificity is both labour-intensive and expensive, it remains an essential step for the validation of new instruments.
Fluorescence in situ hybridisation (FISH) probes specific for meningococcal and gonococcal 16S rRNA were used to demonstrate the expression of the different rRNA genes. Interestingly, the clinical isolate described here expresses both N. meningitidis and N. gonorrhoeae 16S rRNAs, as shown by positive FISH signals with both probes. Over 800 published N. meningitidis 16S rDNA sequences were analysed for intra-species variations as well as the presence of base ambiguities that could point to variations within the four genomic operons of a strain. No sequences with the same attributes as described above could be found in the published 16S rDNA sequences, including the APTIMA Assay binding regions.

Conclusions: Based on the results of this study, the specificity of the Gen-Probe APTIMA Assays for the detection of N. gonorrhoeae is confirmed. The N. meningitidis isolate described must have obtained N. gonorrhoeae-specific DNA containing 16S rDNA by interspecies recombination. Based on the ratio of gonococcal to meningococcal sequences, the N. meningitidis strain seems to have replaced one of its four intrinsic 16S rDNA genes with the gonococcal gene. This is an apparently rare example of lateral gene transfer between two species that hardly ever coexist in the same body site.

**Multicentre evaluation of PNA-FISH test for species identification of selected Gram-negative bacilli, Gram-positive cocci and yeasts from blood cultures**

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**Objective:** Time is crucial for the diagnosis of sepsis. Recently, several tools have been proposed to shorten the turn around time (TAT) for blood cultures. The “peptide nucleic acid fluorescence in situ hybridisation test” (PNA FISH, AdvanDx) allows the identification of some microorganisms directly from the positive blood culture bottles in 90 minutes. Aim of this multicenter study was to evaluate the performances of the PNA-FISH in three different Italian hospitals (Bergamo, Modena and Pavia).

**Methods:** Two centers used the BacT/Alert system, one the Bactec system. Four PNA-FISH assays were evaluated: one distinguishes between Staphylococcus aureus and coagulase negative staphylococci (CNS), one between Enterococcus faecalis and E. faecium, one among Escherichia coli, Klebsiella pneumoniae and Pseudomonas aerugiosa, and one among Candida albicans/C. parapsilosis, C. glabrata/C. krusei and C. tropicalis. A total of 384 isolates from different blood culture samples were included in the study. According to the Gram stain results, one drop of a positive blood culture was examined with the appropriate PNA-FISH assay. PNA-FISH slides were read independently by different test operators and blinded to the final results with traditional techniques. **Results:** Phenotypical tests allowed the identification of 196 Gram negative bacilli (GNB): 96 E. coli, 12 K. pneumoniae, 17 P. aeruginosa and 71 GNB belonging to genus or species not detected by PNA-FISH; 114 Gram positive cocci in clusters (20 S. aureus and 94 CNS); 33 Gram positive cocci in pairs and chains (10 E. faecalis, 7 E. faecium and 16 other genus or species); 41 yeasts: 24 C. albicans/C. parapsilosis (respectively, 15 + 9), 11 C. glabrata/C. krusei (respectively, 10 + 1), 4 C. tropicalis, and 2 other genus or species. Except for one strain of CNS not detected by PNA-FISH, all the isolates were correctly identified by the PNA-FISH assays. In two cases PNA-FISH allowed the identification of mixed enterococcal cultures (E. faecium + E. faecalis) reported as E. faecium infections.

**Conclusion:** This multicenter study confirms the excellent agreement between PNA-FISH and standard laboratory techniques in identifying the microorganisms most frequently involved in bloodstream infections. PNA-FISH is quick, reliable and easy to perform, reducing the TAT in septic patients. In particular, genus and species identification allows to better finalize the empirical therapy in these patients.

**Escherichia coli of the phylogenetic group B2 from patients with inflammatory bowel disease compared with uropathogenic B2 isolates**

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**Background:** Escherichia coli of the phylogenetic group B2 with specific virulence genes known from uropathogenic E. coli are found more frequently after faecal culture among ulcerative colitis patients with active disease compared to patients with inactive disease. This study aimed to characterise possible differences in extraintestinal pathogenic E. coli (ExPEC) genes in faecal E. coli iso-lates from different groups of IBD patients and controls compared to uropathogenic E. coli.

**Methods:** The presence of six ExPEC genes, papA, papC, afa, sfa/foc, iut and kpsM was deter-mined. Disease activity was evaluated by disease activity indexes (Harvey Bradshaw Index and Clinical Activity Index); data were analyzed by chi-square test, two-sided.

**Results:** E. coli were isolated from 92 patients and from 18 healthy controls and B2 IBD isolates were compared to 15 uropathogenic B2 isolates. E. coli strains of the phylogenetic group B2 were cultured from 56% of patients with ulcerative colitis, from 42% patients with Crohn’s disease compared to 11% of healthy controls (p < 0.05). Furthermore, when comparing B2 E. coli strains with at least one positive ExPEC gene among different groups, 79% were found positive among active UC, 86% among active CD patients, significantly more than 25% among inactive IBD patients (p < 0.05). When comparing B2 isolates with IBD patients with active disease with positive ExPEC genes the distribution was not different from uropathogenic isolates, in both groups the most frequently detected ExPEC genes were PapC and KPSMII.

**Conclusion:** In conclusion, E. coli of the phylogenetic group B2 with ExPEC genes were found more frequently among IBD patients with active disease compared to patients with inactive disease, and no difference in ExPEC gene distribution could be determined compared to uropathogenic isolates.

**Evaluation of the new GNR Traffic Light™ PNA FISH® fluorescence in situ hybridisation probes for rapid same-day identification of Gram-negative rods from positive blood culture bottles**

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**Objectives:** Early diagnosis of bloodstream infections is essential for rational antibiotic therapy and minimizing morbidity and mortality. Routine identifications of bloodstream organisms must await subculture and are therefore not available on a same day basis. The PNA FISH® (AdvanDx) assay is performed directly on smears from positive blood cultures using fluorescence labeled peptide nucleic acid (PNA) probes targeting rRNA of the microorganisms, and takes 1-2 h for the whole process. In a previous study on direct identification of positive blood cultures we evaluated five sets of PNA FISH® probes including two for
Diversity of emerging Acinetobacter species in a tertiary university hospital in northern Spain

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Objectives: We evaluated the diversity of Acinetobacter species in a tertiary university hospital in northern Spain.

Methods: 86 clinical isolates, one of each being representative of a different Rep-PCR pattern, were selected from a total of 603 isolates reported as Acinetobacter baumannii-calcoaceticus complex during 2004–2008 period using MicroScan systems. A PCR screening using primers for blaOXA-51-like was used for A. baumannii confirmation. For all clinical isolates, Acinetobacter species identification was carried out by amplified rRNA gene restriction analysis (ARDRA) using enzymes CfoI, AluI, Mbol, Rsal, MspI. To differentiate among isolates with the same ARDRA profile, a further restriction analysis was made with enzymes BfaI and BsmI. PCR for blaOXA-23-like, blaOXA-24-like and blaOXA-58-like followed by sequencing was also performed for all isolates.

Results: Only 38 out of 86 isolates (44.2%) were positive for the blaOXA-51-like, representing 515/603 isolates (85.4%). All blaOXA-51-like-positive isolates were identified as A. baumannii (Acb) by ARDRA, profiles 11121 (n = 20), 11123 (n = 15) and 11121-3 (n = 3).

The blaOXA-51 genes found were: blaOXA-64, blaOXA-65, blaOXA-66, blaOXA-67, blaOXA-69, blaOXA-70, blaOXA-71, blaOXA-94, blaOXA-98, blaOXA-106 and blaOXA-117. Other 8 new variants of blaOXA-51-like were also identified. The carbapenem-resistant Acb isolates harbored the gene blaOXA-24 or blaOXA-51-like + ISAba1. A great diversity of Acinetobacter species has been found among blaOXA-51-like-negative isolates: 41.8% (n = 36) were identified as A. genomic species 3 (Ag3), profile 21213, being the second most frequently Acinetobacter species identified (70/603, 11.6%). The gene blaOXA-58 was found in 19 out of 24 isolates of a carbapenem-susceptible Acb clone. Other 7 different species were also identified: A. calcoaceticus (1 pattern/1 isolate, profile 22113), A. phenon 3 (2 patterns/2 isolates, profile 14143), A. phenon 5 (2 patterns/2 isolates, profile 25113), A. genomic species 10 (3 patterns/9 isolates, profile 42123 and BsmI 1), A. genomic species 13BJ (2 patterns/2 isolates, profile 14122 and BfaI 1-2), A. genomic species 13TU (1 pattern/1 isolate, profile 21113) and A. genomic species 16 (1 pattern/1 isolate, profile 12142).

Conclusion: Emerging Acinetobacter species have been identified in our hospital and their actual incidence could be underestimated by using only automated identification systems.

Introduction: Brucellosis remains the commonest anthropozoonosis worldwide and it was a very important health problem in Spain during the past century. Even now the brucellosis still persists in some regions of Spain with a human incidence higher than 3/100,000, where B. melitensis causes more than 99% of the human cases. In these areas, molecular epidemiology could be a very useful tool to know the origin and evolution of Brucella epidemic.

Objective: The aim of this study is to know the genetic variability and to assess the clonal evolution of Brucella melitensis strains isolates from humans from 1974 till 2008 in Spain.

Material and Methods: The genomic profile of 172 strains of Brucella melitensis was determined by Multiple Locus VNTR Analysis-16 (MLVA-16). The most relevant genotypes characterized by MLVA were selected for MLST analysis. The sequence type (STs) and their allelic profiles were compared using the goeBURST algorithm, a globally optimized implementation of the eBURST algorithm.

Results: We have identified five sequence type (ST): ST7, ST8, ST11, ST28 and ST29; and the most prevalent was ST8. Using the microsatellite MLVA analysis we found 7 different genotypes, two of these, genotype 42 and genotype 60 were the most prevalent. The strains corresponding to genotype 42 belong to the ST8 (described by Whatmore et al.), the main group in this study. Among strains belonging to genotype 60 we have detected the presence of two new alleles in the glk and gyrB genes. Due to the emergence of these new alleles we have described two new ST, ST28 and ST29. These two new allelic profiles are related to the main ST found in this study (ST8). Using the algorithm goeBURST, joined all the main clones in one clonal complex (Figure 1). The figure shows that the ST7 belongs to the same clonal complex but its evolutionary line was different.

Conclusions: MLST population analysis shows that all the strains belong to a single clonal complex. The new ST28 and ST29 belong to the same evolutionary line that ST8, the majority in this study (Figure 1), these new STs therefore appear to have evolved from ST8.

Both the ST8 as the new ST28 and ST29 have the same common ancestor, ST11, connected to genotype 51.

Figure 1: Evolutionary relationships among the ST found in this study.
Molecular diagnosis of respiratory tract infections – Upper and lower

**P1924** Molecular differentiation of *H. influenzae* and non-haemolytic *H. haemolyticus* in bronchoalveolar lavage fluids of children with lower respiratory tract infections

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Objectives: Classical microbiological methods do not reliably distinguish non-haemolytic *Haemophilus haemolyticus* from *Haemophilus influenzae*. *H. haemolyticus*, a strict commensal, is frequently misidentified as *H. influenzae* in upper respiratory tract (URT) samples and sputum. In bronchoalveolar lavage fluid (BALF) its presence has never been verified. We used a PCR-based approach to assess the frequency of non-haemolytic *H. haemolyticus* among routinely identified *H. influenzae* isolates from BALF of children with lower respiratory tract infections (LRTI). To enable comparison the misidentification rate was also evaluated among presumed *H. influenzae* strains isolated from tonsillar smears.

Methods: We investigated presumed *H. influenzae* isolates from 152 quantitative BALF cultures obtained by flexible bronchoscopy from children with LRTI and from 33 tonsillar isolates. A 3-step algorithm was used: first, a PCR for the fuculokinase (fucK) gene was performed as fucK is consistently present in *H. influenzae* and absent in *H. haemolyticus*. The fucK-negative isolates underwent further study with 2 PCR reactions using species specific 16S rRNA primers. In case of equivocal results the complete 16S rRNA gene was amplified and sequenced. Confirmed *H. influenzae* isolates were serotyped according to standard procedures.

Results: 145/152 (95.4%) BALF isolates and 25/33 (75.8%) tonsillar isolates were identified as *H. influenzae*. The misidentification rate was significantly higher in tonsillar isolates (24.2%) compared to BALF isolates (4.6%) (P < 0.0001). Quantitative BALF culture did not reach the cut-off level of >10^5 CFU/ml in 3/7 specimens for *H. haemolyticus* and in 4/145 specimens for *H. influenzae*. Taking only significant BALF cultures into account, the misidentification rate was even lower (4/145; 2.8%). All 145 and 25 confirmed *H. influenzae* isolates from BALF and tonsillar specimens, respectively, were shown to be nonencapsulated or non-typeable *H. influenzae* (NTHi) strains.

Conclusion: Using a PCR-based algorithm we demonstrated that the frequency of non-haemolytic *H. haemolyticus* among presumed *H. influenzae* isolates in BALF is very low as compared to the frequency in tonsillar smears. The low prevalence of *H. haemolyticus* in BALF demonstrates the limited risk of contamination by URT secretions and emphasizes its enhanced value as a diagnostic specimen for LRTI. In tonsillar smears. The low prevalence of *H. haemolyticus* in BALF demonstrates the limited risk of contamination by URT secretions and emphasizes its enhanced value as a diagnostic specimen for LRTI.

**P1925** Diagnostic utility of BinaxNow for detection of *S. pneumoniae* among children with suspected IPD

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Objectives: The aim of this study was to evaluate the Binax NOW immunochromatographic pneumococcal antigen test (ICT) (Leti Diagnostico, Spain) for the identification of *S. pneumoniae* in pleural fluids (PF) and cerebrospinal fluids (CSF) from children with suspected invasive pneumococcal disease (IPD). The results were compared with those obtained by PCR.

Methods: A total of 219 samples (12 CSF and 207 PF) were collected from May 2007 to May 2010 by 35 hospitals of Madrid, Spain. The ICT was applied to these samples as recommended by manufacturer for urine and CSF samples. Detection of pneumococcal DNA was performed by real-time PCR assay targeting the autolysin gene (lytA). Those ICT results didn’t agree with lytA PCR results, were confirmed by ply PCR.

Results: A total of 200 samples were tested by both techniques, whereas 19 were not analyzed by ICT because of small sample volume. Results are shown in table. Of the 200 samples, 131 were positive by both ICT and lytA PCR and 37 samples were negative by both techniques. Using the real-time PCR as the gold standard method for detection of *S. pneumoniae*, the sensitivity and specificity of Binax NOW were 88% and 72.5%, respectively.

Conclusions: Laboratory diagnosis of IPD relies on culture based-methods, but detection of pneumococcal antigen by Binax Now is an easy and fast method to identify *S. pneumoniae* in PF and CSF from children with suspected IPD.

**P1926** Implementation validation of a real-time Mycoplasma pneumoniae assay (Diagenode) on the Rotorgene 6000 platform

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Objectives: To validate the Diagenode Mycoplasma pneumoniae (MP) real-time PCR assay (CE-IVD) on the Rotorgene platform (Qiagen) for diagnosis of MP on nasopharyngeal eSwabs and BAL (Broncho/Alveolar Lavage) liquids.

Methods: All validation experiments started with a pre-treatment by incubating a mixture of 250 µl sample and 250 µl of proteinase K (Merck) for 1 hour at 56°C (700rpm). The pre-treatment was followed by an extraction on the Nuclisens EasyMAG (Biomérieux) and afterwards real-time PCR was performed on the Rotorgene using the Quantifast Probe PCR Master Mix (Qiagen). The assay was checked for analytical sensitivity, specificity, accuracy and precision following international publications on molecular validation methods (e.g. Rabenau, 2007).

Results: 1. Analytical sensitivity: To determine the limit of detection (LOD with a 95% hit rate) of the MP kit in combination with the Quantifast Probe PCR Master Mix, a Vircell DNA control (MP type 2) was used. Known concentrations of MP were spiked in eSwab medium and BAL. For eSwabs, the lowest concentration with 20 positive samples (≥LOD) was 3000 copies/ml (mean Ct-value = 37.09). For BAL, the LOD was 7500 copies/ml with a mean Ct-value of 36.59. Based on literature (Nilson et al., 2010) there is a mean bacterial load of 1600 geq/ml throat secretion for hospitalized patients and 170 geq/ml for non-hospitalized patients. The Diagenode assay meets these specifications.

2. Specificity: According to previous validation papers, specificity was sufficiently documented by the manufacturer: the assay was tested on a broad range of viruses and bacteria that are causative agents of respiratory diseases. No cross-reactivity was found with any of these organisms.

3. Accuracy: The 2010 QCMD panel for detection of MP was used to check the accuracy. All samples showed a perfect correlation (see table). The Diagenode MP assay meets the accuracy validation criterion of 100% agreement for positive samples and 90% agreement for weak positive (LOD – 1 log) samples.

4. Precision: Four samples (2 × BAL & 2 × eSwab) were extracted in triplicate to these samples as recommended by manufacturer for urine and CSF samples. Detection of pneumococcal DNA was performed by real-time PCR assay targeting the autolysin gene (lytA). Those ICT results didn’t agree with lytA PCR results, were confirmed by ply PCR.
Detection rates of Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catarrhalis in children with otitis media with effusion and comparison of the results obtained by culture and PCR

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Objectives: To detect the rates of some common bacteria known as pathogens in the middle ear effusions (MEE) of patients with otitis media that underwent tympanostomy.

Methods: Standard and multiplex polymerase chain reaction techniques have been applied to the detection of bacterial DNAs of these three bacteria in MEE specimens and their diagnostic values were evaluated in comparison to conventional culture method accepted as the “gold standard”.

Results: A total of 67 samples from otitis media with effusion (OME) suspected children were included. Two Haemophilus influenzae and two Moraxella catarrhalis were isolated by conventional culture method (6.0%; 4/67). PCR techniques detected DNAs of 3 S. pneumoniae, 6 H. influenzae, 8 M. catarrhalis and 5 mix bacteria. Sensitivity, specificity, positive predictive value and negative predictive value rates of PCR techniques were 100.0%, 71.4%, 18.2% and 100.0%, respectively. The results obtained by using Mc Nemar tests separately for each bacterium showed that there is no significant differences between culture and PCR methods (p > 0.05). Kappa coefficient was calculated as 0.230. So, the agreement between culture and PCR was inadequate.

Conclusion: Although the specificity and positive predictive value are low, at least for detecting the most common fastidious bacteria that lead to OME, PCR methods could be considered as feasible and rapid screening tests.

Weak mutants within first positive Pseudomonas aeruginosa isolates recovered from bronchial secretions of cystic fibrosis patients

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Objective: Hypermutation is a well-recognized mechanism facilitating the development of multi-drug resistance in Pseudomonas aeruginosa from cystic fibrosis (CF) patients with chronic pathogenic colonization. However, its prevalence is not well established in P. aeruginosa isolates recovered during initial colonization stage. The aim of this study was to establish the mutation frequencies of these first positive culture isolates.

Material and Methods: A collection of 37 first positive P. aeruginosa isolates recovered from sputum samples of 23 CF-patients attended at our CF-Unit in our Hospital along the past 15 years (1994–2009) were studied. First colonizer isolates were each new colonizing P. aeruginosa isolate and subsequent isolates with different PFGE from previous ones. Isolates had non-mucoid morphotypes including 60% rough, 15% metallic, 13% enterobacteria-like and 12% small colony variant (SCV). Genetic relationships were analyzed by PFGE-Spel. Mutation frequencies (MF) were determined using 300 mg/l of rifampin as selector agent.

Results: Although no strong mutants were detected, 7/37 (19%) isolates showed increased MF and were classified as weak-mutator (MF range: 3.0×10^8–1.0×10^7) when compared with the rest of isolates (MF range: 3.0×10^8–1.0×10^7). Interestingly, 6/23 CF-patients (26%) had weak mutant strains in early colonization stage. No association between increased MF and particular morphotypes could be established (3 rough, 2 SCV, and one each metallic and enterobacteria-like). PFGE studies confirmed different strains in each patient but also the presence of three different mutator/non-mutator coexisting sub-populations in one patient during first colonization stage (MF: 1.34×10^5; 6.51×10^4; 1.04×10^7).

Conclusion: This study shows a high proportion of P. aeruginosa isolates with increased MF during first colonization state in CF-patients, which represents the highest proportion (19% of weak mutators) described until now. Most importantly, when considering the studied CF-patient population, 26% of them were colonized with these weak mutators. The finding suggests that colonization stress, and not only antibiotic exposure or chronic conditions, favors the implantation of hyper-mutable strains. This data are relevant for the accurate implementation of early antimicrobial treatment since first positive P. aeruginosa culture in CF-patients.

Molecular diagnosis on culture-negative infections

Identification of bacteria by sequencing in routine laboratory practice

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Objectives: Sequencing of 16s RNA gene (1540 pairs of nucleotides) presents the valuable tool in identification of most bacteria. The aim of the present study was to investigate the efficiency of sequencing of 500 pairs of nucleotides and the use of MicroSeq ID 16s rDNA500 Library v2.0 in identification of bacteria in routine laboratory practice.

Methods: Bacterial strains, isolated from patients with nosocomial infections in multidisciplinary medical center in Saint-Petersburg, Russia, were identified routinely with phenotypic microbiological techniques and sequencing (ABI Prism 3130, MicroSeq ID v2.0 Software, MicroSeq ID 16s rDNA500 Library v2.0, Applied Biosystems).

Results: Sequencing of 16sRNA gene was introduced in routine clinical practice of microbiological laboratory in September, 2010. 229 strains, included in the study, were isolated from blood (47), valves (5), bronchoalveolar lavage (20), sputum (35), pleural exudates (5), lung tissue (4), urine (45), wounds (33), oral swabs (23), endometrial brush-biopsy (9), feces (3).

Totally 19 species and 15 genera were registered by routine techniques compared to 57 species of 27 genera, identified by sequencing. S. aureus, S. pneumoniae, E. faecium, P. aeruginosa, and A. baumannii were the only species, correctly identified by routine phenotypic methods in all cases. Errors in identification of E. faecalis occurred in 2.8%, E. coli in 5.9%, S. epidermidis in 18.1%, P. mirabilis and S. maltophilia in 20%, B. thuringiensis in 25.0%, K. pneumoniae in 28.5% of isolates.

Identification by sequencing to species/subspecies with MicroSeq ID 16s rDNA500 Library v2.0 was successful in 97.9% cases. It failed in 3 (7.8%) Streptococcus strains, 1 Paenibacillus strain, and 1 bacterium remained unidentified.

Conclusions: 1. Sequencing of 500 pairs of 16sRNA gene with MicroSeq ID v2.0 Software, MicroSeq ID 16s rDNA 500 Library v2.0, Applied Biosystems, was effective in identification of the majority of bacterial isolates in multidisciplinary medical center 2. The method presents the valuable tool in routine identification of etiologic agents of bloodstream infections, nosocomial pneumonia, infectious endocarditis, when exact data are of high value. 3. The perfect data on the diversity of microbial species, causing nosocomial infections, can be obtained with sequencing, but not routine microbiological methods.

Implementation of a novel multiple-target real-time assay into a clinical microbiology laboratory

L. Thomas*, T. Olma, J. Iredell (New South Wales, AU)

Objectives: Real-time PCR provides clinically important results in a timely manner, but considerable expertise in set-up and interpretation potentially negates the effectiveness of rapid technology. Multiplexed
Molecular diagnosis on culture-negative infections

Tandem-PCR (MT-PCR; AusDiagnostics, Australia) is a real-time assay that allows simultaneous detection and identification of numerous pathogens using a liquid handling robot and analysis software. We incorporated and evaluated this technology within a clinical microbiology laboratory.

Methods:
- The MRS4 assay detects mec, mecA and attB genes. Its suitability as a screening assay for MRS4 directly from patient swabs was assessed and compared to enrichment/culture.
- The vancomycin-resistance 5 assay targets vanA, vanB, E. faecalis, and E. faecium and was evaluated for detection of VRE from enrichment broth.
- The Gram Positive 12 assay targets Gram-positive bacterial genes of clinical significance in blood cultures: pan-Staphylococcus, pan-Streptococcus, pan-Enterococcus, mecA, lyA, gseA, vanA, vanB, E. faecalis ddl, and E. faecium ddl. Blood cultures with Gram positive cocci seen in Gram-stain were extracted and tested.
- The Faecal profile 6 assay detects Campylobacter, Salmonella spp., Shigella spp., and Clostridium difficile toxin B. Stool samples were extracted and compared to either i) culture or ii) TECHLAB C Diff Quick Chek Complete antigen and Toxins A/B kit (Princeton NJ08540).

Conclusion: MT-PCR is a versatile, simple and robust assay that allows simultaneous detection and identification of numerous pathogens using a liquid handling robot and analysis software. These methods were successfully incorporated into routine use and provided significantly more rapid results than culture.

Results:

| Assay                  | Sample source | Template used | Sensitivity | Specificity | PPV % | NPV % |
|------------------------|---------------|---------------|-------------|-------------|-------|-------|
| MRS4                   | nose/throat swab (36) | Direct Swab in PBST | 87         | 99         | 86.6 | 99.4 |
| vancomycin-resistant/ud | rectal swab (254) | Overnight broth in PBST | 93.5        | 97.3       | 92   | 97.9 |
| Gram Positive/12       | Bacter FX blood broth (703) | NA extracted | 96.4        | 98.2       | 95   | -    |
| Faecal profile 6       | Stool (144) | NA extracted | 99         | 99         | 96.4 | 84.6 |

1 Phosphate Buffered Saline
2 NA extracted using EZ Advanced XL and DNA Tissue kit (Cogen)

Objective: Direct sequencing based on the 16S rRNA gene is advancing as a supplement to cultivation for identification of the infectious microorganisms in Danish hospitals. However, direct sequencing is being reported not to be suitable for diagnosis of polymicrobial infections. The aim of this study was to apply different molecular biological methods for identification of microorganisms in patients with bacteremia and compare the findings with cultivation based results.

Methods: Aerobic and anaerobic blood cultivation was performed using the BACTEC 9240 system. Culture-positive samples were subject to further cultivation steps and subsequent cultivation-dependent colony identification as well as different molecular biological analyses. These included direct 16S sequencing using the 3130xl Genetic Analyzer and MicroSeq system (Applied Biosystems), construction of clone libraries and subsequent sequencing and in some cases specific quantitative polymerase chain reaction (qPCR).

Results: Blood cultures from 36 patients were analyzed, and in 20 cases one single bacterium was identified by direct sequencing while construction of clone libraries identified polymicrobial communities. Direct sequencing failed for 10 patients where construction of clone libraries identified polymicrobial communities for all. In 6 cases concordant results were obtained with direct sequencing and the clone library method (both techniques identified one single bacterium in 5 patients and 1 patient had negative results). In Table 1 data are illustrated.

Conclusion: Direct sequencing is a promising method for fast diagnosis of patients with bacteremia, and in most polymicrobial samples the technique was able to identify the dominant microorganism. Establishment of clone libraries was most suitable for detection of polymicrobial infections; however, it is too laborious to be used in routine diagnosis. The results obtained will be compared with the use of the new algorithm RipoSeq (by Insentio) on failed direct sequencing results to evaluate if the success rate can be further increased.

Table 1: Results obtained using direct sequencing, clone libraries and species-specific qPCR. "+" and "-" indicates if the sample was grown under aerobic/anaerobic conditions, respectively.

| Sample | Direct sequencing | Clone libraries | qPCR | Species |
|--------|-------------------|-----------------|-------|--------|
| Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
| Aerobic | Anaerobic | Aerobic | Anaerobic | Aerobic |

E. van Zanten*, A.V. Möller, A.M. Kooistra-Smid (Groningen, NL)

Objective: Bloodstream infections may be life-threatening with pathogenic bacteria as the most common causative agent. Blood culture is the “golden standard” for microbiological diagnosis of these pathogens. Although blood culturing is a sensitive detection method it has limitations. Antibiotic use, fastidious-, anaerobic- or autolytic-growing microorganisms make detection and identification difficult. Our laboratory supplements blood culturing with 16S rDNA sequence analysis in case of a culture-positive result without bacterial growth or fastidious growth or in case the blood culture remains negative and yet strong clinical suspicion of bacteremia persists.

Methods: From January 2008 till September 2010, a total of 329 blood cultures, performed using BacT/ALERT 3D (bioMérieux), were requested for additional 16S rDNA sequence analysis. Of these, 140 yielded fastidious growths after blood culturing was detected culture-positive and 189 yielded no growth or remained negative. DNA isolation on blood cultures was performed using EasyMAG (bioMérieux) and for bacterial isolates PrepMan Ultra (Applied Biosystems) was used. A 1500 bp amplification of the 16S rDNA gene was carried out, of which 500 bp were sequenced.

Results: The 140 fastidious growing samples yielded 99 different species belonging to 51 genera. The most prevalent genera were: Streptococcus spp. (n=31), Clostridium spp. (n=7), Actinomyces spp. (n=6) and Acinetobacter spp. (n=6) and the most prevalent species: Fusobacterium nucleatum (n=5), Streptococcus mitis (n=5), Streptococcus thermophilus (n=4), Streptococcus sanguinis (n=4). In 57 of the 189 blood culture-negative samples 16S rDNA could be detected and sequence analysis performed. Thirty-six different species were identified belonging to 21 genera. The most prevalent genera were: Streptococcus spp. (n=13), Fusobacterium spp. (n=8) and Capnocytophaga spp. (n=5) and the most prevalent species were: Capnocytophaga canimorsus (n=5), Streptococcus pneumoniae (n=4), Aggregatibacter aphrophilus (n=4), Fusobacterium necrophorum (n=4).
Molecular diagnosis of bacterial endocarditis by 16S rDNA

**P1933**

Molecular diagnosis of bacterial endocarditis by 16S rDNA

**Application of PCR, FISH and culture methods in rapid detection of bacterial joint fluid infection**

M. Balanda*, T. Goslowski, A. Chmielarzycz, D. Romaniszyn (Cracow, PL)

**Objectives:** Aim of this project was checking of possibility of applying PCR, FISH and culture methods in fast detection of bacterial joint fluid infection.

**Methods:** Twenty one joint fluid samples were tested. The samples originated from patients after joint orthopedic surgery who were hospitalized in Cracow Rehabilitation Centre, Poland. The samples were tested to presence of bacteria using PCR, FISH and blood culture method in BACTEC machine. In PCR examination were applied two pair of starters: DG74 and 143SA − for Gram positive bacteria; DG74 and ENT183 for Gram negative bacteria which enable detection of infection starters: DG74 and 143SA − for Gram positive bacteria; DG74 and ENT183 for Gram negative bacteria which enable detection of infection.

**Results:** Percentage of positive joint fluid samples was showed in table 1. Sensitivity of PCR let indicate 6×10⁶ CFU/ml bacteria cells in a sample. For FISH method this level of detection was 6×10⁴ CFU/ml. Moreover, time needed to receive results of samples examination using PCR and FISH was about 4–5 hours opposite to culture method when laboratory needs even five days to confirm infection.

**Conclusions:** 1. Molecular methods like PCR and FISH are much more sensitive than culture method in BACTEC system.
2. Applied methodology of preparation of samples lets on indication bacteria cells in joint fluid.
3. PCR and FISH allowed indicate bacteria in joint fluid in a larger number of samples compared to the culture method.
4. Application of PCR and FISH methods considerably shorten waiting time for results even to few hours.

**Molecular characterisation of the viable bacterial content of ascitic fluid from patients with cirrhosis**

G.B. Rogers*, P. Marsh, L. Fraser, J. Collins, K.D. Bruce, M. Wright (London, Southampton, UK)

**Objectives:** Spontaneous bacterial peritonitis (SBP) is a life threatening development in advanced cirrhosis. Accurate diagnosis of SBP can be difficult, with the causative bacteria identified in only a minority of cases using conventional microbiology. This inability to identify a causative agent hinders rational antibiotic therapy and prophylaxis. A number of recent studies have used molecular methods to detect bacteria in ascites. Whilst this generally results in a greater detection of species, the contribution of naked circulating DNA to positive PCR results has raised concern about the clinical insight such approaches provide. We present the application of a PCR-based approach that is specific to structurally intact bacterial cells.

**Methods:** 106 time series ascites samples collected from 30 patients with advanced cirrhosis were analysed by conventional diagnostic microbiology and culture-independent profiling. Diagnostic microbiology was performed on ascites and blood samples in accordance with routine practice. For molecular profiling, cell pellets were resuspended and incubated with propidium monoazide for 30 min prior to exposure to light. DNA was extracted from samples and a region of the 16S ribosomal gene amplified using primers conserved for Bacteria, with species resolved through 16S ribosomal Terminal Restriction Fragment Length Polymorphism (T-RFLP) profiling. Data obtained through culture-based microbiology and molecular analysis were compared.

**Results:** 104 of the samples were culture negative, with Gram positive cocci isolated in two instances. In contrast, 60 of the samples were PCR positive. T-RFLP analysis detected multiple bacterial species present in all samples, with an average of 2.0 separate T-RF bands per sample (± 2.6 std dev). Cell free ascites fluid spiked with heat-killed bacterial cells were PCR negative when PMA treatment was performed. Significant temporal stability was observed across time series samples, with correlations made to clinical parameters and antibiotic therapy.

**Conclusion:** The combination of culture-independent molecular techniques with propidium monoazide pre-treatment may offer a sensitive and specific means to identify causative agents in SBP. The presence of temporarily stable bacterial assemblages in ascites fluid suggests further investigation into their potential role in the development of SBP is warranted.

**Contribution of molecular methods to diagnostics of prosthetic joint infections**

T. Freiberger*, B. Zaloudikova, J. Noval, M. Filipovic, J. Jurankova, E. Nemcova, M. Stechova (Brno, CZ)

**Objectives:** Prosthetic joint replacement has shown great success in restoring the function of joints in persons disabled by arthritis or osteoarthritis. However, in a subgroup of patients, implantation of a foreign body into the joint carries a high risk of bacterial infection that may result in serious complications and even in the patient’s death. Fast and accurate microbiological diagnostics are needed to detect the infectious agent and initiate targeted antibiotic therapy as early as possible. We aimed to compare sensitivity and specificity of molecular and conventional methods of pathogen detection in prosthetic joint infections. (PJI)

**Methods:** One hundred and seventy-five synovial fluids or periprosthetic tissue samples from patients with prosthetic joint failure, examined in our laboratory from 1.2.2004 to 31.5.2008, were included into the study. Clinical and laboratory markers of infection were recorded. PJI was diagnosed if at least one of the following criteria was present: visible synovial fluid purulence at the time of arthroscopy or during surgery; acute inflammation on histopathologic examination of periprosthetic tissue sections; or presence of a sinus tract communicating with the prosthesis. Aseptic failure (AF) was defined as prosthesis failure not meeting the criteria for PJI. Microbiological diagnostics was performed using routine conventional cultures and broad-range 16S rRNA PCR and sequencing.

**Results:** Seventy-eight samples were classified as PJI and remaining 97 samples as AF. Mean CRP levels were 112.9 in PJI group and 23.0 in AF group. Forty-three (55.1%) and 23 (23.7%) samples were culture positive, while 58 (74.4%) and 21 (21.6%) samples were PCR positive in PJI and AF group, respectively. Thus, PCR showed sensitivity 74.4% and specificity 78.4% compared to sensitivity 55.1% and specificity 76.3% for conventional culture techniques. A discordance of both culture and PCR positive results was present in 4/37 (10.9%) PJI and 1/11 (9.1%) AF cases.

**Conclusion:** Broad-range 16S rRNA PCR followed by sequencing seems to be a very useful tool for pathogen detection in PJI and should be routinely used in addition to conventional microbiological techniques. Discordant results of both methods need to be evaluated carefully with respect to other clinical and laboratory signs of infection and possible contamination.

**Molecular mycology**

**P1938** Aetiology of invasive fungal infections: results of a two-centre study in tissue biopsies from patients with proven infection

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**Objectives:** The objectives of this work were to know the frequency of fungal species as cause of IFI in two different Spanish hospitals, a large onco-haematological reference centre (OHRC) and a tertiary general hospital (TGH).

**Methods:** Hospitals sent all paraffin-embedded biopsies taken between 2004 and 2009 which were considered as proven IFI to the Spanish Mycology Reference Laboratory for molecular analysis. Information about results of microbiological cultures and microscopic examination was also collected. A total of 85 biopsies were analyzed, 49 biopsies were sent from the OHRC and 36 from the TGH. Samples were tested by PCR-based methods. When *Aspergillus* or Zygomycetes species were suspected, genus specific PCR-based techniques were used on tissues. A panfungal PCR targeted the Internal Transcriber Spacer (ITS) regions was performed when specific PCRs were negatives. Tests were done in duplicate on different days.

**Results:** Microbiological cultures were done in 68 patients (80%). Cultures were positive in 59% (40/68). The PCR techniques detected fungal DNA in 89.5% (76/85) of biopsies analyzed. In nine cases PCRs were not capable to detect DNA. Regarding species, 67% (51/76) of tissue biopsies with PCR positive had species belonged to *Aspergillus* genera followed by *Candida* 13% (10/76) and Zygomycetes 9% (7/76). Rare fungi represented 11% (8/76). There were significant differences in frequency of species between OHRC and TGH. In OHRC, *Aspergillus* were in 87% of samples (40/46), but *Candida* spp. (4%), Zygomycetes (4%) and uncommon fungi (4%) were uncommonly identified, while in TGH, *Aspergillus* represented 37% (11/30) of IFIs followed by *Candida* 27% (8/30), rare fungi 20% (6/30) and Zygomycetes 17% (5/30).

**Conclusions:** (1) The PCR-based techniques showed higher performance than cultures to identify the IFI's cause. (2) The PCR-based methods could become reference technique for IFI identification. (3) *Aspergillus* spp. were the most common cause of IFI but the percentage of Zygomycetes and rare fungi was around 20% (4) The etiological profile was different depending on the hospital and individualized epidemiological surveys should be compulsory.
**P1939** Development of an inhalation model of invasive pulmonary aspergillosis by *Aspergillus fumigatus* for evaluating the efficacy of detection of non-invasive markers for early diagnosis of infection

Z. Khan* A. Al-Shaikh, S. Ahmad (Safat, KW)

**Objectives:** Invasive pulmonary aspergillosis (IPA) caused by *Aspergillus fumigatus* in immunocompromised patients is often fatal. Early diagnosis of IPA is crucial for selection of appropriate antifungal therapy. We developed an inhalation model of IPA for evaluating the efficacy of four non-invasive markers ([1 → 3]-β-D-glucan (BDG), galactomannan (GM), *A. fumigatus* DNA and 18 kDa secreted antigen (mitogillin, MIT)) in serum and bronchoalveolar lavage (BAL) specimens for early diagnosis of IPA.

**Methods:** GM was detected by sandwich ELISA by using Platelia Aspergillus kit while BDG was detected by using the Fungitell kit. *A. fumigatus* DNA was detected by a sensitive PCR assay and rat 18S rRNA gene amplification was used to rule out presence of PCR inhibitors in clinical specimens. The MIT protein was obtained by cloning *A. fumigatus* MIT gene in *E. coli* and purification of expressed protein to homogeneity. Polyclonal anti-MIT antibodies were raised in rabbits by using purified MIT protein. The presence of MIT protein in serum and BAL specimens was detected by using anti-MIT antibodies. The expression of MIT protein was also studied at different time points during in vitro growth of *A. fumigatus* in liquid cultures by blotting.

**Results:** Inhalation model of IPA was successfully developed and animals were followed for up to five days post-infection. The lungs of all animals yielded *A. fumigatus* in culture. The fungus was demonstrable in KOH-calciofluor mounts of lung tissues of 19 of 24 (79%) animals. Animals were followed for up to five days post-infection. The lung of all animals was 100%, 100% and 96%, respectively. Expression and secretion of MIT protein was obtained by cloning *A. fumigatus* MIT gene in *E. coli* and purification of expressed protein to homogeneity. Polyclonal anti-MIT antibodies were raised in rabbits by using purified MIT protein. The presence of MIT protein in serum and BAL specimens was detected by using anti-MIT antibodies. The expression of MIT protein was also studied at different time points during in vitro growth of *A. fumigatus*.

**Conclusions:** Our data show that BAL is superior than serum and combined detection of BDG, GM and *A. fumigatus* DNA provide a sensitive diagnosis of IPA in a rat model. Although MIT protein was readily detectable in culture filtrate during in vitro growth of *A. fumigatus* in three different media, it was not detected in any of the serum or BAL specimens of infected rats. It appears that MIT is not a good marker for the detection of IPA.

**P1940** Metabolomics of *Scedosporium* and *Aspergillus* by mass spectrometry

V. Hlacíček*, M. Sulc, K. Peslova, M. Volny, P. Nocak, J.-P. Bouchara, M. Strohalm (Prague, CZ, Angers, FR)

**Objectives:** Small molecular weight fungal metabolites are used as biomarkers of scedosporiosis and/or pseudallescheriasis.

**Method:** Mass spectrometry has already been recognized as an indispensable molecular tool for clinical microbiologists particularly useful in high-throughput bacterial, yeast and fungal strain identification. With the growth of microorganism database the major remaining obstacle has been represented by strain mix analysis. In fungal analysis, other obstacles have been the longer cultivation period mandatory for sufficient material production and visualization of fungal ribosomal protein equipment by MALDI typing as well as the interfering presence of signal scavenging molecules (melanin).

**Results:** We have introduced an alternative diagnostic molecular way based on the knowledge of specific fungal metabolite structures. This approach can be used in fungal mixture analysis and represents a similar added value to that we know from proteomics: going from peptide mapping to peptide sequencing gives better identification rates. Strain-specific fungal products of non-ribosomal origin have been documented on a diverse Pseudallescheria boydii sensu lato complex as well as *Aspergillus* genus. Fungal spores have been used as a source of biomarkers useful for early stage diagnosis. Metabolites were found at picogram quantities on the spores, hence within the dynamic range of accurate-mass spectral analysis (FTICR) combined with high performance liquid chromatography. With *Scedosporium apiospermum* spores cyclic peptide pseudacyclin A, tyroscherin and its analogue YM-193221 were detected. The same components have been searched for in sera of patients suffering from scedosporiosis. Pseudacyclin labelled with stable isotopes has been used as an internal standard in the corresponding quantitation experiments. Lipid structures have been found on the spores of *Scedosporium prolificans*. These were sphingolipids, mono- and dihexosylceramides and particularly glycerophosphocholines. The assignment of the corresponding structures has been based both on exact mass, isotopic profile and aid of in-house software Mnass (www.mnass.org) employing the Lipidmaps.org database. *Aspergillus fumigatus* metabolome has also been explored.

**Conclusion:** Implementation of small molecules into current databases could give higher identification rates of pathogenic microbial strains even in mixture strain analysis.

**P1941** Comparison of MycAssay™ *Aspergillus* test with galactomannan detection in bronchoalveolar lavage fluid samples of haematological and ICU patients

R. Torelli, B. Posteraro, E. De Carolis, G. De Pascale, M. Cairi, G. Bello, M. Antonelli, L. Pagano, G. Fadda, M. Sanguinetti* (Rome, IT)

**Objectives:** To assess the reliability of MycAssay™ *Aspergillus* molecular diagnostic test for the detection of *Aspergillus* DNA in BAL samples of patients with suspected invasive aspergillosis (IA).

**Methods:** BAL fluid samples of consecutive patients with suspected invasive fungal infection were collected for analysis using MycXtra™ DNA extraction and MycAssay™ *Aspergillus* real-time PCR. PCR results were evaluated in comparison with clinical diagnosis and conventional diagnostic tests such as culture and galactomannan (GM) detection. Fungal infections were classified according to the EORTC classification on the basis of clinical, microbiological and radiological findings.

**Results:** One hundred and twenty-two samples from 52 haematological and 70 ICU patients were investigated for the presence of *Aspergillus* DNA by the MycAssay™ *Aspergillus* test. Two and 11 patients had proven and probable IA, respectively. All the 13 patients with IA were PCR-assay positive, whereas all the remaining patients without IA (n = 109) had a negative PCR result. Twelve of 13 patients with IA and 3 of 109 patients without IA had a GM index value of BAL fluid ≥1. Interestingly, the last 3 patients were diagnosed with pulmonary fusariosis.

**Conclusions:** The MycAssay™ *Aspergillus* molecular diagnostic test was able to detect *Aspergillus* DNA in BAL fluid samples from patients with IA with high sensitivity and specificity rates and had a superior performance to GM. MycAssay™ *Aspergillus* has the potential to improve the diagnosis of aspergillosis in haematological and ICU patients and warrants incorporation into routine work up to establish its role in the management of these at risk patient groups.

**P1942** Evaluation of MycAssay™ *Aspergillus* molecular diagnostic test and automated EZ1 DNA extraction for detecting *Aspergillus* spp. DNA in bronchoalveolar samples of patients with risk factors for pulmonary invasive aspergillosis

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**Objectives:** Evaluation of a new real time PCR technique (MycAssay™, Mycosonostics) combined with automated DNA extraction (EZ1, Qiagen) to detect *Aspergillus* DNA in BAL samples of patients with pulmonary Aspergillosis (PA) risk factors in comparison with the Aspergillus galactomannan antigen (AGA) detection and conventional culture.
CandidaFinder: an innovative multiplex diagnostics for the Resolution of Candida mesorugosa viable tested by performing spiking experiments of two-fold serial dilutions of Candida determined between 2 and 15 copies per reaction. This demonstrates blood was used for extraction. The sensitivity of all targets could be determined between 2 and 15 copies per reaction was determined, demonstrating that the CandidaFinder shows equal sensitivity to singleplex PCR. This corresponds with 40 to 600 copies per ml blood. As clinical relevant Candida infections are estimated to even occur at concentrations <10 copies per ml the use of larger blood samples for further enrichment of Candida DNA is required.

**Results:** Seven probable cases of PA were diagnosed in 5 lung transplantation and 2 haematologic patients. Conventional culture was positive in 75% of their samples. If detection of AGA in BAL is not taken into account as a micologica criterion, the sensitivity and specificity of this technique — cut off for positive >1(OD) — was 85.7 and 91% respectively. PCR technique with EZ1 DNA extraction system showed a 100% sensitivity and 93.3% specificity. The extraction method did not include any steps for fungal cell wall lysis (for example bead beating) and was rapid (45 mins) giving patient results in ~3 hrs. In four false positive AGA cases, the PCR was negative. During the study period one case of zygomycosis and one of fusariosis were diagnosed by conventional culture and no PCR cross reactions were detected.

**Conclusion:** The MycAssay™ Aspergillus molecular diagnostic test performed in BAL samples combined with the EZ1 automated DNA extraction system provides greater sensitivity and specificity in diagnosing PA than AGA detection or conventional culture. Automated EZ1 DNA extraction system is fast, simple and sensitive, despite including any fungal cell wall disruption step.

**Methods:** 53 BAL samples belonging to 42 patients with PA risk factors were analyzed: 29 solid organ transplants, 9 with haematologic diseases and 4 with primary or secondary immunodeficiencies. After vortexing the sample, three aliquots were prepared to perform: AGA detection (Platelia Aspergillus®; Bio-Rad); conventional culture and automated EZ1 DNA extraction (Qiagen®) following the 2.0 virus protocol with 400 microlitres of sample. All PCR (two per sample) were performed with the same real time thermocycler (Cepheid SmartCycler system). The PA criteria to classify cases of aspergillosis were those of the European Organization for Research and Treatment of Cancer.

**Objectives:** To evaluate the genetic heterogeneity within a Brazilian outbreak of C. rugosa bloodstream infection in ICU patients.

**Methods:** We analysed a total of 11 C. rugosa strains including: 5 clinical isolates, 3 environmental isolates and 3 reference strains (C. rugosa ATCC 10571, C. rugosa CBS 1948 and C. pseudorugosa CBS 10433). All isolates were phenotypically identified with the ID32 C® system. The clinical isolates were typed using RAPD and minisatellites markers with primers B-14 and (GACA)4, respectively. Band patterns were analysed with dendrogram constructions using Gel Compar II version 4.0 Software. All strains were analysed by sequencing of the ITS and D1/D2 regions. A Neighbor-joining phylogenetic tree was constructed with Clustal W software.

**Invasive fungal infections have become major causes of morbidity and mortality in hospitalized patients and Candida species account for 70−80% of fungal bloodstream infections. Candida spp. are the fourth most common cause of nosocomial bloodstream infections. Current tests are mostly targeting regions of rRNA genes, or internal transcribed spacer regions, which can give difficulties in discrimination and may result in cross-reactivity in PCR assays. This results in an urgent need of high discriminatory and accurate detection methods for clinical purposes using novel probes. The aim of this project is to improve molecular diagnostics of detection of Candida spp. using the CandidaFinder assay.**

**CandidaFinder can detect and differentiate 8 Candida species; C. albicans, C. dubliniensis, C. krusei, C. tropicalis, C. parapsilosis, C. glabrata, C. guilliermondii and C. lusitaniae. The specificity of the CandidaFinder was tested by screening 95 Candida clinical isolates and phylogenetically related species, including 31 Candida spp., 4 Pichia spp. and S. cerevisiae. In addition, analytical sensitivity in blood was tested by performing spiking experiments of twofold serial dilutions of viable Candida cells to sterile blood and negative blood cultures. Two different DNA extraction protocols were evaluated to identify the best method for obtaining inhibitor-free nucleic acids from blood culture in combination with the CandidaFinder assay.

The CandidaFinder showed 100% concordance with ITS region sequencing results and AFLP fingerprints. To determine the analytical sensitivity in blood, MolYsis Complex5 (Molzym) and EasyMag (Biomerieux) extraction methods were compared. For both methods 1 ml blood was used for extraction. The sensitivity of all targets could be determined between 2 and 15 copies per reaction. This demonstrates that the CandidaFinder matches the sensitivity of singleplex PCR.

We demonstrated that the CandidaFinder was able to identify 8 Candida spp. in one reaction with a 100% concordance with ITS sequencing and AFLP fingerprinting. For all targets an analytical sensitivity of 2−15 copies per reaction was determined, demonstrating that the CandidaFinder shows equal sensitivity to singleplex PCR. This corresponds with 40 to 600 copies per ml blood. As clinical relevant Candida infections are estimated to even occur at concentrations <10 copies per ml the use of larger blood samples for further enrichment of Candida DNA is required.

**Conclusion:** Therefore we propose the C. rugosa species complex, including the recently described species C. pseudorugosa and C. mesorugosa sp. nov., which comprises the clinical isolates from Brazil.

**Candida rugosa is a yeast which is apparently emerging as a causative agent of invasive infection, specifically in Latin America. This taxon presents high genotypical heterogeneity and may be resistant to various antifungal drugs.**

**Objectives:** To evaluate the genetic heterogeneity within a Brazilian outbreak of C. rugosa bloodstream infection in ICU patients.

**Methods:** We analysed a total of 11 C. rugosa strains including: 5 clinical isolates, 3 environmental isolates and 3 reference strains (C. rugosa ATCC 10571, C. rugosa CBS 1948 and C. pseudorugosa CBS 10433). All isolates were phenotypically identified with the ID32 C® system. The clinical isolates were typed using RAPD and minisatellites markers with primers B-14 and (GACA)4, respectively. Band patterns were analysed with dendrogram constructions using Gel Compar II version 4.0 Software. All strains were analysed by sequencing of the ITS and D1/D2 regions. A Neighbor-joining phylogenetic tree was constructed with Clustal W software.

**Results:** All clinical isolates used in this study were phenotypically identified as C. rugosa. They were phenotypically different from C. rugosa ATCC10571 by RAPD and microsatellite analyses that revealed less than 80% similarity between our clinical isolates and the type strain. Comparing our isolates to the type strains C. rugosa ATCC10571 and C. pseudorugosa CBS10433, ITS sequences showed identity ranging from 89 to 91% and D1/D2 region ranging from 94% to 98%. Surprisingly, very low similarity was observed among ITS and D1/D2 sequences from our clinical isolates and C. rugosa CBS1948. Further BLAST analysis identified this type strain as C. pararugosa. The ITS Neighbor-joining tree confirmed lower relatedness of our strains compared to the C. rugosa type strain ATCC10571 and other C. rugosa and C. pseudorugosa sequences obtained from GenBank.

**Conclusion:** Therefore we propose the C. rugosa species complex, including the recently described species C. pseudorugosa and C. mesorugosa sp. nov., which comprises the clinical isolates from Brazil.
C. dubliniensis was identified as bright green fluorescent cells while negatives were determined by an absence of fluorescence.

Results: C. dubliniensis PNA FISH correctly resolved 18 reports of C. albicans positive results by biochemical testing. All 18 isolates tested produced a positive result with the C. dubliniensis PNA. Testing of known yeast isolates with the C. dubliniensis PNA probe produced an accuracy of 100%.

Conclusion: C. dubliniensis PNA FISH® is a rapid and accurate method for the identification of C. dubliniensis. This method allows for resolution of identification when standard laboratory methods cannot provide a definitive identification.

**P1946 Rapid discrimination between Candida glabrata, Candida nivariensis and Candida bracarensis using a simple-plex PCR**
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Within the Nakaseomyces clade, two new species, C. nivariensis and C. bracarensis, closely related to C. glabrata, have recently been described as emerging fungal pathogens. Differentiation between these species and C. glabrata is important to better understand their epidemiologic and clinical role in candidiases. The specificity of routine identification based on conventional (phenotypic) methods may be insufficient. In this work, we aimed at developing a simple molecular method to differentiate C. glabrata, C. nivariensis and C. bracarensis with no need of sequencing.

Among highly conserved genes between C. glabrata and Saccharomyces cerevisiae that contain intron(s), we found RPL31, a gene coding for a protein component of the large ribosomal subunit. A single primer pair was designed to amplify a nucleotide fragment of 1136 and 780 bp in C. glabrata and S. cerevisiae, respectively. A touch down protocol using these primers was applied to a panel of 43 strains previously identified using sequencing of the ITS region as C. glabrata (n=5), C. bracarensis (n=6), C. nivariensis (n=27) and, S. cerevisiae, Candida albicans, Candida tropicalis, Candida parapsilosis and Candida krusei (one each).

A PCR product differing by size according to the species was obtained for C. glabrata (1100 bp), C. bracarensis (950 bp), C. nivariensis (740 bp) and S. cerevisiae (780 bp), while the amplification failed for the other Candida species tested.

The method appears reliable and fast making it a valuable tool for either retrospectively investigating collection of strains previously identified as C. glabrata or confirmatory identification.

**P1947 Molecular investigation of Fusarium isolates from keratomycosis**
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Objectives: Cases of keratomycosis caused by the members of the genus Fusarium has increased significantly over the past several years in South India. This type of infection often results in blindness due to inappropriate treatment and misidentification of the causal agent. Therefore, identification of these Fusarium strains at species level would be important for epidemiological and clinical purposes as the pathogenic potential and clinical features vary between species, subspecies and species complexes of the genus.

Methods: A total of 55 Fusarium isolates derived from human keratomycosis (Tamil Nadu and Kerala States, India) was subjected for molecular identification and was compared with similar sequences of the internal transcribed spacer region (ITS), b-tubulin (b-tub) and translation elongation factor 1a gene (tef1) available in the NCBI and Fusarium- ID databases. A combined phylogenetic tree was generated using the maximum-likelihood and Bayesian method based on the tef1 and b-tub sequence data. The in vitro antifungal susceptibilities of the isolates to amphotericin B (AMB), itraconazole (ITZ) and terbinafine (TBF) were determined employing the standard Clinical and Laboratory Standards Institute (CLSI) M38-A2 microbroth dilution method.

Results: Most of the isolates were confirmed as F. solani (52; 94.5%) followed by F. incarnatum (2; 3.6%), and F. sporotrichioides (1; 1.8%). The minimal inhibitory concentrations (MICs) of AMB, ITZ and TBF varied between 4 and >64 lg/mL, and based on the susceptibility data, TBF proved to be the most effective antifungal drug in vitro. Most of the isolates examined were resistant or less susceptible to the antifungal agents used in the present investigation. These isolates formed separate clades on the phylogenetic tree.

Conclusion: Our results indicate that F. solani is the main causal agent of human keratomycosis in South India and several strains of this species show resistance or low susceptibility towards the conventional antifungal agents. Based on the MIC data, TBF was found to be an effective drug against Fusarium keratomycosis in South India. This work is part of the INSA-HAS interacademical bilateral project. L.G. holds a postdoctoral fellowship from the Hungarian Scientific Research Fund (OTKA; grant reference number PD 83355).

**P1948 MALDI-TOF mass spectrometry proteome profiling for identification of clinically relevant moulds**
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Objectives: Pediatric populations are currently at high risk for mould opportunistic infections due to the high impact of changes in medical, intensive care and organ transplantation practices. We designed and set-up an analytical MALDI-TOF MS-based assay to identify the most isolated (e.g. Aspergillus spp.) and emerging moulds (e.g. Scedosporium spp.; Geosmithia spp., Fusarium spp. and Penicillium spp.) from a pediatric setting. The aim was achieved by generating a dedicated customized library of reference spectra from solid media cultures which are currently used in daily clinical mould identification (ID).

Methods: Solid cultures of 230 isolates from 18 different mould species (12 Aspergillus spp., Fusarium oxysporum, Penicillium chrysogenum, Geosmithia argillacea, Scedosporium prolificans, Scedosporium apiospermum, Pseudallescheria boydii) were isolated during routine diagnostic efforts. A growth time-course at 30°C was run at 24, 48, 72, 96 and 120 hours for all 18 reference species to assess the best reproducibility. Overlapping spectra were evaluated for variance by principal component clustering analysis (PCA); a first and second derivative spectra generations were produced. ID concordance analysis for the 230 isolates was assessed between MS and phenotypic IDs. When discordant, the ID was achieved by gene sequencing at single and multiple loci level (β-tubulin; ribosomal LSU D2 region; ITS). The time-course at 120 hours was selected for the highest spectra reproducibility and quality. On visual inspection, similarity of spectra produced by different species was recognized and spectra differences between diverse genera were also registered. The spectra reproducibility was proved by high similarity and PCA outcome for all species. After customized database compilation and probing, only the score range 1.74 ≥ ID ≥ 2.72 was used for reliable species ID for pattern matching. Concordance between phenotypic and MT IDs was provided for 200/230 clinical isolates.

Conclusion: Proteome profiling-based assay for mould IDs may be an optimal diagnostic approach to overcome culture-based methods, to encompass multiple fungal genera, to reflect the variety of morph types, growth phase and fungal microenvironment.
**P1949** Fast and sensitive detection of the mycotoxin gliotoxin and related compounds during human aspergillosis

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**Objectives:** Invasive aspergillosis, a nosocomial opportunistic infection caused by pathogens of the genus *Aspergillus* (mainly by *Aspergillus fumigatus*), is associated with significant mortality. This is due in part to the absence of optimal diagnostic modalities, which hamper early disease detection. Gliotoxin (GT) and bis-methylthio-gliotoxin (mGT) are secondary metabolites produced by pathogens of the genus *Aspergillus*. We have previously shown that GT is a virulence factor during experimental aspergillosis induced by *Aspergillus fumigatus* that helps mold to establish infection and survive within the host. Thus, we hypothesise that GT and mGT should be produced during the first stages of infection, representing a potential early diagnosis marker to reveal mold presence. A method to help with early diagnosis of aspergillosis based on the detection of GT and mGT during invasive aspergillosis is presented.

**Methods:** Our detection system is based on quantitative High Performance Thin Layer Chromatography (HPTLC). In order to establish the detection limit of this system, samples of serum from healthy donors are spiked with known amounts of GT and mGT, extracted with dichloromethane and separated on HPTLC plates. GT and mGT are detected and quantified by using fluorescence dyes or under UV light exposure. Subsequently this method is used to analyse GT and mGT presence in serum samples of patients with probable and proven aspergillosis.

**Results:** A simple, accurate, fast and sensitive technique for simultaneous separation, detection and quantification of GT and mGT in human serum (detection limit lower than 5ng/ml) is achieved. Both GT and mGT are found in real samples of serum from patients with probable and proven aspergillosis.

**Conclusions:** Fast and sensitive detection methods of these mycotoxins would be very useful in order to improve the protocols for early diagnosis of aspergillosis in humans and to design therapies that target gliotoxin activity. Our method has been used to detect and quantify gliotoxin and bis(methylthio)gliotoxin presence in human samples (serum) from probable and proven aspergillosis. Because of its simplicity, speed and accuracy, it could be easily implemented in clinical diagnosis laboratories and would complement or even improve current diagnosis methods of aspergillosis.

**P1950** Usefulness of the 1,3-β-D-glucan and antibody germ tube *C. albicans* for early diagnosis of invasive candidiasis in non-neutropenic critically ill adult patients

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**Objective:** Candida colonization is a significant risk for invasive candidiasis. The value of serum levels of 1–3-β-D-glucan and the antibody germ tube *C. albicans* (CAGTA) to diagnose invasive candidiasis and to differentiate infection from *Candida* colonization was evaluated.

**Material and Methods:** We studied 180 non-neutropenic critically ill adult patients with complicated abdominal conditions admitted to 20 Spanish ICUs participating in a prospective study. The following was performed twice a week: surveillance screening cultures, *Candida* score, and measurement of serum levels of 1–3-β-D-glucan (Fungitell® assay) and CAGTA (Vircell® Kit). Patients were grouped into invasive candidiasis, *Candida* colonization, and neither colonized/nor infected. Biomarkers were expressed as the maximum value either before the event (invasive candidiasis) or across all observations. The discriminatory ability of 1–3-β-D-glucan and CAGTA was assessed by the area under the ROC curve (AUC). A prediction model of invasive candidiasis was developed by CART (Classification and Regression Trees) including biomarkers, *Candida* score, and the patient’s clinical status. The concomitant effect of biomarkers was determined by logistic regression analysis.

**Results:** There were 31 (17.2%) patients with invasive candidiasis, 56 (31.1%) colonized, and 93 (51.6%) neither colonized/nor infected. According to CART, the probabilities of invasive candidiasis were 82.3% for the terminal node of 1–3-β-D-glucan >253pg/mL, 14.3% for 1–3-β-D-glucan <253pg/mL and CAGTA negative or limit NP, and 39.3% for 1–3-β-D-glucan <253pg/mL and CAGTA positive. Using this probability as cutoff, the predictive rule for invasive candidiasis showed 86.2% sensitivity and 54.5% specificity, with an AUC of 0.78 (95% CI 0.7–0.8).

**Conclusions:** Serum levels of 1–3-β-D-glucan >253pg/mL associated with positive CAGTA assay accurately differentiated *Candida* colonisation from invasive candidiasis.

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**P1951** Diversity of proteome profiles in neutropenic patients developing versus not developing invasive pulmonary aspergillosis

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**Objectives:** Invasive Pulmonary Aspergillosis (IPA) in neutropenic patients occurs in 5% to 25% of serial aapsia and remains a devastating infection with attributable mortality of 40%. Early diagnosis is essential to improve survival rates. Diagnosis of IPA generally results from a multiparametric approach bringing together clinical, radiological and biological data. However, clinical signs are not specific and biological diagnostic tools still lack sensitivity and specificity. A proteome profiling approach was conducted to (1) study the proteome patterns of neutropenic patients having (or not) developed IPA and (2) identify diagnostic and prognostic biomarkers for IPA.

**Methods:** For each patient enrolled in this study and for each period of aapsia, sera and plasma were prospectively collected 5 days a week and conserved in liquid nitrogen since 2005. A total of 330 patients were included in two hematologon units (Dijon and Strasbourg, France) among whom 37 developed pulmonary invasive fungal infections in at least 1 period of aapsia. For each of these patients, serial sera and plasma were processed for proteome profiling analyzes. A sub proteome was purified from blood samples and analyzed by mass spectrometry (MALDI-TOF). The profiles of peptides mass generated were pre-processed and analyzed by statistical methods aimed at (1) studying the proteome variability in patients during several periods of aapsia (including periods with versus without ongoing aspergillosis), (2) identifying specific markers whose kinetics correlated to the invasive fungal infection process.

**Results:** Preliminary descriptive analyses conducted on 10 patients showed the capacity of peptidomics approaches combined with specific statistical methods (like k-means clustering) to separate different groups of samples in infected patients: (1) samples from the periods of aapsia: without versus with ongoing aspergillosis; (2) in the period of aapsia with ongoing aspergillosis: samples before versus after EORTC diagnosis of aspergillosis.

**Conclusions:** The validation of these preliminary results will be conducted on the remaining samples available.

**P1952** Detection of galactomannan in serum and isolates of *Fusarium* species from patients with proven invasive *Fusarium* infection

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**Objective:** Cross-reactivity of *Fusarium* species and serum from patients with invasive *Fusarium* infection (IFI) is rarely reported in the Galactomannan enzyme immunoassay (GM-EIA). Isolates of *Fusarium* spp. from 2 patients with proven IFI and their serum samples were studied for their ability to produce positive results with GM-EIA.
Methods: *F. oxysporum* was isolated from a dermic biopsy and a liver necropsy from the same patient and *F. proliferatum* was cultured from blood of another patient. *Aspergillus* spp were not isolated from any sample of both patients. Identification was based on morphology criteria and sequencing the transcription elongation factor 1α gene. Serum samples were tested under clinical demand. Isolates preparation was done by two ways: (1) One loop of biomass grown on Sabouraud cloramphenicol media for several days was suspended in 0.5 ml of sterile distilled water and (2) Preparation and dilutions (10⁻²–10⁻⁵) were performed as described by Swanink C (J Clin Microbiol 1997) with some modifications. GM-EIA assay was performed on serum and by duplicate on both isolates preparation, according to the manufacturer’s instructions (Platelia™ Aspergillus GM EIA). *A. fumigatus* was used as positive isolate control. Results were considered positive when serum samples or duplicate isolates samples had a GM index value >0.5.

Results: Serum from patient with invasive *F. oxysporum* infection tested repeatedly positive for galactomannan and 1,3-D-glucan. GM-EIA and *A. fumigatus* isolates tested directly from sterile water suspension, yielded positive results with GM-EIA (F. oxysporum:1.09 and 8.5; *A. fumigatus*:11.5). None isolates but *A. fumigatus*, yielded positive results with the dilution protocol (10⁻² and 10⁻³). Blood isolate and serum samples from patient with invasive *F. proliferatum* infection yielded negative GM-EIA results.

Conclusions: Serum GM-EIA positive results caused by *F. oxysporum* should be added to the list of possible invasive non-*Aspergillus* fungal infection detected by this immunosassay. Differences in results obtained with different in vitro exoantigens preparation make difficult to establish the clinical impact of these results and indicate the need for additional studies.

**P1953** Laboratory diagnosis of invasive aspergillosis in haematological patients: comparison between galactomannan, 1,3-β-D-glucan and *Aspergillus* IgG test

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Objectives: The diagnosis of invasive aspergillosis still remains problematic and challenging despite the availability of different culture and non-culture based tests. We compared the value of galactomannan (GM), 1,3-β-D-glucan (BG) and *Aspergillus* IgG detection in a haematological population.

Methods: In total 38 haematological patients were included. They were divided in 4 groups according to clinical, microbiological and radiological criteria. Group 1 consisted of 14 patients with positive serum GM (1 proven, 9 probable and 4 clinically non-classifiable according to the revised EORTC/MSG criteria 2008 for diagnosis of invasive aspergillosis). In order to be able to compare the diagnostic sensitivity of GM and BG, at least 3 consecutive samples per patient were analyzed with both tests. Group 2 included 12 patients with only BAL GM positivity (8 probable and 4 non-classifiable). In group 3 patients with suggestive clinical criteria, but negative serum and/or BAL GM were included (n=6). In group 4 patients on therapy but without clear laboratory or radiological criteria were listed (n=6). As controls we selected 15 healthy donors and 10 haematological patients without clinical and microbiological criteria of invasive aspergillosis. GM (Platelia Aspergillus ELIA, BioRad), BG (Fungitell®, Associates of Cape Cod) and *Aspergillus* IgG (Platelia *Aspergillus* IgG Ig, BioRad) were performed according to manufacturer’s instructions.

Results: Results are summarized in Table 1. No diagnostic advantage of GM or BG on consecutive clinical samples was found. In case of negative serum GM results also all BG results were negative. Remarkably, in 2 out of 6 patients with suggestive clinical criteria but without any microbiological criteria, *Aspergillus* IgG was positive.

Conclusion: In our study, serum galactomannan and 1,3-β-D-glucan had a comparable sensitivity for diagnosis of invasive aspergillosis in haematology patients. Due to the higher specificity, galactomannan is preferred for routine screening. *Aspergillus* IgG is not a sensitive diagnostic laboratory tool for the diagnosis of invasive aspergillosis as compared to galactomannan and 1,3-β-D-glucan. In the group of patients with suggestive clinical picture but negative serum galactomannan values, *Aspergillus* IgG may represent an added value as the next laboratory parameter to be tested prior more invasive methods of diagnosis.

The study was supported by an unrestricted grant from MSD Belgium.

**P1954** A clinical evaluation of two novel PCR-based analyses for the diagnosis of dermatophytosis in skin and hair

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Objectives: Classic diagnosis of dermatophytosis may require several weeks due to the slow-growing nature of dermatophytes. We implemented a multiple PCR detecting any dermatophyte and *Trichophyton rubrum* specifically in 2006 and subsequently developed and evaluated species specific PCRs for the other common human pathogenic species which were implemented in the spring 2010. Here we present the performance of PCR-based analyses of skin and hair specimens compared with traditional diagnostics.

Methods: Positive rates and species distribution were retrospectively evaluated for samples received in the period from July 15th to October 1st in 2009 (before implementation of new PCR tests) and in 2010 (after implementation of new tests). The diagnostic algorithms for PCR tests were based on epidemiological statistics. Thus, skin samples (other than from feet) were channeled to two specific PCR assays detecting all *Trichophyton* sp., *Microsporum canis/audouinii* and all species within the *T. mentagrophytes* complex (*T. interdigitale, A. vanbreuseghemii, A. benhamiae* and *T. erinacei*). Hair/scalp samples were subjected to *Trichophyton* sp., *M. canis/audouinii, M. canis* and *T. violaceum* specific PCR assays. Samples negative for *T. rubrum* but positive in the pan-dermatophyte PCR were subjected to the *T. mentagrophytes* complex specific PCR.

Results: A total of 3148 and 3266 samples were collected in the 2009 and the 2010 periods respectively (Table). Overall, more than 75% of the samples were subjected to the previously described PCR analysis detecting all dermatophytes and *T. rubrum* specifically and no significant changes between 2009 and 2010 in sample number or species distribution were observed. Samples submitted to the two novel PCR assays constituted 6.5% and a total number of 213 analyses were ordered. Comparing again 2009 and 2010 we demonstrate that except an increase in detected *T. rubrum* the species distribution statistically was not different indicating that the performances of the novel analyses were as intended.

Conclusion: Dermatophytosis is by far the most prevalent fungal infection and many are affected by a fast and confident diagnosis of such infections. Here we demonstrate that the implementation of two novel PCR-based analyses for diagnosis of dermatophytosis in skin and hair potentially can replace culturing and thereby reducing the diagnosis-time from several weeks to a few days.

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**Table 1 (Continuation) 10 – not intact**

| Population | Normal | Disease | GM | GM-EIA | dawn and samp. | M-Ag | S-Ag | S-Ag | S-Ag |
|------------|--------|---------|----|--------|----------------|-----|-----|-----|-----|
| Group 1 (10 samples) | 100% | 100% | 0% | 0% | 0% | 0% | 0% | 0% |
| Group 2 (5 samples) | 100% | 100% | 0% | 0% | 0% | 0% | 0% | 0% |
| Group 3 (8 samples) | 100% | 100% | 0% | 0% | 0% | 0% | 0% | 0% |
| Group 4 (3 samples) | 100% | 100% | 0% | 0% | 0% | 0% | 0% | 0% |
Clinical management challenges in hepatitis in an era of changing treatment options

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Objectives: Hepatitis C virus (HCV) displays considerable levels of nucleotide and amino acid (aa) diversity. 7 genotypes and >80 subtypes have been described worldwide. Genotype and subtype-specific natural polymorphisms were identified within HCV NS3 protease, the target enzyme of future specifically-targeted antiviral therapy for HCV (STAT-C), at aa sites associated with resistance to NS3 protease inhibitors (NS3-Pis) or with compensatory mutations. We analyzed the natural genetic and aa diversity of HCV NS3 protease from NS3-Pis-naïve patients (pts) monitored for their HCV chronic infection in public hospitals of Marseille, southeastern France.

Methods: 328 serum samples collected in 2004–2010 from 302 pts chronically-infected with HCV were analyzed. Amplification/direct sequencing of the HCV NS3 protease gene were performed using house protocols with primers designed using the SVARAP tool. HCV NS3 protease genotypes were determined by phylogenetic analysis. Divergent NS3 sequences were identified based on a nucleotide identity <85% with their best BLAST hit in the NCBI GenBank and/or the Timone sequence databases. Aa diversity at sites associated with resistance to NS3-Pis, compensatory mutations, enhanced viral replication, or corresponding to functionally and/or structurally-critical aa residues was analyzed using Microsoft Excel.

Results: HCV NS3 genotype was 1a in 144 (48%) of 302 pts, 1b in 67 (22%) pts, 2 in 8 (2.6%) patients, 3a in 61 (20%) pts, 4a in 9 (3%) pts, 5 in 1 (0.3%) pt, and 6 in 2 (0.7%) pts. Divergent NS3 sequences were identified in 20 (7%) pts, and included sequences of genotypes 2k and 3h. In NS3 protease from the 302 pts, 8 of the 11 aa positions implicated in resistance to NS3-Pis harbored ≥2 different residues (mean±SD, 2.5±1.4). Drug resistance aa substitutions were observed at 7 positions (V36L/M, T54S, V55A, Q80K, R155K, V158I, D168E/Q) in a number of NS3 sequences ranging from 1 (R155K, V36M) to 9 (V36L). Additionally, ≥2 (3.5±2.2) different aa residues were observed at 13 other relevant positions of NS3 protease.

Conclusions: We identified substantial genetic and aa diversity within NS3 protease from HCV chronically-infected pts followed-up in our institution and who never received NS3-Pis. These data prompt to...
IL28B-gen polymorphic variants as pharmacogenetic markers of HCV treatment response in HIV-infected patients

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Background: HIV infection modifies the natural history of chronic HCV, thus promoting more rapid progression to cirrhosis and end-stage liver disease. The combination of pegylated interferon plus ribavirin permits to achieve sustained HCV clearance in no more than 40% of HIV-HCV co-infected patients. Recent studies showed that genetic variation in the IL28B gene, which encodes IFN-lambda, is associated with HCV good treatment response in individuals infected with HCV genotype 1. These studies have been performed in HCV population, and no data are available regarding HIV-HCV patients.

Methods: A prospective study of consecutive HIV-HCV adult patients in a tertiary teaching hospital in Barcelona (Spain). Blood samples were genotyped for IL28B SNP rs12979860 and rs12980275 using TaqMan SNP Genotyping Assays (Applied Biosystems). All patients provided consent for genetic studies. We studied two SNP’s in a HCV-HIV population and correlated the demographic data and viral response.

Results: 173 HCV-HIV patients were included in demographic analysis. 63% men. Age 46 ± 8.3 (24–65) year. HCV disease evolution 15-6 (0–26) ys. 70% were IDU. 95% follow treatment with ARV drugs actually, wht a mean of 4 different treatments during 10 a mean of years. 82% with undetectable HIV viral load, mean CD4 count 606±321 (89–1843) cell/mm³. Mean basal HCV viral load: 4.200,000 U/mL. Mean basal ALT 56 U/L. Genotype 1 57%. Mean basal fibrosacan were 8.8±5.7 (3.4–29) Kpa. 12% were in cirrhosis. SNP rs12979860 allelic distribution were: CC, CT and TT in 45.5, (protective allele) 44.2 y 10% respectively. SNP rs12980275 Allelic distribution (AA, AG y GG) show the same percentages. Basal protective allele distribution was not correlated wht hepatic cirrhosis and fibrosacan data (Mean of 9 Kpa for protective allele carriers and no protective carriers). Protective allele (CC) was correlated wht HCV genotype (G1:30%, G3: 78.6%, G4 33,3%) (p=0.007). 50 patients were treated wht peg interferon and RBV, wht 43% of sustained viral response. High correlation between CC protective allele and SVR was observed: 83% of patients wht SVR were CC allele carriers (OR: 20).

Conclusions: Our findings suggest that the genetic variation of IL28B have high influence wht SVR. Protective CC carriers was correlated wht G3 HCV but not wht fibrosacan data and cirrhosis stage. Results: 62 hepatitis E were diagnosed during the study period. Three (4.8%) FHE occurred, and two patients died, despite liver transplantation (LT) in one case. Patients were admitted 2–4 weeks after the onset of clinical signs. Their mean age (±SD) was 43±17 years (range, 29–58). Mean ALT was 1716±1167 IU/L, mean bilirubinemia was 450±166 micromol/l, mean PI was 261±8%, and mean factor V level was 0.32±0.12. Case no. 1 had non insulin-dependent diabetes mellitus and was receiving azathioprine for Crohn disease. Transjugular liver biopsy revealed preexisting fibrosis. He developed encephalopathy and acute respiratory deficiency syndrome then died. Cases no. 2 and no. 3 had no underlying chronic liver disease. Both received LT; case no. 3 died 36 days after surgery. All patients were infected with genotype 3f, which is the most commonly found in autochthonous cases in France. None had traveled abroad during the 2–9 weeks incubation period of the disease. Consumption of pig or wild-boar meat was identified in 2 of 3 patients.

Conclusion: Fulminant hepatitis occurred in about 5% of HEV infections diagnosed in our lab and was fatal in 2 of 3 cases (3.2%). Underlying chronic liver disease, which has been associated with poor prognosis, was only evidenced in one patient. These data prompt to improve prevention of HEV infection in our geographical area and raise the question of treatment by ribavirin of such cases.

Seroepidemiology of hepatitis virus infections among men who have sex with men aged between 18 to 40 years in Taiwan

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Objective: Men who have sex with men (MSM) are reportedly at increased risk for hepatitis A virus (HAV), hepatitis B virus (HBV), and hepatitis C virus (HCV) infection than the general population. This study aimed to compare the seroprevalence of hepatitis infections among MSM with and without HIV infection who were aged less than 40 years and to examine the associated factors with hepatitis virus infections.

Methods: From June 2009 to June 2010, clients aged 18–40 years who were MSM and sought voluntary counseling and testing (VCT) for HIV infection at the National Taiwan University Hospital were provided serological tests for HAV, HBV, HCV, and syphilis that is diagnosed by elevated Venereal Disease Research Laboratory/Rapid Plasma Reagin (RPR) titers and positive for Treponema pallidum hemagglutination antibody (TPHA). HIV-infected MSM of the same age group who sought HIV care were tested for the same serological markers.

Results: During the 1-year study period, 690 VCT clients and 438 HIV-infected patients were tested for anti-HAV antibody, HBV surface antigen (HBsAg), and anti-HCV antibody. HIV-infected patients were significantly older than HIV-negative VCT clients (30.5±5.4 vs 25.8±4.7, P < 0.01). The overall seroprevalence for HAV, HBsAg, and HCV in VCT clients were 7.4% (51/690), 6.2% (43/690), 0.4% (3/690), while that in HIV-infected MSM were 15.3% (67/438), 16.4% (70/428), 5.5% (24/433), respectively. In multivariate analysis, age was significantly associated with seropositivity for HAV (OR, 1.15; 95% CI, 1.1 –1.2), HBsAg (OR, 1.12; 95% CI, 1.1–1.2), and HCV (OR, 1.09; 95% CI, 1.0–1.2). HCV infection was independently associated with HBsAg (OR, 1.75; 95% CI, 1.1–2.7), anti-HCV seropositivity (OR, 9.07; 95% CI, 2.6–32.0), and RPR/VDRL titers of 4 or greater within 1 year of testing (OR, 7.03; 95% CI, 4.5–10.98).

Conclusions: Among MSM aged less than 40 years in Taiwan, seroprevalence for HAV, HBsAg, and HCV increased with age, while, because of sharing transmission routes, HIV infection was associated with increased seroprevalence of HAV, HCV, and syphilis.
**P1961** Impact of the use of epoetin β on sustained virological response in patients with hepatitis C virus treated with pegylated interferon alfa-2a plus ribavirin

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Objectives: The aim of this pragmatic (α-risk = 100% and β-risk = 0%, with no test at the end), multicenter, randomized trial was to demonstrate if epoetin β (EPO) could enhance sustained virological response (SVR) by maintaining an optimal dose of ribavirin (R). Two therapeutic strategies were compared: use or non-use of EPO in chronic hepatitis C patients treated with pegylated interferon alfa-2a (IFN) plus R.

Methods: HCV genotype 1, 4 or 5 infected patients on combination therapy (IFN 180 μg weekly and R 1000–1200 mg daily during 48 weeks (W)) were included with a randomization 1:2 in two groups: A, non-use of EPO and B, use of EPO (30,000 U/W) when blood concentration of Hb was below 12 g/dL in men and 11 g/dL in women (anemia and ITT population definition in this study).

Results: 227 patients were randomized (group A: N=78 and group B: N=149). Mean age was 48.8±12.4 years and sex repartition (male/ female) was 61.0% and 39.0%. Distribution of HCV genotypes 1, 4, 5 was 81.9%, 15.5%, 2.6%, respectively. 17.2% of patients had cirrhosis. Baseline mean haemoglobin level was 14.7±1.4 and 14.6±1.3 g/L and baseline mean HCV RNA was 6.0±0.7 and 6.0±0.8 log10 IU/mL in groups A and B, respectively. Among the overall population, 164 (ITT population) presented anaemia (63 in group A and 101 in group B). Among the overall population, 164 (ITT population) presented anaemia (63 in group A and 101 in group B). Globally, the rate of SVR was 52.0% (118/227). Considering the ITT population, the proportion of patients with undetectable HCV RNA was 74.6% and 80.2% at W48, and was 52.4% and 57.4% at W72, in groups A and B, respectively. The rate of relapse six months after the end of treatment was 29.8% and 28.4% in groups A and B, respectively.

Conclusion: The SVR rate in the standard group (non use EPO) is consistent with those of the literature in this population. In this pragmatic trial, the EPO group shows an increased rate of SVR with a γ-risk at 27%. Further analyses are in process to refine these results.

**Clostridium difficile**

**P1962** *Clostridium difficile* in Dutch animals: presence, characteristics and comparison with human isolates

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Objectives: The aim of this study was to determine the presence and molecular diversity of *Clostridium difficile* isolated from Dutch animals and to examine the genetic relatedness by multi-locus variable number of tandem repeats analysis (MLVA) with those causing human infections in the Netherlands.

Methods: Stool samples from various animal species (dogs, cats, horses, pigs, cattle, sheep, and poultry) were examined for the presence of *C. difficile*. Samples were collected from apparently healthy animals at abattoirs (n=400) and from diarrheic animals (n=439) which were obtained from two Dutch veterinary microbiological diagnostic labs. After heat shock treatment, stool samples were directly inoculated onto selective agar media (CCFA and Bazierns medium) as well as after enrichment and incubated for 7 days at 37°C. Confirmation of *C. difficile* suspected colonies was done by PCR for the presence of glutamate dehydrogenase gene. Isolates were characterized by PCR ribotyping and tested for the presence of toxin genes encoding enterotoxin A (tcdA), cytoxin B (tcdB), binary toxin A (cdtA) and binary toxin B (cdtB). Genetic relatedness of 70 isolates was examined by MLVA. For ribotype 012 and 014, MLVA patterns of animal and human strains were compared.

Results: The mean prevalence of *C. difficile* in animal samples was 11.4%, ranging from 1.0% (dairy cattle) to 25.0% (dogs). Of all isolates, 53% were toxigenic. Among 96 animal isolates, a total of 22 different ribotypes were identified, the majority of which were shared by human isolates. Among the toxigenic ribotypes found in animals, 014 was most common (13.5%), followed by, ribotype 012 (10.4%), and 078 (9.4%). Minimum spanning trees based on MLVA patterns show that the distribution of *C. difficile* ribotypes is associated with, but not restricted to distinct animal species. MLVA results from ribotypes 012 and 014 showed no genetic relatedness between human versus animal isolates.

Conclusions: The distribution of *C. difficile* PCR ribotypes follows a host species association, although an overlap is seen among various animal species and human isolates. The limited genetic relatedness found by MLVA of human and animal ribotype 012 and 014 indicate that zoonotic transmission of these types is unlikely to occur on an extensive scale.

**P1963** Validation of the time and temperature relationships of *Clostridium difficile* to healthcare infection-associated bacteria

J. Holton*, N. Lakdawalla (London, UK)

Objective: There is little current evidence that the recommended time/ temperature relationships for laundry as given in UK HSG (95) 18 is efficacious for organisms of particular current concern in nosocomial infection. As the guidance is under review and will be published as HTM 01–04 a study was undertaken to define the laundry conditions that lead to decontamination of contaminated linen.

Methods: Swatches of uniform fabric and bed linen were artificially contaminated with 107 cfu/ml of MRSA; multi-resistant *Acinetobacter* and *Klebsiella*, *E. coli*, vancomycin-resistant *Enterococcus* and *Clostridium difficile* (CD) spores. The swatches were contaminated with 2 mls of the organism suspended in PBS with 3.5% bovine serum albumin, dried at room temperature. The swatches were laundered in an Electrolux FOM 71 machine with a Claris controlled system providing control of the time and temperature of each wash cycle. The wash load was 5Kg. The only variables were the temperature and wash cycle time and the presence or absence of a commercial detergent. The following temp(°C)/time(min)
conditions were tested: 90°C: 71/3; 65/10; 50/10; 40/20; 40/10; 30/20 and 30/10. Post laundry the swatches were massaged for 15 minutes in 10mls of PBS. Total viable counts were performed and the agar plates were incubated aerobically at 37°C and anaerobically for CD.

Results: Our study indicates the mechanical action of washing alone, without detergent, reduced the organisms by a factor of 102−3. At 40°C the organisms were generally reduced by a factor of 103−4, at 65°C for 10 minutes no organisms were isolated. The only exception to this was Cl. difficile where temperature and detergent had no additional effect over and above the mechanical washing action. In the presence of either a biological or non-biological detergent even at a low temperature wash (30°C for 10 minutes) the Gram positive bacteria were eliminated from the material but the Gram negative bacteria were more difficult to remove.

Conclusions: The laundry cycle conditions of time and temperature relationships as given in HSG (95) 18 are sufficient to eliminate hospital pathogens with the exception of Clostridium difficile spores. However, the low numbers of Cl. difficile spores remaining on the linen are unlikely to present a pathogenic risk to patients. The addition of a detergent allows a low temperature wash to effectively remove staphylococci and enterococci.

Survival of Clostridium difficile on bed linen after processing in a commercial continuous batch (tunnel) washer according to HSG(95)18

N. Lakdawalla, R. Morris, J. Holton* (London, UK)

Objectives: Clostridium difficile (CD) is a major healthcare associated problem causing Cl. difficile-associated diarrhoea (CDACD) or life-threatening pseudomembranous colitis. Patients may be colonized by toxigenic CD yet not have symptoms and may act as a source of infection for others. Sheets from these patients will normally be laundered in CBW as the linen will not be categorized as foul or infected. Therefore, it is necessary to assess the efficacy of CBWs in removing CD spores. Our objective was to determine if Clostridium difficile could be detected on bed linen following a commercial washing process in a CBW satisfying HSG(95)18 (71°C, 3 minutes) followed by a steam press. HSG(95)18 is soon to be replaced by new guidance in the form of HTM 01−04, however, the cleaning conditions described here remain relevant to the revised work.

Methods: Six patients were identified as having CDAD with diarrhoea and a positive stool toxin test. Naturally-contaminated linen was marked and processed through a CBW. All wash experiments were carried out using a 14-compartment Voss CBW operating to HSG(95)18 requirements. Washed product was dewatered (hydraulic press) and out using a 14-compartment Voss CBW operating to HSG(95)18 processing in a commercial continuous batch (tunnel) washer according to HSG(95)18.

Results: Our results demonstrated that we could detect 101−103 CFU/100cm2 from the bed linen. The ribotype of the patients’ isolates matched the ribotype isolated post laundry. In one case an additional ribotype was isolated.

Conclusion: These results suggested that a standard industrial washing process and subsequent steam ironing did not fully eradicate CD spores from bed linen. The low numbers of spores are unlikely to present a pathogenic risk to patients. These results also suggested that cross contamination of laundry may occur during the laundry process.

Epidemiologic study of Clostridium difficile culture positive cases using repetitive-sequence based PCR fingerprinting and PCR-ribotyping in 3 tertiary care hospitals in Korea from 2004 to 2010

B. Shin*, S. Moon (Seoul, KR)

Object: Clostridium difficile is a common pathogen of health care associated Clostridium difficile infection (HA-CDI). Community associated CDI (CA-CDI) seems to be increasing as well. Therefore, we need DNA typing techniques which are more discriminatory to differentiate the origin of strains and to control of spreading of C. difficile. Repetitive-sequence based PCR fingerprinting (rep-PCR) is a typing technique that uses primers that target noncoding repetitive sequences interspersed throughout the bacterial genome. Semi-automated rep-PCR (Diversilab, bio-Merieux) could offer good standardization and useful discrimination power.

Method: We performed C. difficile culture and compared rep-PCR with PCR-ribotyping using 378 C. difficile strains isolated in 3 tertiary care hospital in Korea from 2004 to 2010. We also amplified tcdA, tcdB and tcdC genes in 578 C. difficile strains.

Results: The number of tcdA-tcdB+, tcdA-tcdB+ and tcdA-tcdB− strains was 246, 95 and 37. Among them, 20 (5.3%) tcd C negative strains were found and 4 of them were tcdA−tcdB+ strains. In tcdA−tcdB+, tcdA−tcdB+ and tcdA−tcdB− strains, 37, 10 and 15 patterns were identified with rep-PCR and 11, 2 and 8 patterns with PCR-ribotyping, respectively. We observed strain shift among 2004–2005 and 2009–2010 C. difficile strains. Ribotype KS1 and KS2 were the major ribotype, but KS2 and KS3 were the major in 2009–2010. There were common strains among 3 hospitals (KS2 and KS 4, 6 and 8).

Conclusion: We found C. difficile strain shift from 2004/2005 to 2009/2010 and common strains distributed among 3 hospitals. This results suggested that several major strains are distributed and major strain shift were occurred during 5 years in Korea. More diverse patterns were observed in rep-PCR than in PCR-ribotyping and most of tcdA−tcdB+ strains (97%) showed same pattern (PCR-ribotype 017) in PCR-ribotype but more diverse patterns in rep-PCR, which could be useful with the infection control in tcdA−tcdB+ strain prevalent areas. This suggested rep-PCR is a reliable and valuable tool for the discrimination and delineation of C. difficile strains.

Molecular epidemiology of Clostridium difficile at a medical centre in Taiwan: an increasing prevalence of toxin A-positive and toxin B-positive strains

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Objectives: The incidence and severity of Clostridium difficile infections have been alarmingly increasing over the recent years world-wide. However, the recent situation of C. difficile infection in Taiwan was not systematically studied. In the present study, we investigated the prevalence of C. difficile infection at Chang Gung Memorial Hospital (CGMH), a 4,000-bed medical center in Taiwan. The genetic relatedness and the antimicrobial susceptibility to metronidazole and vancomycin were also examined.

Methods: A total of 181 non-repeat C. difficile isolates were collected at CGMH during 2002 and 2007. The presence of toxin genes (tcdA, tcdB, cdA and cdB) was determined by PCR. Further characterization of the toxigenic strains included antimicrobial susceptibility testing by the standard agar dilution method, genotyping by multilocus variable-number tandem-repeat analysis (MLVA) and multilocus sequence typing, tcdC genotyping by PCR and DNA sequencing, and toxinotyping by PCR and restriction-fragment-length polymorphisms.

Results: The isolation rate of C. difficile from stool samples increased from 3% in the year 2002 to 11% in 2007, with a range of 10%. Toxin gene was identified in 110 (60.8%) isolates, including 70 (38.7%) toxin A-positive/toxin B-positive (A+B+) and 40 (22.1%) toxin A-negative/toxin B-positive (A-B+). The annual prevalence rate of
Clostridium difficile

the A+B- strains increased significantly over the study period (p < 0.05). A total of 72 different genotypes were identified by MLVA and 24 isolates (21.8%) formed a major cluster 1, varied by less than one allele. The 24 isolates were all A+B- strains, toxinotype VIII/dCloST-c7, sequence type ST-45. Two isolates were found to be metronidazole-resistant and were genetically related A+B+ strains. Another four were vancomycin-resistant and two of them belonged to the major cluster 1 A+B- strains. Conclusion: C. difficile infections are increasing in Taiwan, accompanied with the persistence of a MLVA cluster 1, A+B- strains and significantly increased in A+B+ strains. Moreover, strains resistant to metronidazole or vancomycin start to emerge. Continuous surveillance is warranted to monitor the C. difficile infections, especially among the hospitalized patients.

P1967 Surveillance analysis of C. difficile genotypes demonstrates decreasing frequencies of 027 infections in a tertiary care hospital

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Objective: Clostridium difficile infections (CDI) have become an emerging hazard in hospitals and also in the community. Since 2007 infections with a "highly virulent" strain identified as ribotype 027, toxinotype III, PFGE NAP1 have been reported. New 027 outbreak strains are characterised by Toxin A, B and binary toxin expression, mutations of a potential toxin repressor gene (tdcC), and increased toxin production in vitro. Severe infections with pandemic 027 strains were identified in Germany for the first time in 2007. Single locus sequence typing of surface layer protein A (slpAST) is a new standardized method for typing of C. difficile outbreak strains which allows comprehensive identification of various outbreaks strains which is important for follow-up studies. Methods: The University of Saarland Hospital is a 1300-bed (150 ICU-beds) tertiary care facility. Consecutive C. difficile isolates (n = 666) of 431 adult patients with diarrhoea were propagated by standard anaerobic stool cultures using CCFA media (CLO-Agar). SlpAST was analyzed as previously described (Kato et al., JMM 2005). The sequence data were analysed by BLAST search and also by sequence alignment with a growing institutional database. In the present single centre retrospective study the appearance of different C. difficile genotypes was analyzed between March 2008 and June 2010. Results: Fluctuating numbers of CDI were found without seasonal clusters (Table 1). At the initial phase of follow-up (October 2008 and June 2009) high numbers of 027 positive patients were identified in our institution (up to 50%). The severe infections were found both in groups with 027 and with other C. difficile genotypes. Since July the frequency of 027 is decreasing with significant differences between the plateau phase in 2008/2009 and the consecutive replacement phase in 2009/2010 (Fisher’s exact test).

Figure 1: Genotypes of C. difficile isolates of adult patients with symptoms of infection

P1968 Clostridium difficile recurrence, alcohol consumption, and the effect of fidaxomicin vs vancomycin

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Objectives: Despite of successful therapy of the initial episode of Clostridium difficile infection (CDI), recurrence rates remain high and represent a major unmet need in the management of CDI. While the effect of alcoholism on the gut flora is not well studied, alcohol abuse (AA) may increase the risk of CDR. We examined the role of AA in CDR.

Methods: We used data from 2 large, randomized phase 3 clinical trials in which adults with CDI symptoms and a positive toxin test received oral fidaxomicin or vancomycin for 10 days. AA was defined as the regular consumption of >2 alcoholic drinks a day (>30g/day). CDR was defined as the reappearance of diarrhoea and a positive stool toxin test within 1 month following completion of therapy. Risk factors for CDR, identified using univariate analyses, were used in a multivariate analysis to adjust the effect of AA on CDR.

Results: Of the total of 737 patients in the per protocol population who were cured of the initial CDI episode and had information on alcohol consumption, 54 (7%) reported AA and 150 had CDR (19%). CDI severity was similar among those with AA (57% with moderate or severe CDI) and those without AA (66%). AA was associated with a higher rate of overall CDR (32% vs 18%; p 0.015), and particularly early CDR (days 1–15 of followup) (26% vs 13%; p 0.008). Highest CDR was observed among those who: are older than 64 years (AA: 41%, no AA: 20%; 0.015), infected by a none-BI/NAP1 strain (35% vs 12%; 0.002), or had a prior CDI episode (AA: 40% vs 26%; 0.483). In multivariate analysis, after adjustment for CDI treatment, albumin level, age, CDI type, prior CDI occurrence, kidney dysfunction and receipt of antibiotics during the followup period, AA remained independently associated with high CDI recurrence (adjusted OR 2.2, 96% CI 1.1–4.5). Compared to vancomycin, fidaxomicin therapy was associated with lower rates of overall CDR (13% vs 25%; <0.001). CDR among those with and without AA, and particularly, early CDR (AA: fidaxomicin 10%, vancomycin 35%; 0.05, no AA: 6% vs 19%; <0.001).

Conclusion: Independent of other risk factors, AA is associated with increased risk of CDR. Recurrence rates are especially high among those with AA and other risk factors for CDR. Fidaxomicin therapy is associated with a substantially lower rate of CDR compared to AA.
Results: Sixty-five clinical isolates were identified from the private (n = 43) and public (n = 22) hospitals. At the private hospital, 32 different REP-PCR patterns were identified among 43 clinical isolates and 19 different REP-PCR patterns were identified among 22 clinical isolates at the public hospital. No single unique strain accounted for more than 10% of isolates at either hospital although 6 REP-PCR patterns from 14 isolates were identified at both hospitals. Eleven of 65 (17%) isolates were a known toxinoype or PFGE pattern. Three of 65 (4.6%) isolates were identified as the NAP-1 strain.

Conclusions: There was no evidence of a clonal epidemic strain at these two hospitals although a number of C. difficile strains were present at both hospitals. These results could have significant implications for community-wide infection control measures to control CDI.

**P1970 C. difficile in patients with chronic heart failure**

N.A. Bylova*, G.P. Arutunov, L.I. Kafarskaya (Moscow, RU)

Patients with III-IV functional classes (FC) of chronic heart failure (CHF) are usually hospitalized for pneumonia or pyelonephritis. This group of patients commonly has 3 or more hospitalizations a year. In all cases they receive a lot of different classes of antibiotics. It is well known, that in case of treating with antibiotics we risk with growth of Clostridium difficile, which associated with a very poor prognosis. Now, from literature we know that usually patients with co-morbidity, who receive antibiotics, develop clostridial infection without morphology changes corresponded to pseudomembranous colitis.

**Aim:** Analysis of changes of large intestinal microflora in patients with III-IV FC of CHF hospitalized due to decompensation of heart failure.

**Methods:** Eighty-four patients with decompensation of III-IV FC CHF of ischemic genesis (group 1) were enrolled in study. All of included patients have more then 3 hospitalizations in previous 12 months. Also forty patients with I-II FC of CHF (group 2) were included into the study. All patients underwent of EF (echoCG); measurement of blood CRP (EIA); endotoxin (LAL); faeces plating on growth media; colonoscopy with fecal biopsy and sample plating on growth media.

**Results:** Patients of group 1 showed a significant (p < 0.05) increase in total number of enterobacteria (1010 CFU/g) as compared to the control group (10 CFU/g). The pool of enterobacteria grew primarily due to growth of E. coli, Klebsiella spp., citrate-assimilating enterobacteria and citrobacters. Total number of Clostridium difficile also were significantly increase in group 1 (107 CFU/g) as compared to the control group (105 CFU/g), p < 0.05. Comparison of the cavity and parietal microflora did not reveal significant differences. Analyses of blood from patients with III-IV FC CHF revealed significantly (p < 0.05) higher levels of endotoxin (LAL test) (1.2±0.02 EU/ml) and CRP (9.02±0.2 UI), then in patients with I-II FC CHF: 0.46±0.02 EU/ml and in healthy volunteers – 0.4±0.01 EU/ml; CRP levels were 2.9±0.11 and 2.2±0.09 UI, respectively. Results of endoscopy show no sing of pseudomembranous colitis.

**Conclusions:** The revealed alterations indicate the presence not only of high levels of Gram-negative flora in the large intestine of patients with III-IV FC CHF, but also high levels of Clostridium difficile, that may cause a poor prognosis of these patients.

**P1971 Demographic and clinical characteristics of patients with Clostridium difficile associated diarrhoea: a retrospective study**

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**Background:** Clostridium difficile (CD) is responsible for up to 30% of cases of diarrhea occurring after antibiotic (AB) treatment: Clostridium difficile associated diarrhoea (CDAD). CD is the leading cause of hospital acquired diarrhea (HA-CDAD), although recently there were reports on an increased incidence of community acquired cases (CA-CDAD). Studies aimed to detect risk factors for the development of CDAD founded in addition to AB use, antisaccids and chemotherapy treatment, together with inflammatory bowel disease (IBD).

**Objectives:**
1. Identify risk factors for development of CA and HA-CDAD.
2. Compare clinical and demographic characteristics, of both groups.
3. Examine the rate of acquisition of CDAD during hospitalization.

**Methods:** All pts with positive CD tests in stool samples identified at Ha’Emek Medical Centre between 1/1/2008 and 31/12/2009 were included. Demographic and clinical data (age, comorbidities, prior AB use, hospitalization, 30 days mortality) were obtained from hospital and community electronic medical records. Pts were classified by acquisition place:
- CA-CDAD: pts without hospitalization within prior month.
- HA-CDAD: pts developing diarrhea 48 hours from admission until a month after discharge.

**Results:** From 1/1/2008 to 31/12 2009, 1101 fekal samples were sent to the microbiology lab: 115 (10.4%) were positive to CD toxin. Fifty five (55.7%) were HA-CDAD (0.065/100 admissions) and 40 (44.3%) CA-CDAD. Pts with HA-CDAD were older than those with CA-CDAD (65.13 ± 51.5y, p < 0.0006). Moreover, bed ridden pts had HA-CDAD more frequently than CA-CDAD (35 vs. 19, p < 0.001). Fifteen pts with HA-CDAD had a nasogastric feeding tube (24.6%), but none of those with CA-CDAD (p < 0.001). Twenty two pts (19%) suffered from IBD; 16 of them (72.7%) CA-CDAD, and only 6 (27.3%) HA-CDAD (p < 0.007). More pts in the HA-CDAD group received AB treatment in the prior 3 months than pts in the CA-CDAD group (77.1% vs. 51.9%, p < 0.005). Mortality (30 days) was higher in the HA-CDAD group (19.7%) than in the CA-CDAD group (5.6%; p < 0.02).

**Conclusions:**
1. Risk factors for HA-CDAD: age, bed ridden pts, feeding by nasogastric tube and previous AB treatment.
2. Risk factors for CA-CDAD: IBD and previous AB treatment.
3. Higher mortality rate was found in HA-CDAD.
4. About half of the cases were CD-CDAD. Community health teams have to be aware of these findings.
5. IBD is a common comorbidity in CA-CDAD. Diarrhea in these pts could be miss-interpreted as a flare-up of the disease.

**P1972 Detection of Clostridium difficile PCR ribotype 027 from hospitalised patients in Sydney, Australia**

PG. Huntington*, A.A. Darbar, G. Kotsios (St Leondards, AU)

**Objectives:** Clostridium difficile is an anaerobic Gram positive rod that is a recognised cause of antimicrobial-associated diarrhoea and pseudomembranous colitis. Hypervirulent strains of C. difficile with deletions in the tcdC gene have been responsible for outbreaks in USA, Canada and UK marked by severe disease and increased mortality. C. difficile BI/NAP1/027 was first reported in Australia in Victoria in 2008 in a returned traveller. Further cases of hypervirulent C. difficile were reported in Victoria during 2010. There have been no previous cases of C. difficile 027 reported in New South Wales, Australia.

**Methods:** From March 2009 to December 2010 all faeces at Pacific Laboratory Medicine Services that requested C. difficile were cultured directly onto selective agar plates and incubated anaerobically for 48 hours. Plates were examined for growth of C. difficile and isolates were collected and stored frozen at −80°C until required. Isolates were characterised by PCR ribotyping using previously published methods and capillary electrophoresis of PCR products. Electrophoresis patterns were compared to known strains of C. difficile ribotype 027.

**Results:** Of 413 isolates available for study, 22 were determined to be ribotype 027. All 22 of these isolates showed an 18 bp deletion at position 330 in the tcdC gene and a single bp deletion at position 117. All ribotype 027 isolates also had moxifloxacin MIC ≥32µg/mL. The 027 isolates were obtained from 21 different patients across four different public healthcare facilities during the 22 month period. The mean age of the patients with ribotype 027 was 82 years old. Infection with C. difficile
Microbial factors in recurrent Clostridium difficile infections in Stockholm, 2010
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Objectives: Clostridium difficile is the major cause of nosocomial diarrhoeal diseases in elderly patients after treatment with antimicrobial agents. About 20% of the patients develop a recurrent infection after the primary episode. The aim of this study was to investigate microbial factors that may contribute to the recurrences of C. difficile infection (CDI).

Methods: Thirty-five patients with a primary CDI were enrolled in this study. Faecal samples were collected at 1, 2, 4, 6 and 12 months after the primary infection and analysed for the presence of C. difficile and toxin B. All isolates were investigated by PCR-rhibotyping and antimicrobial susceptibility.

Results: The mean age of the 35 patients was 74 years and 20 were females and 15 males. Five of the patients died during the follow-up period due to underlying diseases not directly attributed to CDI. Seven patients harboured the same PCR-ribotype of C. difficile up to 6 months after the initial episode without diarrhea. However, from four patients a new C. difficile PCR-ribotype was isolated after a period of negative samples. Patient 1 changed the ribotype from 001 to 020; patient 2 changed from 014 to 026; patient 3 changed from 014 to SE36, and patient 4 changed from 014 to 020 and then to 023. Four patients had PCR-rhibotype 078 in their initial samples and the same isolate was also found in samples 2–4 months after the primary episode. No PCR-ribotype 027 was found in any samples. Fifty-eight isolates were sensitive to metronidazole, vancomycin, tigecycline, fusidic acid, linezolid and fidaxomicin. Two isolates were resistant to moxifloxacin, tetracycline and rifampicin. Eighteen C. difficile isolates were resistant to clindamycin.

Conclusion: Among the 35 patients enrolled in this study, 11 were positive for C. difficile after the initial episode. During the study period, 4/35 (11%) changed the PCR-ribotype. These patients were re-infected with an other C. difficile ribotype. The 078 PCR-ribotype was isolated from four patients. No PCR-ribotype 027 was detected in any samples. All tested strains were sensitive to metronidazole, vancomycin, tigecycline, fusidic acid, linezolid and fidaxomicin and 18 strains (30%) were resistant to clindamycin.

Molecular characterisation and antimicrobial susceptibility in clinical isolates of Clostridium difficile in a university hospital, Split, Croatia
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Objective: Clostridium difficile is the leading cause of nosocomial diarrhea. However, data on C. difficile infection (CDI) in Croatia are still scarce.

The aim of this study was to perform a phenotypic and molecular characterization of toxigenic clinical isolates collected over six months.

Methods: The period of study was between January and June 2010. A total of 97 faecal specimens from patients suspected of having CDI were submitted to the laboratory and tested for C. difficile toxins A and B by Immunoccard Toxins A/B, Meridian. All toxigenic samples were cultured on commercial selective C. difficile agar plates (CLO; bioMérieux) after alcohol shock. Strains were identified according to cultural morphology and confirmed as C. difficile by commercial biochemical identification system (RAPID ANA; bioMérieux and Vitec 2 Compact ANA Card; bioMérieux).

MIC values for moxifloxacin (MX), ciprofloxacin (CI), gatifloxacin (GA), levofloxacin (LE), erythromycin (EM), clindamycin (CM), meropenem (MEM), metronidazole (MZ), vancomycin (VA) and piperacillin/tazobactam (PTc) were determined by E test (AB biodisk) on Brucella agar plates containing vitamin K, haemin and 5% defibrinated sheep red blood cells, according to the manufacturer’s instructions. Isolates were characterized by PCR-rhibotyping using the method of Bidet et al. (FERMS Microbiol. Lett. 1999. 175: 261–266). DNA fingerprinting patterns produced by PCR-rhibotyping were compared to a collection of the predominant PCR-ribotypes circulating in Europe (gently provided by ECDC).

Results: Among tested samples, 13.4% were toxins positive and 13 C. difficile strains were isolated. All isolates were susceptible to MZ, VA, MEM and PTc. One strain exhibited multiresistance (MR) to EM, CM and to all tested fluoroquinolones. Six strains were resistant and one was intermediate to CT. Five strains were intermediate to LE.

The strains CDI testing to 8 different PCR ribotypes. Two strains were ribotype 003, two ribotype 012, one ribotype 150, while eight strains showed a pattern not comparable with the reference strains (including the MR strain).
**Conclusion:** The first characterization of *C. difficile* in South Croatia shows the presence of an heterogeneous population and high resistance to fluoroquinolones. We found no clear association between antimicrobial profiles and ribotyping. Further studies of incidence of CDI including patients clinical data and antibiotic usage data in the hospital wards are warranted.

**Risk factors for recurrence of *Clostridium difficile* infection: impact of stool vancomycin-resistant enterococci colonisation**

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**Objectives:** Recurrent *Clostridium difficile* infection (CDI) is one of the most difficult problems in healthcare infection control. We evaluated the risk factors associated with recurrence in patients with CDI.

**Methods:** A retrospective cohort study was performed at Pusan National University Yangsan Hospital from December 2008 through October 2010. Variables associated with clinical findings, treatment and laboratory findings were analyzed to identify risk factors related to CDI recurrence.

**Results:** A total of 84 patients were included in this study. Recurrence occurred 14.1% (11) cases. Stool vancomycin-resistant enterococci (VRE) colonization (p = 0.006), number of exposed antibiotics ≥3 (p = 0.009), continued use of previous antibiotics (p = 0.05) and lower hemoglobin level (p = 0.025) were more frequent in recurrent group. On multivariate analysis, stool VRE colonization was independently associated with recurrence of CDI (OR 14.519, 95% CI 1.157–182.229, P = 0.038).

**Conclusion:** These data suggest that stool VRE colonization is a significant risk factor for CDI recurrence. Preventing the initial acquisition of VRE and *C. difficile*, both in terms of VRE and CDI control, should be emphasized.

**Diagnosis of *C. difficile* infections**

**P1977** Analytical comparison of the BD GenOhm Cdiff assay to the Ridascreen *C. difficile* toxin A/B ELISA for detection of *C. difficile* associated disease

I. Müller*, O. Coste, K. Schmidt, K.P. Hunfeld (Frankfurt/Main, DE)

Rapid detection of toxin producing *Clostridium difficile* is essential in CDAD patients. This study compares the performance of a new PCR assay (BD GenOhm Cdiff Assay) and a conventional ELISA (Ridascreen *C. difficile* Toxin A/B) for rapid detection of toxin-producing *C. difficile* strains directly from stool.

531 stool samples obtained from 411 patients with suspected CDAD from a single centre were analysed by real-time PCR and ELISA. Only samples in accordance with the criteria for diarrheal stool (Bristol Score) were eligible for the study. Anaerobic cultures were also performed (George’s agar). The study was approved by the institutional review board.

Using the ELISA as an analytical gold standard PCR yielded 106 concordant positive results (93.8%) and 352 concordant negative results (84.2%). In 73 stool samples both assays showed discrepant results. Including culture results into the discrepant analysis, PCR resulted in 165 (31.1%) true positives and 359 (67.1%) true negatives. One false positive and 7 false negative PCR tests were observed yielding an analytical sensitivity and specificity of 99.4 respective 98.1% compared to 64.5% and 98.4% for the ELISA. Positive and negative predictive values for PCR testing were 95.9% and 99.7%, respectively.

Although, studies dealing with cost effectiveness of PCR in the management of CDAD patients are sparse, the BD GenOhm Cdiff assay (PCR) has greater analytical sensitivity than a conventional toxin ELISA maintaining comparable specificity in samples from CADA patients.

**P1978** Development of a multiplexed LATE-PCR assay for *Clostridium difficile* identification and characterisation

S. Goldenberg*, K. Pierce, H. Khan, R. Mistry, J. Levengood, L. Wang, G. French (London, UK, Waltham, US, Watford, UK)

**Objectives:** *Clostridium difficile* (Cd) causes significant morbidity & mortality and its prevalence/severity has increased recently. This is partly due to emergence of virulent strains which have potential to spread rapidly causing outbreaks. Many laboratories use toxin Enzyme Immunoassay for laboratory detection, however these are suboptimal. Cell Cytotoxin Neutralisation is the gold standard but can take up to 72 hours and is labour intensive. Thus there is a need for a rapid, highly sensitive/specific test that can detect Cd and identify potential clusters of cases. LATE-PCR (Linear After The Exponential-PCR) is a molecular method that can meet these requirements.

**Methods:** We have developed a multiplexed LATE-PCR assay for Cd to be run on the Clinical Bio-Seq® System, a Point-of-Care (POC) PCR platform that provides “sample in, answer out” capability. LATE-PCR is an advanced form of asymmetric PCR which is able to give greater levels of multiplexing, strain level discrimination with Mismatch Tolerant Probes, and quantitative endpoint detection. Single-stranded PCR products from LATE-PCR allow probe hybridization at endpoint, including at temperatures well below that used for primer annealing. Probes are designed to bind at different temperatures providing multiplex detection based on temperature, as well as colour. Strain level discrimination can be achieved at each site using a single probe which hybridises to target sequence with greater nucleotide mismatches as the temperature is lowered.

**Results:** The assay has been tested on >300 clinical isolates of Cd with a high degree of reproducibility. There was complete agreement with resulted obtained for LATE-PCR targets and traditional PCR using previously published primers. The assay is able to simultaneously identify genes for toxins A, B and binary and distinguish >8 tcdC variants. The assay has high specificity against non-Cd gastrointestinal commensals and pathogens.

**Conclusion:** The test is highly sensitive and specific for tcdB (toxin B) and can identify tcdA (toxin A) and tcdB (binary toxin), and characterise strains according to multiple tcdC alleles. These additional virulence factors may be associated with increased disease severity. Strain characterisation may also aid epidemiological monitoring and rapid identification of transmission between patients. This information can direct clinicians and infection prevention staff in the optimal management of patients and rapid termination of outbreaks.

**P1979** Development of a novel multiplexed molecular assay for detection of the tcdA and tcdB genes in pathogenic *Clostridium difficile* using room temperature stable reagents

N. Nassir*, H. Liang, V. Armendarez, T. Ott, T. Pack, R. Lollar, T. Ranalli, T. Stenzel (San Diego, US)

**Background and Objectives:** Cytotoxic *C. difficile* is a Gram-positive, anaerobic, spore forming bacillus capable of producing *Clostridium difficile* associated diarrhea (CDAD), or pseudomembranous colitis. These symptoms are the result of the presence of either one or both of two related cytoxins – toxin A and toxin B. The genes for toxin A and toxin B (tcdA and tcdB, respectively) are located together within...
Development of a rapid and simple isothermal molecular assay for the qualitative detection of Clostridium difficile from faecal specimens in a non-instrumented platform with room temperature stable reagents

W. Tang, M. Wilson, K. McCarthy, T. Pack, N. Nasser, T. Ranalli*, R. Lollar, H. Kong, T. Stenzel (Beverly, San Diego, US)

Background and Objective: Clostridium difficile (C. diff) is a Gram-positive, anaerobic, spore-forming bacillus that produces two major toxins, toxin A and toxin B, resulting in severe diarrhea and may lead to complications such as toxic megacolon and death. Traditional methods that are currently employed to diagnose C. difficile infection (CDI) and outbreaks. The slpA gene of C. difficile involves in severe CDI and outbreaks.

Methods and Results: Helicase dependent amplification (HDA) is an isothermal nucleic acid amplification platform that utilizes a helicase to separate strands of DNA or RNA, rather than heat, allowing for efficient amplification of specific targets in the absence of thermocycling. The C. difficile assay combines HDA with a disposable self-contained detection device (BEST™ cassette) that allows for the rapid detection of labeled amplicons generated by primers and probes specific for a region present in the tcdA gene found in all toxigenic strains of C. diff. A swab from a patient sample is placed into a dilution tube, briefly heated to lyse the cells, and then transferred to a reaction tube containing the lyophilized HDA reagents. The sample is then incubated in a heat block at 64°C for 45 minutes and transferred to the BEST™ cassette wherein the result is read via a lateral flow strip. The entire assay is performed in less than 1.5hrs and can be performed “on-demand” without the need for batching of samples. Initial analytical sensitivity testing has determined that the C. difficile assay can detect down to 20 copies of genomic DNA, does not cross-react with any non-C. difficile organisms that have been evaluated (11/11), and is capable of detecting 6/6 of the toxigenotypes. A preliminary clinical study performed with previously frozen samples has found that the assay correctly identified 9/9 positive samples and 10/10 negative samples.

Conclusion: The HDA-based assay for the qualitative detection of C. difficile is a simple and sensitive assay that can be performed in a wide variety of hospital settings without the need for costly instrumentation.

Loop-mediated isothermal amplification technique for routine detection of Clostridium difficile

V. Zidaric, N. Oresic, M. Rupnik* (Maribor, SI)

Objectives: Rapid and accurate diagnosis of C. difficile is crucial for effective patient management and infection control. Today, variety of commercial diagnostic tests is available, differentiating in turnaround time, sensitivity, specificity, costs, workload and availability. Recently, highly sensitive and specific molecular assays have been applied directly to faecal samples and can represent a useful tool in diagnosis of C. difficile. The aim of this study was to compare the novel loop-mediated isothermal amplification technique (LAMP; Illumogene, Meridian Bioscience Inc.) to routine toxigenic culture.

Methods: Between October and December 2010, altogether 138 samples sent for C. difficile diagnosis had been tested in our routine laboratory. Diagnosis included culturing on selective CLO agar plates (bioMérieux) after alcohol treatment, direct toxin testing by VIDAS CDAB assay (bioMérieux) and Illumogene C. difficile test (Meridian). Illumogene C. difficile test was performed additionally on all 31 known reference toxigenotypes, according to manufacturers instructions with an easy sample processing and short turnaround time (~1 hour). Results were given as Positive, Negative or Invalid.

Results: Of the 138 tested stool samples, 19 (13,8%) were toxigenic culture C. difficile positive, while one strain was nontoxigenic. Of 19 samples with toxigenic C. difficile 18 were positive by new LAMP test and 15 were positive with VIDAS toxin test. Three culture negative samples had to be repeated in order to resolve the invalid result and were finally negative with Illumigene test. Using toxigenic culture as gold standard, specificity, sensitivity, positive and negative predictive value for LAMP were 100,0%, 94,7%, 100,0% and 99,2%. Of 31 reference toxigenotypes only four (Xla, Xlb both toxon non-producers), XXX and XXXI were not detected by the molecular test, due to absence of target sequence within tcdA region of the C. difficile pathogenicity locus (PaLoc).

Conclusions: New commercial molecular diagnostic kit is rapid and sensitive also in the settings with low C. difficile prevalence. It recognizes all known variant forms of C. difficile except some rare types with tcdB gene only.

Low variability of the surface layer proteins between two high virulent Clostridium difficile PCR-ribotypes

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Objective: To analyze and compare the surface-layer (S-layer) proteins, high and low molecular weight (HMW and LMW), of predominant Clostridium difficile PCR-ribotypes involved in severe C. difficile infection (CDI) and outbreaks.

Methods: The slpA gene of C. difficile strains belonging to different PCR-ribotypes (001, 012, 014, 017, 027 and 078) were sequenced for the slpA gene using different sets of primers, the DNAStar’s Lasergene v.8.0 software, the National Center for Biotechnology Information BLAST server and the European Bioinformatics Institute Clustal W server. Amino acid sequences were analyzed using the SignalP 3.0 Server and the ExPASy Proteomics Server. Amino acid comparisons were accomplished using the European Bioinformatics Institute ClustalW server and the output was used for the construction of the phylogenetic tree by TreeView 1.6.6. Visualization of the LMW structure for the identification of surface exposed regions was performed using Swiss-Pdb Viewer. The S-layer proteins of representative strains were obtained by a low pH glycine extraction and separated by SDS-PAGE. Immunoblotting
Evaluation of new real-time PCR assay using stool specimens compared with conventional real-time PCR assay, toxigenic culture and enzyme immunoassay for rapid diagnosis of Clostridium difficile infection

B. Shin*, S. Man (Seoul, KR)

Objectives: The pathogenesis of C. difficile infection (CDI) is mediated by two toxins, enterotoxin A (TcdA) and cytotoxin B (TcdB), and efficient and effective identification of these toxins is important. The sensitivity of toxin EIAs is 50–90%, and CCNA is labor-intensive, subjective and requires tissue culture facilities. Therefore, assays for the rapid and sensitive tools for diagnosis of CDI are required. We evaluated a new real time PCR assay using stool specimens that targets the tcdA and tcdB genes and compared it with the commercially available BD GeneOhm PCR and an EIA assay for TcdA and TcdB.

Methods: A total of 450 fecal specimens collected from patients clinically suspected to have CDI. We performed C. difficile culture and an enzyme linked fluorescent immunoassay and evaluated a real time PCR assay that targets the tcdA and tcdB genes (AdvAnSure CD real time PCR, LG Life Science, Seoul, Korea) and compared it with the commercially available BD GeneOhm PCR that target the tcdB gene alone. We also amplified tcdA and tcdB genes in culture positive cases to differentiate tcdA-tcdB+, tcdA–tcdB+ and tcdA–tcdB− strains.

Results: The number of culture positive and negative cases was 191 and 259, respectively. The concordance rate between BD GeneOhm and LG AdvAnSure was 92.2% (415/450). We placed tcdA-tcdB+ and tcdA–tcdB+ strains among culture positive cases in a hypothetical toxin positive group, and tcdA–tcdB− strains among culture positive cases and culture negative cases in a hypothetical CDAB negative group. Based on these criteria, the sensitivity and specificity, the positive predictive value (PPV) and negative predictive value (NPV) of LG AdvAnSure were 80.1%, 96.2%, 95.5% and 83.9%, respectively. The sensitivity, specificity, PPV and NPV of BD were 89.7%, 92.1%, 92.8% and 88.8%, respectively. The sensitivity, specificity, PPV and NPV of EIAs were 61.3%, 94.8%, 98.1% and 77.8%, respectively. Especially, LG AdvAnsure can differentiate tcdA-tcdB+ strains among toxigenic C. difficile strains (78.9% performance).

Conclusion: A new real time PCR assays (LG AdvAnSure) represented comparable or good performance compared with conventional real time PCR assay (BD) and EIA. LG AdvAnsure can differentiate tcdA–tcdB− strains, in contrary with BD which detect tcdA alone, which can be used as a new rapid diagnostic and epidemiologic tool for toxigenic CDI, especially in areas where TcdA–TcdB− variant C. difficile strains are prevalent.
Correlation between Clostridium difficile toxin genes and the direct toxin detection assay results  

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Objectives: Direct enzyme immunoassays detecting toxin A and/or toxin B are widely used for the rapid diagnosis of Clostridium difficile infection as the rapid detection of toxin-producing strains is essential for optimal management of patients with C. difficile infection (CDI). Our aim was to study in what extend C. difficile isolates harbour toxin genes, that is, their potential to produce toxins and how this compares the results of the direct toxin detection assay.  

Methods: This present study included a total of 2170 routine C. difficile culture positive faecal samples that had already been tested with the direct toxin detection test (Vidas C. difficile toxin A and B (CDBA) assay, BioMérieux) as part of initial evaluation of a suspected case of CDI. C. difficile isolates were analysed with the in-house PaLoc-CDF-multiplex-PCR, detecting genes needed for toxin production and regulation (tcdA, tcdB, cdBe, tcdC and cdu2). The results of the multiplex-PCR and direct toxin detection assay were retrospectively compared.  

Results: In our 3-year clinical material, 2076 (95.7%) of the C. difficile isolates carried the toxin gene(s) and only 94 (4.3%) did not have the genes needed for toxin production (only cdu2 was amplified). On the contrary, the direct toxin detection assay had given negative results in 521 (24.0%) of the corresponding faecal samples and positive results only in 1414 (65.2%) of the samples. By direct detection assay there were also 235 (10.8%) equivocal results of which 228 (97.0%) harboured toxin genes. By using the culture followed by toxin gene detection by PCR as a standard, the sensitivity and specificity of the Vidas CDBA was 67.9% and 88.3% respectively.  

Conclusion: The use of enzyme-linked fluorescence immunoassay alone may lead to a substantial portion of toxigenic C. difficile strains being missed, leading possibly insufficient treatment and spread of infection as isolation procedures may not be imposed.

A new player on the market for detection of Clostridium difficile?  

K. Floré* (Bruges, BE)  

Introduction: Clostridium difficile (CD)-associated diarrhea (CDAD) is a frequent cause of antibiotic associated nosocomial diarrhea. Recently a new molecular technique for detection of toxigenic CD was launched. The loop-mediated isothermal DNA amplification (LAMP) targets the pathogenicity locus of toxigenic CD strains.

In this study we evaluated the test both retrospectively and prospectively.  

Materials and Methods: The LAMP test (Illumigen™ (ILL), Meridian Bioscience Europe) was first validated on 30 selected stool samples, in comparison with culture and a second molecular detection method (GeneXpert (GX), Cepheid, Europe). During an outbreak of CDAD on a geriatric ward we tested 90 consecutive faecal specimens. All samples were processed within 8h or kept at 4°C until processing (max 48h). Samples were cultured on cycloserine-cefoxitin-fructose agar plates (CLO, bioMérieux, France) and incubated for 72h under anaerobic conditions at 37°C. Toxin detection was performed either directly from faecal sample using the ImmunoCard® Toxins A and B (TOXA/B, Meridian Bioscience Europe), or, if negative, from pure cultures.  

Results: In the retrospective study 30 samples were compared with GX:18 toxigenic strains, 5 atoxigenic strains and 7 negative samples. The overall sensitivity of ILL compared with culture is 95% (1 false negative result with ILL), with a specificity of 100%. The overall sensitivity and specificity of GX was 100%. In routine analysis we cultured 21 CD strains (21/90), among those 6 atoxigenic, resulting in a negative result with ILL. All 15 toxigenic strains were positive both on culture and with ILL. No invalid results were noticed. The overall sensitivity and specificity of Illumigen™ compared with culture and TOX A/B was 100%.  

Conclusion: Although the preliminary results of the LAMP technology are promising, this test is still expensive and no reimbursement for molecular detection of CD in Belgium is provided. The high sensitivity is comparable to other molecular tests such as GX. ILL proved to be rapid (<1h) and easy to perform, with 20 minutes hands on time. Strain typing is not possible. Further testing with this promising molecular technique is warranted.

| Table: |

|   | Retrospective study |   |
|---|-------------------|---|
|   | IL positive       | IL negative |
| Toxigenic strain | 17               | 2           |
| Atoxigenic strain | 2                | 9           |
| Sensitivity ILL 95% | 17/19            | 9/11        |
| Specificity ILL 100% | 2/2              | 9/9         |
| Specitivity GX 100% | 17/19            | 9/11        |
| Sensitivity GX 100% | 2/2              | 9/9         |

Evaluation of three real-time PCR-methods for the detection of C. difficile in stool specimens  

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Objectives: Fast and reliable detection of Clostridium difficile toxin positive is crucial for the prevention of outbreaks of this serious infectious agent. We present our evaluation of three real-time PCR-tests (BD GeneOhm™ C diff., BD; RIDA® GENE C. diff. Test A/B, R-biopharm; C. difficile PCR, Astra) for the detection of toxin-producing C. difficile in stool specimens.  

Methods: 247 liquid or semi-liquid stool specimens for C. difficile analysis were examined in two microbiology laboratories. All stool specimens were directly tested with the C. DIFF CHEKTM-60-EIA (Techlab) for the detection of the GDH-antigen of C. difficile and with an EIA for the detection of C. difficile toxins A and B (Premier™ TOXINS A&B, Meridian; RIDASCREEN® Clostridium difficile Toxin A/B, R-Biopharm). Culture on CLO-agar (bioMérieux) was performed for all stool specimens as reference method. Cultured C. difficile-isolates were
tested with the *Clostridium difficile* toxin A&B EIA and the PCR method. All PCR-assays were performed according to manufacturer’s instructions.

**Results:** In total 247 stool specimens were analysed with the GeneOhm PCR test. 40 were congruently positive with the GDH-test and the Toxin A/B-EIA. 153 specimens were congruently negative. Discrepant results (n=54) between the tests were resolved by toxigenic culture. Performance of the GeneOhm PCR test was calculated with 99% sensitivity, 97% specificity, 99% NPV and 94% PPV. Out of 247 stool specimens, 105 were tested with the Astra PCR assay. 9 were congruently positive with the GDH-test and the Toxin A/B-EIA. 60 specimens were congruently negative. After resolution of discrepant results by toxigenic culture, performance of the Astra assay was calculated with 95% sensitivity, 95% specificity, 97% NPV and 93% PPV. For RIDA GENE C. difficile toxin A/B PCR test 79 stool specimens out of 247 in total were evaluated. 5 were congruently positive with the GDH-test and the Toxin A/B-EIA. 45 specimens were congruently negative. In comparison to toxigenic culture the RIDA GENE assay showed 100% sensitivity, 94% specificity, 100% NPV and 88% PPV.

**Conclusions:** The three Real-Time PCR-assays (GeneOhm C.diff, BD; RIDA GENE C. diff, R-biopharm; C. difficile PCR, Astra) evaluated showed rapid, high sensitive and specific results for the detection of *C. difficile* toxin directly from stool specimens.

**P1989**

**External quality assessment for laboratory detection of *Clostridium difficile***  
C. Walton*, E. Fagan, A. Marwaha (London, UK)

**Objectives:** The United Kingdom National External Quality Assessment Service (UK NEQAS) for Microbiology introduced a scheme for *Clostridium difficile* in April 2009 and provides participants with the opportunity to assess the quality of culture, molecular and toxin testing for *C. difficile*. This study analysed the performance of clinical laboratories participating in the *Clostridium difficile* scheme from April 2009 to November 2010.

**Methods:** Fourteen simulated liquid faecal specimens were distributed to between 177 and 276 participating laboratories in seven distributions. Twelve specimens included toxigenic strains of *C. difficile* ribotypes: 001, 002, 005 (x2), 014 (x2), 015, 016, 027 (x3) and one 078 (toxin A-B+); one toxin negative strain (RT 010) and one negative for *C. difficile*, containing commensal organisms only. Results reported by participants were analysed by method to determine their performance with these specimens.

**Results:** For 9 of the 12 specimens containing a toxigenic strain of *C. difficile* 84% to 100% (average 94%) of participants reported correctly on the detection of *Clostridium difficile* and/or toxin. The remaining three specimens contained weakly toxigenic strains (all confirmed positive in reference testing) and participants experienced a higher failure rate (27%, 18% and 25%). Ability to detect *C. difficile* and/or toxin was higher in culture (98.2%) than in EIA or membrane assays (mean 92%). The non-toxigenic strain was successfully identified by 98% of participants and 97% for the specimen containing only commensals. The number of participants using culture increased from 14 to 50 but overall toxin testing using an EIA and/or membrane assay was the most popular way of examining. The number using kits detecting GDH has increased from 9 to 74 and the number using PCR toxin gene detection increased from 2 to 27. Results reported for both GDH and molecular tests were 98% and 100% accurate respectively.

**Conclusions:** Many participants used a single assay and toxin levels produced by some strains may have been at the lower limit of detection for some assays thereby affecting participant performance. Current opinion on performance of *C. difficile* toxin detection assays indicates that laboratories should not be using these assays in isolation. There were no discrepancies with PCR test results. Results from the UK NEQAS *C. difficile* scheme will help participants monitor the performance of their methods.
**P1991** Evaluation of culture methods for isolation of *Clostridium difficile* and comparison of phenotypic identification with 16S ribosomal DNA sequencing and matrix-assisted laser desorption ionisation – time-of-flight mass spectrometry

A. Jain*, C. Pope, M. Wilks, M. Pond, J. Haigh, T. Planche (London, UK)

Objectives: Stool culture is generally agreed to be the most sensitive method for detection of *Clostridium difficile*. It is essential for epidemiological investigations and to monitor emerging resistance to antibiotics. Identification of *C. difficile* in culture by phenotypic methods is common but is time consuming.

We compared different culture methods for isolation of *C. difficile* from human stool samples in order to optimise laboratory diagnosis of *C. difficile* infection. Additionally, we evaluated identification of *C. difficile* using phenotypic methods, 16S ribosomal DNA (rDNA) sequencing and matrix-assisted laser desorption ionisation – time of flight mass spectrometry (MALDI-TOF MS).

Method: 163 faeces samples were analysed by simultaneously performing six culture methods over a one month period. Samples included 150 fresh stools and 13 stored *C. difficile* positive stools.

Stools were alcohol-shocked and cultured on 2 different selective media – cycloserine cefoxitin fructose agar (CCFA) and Brazier’s cycloserine cefoxitin egg-yolk agar (CCEY) for 48 hours. Additionally, samples were also enriched with brain heart infusion (BHI) broth for 24 hours either before or after alcohol shock followed by culture on these 2 media. Colonies were identified using standard phenotypic methods (UK HPA BSOP10i1.3) for *C. difficile* (colonial morphology, fluorescence, latex agglutination and lecithinase activity). Colonies were also identified by 16S rDNA sequencing and MALDI-TOF MS.

Results: The methods of culturing alcohol-shocked stools with or without enrichment on Brazier’s CCEY were best with sensitivity and specificity being 100% (95% confidence interval [CI] 79–100%) and 99% (95% CI 95–100%) respectively, with 16S rDNA sequencing as the reference standard (Table).

*C. difficile* was phenotypically identified in 29 samples out of 163. Only 16 out of these 29 samples (55%) were confirmed as *C. difficile* by both sequencing and MALDI-TOF MS, both of which showed full agreement on these 16 samples.

Conclusion: The additional step of enrichment of alcohol-shocked stool did not significantly increase the rate of isolation of *C. difficile* on Brazier’s CCEY media. This will need confirmation using a larger sample size. Phenotypic methods for identifying *C. difficile* culture may misidentify other clostridia species like *C. bifermentans* as *C. difficile*.

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**P1992** Lack of impact of strain type on detection of *Clostridium difficile* using PCR and glutamate dehydrogenase

S. Goldenberg*, M. Gumba, A. Hall, A. Patel, G. French (London, UK)

Objectives: The poor performance of Toxin Enzyme Immunoassays (EIAs) for the laboratory diagnosis of *Clostridium difficile* (Cd) Infection has resulted in a renewed interest in incorporating detection of Glutamate Dehydrogenase (GDH) into two and three step testing algorithms. Some recent work has suggested that the sensitivity of GDH may vary according to ribotype, and specifically that GDH is less sensitive than PCR in detecting PCR ribotypes 002, 027 and 106. We undertook a proof of concept study to determine the effect of strain type on detection using GDH (TechLab CDiff Chek-60) and PCR (Cepheid GeneXpert).

Methods: Suspensions of pure Cd of known ribotypes (n=62) were made to a 0.5 McFarland standard in nuclease free sterile water. This was diluted 1:100, 1:200, 1:400 and 1:800 and tested by GDH (CDiff Chek-60 using the automated Dynex 2 Platform) and Cepheid GeneXpert PCR methods. It is important to note that the manufacturers recommend using these assays with stool samples and not cultured isolates as we have done. The mean optical density (OD) (450/630 nm) for the GDH and Cycle threshold (Ct) value for the PCR were compared for different ribotypes (n=62, 10 each of ribotypes 002, 005, 023, 078 and 106 and 12 of ribotype 027).

Results: All samples were positive by both methods in at least one dilution using manufacturers’ cut off values. We found no significant difference amongst mean values obtained for GDH and PCR for different PCR ribotypes (see table).

Conclusion: We did not find any differences in the sensitivities of GDH and PCR tests for different PCR Ribotypes. All samples were positive in at least one dilution using the manufacturers’ cut-off values. All dilutions for PCR were positive, however some of the higher dilutions for GDH were negative, indicating that (as expected) PCR is more sensitive than GDH. We tested isolates of Cd in pure culture, which is outside of the manufacturers’ recommendations for these tests and results should be interpreted with caution. A larger study using stool samples should be conducted.

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**P1993** Evaluation of five methods for rapid detection of *Clostridium difficile* in diarrhoeal stool samples from paediatric patients

C. Burnham*, D. Tasler, R. Craven, L.F. Westblad, W.M. Dunne (St. Louis, US)

Objectives: Many new methods, including molecular diagnostics, have become commercially available to assist in making the diagnosis of *Clostridium difficile*-associated disease. Few studies have evaluated the performance of these assays in samples submitted from paediatric patients. The purpose of this study was to compare five methods for the detection of *C. difficile* (CD) in this patient population. Methods amenable to small volume testing were selected for evaluation as CD is typically a low-volume test for a stand-alone children’s hospital.

Methods: All samples included were diarrheal stools submitted to the St. Louis Children’s Hospital Microbiology laboratory for CD testing. Fresh samples were tested using the Wampole Tox A/B QuikCheck rapid immunoassay as part of routine testing in our laboratory. Positive specimens and an equal number of negative specimens were frozen at −80°C prior to subsequent testing. All samples were then analyzed using the Techlab CDiff QuikChek GDH (Inverness Biomedical), TechLab CDiff QuikChek Complete Tox A/B (Inverness Biomedical), Xpert C. difficile (Cepheid) and illumigene C. difficile (Meridian Biosoiences) assays. Toxigenic culture was performed on all samples and was considered the Gold Standard for comparison. Our historical CD
positivity rate of 5.5% was used for positive and negative predictive value (PPV and NPV) calculations. Typing was performed on all of the CD isolates using a novel typing method combining ribotyping and Diversilab bacterial barcodes analysis. Results: The two molecular methods tested in this study (Xpert and illumigene) demonstrated improved sensitivity and specificity compared to rapid immunoassay-based detection of Toxins A and B. See Table 1 for sensitivity, specificity, PPV and NPV for each method. Molecular typing of the isolates revealed six unique clusters, and six of the isolates did not cluster, suggesting the study was not biased due to a clonal population of isolates.

Conclusions: Rapid enzyme immunoassays for Toxin A/B detection are less sensitive than nucleic acid-based methods or GDH antigen detection. The findings of this study suggest that either a “two-step” algorithm of screening samples with GDH followed by the Xpert C. difficile assay, or the Xpert C. difficile assay alone, would be a sensitive and specific approach for the detection of CD in the stool of paediatric patients.

Table 1. Performance characteristics of assays evaluated in this study (n=47)

| Assay          | Wangene Top A/B QuickCheck | TechLab Top A/B Diff QuickCheck | Illumigene Top A/B Diff QuickCheck | Cepheid GeneXpert PCR | C. difficile | Toxigenic Cultures |
|----------------|-----------------------------|---------------------------------|-----------------------------------|-----------------------|-------------|-------------------|
| Sensitivity    | 88                          | 100                             | 91                                | 100                   | 86          | 100               |
| Specificity    | 95                          | 87                              | 100                               | 100                   | 100         | 100               |
| PPV            | 50                          | 50                              | 100                               | 100                   | 100         | 100               |
| NPV            | 99                          | 99                              | 99                                | 99                    | 100         | 100               |

* Gold Standard, therefore 100% sensitive and specific by definition.

**Frozen human foreskin fibroblasts provide a convenient alternative to conventional cell lines for diagnosis of Clostridium difficile by cell cytotoxicity assay**

S. Goldenberg*, N. Price, D. Tucker, P. Wade, G. French (London, UK)

Objectives: Widely used toxin Enzyme Immunoassays (EIAs) for Clostridium difficile (Cd) detection do not perform well enough for use as single tests. Several molecular tests are now available promising much improved sensitivity and specificity. We describe our experience of changing from a toxin EIA to a two-step method comprising screening with GDH (Glutamate Dehydrogenase) followed by confirmation with Cepheid GeneXpert PCR which detects toxin B gene.

Methods: We changed our laboratory methods for detecting toxigenic Cd as described above on 1st September 2010. All samples positive by the two-step algorithm were also tested by the gold standard Culture-Cytotoxicity Neutisation Assay (CCNA). We monitored for changes in disease prevalence, laboratory turnaround time, antibiotic prescribing and environmental contamination with Cd for the following 3 months.

Results: Between 1st Sep. and 30th Nov. the two-step algorithm identified 66 positives, 60 were confirmed by CCNA (positive predictive value = 91%). A number of repeat specimens which were initially CCNA negative subsequently became positive, suggesting that PCR can detect disease earlier. (These specimens were not excluded from the performance analysis). All cultured isolates were Ribotyped: no 027 strains were identified during this period and typing did not reveal any significant transmission between patients. Mean turnaround time was unaffected (6.3 hours for EIA and 6.5 hours for two-step). There was no significant change in antibiotic prescribing or environmental contamination (9/120 samples = 7.5%). Reportable rates of Cd infection have increased from 0.18 to 0.45 cases per 1000 occupied bed days.

Conclusion: Following a change to an improved testing method, our reportable Cd rates increased significantly. We excluded several factors which might have contributed to this. We conclude that the increase in rates was a consequence of using a better diagnostic method. Having introduced a test which improves patient safety, we are now at risk of financial penalties from the UK Department of Health for exceeding the reportable Cd infection target set for us in the upcoming year. We believe this is unreasonable since the calculated targets were based on the previous suboptimal, poorly sensitive EIA method. PCR allows sensitive, robust and rapid detection of Cd but regulatory authorities must recognise that a change to a more sensitive and accurate test will increase reportable Cd infection.
**Mycobacterial disease: diagnosis**

**P1996** Evaluation of a new molecular oligochromatographic assay for direct mycobacterial detection in human respiratory clinical specimens

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**Objective:** To evaluate a novel assay, namely “Speed-Oligo® Direct Mycobacterium tuberculosis” (DirectMB), based on PCR method attached to a dipstick, for detection of Mycobacterium spp. and specific identification of Mycobacterium tuberculosis complex (MTB) in human respiratory clinical specimens.

**Methods:** A total of 382 N-acetylcysteine-4%NaOH decontaminated specimens in 3 different sets were blindly tested with DirectMB:

- **Set A:** 327 fresh, prospective respiratory specimens, from 270 patients suspected of having a type of mycobacterial disease. Set B: To assess the sensitivity of the test, 44 selected respiratory smear positives specimens with different load of acid-fast bacilli (AFB/field 200×) were assayed. The clinical specimens previously decontaminated (20 culture-pos. specimens). Set C: to investigate the cross-reactivity, 16S rRNA Mycobacterium genus-specific sequence and IS6110 fragment MTC-specific. PCR detection: PCR product was added to a dipstick with probes bound to colloidal gold and to the membrane (5 min incubation).

**Results:** Culture of set A: 23 MTC; 6 NTM; 273 neg.; 5 and 1 neg. with another specimen from the same patient MTC or NTM, respectively; and 19 contaminated. The DirectMB showed no reactivity with all of the Mtb strains. No result (INV) could be obtained for 13 specimens culture-neg. due to the absence of the amplification control line (poor specimen collection or presence of PCR inhibitors). The sensitivities (S) of the DirectMB were: 0.92 for smear-pos. (0.76 for low-pos. and 0.97 for heavy-pos.), 0.86 for MTC pos., 0.74 for NTM pos. and 0.46 for smear-pos. The specificity was 0.99, the PPV 0.92, and the NPV 0.96 (prevalence 0.12). DirectMB produces reliable results, and may be a valuable tool for rapid diagnosis of mycobacterial lung infection.

| MTB | NMT | CB | CB NMT | CB | CB NMT | CB | CB NMT | CB | CB NMT | CB | CB NMT |
|-----|-----|----|--------|----|--------|----|--------|----|--------|----|--------|
| N BK | BK | BK | BK N BK | BK | BK N BK | BK | BK N BK | BK | BK N BK | BK | BK N BK |
| N BK | 29 | 2 | 5 | 5 | 5 | 5 | 2 | 10 | 9 | 9 | 9 | 9 |
| N BK | 275 | 0 | 19 | 19 | 19 | 19 | 19 | 19 | 19 | 19 | 19 | 19 |

Conclusions: Analyzing the mutations of the embB gene, the MTBDRsl assay does not appear sufficiently sensitive for use as a rapid screening test for detection of resistant to EMB at 5 μg/ml (sensitivity 20%). It is likely that MTB isolates without an embB resistance-determining region mutation likely represent the first level on the multi-step phenomenon of EMB resistance acquisition. There is a need for additional data pointing on the association between EMB resistances, associated mutations and MICs of the drug.

**P1998** Could negative T Spot-TB exclude an active tuberculosis suspect?

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**Objective:** Mycobacterium tuberculosis complex (MTC) infection remains a problem for public health. There are commercial blood test (IGRA: interferon-γ release assay) based on the release of this cytokinin (IFN-γ) after the antigenic stimulation with specific peptides (ESAT-6 and CFP-10) for the diagnosis of latent tuberculosis that are more sensitive and specific than Mantoux test. The purpose of this study is to evaluate the usefulness of T SPOT-TB (Oxford Immunotec) for the exclusion of active tuberculosis.

**Methods:** The dates were extracted from the our laboratory database by the software Virtuosio Plus (Metaphora Computer Ltd.). We enrolled all patients with negative T SPOT-TB and selected those with the notification of suspicious tuberculosis within one year from the execution of IGRA test.

**Results:** Between November 2005 and December 2009, 1442 patients were investigated and 760 (53%) with negative T SPOT-TB were enclosed in this study. Tuberculosis was diagnosed in 8/760 (1%) by microbiological isolation or clinical features only. Table 1.

**Conclusion:** Eight patients with negative T SPOT-TB and notification of tuberculosis should be considered as a failure of immunological test in recognizing active infection. In these cases, it would be however required further evaluation, considering additional factors that can weaken the immune system as HIV infection, pregnancy and old age. From clinical

**P1997** Evaluation of the new GenoType MTBDRsl® assay for rapid detection of resistance in Mycobacterium tuberculosis isolates in a low-incidence community

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**Objective:** The new developed assay GenoType MTBDRsl (Hain Lifescience, GmbH, Nehren, Germany), permits the identification of the M. tuberculosis (MTB) complex and the detection of specific mutations in the genes gyrA, rrs and embB associated with resistance to fluoroquinolones, aminoglycosides/cyclic peptides and ethambutol (EMB) respectively. In this study we examined this molecular assay all MTB strains, isolated in our laboratory for a period of nine-years.

**Methods:** M. tuberculosis isolates included in the study were collected from 221 patients cared for or referred to the University Hospital of Heraklion from January 2000 to May 2009. The conventional drug susceptibility testing (DST) was routinely performed in every case of a positive culture for M. tuberculosis with Bact/Alert 3D system for initial screening of drug resistance and on LJ slants. For EMB, the high- and low-level resistance were defined by MIC >5 μg/ml and MIC = 2 μg/ml, respectively. Shortly after isolation, the isolates had been stored at −70°C. All MTB isolates were retrospectively studied with the GenoType® MTBDRsl assay, according the manufacturer's instructions.

**Results:** All the 221 MTB strains (100%) correctly identified by the specific probe of the MTBDRsl assay. Among the 221 MTB isolates tested with the conventional DST for EMB, 208 were susceptible, 5 were high-level and 8 were low-level resistant. The MTBDRsl assay detected a mutation in the embB gene (mutation M306I) and a lack of a wild type (embBWT) band in only one high-level EMB-resistant strain. This is an MDR strain with mutations also in the rpoB and katG genes. None of the other resistant and susceptible strains contained any embB mutation. Furthermore, none of the isolates tested showed mutations in the gyrA and rrs genes, associated with resistance to quinolones and aminoglycosides.

**Conclusion:** Eight patients with negative T SPOT-TB and notification of tuberculosis should be considered as a failure of immunological test in recognizing active infection. In these cases, it would be however required further evaluation, considering additional factors that can weaken the immune system as HIV infection, pregnancy and old age. From clinical
data, our results show that the T SPOT-TB, which is normally used for the diagnosis of latent tuberculosis, has a negative predictive value of 99.0% (760/760 + 8) and would therefore exclude a pulmonary or extra-pulmonary tuberculosis in differential diagnosis with other pathological forms.

**P1999** Performance of the GenoType® MTBDRplus assay for detection of *Mycobacterium tuberculosis* drug resistance in routine settings: a multicentre study

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**Objectives:** Spread of multidrug resistant TB (MDR-TB) poses a major public health problem in Eastern Europe. We conducted a retrospective multicenter analysis of the performance of a commercial line-probe assay (LPA) (GenoType® MTBDRplus, Hain Lifescience, Nehren) at four sites in a non-trial context. The process of analysis and results were used to support the development of a clinical/diagnostic trial network.

**Methods:** Analysis of retrospective (2008–2010) data comparing molecular LPA and routine rifampicin (RIF) and isoniazid (INH) resistance on 1045 consecutive primary specimens (Table 1) collected at four TB reference laboratories (Samara, Russia; Tartu, Estonia; Vilnius, Lithuania; and Riga, Latvia) was performed using a developed electronic data collection forms. Decontamination, smear microscopy, culture identification and drug susceptibility tests (DST) were performed using internationally established standard methods.

**Results:** Sputum specimens (N = 1024) comprised the majority (98.0%) of all tested primary samples. Based on the acid-fast bacilli (AFB) smear microscopy results, 22 specimens (2.1%) were graded negative, 107 specimens (10.4%) – scanty; 218 (21.1%) – +; 235 (22.8%) – ++; and 450 (43.6%) – +++.

**Molecular assay readability** was associated with smear positivity; with the lowest (74.8%) in specimens having 1–9 AFB/100 fields and the highest (96.7–97.0%) in specimens graded +++, and 450 (43.6%) – +++.

Molecular assay readability was associated with smear positivity; with the lowest (74.8%) in specimens having 1–9 AFB/100 fields and the highest (96.7–97.0%) in specimens graded +++ and ++++. Seventeen smear-negative sputum specimens (77.3%) also produced readable result.

**Performance characteristics** of the MTBDRplus assay have been calculated against phenotypical DST as the gold standard (Table 1). Total agreement rates for the detection of RIF and INH resistance exceeded 90% at all sites with the highest in Vilnius (96.0% and 100.0%) respectively. Sensitivity and specificity values varied across sites (91.9–100.0% and 80.2–100.0%) with sensitivity values being generally higher, particularly in Vilnius (100.0%) and Samara (95.8% and 96.1%) for RIF and INH respectively.

**Conclusion:** Performance characteristics of the MTBDRplus assay when used routinely on direct patient specimens at four sites located in medium TB incidence countries with high TB drug resistance rates are close to those previously published for validation study. The current study has formed the basis for the establishment of the integrated network of sites for conducting clinical and diagnostic trials in Eastern Europe.

The study was funded by EU FP7 “TB PAN-NET” grant.

**Table 1. Performance characteristics of MTBDRplus assay across four sites.**

| Site          | No of samples tested | Drug | Total agreement, % | Sensitivity, % | Specificity, % | PPV, % | NPV, % |
|---------------|----------------------|------|--------------------|----------------|----------------|--------|--------|
| Riga          | 133                  | RIF  | 94.40              | 91.09          | 95.45          | 89.47  | 96.55  |
|               |                      | 95.70 |                   | 93.24          | 88.96          | 98.96  | 95.00  |
| Vilnius       | 27                   | RIF  | 94.05              | 100.00         | 91.67          | 92.88  | 100.00 |
|               |                      | INH  | 100.00             | 100.00         | 100.00         | 100.00 | 100.00 |
| Tartu         | 105                  | RIF  | 94.29              | 100.00         | 93.53          | 71.43  | 100.00 |
|               |                      | INH  | 93.33              | 88.09          | 94.87          | 87.71  | 96.50  |
| Samara        | 780                  | RIF  | 91.31              | 95.77          | 88.33          | 90.27  | 93.86  |
|               |                      | INH  | 91.05              | 96.06          | 88.18          | 91.32  | 90.36  |

**P2000** Clinical utility of immunochromatographic assay based on mouse monoclonal anti-MPT64 antibody for simple and rapid discrimination between *M. tuberculosis* complex and non-tuberculous mycobacteria in clinical isolates of extrapulmonary tuberculosis

A. Maurya*, S. Kant, V. Nag, R. Kashwaha, S. Jain, T.N. Dhole (Lucknow, IN)

**Objectives:** Mycobacterium tuberculosis and nontuberculous mycobacteria (NTM) are different, therefore prompt detection, isolation, and discrimination is necessary for suitable management. Conventional methods are able to identify mycobacterial species; but they are troublesome, time-consuming and laborious. Simple, quick and reliable identification of mycobacteria may provide patients with earlier and proper treatments, and therefore may spare patients from unnecessary treatments in cases of growth of environmental NTM. The aim of study presented here was to investigate simple, rapid and easy discrimination between *Mycobacterium tuberculosis* complex and Non tuberculosises Mycobacteria by Mouse Monoclonal anti-MPT64 based Immunochromatography Assay in clinical isolates of extra pulmonary tuberculosis in Northern India.

**Methods:** A total of 250 BACTEC Culture (BACTEC 12 B vial), which were positive in BD 460 TB System (Becton Dickinson, Sparks, MD, USA) from clinical isolates during July 2009 to Nov 2010 has been further evaluated on the ICA assay. BACTEC Media was subjected to SD TB Ag MPT64 rapid detection kit (Standard Diagnostics). Briefly, 100 ul aliquot of the Media were placed in to the ICA well, followed by 15 minutes to incubation. Results were read as positive for *M. tuberculosis* complex by observing presence of line of precipitation in the control as well the test well.

**Results:** Out of 250 BACTEC Culture positive isolates were tested by ICA test; 165 (65%) were positives (*M. tuberculosis* complex) and remains 85 (35%) were considered as Nontuberculous mycobacteria. We have compare ICA test with the NAP Test for the detection of *M. tuberculosis*. The sensitivity, specificity, and positive/negative predictive values of ICT for identifying *M. tuberculosis* were 98.7%, 100%, 100%, and 97.7% respectively. ICA performance was excellent, simple, easily disseminate, rapid and less time consuming.

**Conclusion:** ICA is of a new creation and is one of the simplest and fastest tests to perform. Its methodology is simple and does not require a high level of skill or equipment. The cost is approximately one third of the price of the current molecular probe method, and the time required to perform the assay is approximately 15 min, compared to 5 days for the NAP Test. It can be easily discrimination between *M. tuberculosis* complex and non tuberculosis mycobacteria. It can help in early diagnosis and appropriate management of tuberculosis.

**P2001** Ambiguous Quantiferon-TB gold test results in Swiss healthcare workers: analysis of IL-2/IFN-g ratios

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**Objectives:** More and more occupational health programs use Interferon-γ (IFN-g) release assays (IGRA), such as the Quantiferon-TB Gold In-Tube test (QFT; Cellestis Inc., Australia), to screen employees for latent *Mycobacterium tuberculosis* (MTB) infection (LTBI). A correlation exists between the IFN-g plasma concentration released upon MTB-specific stimulation and the risk of developing active tuberculosis disease. However, data concerning the interpretation of weak-positive IGRA results that, in our program, contribute >50% of all positive IGRA results, are scarce. In analogy to viral infections, where antigen clearance is associated with Interleukin-2 (IL-2) functional T-cell signatures, we tested if in LTBI there was an inverse association between the IFN-g plasma concentration and the relative proportion of IL-2 released upon MTB-specific stimulation.

**Methods:** Plasma concentrations of IFN-g and IL-2 were assessed in duplicates by ELISA (Mabtech AB, Germany) in QFT-plasma
supernatants (kept at −20°C) after MTB-specific stimulation of the T-cells. QFT-positive healthcare workers were stratified into three groups on the basis of the QFT screening test result (IU/ml): weak responders, >0.35 to <1.0 (n = 23); intermediate responders, 1.0 to 5.0 (n = 32); strong responders, >5.0 (n = 21). Data were log-transformed and analyzed statistically. Significance was determined by one-sided ANOVA and the Tukey-Kramer post test.

**Results:** We found a significant inverse association of the QFT test results and the corresponding IL-2/IFN-γ ratios (see figure). Means proved to be different (p < 0.001). Tukey-Kramer post test comparison of all pairs of columns revealed significant differences between group A and B (p < 0.05) and group A and C (p < 0.001).

**Conclusion:** Screening a low prevalence population often generates difficult to interpret positive test results around the cut-off value. IL-2/IFN-γ ratios revealed indirect evidence of IL-2 dominant T-cell responses. However, the clinical relevance of these findings needs to be further investigated by analyzing more samples and by following patients over time.

**Methods:** 39 M. avium were collected from clinical samples, mostly lymphonodal biopsy, of patients recovered at the Bambino Gesù Children Hospital and at the Mycobacterial Reference Center of the Tuscany Region, while reference strains were provided from Prof. Böttger (Zurich University). The whole set of strains were grown on 7H10 medium and CLM susceptibility was assessed by broth dilution assay. Protein profiles were provided by using MALDI-ToF MS Biotyper for both clinical and reference strains. Since M. avium was not present in the MALDI-ToF database, 20 spectra were collected from each strain in order to create a reference database suitable for M. avium identification. Spectra were analyzed even to detect differences in susceptible and resistant CLM strains.

**Results:** Using MIC broth dilution assay 29 CLM susceptible M. avium strains and 10 resistant strain were identified. MALDI-ToF MS provided the correct M. avium identification with a high sensitivity and specificity showing a 99% of concordance compared to 16S rRNA based sequencing analysis for all the analysed isolates.

**Conclusion:** This study provides the evidence that MALDI-ToF MS is a fast and reliable method for the identification of M. avium in clinical samples and it could be useful for CLM evaluation. Broth dilution assay is timeconsuming and end-point reading is somewhat subjective and consequently open to variation in different settings. Given these limitations, it’s necessary an equally robust methodology, with faster turn-around times and where endpoints determination is objective.

**Objectives:** The incidence of Nontuberculous Mycobacteria (NTM), as well as the number of mycobacteriosis patients is steadily rising. The awareness of NTM as potential pathogen is increasing. While the isolation and identification of these organisms is difficult, the aim of this study is to assess the usefulness of GenoType Mycobacteria CM and AS for the identification of mycobacterial strains isolated from patients living in Crete.

**Methods:** Over a 10-year period (2000–2010) 12971 samples from inpatients and outpatients were collected. Recovery of NTM isolates was accomplished using Lowenstein-Jensen slants and BactAlert 3D (bioMérieux, Durham, NC) system. For identification, the GenoType Common Mycobacteria (GenoType CM) and GenoType Additional Species assays (GenoType, Hain LifeScience, Nehren, Germany) were performed, according to the manufacturer’s instructions. When necessary, 16S rRNA and/or hsp65 gene sequencing. PCR-RFLP analysis of the hsp65 gene and the conventional culture identification methods were performed.

**Results:** During the study period 242 NTM isolates were recovered from different patients, which belonged to 23 species. M. lentiflauum (67/242, 27.7%) represents the most frequent species, probably due to contamination of hospital water, followed by M. gordonaenae (53/242, 21.9%), M. fortuitum (32/242, 13.2%), M. avium (21/242, 8.6%). For one culture with M. interjectum and M. boehemicum the PCR-RFLP analysis of hsp65 detected these two species but the Genotype Mycobacterium CM/AS identified only the M. interjectum. GenoType assay failed to identify 24/242 (9,9%) of the strains, which belonged to new or newly described species when compared with 16S rRNA or hsp65 gene sequencing. Apart from these isolates, all other identified by the GenoType assay, were in concordance with the conventional methods.

**Conclusion:** The results show that the GenoType mycobacterium system is reliable, rapid, easy-to-perform and easy-to-interpret assay. Moreover it appears to be suitable for use in our region, since it identified all but 9,9% of the mycobacterial species. For the unidentified species sequencing analysis is needed.

**Objectives:** The aim of this study was to evaluate the alterations of lymphocyte subpopulations in tuberculosis (TB) patients compared to healthy subjects, particularly related to IFN-γ production in response to a commercially available IFN-γ-related assay and to radiological and microbiological markers of advanced disease in pulmonary tuberculosis (PTB) patients.

**Methods:** In this study, we exploited the MALDI-ToF MS (Matrix-assisted laser description ionization – time of flight-mass spectrometry) Biotyper in order to verify the capability of an accurate and rapid identification of M. avium from clinical specimens. Moreover we evaluated clarithromycin (CLM) susceptibility because macrolides are first line drug used as NTM treatment.

**Results:** Using MIC broth dilution assay 29 CLM susceptible M. avium strains and 10 resistant strain were identified. MALDI-ToF MS provided the correct M. avium identification with a high sensitivity and specificity showing a 99% of concordance compared to 16S rRNA based sequencing analysis for all the analysed isolates.

**Conclusion:** This study provides the evidence that MALDI-ToF MS is a fast and reliable method for the identification of M. avium in clinical samples and it could be useful for CLM evaluation. Broth dilution assay is timeconsuming and end-point reading is somewhat subjective and consequently open to variation in different settings. Given these limitations, it’s necessary an equally robust methodology, with faster turn-around times and where endpoints determination is objective.

**Objectives:** The incidence of Nontuberculous Mycobacteria (NTM), as well as the number of mycobacteriosis patients is steadily rising. The awareness of NTM as potential pathogen is increasing. While the isolation and identification of these organisms is difficult, the aim of this study is to assess the usefulness of GenoType Mycobacteria CM and AS for the identification of mycobacterial strains isolated from patients living in Crete.

**Methods:** Over a 10-year period (2000–2010) 12971 samples from inpatients and outpatients were collected. Recovery of NTM isolates was accomplished using Lowenstein-Jensen slants and BactAlert 3D (bioMérieux, Durham, NC) system. For identification, the GenoType Common Mycobacteria (GenoType CM) and GenoType Additional Species assays (GenoType, Hain LifeScience, Nehren, Germany) were performed, according to the manufacturer’s instructions. When necessary, 16S rRNA and/or hsp65 gene sequencing. PCR-RFLP analysis of the hsp65 gene and the conventional culture identification methods were performed.

**Results:** During the study period 242 NTM isolates were recovered from different patients, which belonged to 23 species. M. lentiflauum (67/242, 27.7%) represents the most frequent species, probably due to contamination of hospital water, followed by M. gordonaenae (53/242, 21.9%), M. fortuitum (32/242, 13.2%), M. avium (21/242, 8.6%). For one culture with M. interjectum and M. boehemicum the PCR-RFLP analysis of hsp65 detected these two species but the Genotype Mycobacterium CM/AS identified only the M. interjectum. GenoType assay failed to identify 24/242 (9,9%) of the strains, which belonged to new or newly described species when compared with 16S rRNA or hsp65 gene sequencing. Apart from these isolates, all other identified by the GenoType assay, were in concordance with the conventional methods.

**Conclusion:** The results show that the GenoType mycobacterium system is reliable, rapid, easy-to-perform and easy-to-interpret assay. Moreover it appears to be suitable for use in our region, since it identified all but 9,9% of the mycobacterial species. For the unidentified species sequencing analysis is needed.
Methods: We analysed 99 patients affected by active TB compared to a control group of 93 healthy subjects; patients affected by HIV and other immunosuppressive pathologies or treatments were excluded. All TB patients had a positive cultural exam and underwent thoracic CT scan and QuantIFERON-TB Gold In-Tube (QFT-IT) (Cellestis Ltd). Lymphocyte subpopulations were studied by using monoclonal antibodies with six different fluorochromes and subsequently by flow cytometry (Becton-Dickinson).

Results: Total and relative lymphocyte counts showed statistically significant reductions in TB patients compared with healthy subjects; CD8+ lymphocytes were more affected by this phenomenon than CD4+, resulting in a significant increase in CD4+/CD8+ ratio. Despite the general lymphocyte decrease, activated (DR+) CD4+ and CD8+ cells were more relevant in proportion and in total count in TB patients. In the TB group we also found a significant proportional increase of CD4+ CD57+ cells. 

A positive QTF-IT result was related to a similar pattern of activated and CD4+ CD57+ cells increase, together with a higher total and proportional lymphocyte count. Smear-positive PTB patients with extended (>2 lobes affected) and cavitary pulmonary involvement showed lower IFN-g response to QFT-IT and significantly reduced NK cell and total lymphocyte counts, with a trend towards higher levels of CD4+ lymphocytes and increased CD4+/CD8+ ratio. 

Conclusion: Our study reveals remarkable alterations in TB patients lymphocyte subpopulations. Contrasting with a general lymphocyte deficiency, we found an increase in activated and CD4+ 57+ lymphocytes. This subset of effector T lymphocytes is able to express IFN-g and in our study was associated with QFT-IT positivity. Severe, smear-positive pulmonary disease was associated to lower levels of total lymphocytes and NK cells, with decreased IFN-g production leading to higher rates of false-negative QFT-IT results. This finding could reflect increased compartmentalization of the immune response to the extensively affected lung parenchyma.

P2005 Direct detection of multiple drug-resistant Mycobacterium tuberculosis in clinical specimens using real-time PCR, multiplex allele-specific PCR and PCR-sequencing

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Objectives: The emergence of drug-resistant Mycobacterium tuberculosis demands rapid diagnosis for better clinical management and public health control.

Methods: A total of 838 respiratory specimens and 622 non-respiratory specimens were collected for in-house single tube nested real-time PCR and COBAS TaqMan (Roche Diagnostics) real-time PCR assays. Samples positive for either PCR assay will be subjected to multiplex allele-specific (MAS)-PCR for katG315 and maba-15; PCR-sequencing for rpoB and gyrA genes. Results were evaluated towards direct AFB smear and mycobacterial culture. Susceptibility test of isolates for isoniazid (INH), rifampicin (RIF), and ofloxacin (OFX) was performed using agar proportional method.

Results: For respiratory and non-respiratory specimens, 231 and 25 were culture positive for M. tuberculosis among which 138 and 2 were also AFB smear-positive. Both in-house and Roche real time PCR exhibited 100% sensitivity and specificity for AFB-smear positive specimens. For culture positive but AFB smear-negative specimens, diagnostic sensitivity of in-house and Roche PCR was 79% and 75%. PCR inhibitors were detected at a rate of 1.5% and 5.5% for respiratory and non-respiratory specimens. Subsequent MAS-PCR detected 83% of INH resistant isolates whereas PCR-sequencing detected 96% RIF resistant and 91% OFX resistant isolates among which 8 strains of MDR-TB and 2 strains of XDR-TB were identified. All PCR results showed complete concordance with mycobacterial culture and anti-mycobacterial susceptibility test.

Conclusion: Both in-house and Roche PCR assays exhibited high sensitivity and specificity suitable for rapid diagnosis of M. tuberculosis. This study also evaluated a molecular strategy to detect multiple drug resistant M. tuberculosis in respiratory specimens. A cost-effective and cascade system will further shorten the turnaround time for identification and drug susceptibility testing of M. tuberculosis in clinical specimens to around 5 days (Fig. 1) suitable for routine diagnostic services in areas with high prevalence of drug-resistant tuberculosis.

P2006 Introduction of 24-loci MIRU-VNTR typing in a region-wide scheme considerably reduces the number of cases requiring epidemiological investigation

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Objective: In 2003, routine 15-loci Mycobacterial Interspersed Repetitive Unit-Variable Number tandem Repeat (MIRU-VNTR) typing commenced at the West Midlands Public Health Laboratory, Birmingham, UK. In January 2010, an additional 9 MIRU-VNTR loci were added to the 15-loci panel to provide a routine prospective 24-loci typing service.
The aim of the study was to determine if the implementation of 24-loci typing reduced the level of clustering and therefore the amount of epidemiological investigation required compared to the 15-loci typing service.

Methods: All available culture confirmed cases of *M. tuberculosis* identified at the West Midlands Public Health Laboratory, in 2010 had DNA extracted for 24-loci typing. All isolates with indistinguishable MIRU-VNTR profiles were considered clustered. The level of clustering observed using 15-loci was compared to that with all 24-loci. The Hunter-Gaston Index (HGI) was used to determine the discriminatory power of each typing method.

Results: A total of 850/893 (95.1%) culture-confirmed cases had isolates available for 24-loci typing. Using 15-loci typing, 60.0% (510/850) of isolates were clustered compared to 35.2% (299/850) of isolates when analysed with all 24-loci. Clusters ranged from 2-25 isolates and 2-18 isolates with 15-loci and 24-loci typing respectively. 24-loci typing reduced the number of large clusters resulting in a greater proportion of smaller clusters, 74% of clustered isolates were contained in clusters with 5 or less isolates compared to 50.2% of clustered isolates with 15-loci typing. The HGI confirmed that 24-loci was more discriminatory than 15-loci typing (0.968 and 0.957 respectively). Overall the addition of 9-loci to the original 15-loci resulted in 21 fewer clustered isolates and ultimately reduced the number of cases requiring further investigation by 41.4%.

Conclusion: The introduction of routine 24-loci typing reduces the amount of cases that require epidemiological investigation and the costs associated with contact tracing. This new typing service therefore provides more accurate molecular epidemiological data for public health teams to initiate cluster investigations.
Mycobacterium tuberculosis complex. Specific probes targeting the hot-spot region of rpoB, the codon 306 of katG, and nucleotides –15 and –8 in the promoter region of inhA were designed for drug resistance detection.

Malaria: Detection of the five human malaria-causing Plasmodium species (Plasmodium falciparum, P vivax, P ovale, P knowlesi and P malariae) is performed using PCR based on 18S rRNA gene followed by hybridization with species-specific probes on the LoC.

**Results:** TB: the performance of the In-Check™ platform was assessed using DNA extracted from both isolates and clinical specimens. Selected probes allowed identification of M. tuberculosis complex, M. avium, M. intracellulare, M. simiae, M. kansasi, M. scrofulaceum, M. abscessus, M. chelonae, M. xroxii, M. haemophilum and M. fortuitum. Concerning drug resistance detection, the assay detects mutations D516V, S531L for rpoB, and c-15 t, t-8c, t-8a for inhA. Mutations at codon 533 and 526 in rpoB and other mutations at codon 315 of katG are identified by a negative signal from wild-type probes. The test sensitivity is 4000 cells/mL. Malaria: the LoC was tested on pure species samples and patient samples and allowed correct identification of all human malaria parasite species. The accuracy of the test is 100% for the identification of all five Plasmodium species and shows a sensitivity equivalent to microscopy and standard PCR assays.

**Conclusions:** This integrated PCR and microarray LoC tool represents an innovation for its simplicity of use, rapidity and cost-effectiveness. Moreover, the In-Check™ platform is particularly suitable for different diagnostics purposes, making this assay indicated for the laboratory routine.

**P2010 Evaluation of GeneXPERT MTB/RIF assay in respiratory and non-respiratory specimens for the rapid detection of Mycobacterium tuberculosis**

Z. Archontakis, A. Dimoulaos, A. Charitakis*, S. Kanaki, S. Foytoyolakis, E. Michailiellis, K. Thymaki, V. Liakou (Harekonl, GR)

**Objectives:** The principle of the Xpert MTB/RIF test running on GeneXpert system, is to detect M. tuberculosis complex (MTBC) and mutations in the gene rpoB that cause resistance to rifampicin. The aim of this study is to compare the detection sensitivity of pulmonary and extrapulmonary MTBC using MTB/RIF assay and GenoType MTBDRplus (Hain Lifescience, Nehren, Germany) against liquid and solid culture (Becton Dickinson BACTEC™X MGIT™, Lowenstein-Jensen).

**Methods:** Each individual provided 3 sputum samples of sufficient quality within 72 hours. Sputum specimens for each patient were processed using NALC (n-acetylcysteine)/NaOH decontamination, were concentrated and tested using smear microscopy, culture, GenoType MTBDRplus and XpertMTB/RIF (smear and Xpert MTB/RIF were done from the same sediment as culture). Concentrated urine and gastric fluids were centrifuged prior to decontamination and addition of lysis buffer. Pleural fluids were not decontaminated but were centrifuged and up to 500 μl distilled water was added in the sediment prior to decontamination and addition of lysis buffer. For liquid culture 500 ml of medium were centrifuged prior to addition of lysis buffer.

**Results:** 250 specimens have been tested. Culture was positive in 50 samples (Mean time of isolation 8.02 days). Twenty six (10.4%) were identified as atypical mycobacterium. None of these 26 samples were positive by the Xpert test. MTBC was isolated from 24 (9.6%) specimens, gastric fluids (n = 8), pleural fluids (n = 1), respiratory samples (n = 14) and urine (n = 1). All of these were positive by the Xpert MTB/RIF test. Twenty three specimens were identified as Mycobacterium tuberculosis and 1 (urine) was identified as Mycobacterium bovis (patient was known to be treated with M. bovis as immunotherapy for bladder cancer).

**Conclusions:** The Xpert MTB/RIF provides rapid and specific detection of MTBC compared with conventional laboratory tests, both in respiratory and extrapulmonary samples. It can be used as an important tool for early diagnosis of TB and efficient patient management.
diagnosis of tuberculosis and rapid detection of rifampin resistance in clinical specimens.

Materials and Methods: A total of 232 clinical specimens of 228 patients were included in the study. The GeneXpert MTB/RIF test was used according to the instructions of the manufacturer. The results obtained by the GeneXpert MTB/RIF assay were then compared with those obtained by culture, clinical diagnosis and phenotypic drug susceptibility testing (DST). Diagnosis of respiratory and nonrespiratory tuberculosis were confirmed by clinical (productive cough, hemoptysis, weight loss), pathological and radiographic findings and/or positive culture and microscopy tests. The final diagnosis for the culture-negative patients was established by the clinicians.

Results: A total of 232 clinical specimens which 28 smear-positive and 204 smear negative were included in the study. Among all culture positive clinical specimens (direct MTB/RIF) test identified 56 of 69 specimens (NPV: %92.4, PPV: %93.3). In 27 of 28 (26 culture positive) smear positive specimens and 30 of 204 (42 culture positive) smear negative clinical specimens were correctly identified by the test. GeneXpert MTB/RIF assay detected 41 of 137 (40 culture positive) respiratory specimens (NPV: %94.8, PPV: %97.6). In 19 of 95 (16 culture positive) nonrespiratory specimens PCR was positive (NPV: %89.5, PPV: %84.2). When we evaluate the performance of PCR for the diagnosis of clinically diagnosed pulmonary tuberculosis (71 culture positive, 12 culture negative) NPV: %87.3 and PPV: %100. It had lower sensitivity in nonrespiratory specimens (Table1). GeneXpert MTB/RIF assay did not detect any RIF resistance and it was confirmed by DST.

Discussion: In conclusion, GeneXpert MTB/RIF assay could be useful to rapidly diagnose M. tuberculosis especially for smear negative and smear positive respiratory specimens. However, in nonrespiratory specimens it has lower sensitivity.

Table 1. GeneXpert MTB/RIF assay performance

| Specimen Type | Sensitive | Specificity | NPV % | PPV % |
|---------------|----------|------------|-------|-------|
| smear+ specimen | 28 | 96 | 91 | 89.4 | 93.3 |
| smear- specimen | 204 | 71.4 | 98.1 | 90 | 90.9 |
| Respiratory specimens | 137 | 88.9 | 99.8 | 94.9 | 97.6 |
| Nonrespiratory specimens | 95 | 66.5 | 95.8 | 89.3 | 84.2 |
| Clinically confirmed tuberculosis | 85 | 73.5 | 100 | 87.5 | 100 |

Conclusion: Urine PCR is helpful in diagnosing tuberculosis especially in HIV positive patients with extrapulmonary TB. This technique has high specificity in diagnosing tuberculosis.

P2015 Specificity of urine PCR in diagnosing tuberculosis

M. Jamshidi*, A. Nejatizadeh, P. Dacoodian, S. Zaare, H. Vahdat, T. Eqbal eftekhaari, M. Bager shiroodi, H. Dadcand, F. Fakhar (Tehran, Bandar-Abb, IR)

Objective: Sputum staining and culture is a standard method of diagnosing tuberculosis. Because of long time elapsed in culture of tuberculosis bacilli, a more rapid test is required for quick diagnosis of tuberculosis to confirm the clinical diagnosis and initiate the standard antituberculosis regimen. Polymerase chain reaction (PCR) of urine appears as the method of choice, particularly when tuberculosis gets associated with HIV infection. Urine PCR seems to be helpful in diagnosing pulmonary TB in patients who cannot expectorate and in patients with extrapulmonary TB.

Method: A prospective study was designed by collection of three early morning urine specimens of 103 patients from Sep, 2008 to Sep 2009, suffering from extrapulmonary and/or pulmonary tuberculosis. The three samples were mixed, and a nested PCR was performed. Data analysis was carried out by SPSS 16, using descriptive statistics.

Results: 28/103 (27.18%) had a positive urine PCR. 72.8% had pulmonary TB. 48% were smear negative. 16.66% of smear negative pulmonary tuberculosis, 30.76% of smear positive pulmonary TB and 37.03% of patients with extrapulmonary tuberculosis had positive urine PCR. 21.35% of studied population was HIV positive. 81.81% of HIV patients had pulmonary TB. Urine PCR was positive in 38.88% of HIV positive patients with pulmonary TB, and in 25% of HIV positive patients with extrapulmonary TB. The most involved organ in extrapulmonary tuberculosis was lymph node in 16 cases (15.5%) Sensitivity of PCR in diagnosing pulmonary tuberculosis was 24%, specificity was 64%, with a Positive predictive value (PPV) of 64%, negative predictive value (NPV) of 24% and accuracy of 34%. Sensitivity of this test in diagnosing smear positive TB was 30%, specificity was 73%, with a PPV of 42%, NPV of 63% and accuracy of 78%. Sensitivity of this test in diagnosing pulmonary TB in HIV positive patients was 38%, specificity was 80% with a PPV of 38%, NPV of 63% and accuracy of 70%. Sensitivity of urine PCR in diagnosing extrapulmonary TB in HIV positive patients is 25%, specificity is 62.5%, with a PPV of 10% and NPV of 83%, and the accuracy is 57%.

Conclusion: Urine PCR is helpful in diagnosing tuberculosis especially in HIV positive patients with extrapulmonary TB. This technique has high specificity in diagnosing tuberculosis.

P2016 Evaluation of an induced sputum service for diagnosis of pulmonary tuberculosis

P. Papineni*, R. Lover, A. Bello, J. Aspinall, M. Brown (London, UK)

Objectives: The diagnosis of pulmonary tuberculosis (TB) is usually on sputum microscopy and culture. However, some patients are unable to expectorate and alternatives include gastric lavage, fibreoptic bronchoscopy and sputum induction. We set up a service for sputum induction (SI) at the Hospital for Tropical Diseases, London, and conducted a retrospective analysis to consider if it is a useful tool for diagnosing pulmonary TB in our patient group.

Methods: The study included patients with suspected pulmonary or pleural TB (i.e. had clinical symptoms or a chest radiograph consistent with TB) who were unable to expectorate. The patients excluded were those with reduced consciousness, uncontrolled obstructive lung diseases, hypoxia, pneumothorax or inability to consent. The technique involves the patient receiving 30mls 3% hypertonic saline via nebuliser in a negative pressure room, with the operator using standard respiratory protection measures. We evaluated data over an 18-month period from Dec 2008-July 2010.

Results: 69 patients were included in the study group. The median age was 36 years (range 12–81 years) and 65% of patients were male. With regards to HIV status, 34 patients were negative, 19 were HIV-positive and 16 were not tested. 16 patients were referred from outpatient clinics; the rest were inpatients.

In 6 patients SI was unsuccessful. The average number of sputum samples sent was 2.4 (range 4–10). 10 patients were smear positive for acid-fast bacilli on SI (all Mycobacterium tuberculosis on culture). 3 patients were smear negative but culture positive for Mycobacterium tuberculosis, and 3 patients were smear negative but isolated non-tuberculous mycobacterium.

Of those patients who were smear negative on SI, 6 proceeded to fibreoptic bronchoscopy and 3 had endobronchial ultrasound. None of these patients had a diagnosis of pulmonary TB made following bronchoscopy (4 had a diagnosis of Pneumocystis jirovecii pneumonia from bronchial washings and 2 patients had a diagnosis of lymph node TB). Smear positive pulmonary TB diagnosed by SI had a prevalence of 20% in the study group. SI for diagnosis of smear positive pulmonary TB had a sensitivity of 0.76 (95% confidence interval 0.45–0.93).

Conclusions: SI is useful in the diagnosis of pulmonary TB in patients who are unable to expectorate. Our service could be expanded to include more outpatients, thus reducing costs with regards to length of hospital stay and the need for invasive investigations.

P2017 Use of Quantiferon-TB gold assay for tuberculosis in immunocompromised patients

M. Fomichev*, K. Levina, N. Fomicheva (Tallinn, EE)

Objectives: To evaluate the response to the Quantiferon-TB Gold Assay (QFT) In Tube in immunocompromised patients.

Methods: 194 patients were routinely tested in QFT assay during the period from August 2007 to October 2010. Patients were divided into
following 3 groups: group 1) consisted of Intensive Care patients (39 subjects), group 2) consisted of hematology patients (both groups had negative serological tests for HIV), and in group 3) 124 HIV patients were included. QFT assay was performed according manufacturer's recommendations. The cut-off value for a positive result was >0.35 IU/ml interferon-γ (IFN-γ).

**Results:** Among of 194 immunocompromised patients positive value was found in 23 (11.9%) patients, negative was in 150 (77.3%) and indeterminate was in 21 (10.8%). In group 1) 8 (20.5%), 95% CI 10.41 demonstrated positive results and 22 (56.4%), 95% CI 55.87% negative. In group 2) patients represented the positive rates in 7 (17.9%), 95% CI 6.30% cases and negative rates in 25 (64.1%), 95% CI 49.79%, 8 patients (6.5%, 95% CI 2.11%) from group 3) were QFT positive and 103 (83.1%, 95% CI 76.90%) were negative.

**Conclusions:** Sensitivity and PPV of QFT for diagnosing active TB in immunocompromised patients were unsatisfactory. Usefulness of QFT for these patients is limited. For the diagnosis of active TB infection QFT must be used in combination with other tests.

**P2018 Multicentre laboratory validation of the colorimetric redox indicator assay for the rapid detection of extensively drug-resistant Mycobacterium tuberculosis**

*A. Martin*, P. Paasch, S. Docx, K. Fissette, B. Imperiale, W. Ribon, L. Gonzalez, W. Werngren, A. Engstrom, G. Skenders, P. Jureen, S. Hoffner, P. Del Portillo, N. Morcillo, J.C. Palomino (Antwerp, BE; LIC, Clinic of Tuberculosis and Lung Diseases, Riga, Latvia; the Dr. Cetrángolo Hospital, Vicente López, Buenos Aires, Argentina; the Swedish Institute for Infectious Disease Control, Solna, Sweden, the Instituto Nacional de Salud, Bogota, Colombia and the Institute of Tropical Medicine in Antwerp, Belgium. In Phase I we tested 149 M. tuberculosis isolates and determined the MIC for each drug and compared results with the conventional proportion method (PM). In Phase II, a set of 30 strains, with different resistance patterns to second line drugs were sent and tested blindly by the study sites to determine the critical concentration of each drug.

**Results:** MICS were obtained after 8 days. Phase I: a strain was considered resistant by the REMA if the MIC >0.5 mg/L for rifampicin, >0.25 mg/L for isoniazid, >4.0 mg/L for ofloxacin, and >5.0 mg/L for kanamycin and capreomycin. Sensitivity was 99.2% for isoniazid and 100% for the other drugs and specificity was 96.7% for capreomycin and 100% for the other drugs. Phase II: the critical concentrations for rifampicin was 0.5 mg/L; for isoniazid 0.25 mg/L; for ofloxacin 2.0 mg/L; and 2.5 mg/L for kanamycin and capreomycin giving an overall accuracy of 98.4%, 96.6%, 96.7%, 98.3% and 90% respectively.

**Conclusion:** Results demonstrate that REMA is an accurate method for the rapid detection of XDR-TB in M. tuberculosis. A very good correlation between results by the REMA and the PM was obtained. REMA is faster that the conventional drug susceptibility testing method using solid medium, has the same turnaround time that the BACTEC MGIT 960 system but is less expensive and could be an adequate method for low-income countries.
**Advances in mycobacterial culture**

**P2021 Selective use of automated liquid mycobacterial culture in an area of low tuberculosis incidence**

**J. Greig** *(Plymouth, UK)*

**Objectives:** Delays in mycobacteriological diagnosis may lead to the avoidable transmission of tuberculosis (TB) and inappropriate treatment of non-TB mycobacterial (NTM) infections. The use of techniques such as automated liquid culture (CAMLiC) can reduce time needed to isolate mycobacteria and is more sensitive than solid culture but the cost of CAMLiC can be hard to justify in areas of low TB incidence. Diagnostic standards such as TB identification within 21 days and first line sensitivities within 30 days cannot be met using only solid culture. An attempt to improve laboratory performance was made using a protocol by which samples likely to yield a mycobacterium were referred off site for CAMLiC.

**Methods:** Smear positive specimens, samples from patients with a history of TB and invasive samples such as bronchial lavage or cerebrospinal fluid were referred off site for CAMLiC whilst all others were cultured locally using existing solid media techniques. Fifty six mycobacteria were isolated in the 34 months before the change in methods and 128 in the following 47 months. The time to identify an isolate as TB or NTM in smear positive samples was measured.

**Results:** After the protocol change, time to useful speciation for all TB isolates identified within 21 days was measured.

**Conclusions:** The selective referral policy had a modest impact on time to speciate mycobacteria and led to some significant diagnostic improvements. Patients with smear positive respiratory disease are those most likely to transmit TB or for NTMs to be treated inappropriately. Time to identify an isolate as TB or NTM in smear positive samples was reduced by over a week. The referral policy led to more TB isolates being identified within 21 days but even for smear positive respiratory specimens diagnostic targets were not met. A policy of CAMLiC for all samples for which mycobacterial culture is indicated remains the ideal diagnostic approach.

**P2022 Enrichment of Mycobacterium spp. in sodium metasilicate solution**

**S. Das** *(Kolkata, IN)*

**Objectives:** There is no enrichment technique for Mycobacteria spp so far. Thus except PCR technique sometimes it is very difficult to get a positive detection result if the number of Mycobacteria spp. in the clinical specimen is too low. Again PCR technique for detection of Mycobacteria spp. in clinical materials often gives false positive results particularly in developing countries. Thus this experiment was done to study the efficacy of sodium metasilicate solution for enrichment of Mycobacteria spp. in general and in clinical samples.

**Methods:** Four concentrations of Sodium metasilicate solution – 0.5 g/dL, 1.0 g/dL, 2.0 g/dL, 4.0 g/dL were used in this study along with normal saline and distilled water as controls. International strains of Mycobacteria spp. – *M. tuberculosis* H37Rv, H37Ra; *M. smegmatis* were used along with 22 wild strains of *M. tuberculosis*, 1 wild strain each of *M. marinum*, *M. scrofulaceum*, *M. flavescens*, *M. gordonae*, *M. avium*, *M. terrae*, *M. triciiale*, *M. fortuitum* were used in different concentrations (cfu) in this study. 50 smear positive and 100 smear negative sputum samples for AFB were also screened by this method. All the tests were also compared with NALC and NaOH decontamination methods.

**Results:** Significantly good enrichment along with decontamination of AFB was found with 1g/dL sodium metasilicate solution. However, other concentrations of sodium metasilicate which were used in this study failed to show any significant result.

**Conclusion:** Sodium metasilicate solution (1g/dL) may be used for enrichment of Mycobacteria spp. present in clinical samples.

**P2023 Bead-based specimen concentration for mycobacterial culture**

**S. Mitarai**, *H. Yamada, K. Mizuno, K. Chikamatsu, A. Aono, T. Sugamoto, R. Kariyama, T. Hatano* *(Kiyose, Matsudo, JP)*

**Objectives:** The centrifugation is a hazardous laboratory process. Genetec Co. Ltd. has recently developed a beads-based *M. tuberculosis* collection and concentration method, named TRICORE, to avoid centrifugation process. The efficiency of TRICORE for the concentration of *M. tuberculosis* from the sputum was evaluated.

**Methods:** A total of 30 sputum specimens were collected from the patients with active tuberculosis. The specimens were mixed well with same volume of NALC-NaOH for the digestion and decontamination, and incubated at room temperature for 15 min followed by neutralisation with phosphate buffer (PB; pH 6.8). To obtain various degrees of smear positivity, 5ml of neutralised specimen was dispensed into two tubes, and 0.5ml of the original was serially diluted with 4.5ml of PB into by 1/10 and 1/100. A total of 180 pre-treated specimens (30 specimens × 3 dilutions, × 2 series) were prepared for the following experiments. One series of specimens were centrifuged at 3,000g for 20 min at 4°C. The supernatant was discarded and the sediment was re-suspended with 1ml of PB. For the TRICORE method, 200μl of bacteria capture beads, 100μl of capture sol, and 100μl of co-precipitation beads were added to each tube, and mixed by inversion rotation for 3 minutes. After 3 minutes’ incubation, the supernatant was removed remaining 1 ml of the specimen attracting the co-precipitated beads by magnet. 100μl...
and 500 μl of each residue were inoculated into 2% Ogawa and MGIT (Becton Dickinson) media, respectively.

**Results:** Among 90 pre-treated sputum specimens, 51 (57.3%) and 55 (61.8%) strains were recovered by MGIT system with the centrifugation and the TRICORE method, respectively (Chi square test: p = 0.5413). The time to detection for centrifugation method was 359.3 ± 117.0 hours, while that of TRICORE method was 377.6 ± 162.3 hours (p = 0.5637). However, the numbers of colonies recovered on solid media were significantly higher in the TRICORE method (p = 0.003). Especially, among the smear-negative specimens, the culture positivity of TRICORE method was 39.6%, while the centrifugation method was 15.1% (p = 0.005).

**Conclusion:** The TRICORE is comparable to the conventional centrifugation method, and considered to collect the tubercle bacilli more efficiently than the centrifugation method especially in paucibacillary specimens. The TRICORE, a new concentration method without using centrifugation, will be safe and could yield more positive culture results.

**P2024 Evaluation of a rapid identification test for Mycobacterium tuberculosis complex on mycobacteria growth indicator tube S. Van den Wijngaert*, G. Stas, W. Chaker, A. Dediste (Brussels, BE)**

**Objectives:** Identification of a positive culture suspected for *Mycobacterium tuberculosis* complex (MTBc) relies on biochemical tests and colony characteristics. Some centres have the privilege to use Nucleic Acid Amplification Techniques (NAT), but these are expensive and are not performed on a daily base. Consequently, both methods delay susceptibility testing.

The purpose of this study was to evaluate 2 rapid chromatographic immunoassays (CIA) on different mycobacteria, and one CIA prospectively on successive positive cultures.

**Methods:** Twenty four different mycobacteria were tested with 2 CIA: the BD GMI(TM) TBc Identification Test (BD) and the SD TABagMPT64 Rapid (BioLine; SDAg). Both tests detect the MPT64 Ag, a mycobacterial protein fraction secreted by MTBc cells during culture. Since results were comparable and costs were in favour of the SDAg, a prospective evaluation between 17 May and 31 August 2010 on 84 successive positive culture media of 84 different patients was performed with this CIA.

**Results:** Both CIA detected all MTBc strains with the exception of 2 BCG. Eleven different Non Tuberculous Mycobacteria (NTM) tested negative with both CIA.

The prospective comparison of the SDAg with the COBAS Taqman assay (Roche) is summarised in table 1. Seven (8.33%) cultures gave an undetermined NAT result. The Belgian National Centre for Mycobacteria isolated 3 of them as NTM and 2 as MTBc. The remaining two had a positive NAT test directly on the sample, and were considered as MTBc. One culture was probably false positive: doubtful Auramine O smear performed on MGIT tube, negative Löwenstein-Jenssen after 10 weeks, re-culturing was negative. SDAg differentiated the remaining 83 positive MTG tubes correctly between MTBc and NTM, giving it a sensitivity and specificity of 100%.

Both CIA were also tested directly on a respiratory sample with a strong positive Auramine O smear but both remained negative.

**Conclusion:** Identification of a positive mycobacterial culture is time consuming and need experienced laboratory technicians. Some manufacturers have developed a rapid immunoassay, which determines the presence or absence of MTBc. The assay is easy to use, fast (less than 15 minutes) and accelerates initiation of susceptibility testing.

This study shows a very high sensitivity and specificity of the CIA compared to a NAT. In fact, the SD TB Ag MPT64 Rapid scored even better than NAT, since 84.3% (7/83) of the NAT were undetermined, probably due to inhibitors.

**Antimicrobial activity against mycobacteria**

**P2025 Synergism of linezolid, levofloxacin and amikacin combination against multidrug-resistant and drug-susceptible isolates of *M. tuberculosis* E. Rey*, G. Tudó, J. González-Martín (Barcelona, ES)**

**Objective:** To evaluate the in vitro effectiveness of linezolid, levofloxacin and amikacin combination against multidrug-resistant and drug-susceptible isolates of *M. tuberculosis*.

**Methods:** Clinical isolates were collected in the Hospital Clinic of Barcelona: 9 multidrug-resistant, 10 drug-susceptible isolates and H37Rv reference strain. The individual MICs of the isolates studied were evaluated with the proportional method in 7H11 solid medium. The linezolid, levofloxacin and amikacin combination was studied crossed 3 concentrations of each antibiotic (corresponding to their MIC and two lesser dilutions), with an adaptation to a three-antibiotic checkerboard assay in 7H11 medium. The fractional inhibitory concentration (FIC) was calculated as follows: FIC index = MIC in combination/MIC of each drug alone + MIC in combination/MIC of each drug alone. Where A, B and C were the three respective antimicrobial agents tested. The FIC index was interpreted as FIC index ≤ 0.5 as synergistic activity, FIC > 0.75 to 4 antagonistic activity. As a control a 1/100 inoculum was seeded in antibiotic-free medium. All the plates were incubated at 37°C, being read at the end of 3 and 4 weeks.

**Results:** Individual MIC of the studied isolates was 0.5 μg/ml, 0.5 μg/ml and 2.5 μg/ml for linezolid, levofloxacin and amikacin respectively. All the linezolid, levofloxacin and linezolid MIC in combination of the multidrug-resistant and drug-susceptible isolates decreased two dilutions compared to their individual MIC displaying synergism of all the isolates with FICs = 0.75.

**Conclusion:** The linezolid, levofloxacin and amikacin combination is synergistic against multidrug-resistant as well as susceptible isolates of *M. tuberculosis*, suggesting that could be used as a multidrug-resistant treatment.

**P2026 Activity of drugs against *Mycobacterium tuberculosis* under hypoxic plus acidic conditions G. Piccaro, F. Giannoni, P. Filippini, L. Fattorini* (Rome, IT)**

**Objectives:** *Mycobacterium tuberculosis* (Mtib) is the etiologic agent of tuberculosis (TB), which causes 1.8 million deaths per year. Furthermore, about 2 billion persons are latently infected with Mtib, with 10% of them reactivating to active disease. Mtib comprises several types of lesions, ranging from caseous granulomas with hypoxic centres (latent TB) to necrotizing granulomas with pH values estimated to be between 5.5 and 6 (active TB). The aim of this study was to test the activity of drugs under hypoxic plus acidic conditions.

**Methods:** Hypoxic Mtib H37Rv cultures were obtained through the self-generated formation of an oxygen gradient in Dubos broth at pH = 5.8 (acidic Wayne model). Aerobic (A), replicating, bacilli were prepared by incubation of the tubes with loosened screw caps for 5 days (A5). Hypoxic (H), dormant, bacilli were obtained by incubation of the tubes for 5, 12, 19 days (H5, H12, H19), and closing them with tight-fitting rubber caps under the screw caps. To determine drug activity, rifampin (RMP), moxifloxacin (MX), linezolid (LZ), metronidazole (MZ), niclosamide (NC) (8, 4, 8, 8, 0.3 μg/ml, respectively, for their Cmax in serum), and 100 μg/ml of pyrazinamide (PZA), were added to A5 and, by syringe, to H5, H12, H19 cultures. After 7, 14, 21 days, 1 ml
Antimicrobial activity against mycobacteria

was withdrawn, washed, and resuspended in Middlebrook 7H10 agar for CFU counts.

Results: CFUs of untreated controls increased up to day 8 then stabilized up to day 26, followed by a drop, and a raise of pH to 5.9. RMP was the most active drug, as shown by the fact that no CFU were found after treatment of A5 and H19 cells for 7, 14, 21 days, in comparison with untreated cultures; however, some H5 and H12 cells survived. On day 21, the log10 CFU decrease of A5, H5, H12, H19 cells were: RMP, > 8, 5.7, 6.0, >6.3; MX, 8.5, 5.3, 1.3, 0.9; LZ, 5.0, 3.8, 0.4, 0.3; NC, 2.8, 0.5, 2.2, 2.6; PZA, 4.9, 0.5, 1.4, 2.6; MZ, 0.3, 2.9, 5.0, >6.3.

Conclusion: A differential activity of drugs was observed, with H5 and H12 being more resistant than H5 and H19 cells to RMP. H12 and H19 cells were the most resistant to NC and LZ. H5 cells were the most resistant to NC and PZA. MZ was inactive against A5 cells, however its activity increased with dormancy, from H5 to H19. These observations can be important to design new drug combinations active under hypoxic plus acidic conditions for treatment of active and latent TB. (This work was supported in part by the European Project StopLatent-TB, grant agreement 200999.

P2027 Antimicrobial susceptibility testing of rapidly growing mycobacteria by microdilution

C. Caussagot*, T. Garpin, T. Ecemis (Izmır, Manisa, TR)

Objectives: In this study, activities of amikacin, cefoxitin, ciprofloxacin, clarithromycin, doxycycline, imipenem, linezolid, sulfamehoxazole, and tobramycin against 25 clinical isolates of rapidly growing mycobacteria (RGM), including the common disease producing species Mycobacterium abscessus, M. chelonae, M. fortuitum and M. peregrinum were tested by Sensititre RAPMYCO (Trek Diagnostic Systems Limited, UK).

Methods: The 25 clinical isolates of RGM belonging to 4 species were included in the study. Organisms were identified to the species level by PCR-reverse hybridization and DNA sequencing of the 441 bp of the 16S rRNA gene. Susceptibilities to amikacin, cefoxitin, ciprofloxacin, clarithromycin, doxycycline, imipenem, linezolid, sulfamehoxazole and tobramycin were determined for 25 clinical isolates of RGM belonging to 4 species (M. abscessus, M. chelonae, M. fortuitum and M. peregrinum) by Sensititre RAPMYCO. The drug susceptibility patterns of the isolates are shown in Table I. M. fortuitum and M. peregrinum was much less drug resistant than M. abscessus, M. chelonae. All isolates of M. fortuitum and M. peregrinum were susceptible to amikacin and ciprofloxacin. About 90% of isolates of M. fortuitum and M. peregrinum were susceptible or intermediate to cefoxitin, clarithromycin, imipenem, linezolid, and sulfamehoxazole. All isolates of M. abscessus and M. chelonae were resistant or intermediate to ciprofloxacin. Isolates of M. abscessus and M. chelonae were much less susceptible to amikacin than M. fortuitum, and M. peregrinum.

| Species          | M. abscessus | M. chelonae | M. fortuitum | M. peregrinum |
|------------------|--------------|-------------|--------------|---------------|
| Amikacin         | 6.5-7        | 6.5-7       | 6.5-7        | 6.5-7          |
| Cefoxitin        | 7.0-7        | 7.0-7       | 7.0-7        | 7.0-7          |
| Ciprofloxacin    | 7.0-7        | 7.0-7       | 7.0-7        | 7.0-7          |
| Clarithromycin   | 7.0-7        | 7.0-7       | 7.0-7        | 7.0-7          |
| Doxycycline      | 7.0-7        | 7.0-7       | 7.0-7        | 7.0-7          |
| Imipenem         | 7.0-7        | 7.0-7       | 7.0-7        | 7.0-7          |
| Linezolid        | 7.0-7        | 7.0-7       | 7.0-7        | 7.0-7          |
| Sulfamehoxazole  | 7.0-7        | 7.0-7       | 7.0-7        | 7.0-7          |
| Tobramycin       | 7.0-7        | 7.0-7       | 7.0-7        | 7.0-7          |

Conclusion: The treatment of serious infections with RGM is a problem and limited by the small number of available drugs with activity at clinically achievable levels in tissue or/and blood. In conclusion, a variability in sensitivity to different antimicrobials exists in all strains; therefore, each species and strain must be individually evaluated, and it is advisable always to perform in vitro sensitivity tests before using the drug for human therapy.

P2028 Antibiotic susceptibility testing of Mycobacterium avium subspecies under iron starvation

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Objectives: Mycobacterium avium ssp. are environmental opportunistic pathogens, that infect a wide range of hosts, including humans. Upon entry into macrophages they swell and replicate inside phagosomes, eventually killing the macrophage. Since macrophage environment is known to be hostile to most of bacteria, especially by limiting available iron, we wanted to test if susceptibility of Mycobacterium avium ssp. to antibiotics is affected by iron concentrations. Previous extensive genomic analysis of Mycobacterium avium ssp. paratuberculosis K-10 genome pointed to existence of potential antibiotic resistance genes under regulation of iron responsive repressor IdeR.

Methods: MICs for different first and second line anti-tuberculous antibiotics under iron replete or depleted growth conditions were measured for Mycobacterium avium strain 104 and several field isolates of Mycobacterium avium ssp. paratuberculosis with resazurin viability assay. Strains were first grown in Sauton's medium with 100 μM iron to late exponential phase. Cells were then washed and diluted in Sauton's medium without iron (< 1 μM) to an OD600 of 0.010 and transferred to 96-well plates. Antibiotic was added at different concentrations in triplicates along with controls and plates were incubated for 6 days at 37°C. Resazurin redox dye was then added to each well and the plates incubated for 24 hours. Fluorescence was measured at 590 nm for each well. MICs and growth inhibition were determined for each antibiotic in iron replete/replete medium.

Results: MICs for antibiotics tested were different between subspecies and compared to MICs, determined for Mycobacterium tuberculosis. However MICs were not significantly different in iron replete compared to iron replete medium. Extent of growth inhibition showed different patterns in presence of iron compared to growth without iron. Subinhibitory concentrations of antibiotics have shown to increase growth of bacteria in iron depleted compared to iron replete medium.

Conclusion: Growth conditions can significantly affect antibiotic susceptibility, therefore it is very important to closely mimic the natural environment of pathogen. Current in vitro antibiotic susceptibility testing for mycobacteria is carried out predominantly in nutrient rich media, optimal for bacterial growth, which by no means resembles host environments. This should be taken into consideration in future development of antibiotics.

P2029 Change in first-line anti-tuberculosis drug susceptibility trends of Mycobacterium tuberculosis clinical isolates over a period (30 years)

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Mycobacterium tuberculosis, remain one of the leading killers worldwide. In several countries, a large number of multidrug-resistant (MDR) and more recently, extremely drug-resistant (XDR) tuberculosis cases have been reported.

Objectives: The assessment of resistance trends to first line anti TB drugs of M. tuberculosis isolates in a tertiary General Hospital serving 240.000 inhabitants.

Methods: Prospective analysis included all drug susceptibility test (DST) performed on 1904 initial isolates of M. tuberculosis between 1981 and September 2010, from newly treated human immunodeficiency virus (HIV) negative patients, devised in five periods. DST was conducted by a national reference laboratory (Dra M. Jiménez, Instituto de Salud Carlos III, Madrid, Spain) using the standardised proportion method, according
Epidemiology and clinical significance of non-tuberculous mycobacteria isolated from pulmonary specimens

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Objectives: The growing awareness and the development of new molecular tools for the diagnosis of nontuberculous mycobacteria (NTM) have led to increased number of cases associated with these pathogens. This study was designed to evaluate the clinical significance of NTM isolated from pulmonary specimens referred to the laboratory of clinical microbiology of a large tertiary care medical center in Northern Israel.

Methods: Clinical specimens were collected from in-hospital population. NTM isolated by standard microbiological cultures were further identified by restriction enzyme pattern analysis of a PCR-amplified 439-bp fragment of the hsp-65 gene using DNA extracted from bacterial colonies. Identification was confirmed by direct sequencing of a PCR-amplified region of 16S rRNA gene flanked by conserved sequences. Following identification of NTM, patients were followed or their records were reviewed. Cases were classified as significant, non-significant and inconclusive, according to the 2007 ATS/IDSA Criteria for disease.

Results: Between January 2004 and December 2010, 215 cases of respiratory isolations of NTM were identified in our hospital. Age range was 11 to 98 years. Mycobacterium xenopi was the most common species (85 isolates, 39.5%) followed by M. simiae (52 isolates, 24.2%). M. simiae was the most common isolate in 2004–6, while M. xenopi is the leading isolate since 2007. 167 of the 215 (77.7%) cases were classified as non-significant. 27 cases (12.6%) were considered inconclusive. Only 21 (9.8%) cases were clinically significant, the majority being M. kansasii (7 isolates, 33% of significant isolates) and M. avium complex (6, 28%). Only one case of M. simiae and two of M. xenopi were clinically relevant. Among patients with significant disease 6 had no underlying conditions. The rest had chronic obstructive lung disease (3), cardiac disease (6), HIV (3), malignancy (2) and CF (1).

Conclusions: Multifocal tuberculosis vertebral osteomyelitis: a re-emerging infection

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Skeletal disease accounts for 5% of all cases of tuberculosis (TB) and 15% of extrapolmonary TB. Multifocal disease is rare and a low index of suspicion leads to a delay in diagnosis and increased morbidity.

Methods: All cases of multifocal tuberculosis vertebral osteomyelitis (TBVO) seen in our institution since 2007 were reviewed. Data recorded included demographic characteristics and comorbidities, clinical and radiologic features, diagnostic methods (microbiologic and pathology), treatment and outcomes.

Results: Since 2000, 77 cases of extrapolmonary tuberculosis were diagnosed in our hospital; six (8%) had TBVO. We report on three patients with multifocal spinal disease that have been diagnosed in the last 4 years. All were men and median age was 33 years (21–35 y); mean Charlson index was 0. All were immigrants from West Africa. The number of affected vertebrae ranged 3 to 16. Back pain and weakness were the most frequent symptoms. All patients had neurologic findings, 67% had visceralomegalias and fever, and 33% had lymph node enlargement. Two patients had pulmonary involvement and other extra-axial manifestations. Magnetic resonance was the best radiologic method in the diagnosis of TBVO as compared to other imaging techniques (spine X-rays, bone scan, CT). Two patients underwent needle biopsy of a localized abscess (spinal or other localization), and in one patient a vertebral body biopsy was performed. Necrotizing granulomas were present in all of them. Culture yielded Mycobacterium tuberculosis in 2 cases. Median time to diagnosis from onset of symptoms was 4 months (4–10), median duration of therapy was 12 months. Two patients required surgery; all of them had spinal instability, epidural abscess and compressive symptoms. Median time to surgery was 20 days (18–21) from onset of therapy. At follow up, one case (33%) showed clinical sequelae. Two are free of active disease, at 31 and 36 months of follow up and the last one is still on therapy.

Conclusions: Tuberculous vertebral osteomyelitis presents frequently with multifocal involvement in African immigrants. Early surgery is safe and effective.

Risk factors of mortality among MDR-TB patients in Iran: 2002–2009

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Introduction: Multi-drug resistant Tuberculosis (MDR-TB) is a major issue in control of TB. The aim of present study was to identify the risk factors of mortality in MDR-TB.

Material and Method: During 2002–2009, all patients with documented MDR-TB in only referral center, Tehran, Iran, who had at least 6 months of follow up, were recruited. All patients received standard treatment consisted of Ofloxacin, Prothionamide, Amikacin and Cycloserine. All demographic and characteristic factors were studied, comparing between the death group and the control group.

Results: 159 patients were included. 88 patients were male. The mean age was 43.6±17.4 and 41.6±18.9 respectively in death and control group. Outcome of treatment was: 118 (74.2%) cured, 4 (2.5%) failure, 21 (13.2%) dead and 16 (10.1%) interrupted of treatment. There was no difference in sex, smoking, opium, co-disease, HIV status and cavitory lesions between death group and the control group (P value >0.05).

In univariate analysis developing adverse effects and using Amikacin less than 6 months were significantly higher in death group. In logistic regression analysis (multivariate), using Amikacin less than 6 month retained the statistical significance.

Conclusion: This study showed that using Amikacin less than 6 months can raise mortality rate in MDR-TB patients.
**P2033** Variations in the occurrence of specific rpoB mutations in rifampicin-resistant Mycobacterium tuberculosis strains isolated from patients of different ethnic groups in Kuwait

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**Objectives:** Frequency of specific resistance-conferring mutations in target genes varies among isoniazid- and ethambutol-resistant Mycobacterium tuberculosis strains isolated from tuberculosis (TB) patients of various ethnic groups within the same country. Similar studies have not been carried out with rifampicin-resistant strains. This study determined the frequency of specific mutations in three rpoB gene regions among rifampicin-resistant M. tuberculosis strains of different genetic background isolated from TB patients belonging to three major ethnic groups in Kuwait.

**Methods:** Rifampicin-resistant M. tuberculosis strains (n=119) isolated from Kuwait (n=55), Southeast Asian (n=23), Middle Eastern (n=39) and other (n=2) patients in Kuwait were analyzed. Rifampicin-susceptible strains (n=107) were also tested. Mutations in N-terminal, hot-spot and cluster II regions of rpoB gene were detected by DNA sequencing. Polymorphisms at katG463 and gyrA95 were detected by PCR-RFLP for genetic group assignment.

**Results:** None of 107 rifampicin-susceptible but 116 of 119 (98%) rifampicin-resistant isolates contained mutation(s) in rpoB gene. While mutations at codon 516 (20%), 526 (24%) and 531 (27%) were evenly distributed among isolates from South Asian patients, majority of isolates from Southeast Asian (78%) and Middle Eastern (51%) patients contained a mutated codon 531. All strains with N-terminal and cluster II region mutations were isolated from Middle Eastern and South Asian patients, respectively. Majority of M. tuberculosis strains isolated from South Asian (84%) and Southeast Asian (70%) patients belonged to genetic group I while nearly all remaining isolates from these two ethnic groups belonged to genetic group II. Isolates from Middle Eastern patients were distributed among genetic group I (46%), genetic group II (33%) and genetic group III (21%).

**Conclusion:** The frequency of specific rpoB mutations varied in rifampicin-resistant M. tuberculosis strains isolated from TB patients belonging to different ethnic groups. Since rifampicin-resistant TB mostly occurs among expatriates, due to reactivation of prior infection, in Kuwait, the variations are likely due to genetic differences in M. tuberculosis strains infecting different populations. The data are also important for designing rapid methods for detecting majority of rifampicin-resistant M. tuberculosis isolates recovered from patients of various ethnic groups.

Supported by KURA grant YM03/06.

**P2034** Genetic analysis of the host-pathogen interaction in tuberculosis (“TB-EURO-GEN”) study – progress and current tuberculosis epidemiology

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**Objectives:** “TB-EURO-GEN” (Genetic analysis of the host-pathogen interaction in tuberculosis) is an FP 7 EU Framework project (2008–2011) aiming to identify major host susceptibility genetic factors and their interaction with Mycobacterium tuberculosis using a genome-wide approach and state-of-the-art genotyping techniques. Functional experiments of the role of identified mycobacterial factors and the effect of this variation on aspects of innate immunity as influenced by newly-identified TB-associated genes will be studied.

**Methods:** Biobanks of human DNA samples from culture-confirmed TB patients and matching healthy controls, along with matching TB strains from the same patients combined with detailed clinical and demographic information have been established at our field site in Samara Oblast, Russia. Further analyses will be performed on a combined collection of Russian (established currently and during a previous pilot study) and Ghanaian (established previously) biobanks.

**Results:** 2763 patients with culture confirmed tuberculosis and 2500 healthy blood donors without active TB were enrolled as a control group representing the general population (in addition to similar numbers recruited from a prior pilot study). While genetic analysis is on-going, the descriptive epidemiological analysis is complete reflecting the current TB epidemiology in Samara. The median age of recruited TB patients was 40 years with over 80% of participants being ethnically Russians. A quarter of patients had a history of imprisonment, 16% admitted recreational drug use, 17% abused alcohol. Almost half of participants (46%) were unemployed. In an earlier pilot study (2005) the rate of HIV-TB coinfection in Samara population was 4%; however in the current cohort 17% of all recruited patients are HIV-positive. Rates of multidrug and extensive drug resistant TB (MDR/XDR TB) were 50% and 9% for all cases, 40% and 5% for new and 70% and 16% for re-treatment cases respectively. Of note, the study of MDR TB conducted in 2002 in the same region showed 0% rate of XDR TB.

**Conclusion:** This is the world’s largest study (5000 controls and 5275 patients) on the genetic susceptibility to TB in humans and host-pathogen interaction. The recruited cohort of TB patients highlights major obstacles in TB control in Russia including a worrying high importation of multidrug and extensive drug resistant TB and of HIV co-infection.

Funded by EU FP7 TB EUROGEN grant

**P2035** Molecular epidemiology of multidrug-resistant (MDR) Mycobacterium tuberculosis revealed by genotype MTBDRs assays in southern China

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**Objectives:** There are urgent needs called for clinical laboratories in Mainland China to establish rapid diagnostic route for MDR TB and serve local epidemiological surveillance, since the condition of lack of laboratory diagnostic resources prevails in Mainland China, the second highest TB burden country. This study investigated the epidemics of MDR TB in a large-sized general hospital in southern China and explored the feasibility of incorporation of Genotype MTBDRs into routine conventional TB procedures for rapid laboratory diagnosis of MDR TB.

**Methods:** The multiplex PCR-based reverse line probe hybridization assays, Genotype MTBDRplus and MTBDRsr, were used to identify the presence of mycobacterium tuberculosis complex and detect mutations conferring resistance to most active first-line and second-line drugs against TB (rifampin [RMP], isoniazid [INH], ethambutol [EMB], fluoroquinolones [FLQ], amikacin [AMK], capreomycin [CAP], kanamycin [KAN] and viomycin [VIO]) from clinical specimens and isolates. 238 smear-positive sputum specimens and 124 clinical isolates were included in the study.

**Results:** Among confirmed mycobacterium tuberculosis strains, MDR TB occurred in an overall rate of 14.6% (49/336). Of 49 MDR TBs, 17 (34.7%) had fluoroquinolone resistance-mediated mutations and 4 (8.2%) harbored mutations conferring cross resistance to AMK, CAP, KAN or VIO. 6.1% (3/49) of MDR-TBs was identified as XDR TB with additional resistance to fluoroquinolones and to at least one of the injectable second-line drugs (AMK, CAP, KAN). In addition, EMB resistance was found in 44.9% (22/49) MDR TB, among which 20.4% (10/49) were resistant to both EMG and FLQ and 4.1% (2/49) were simultaneously resistant to EMG and one of the injectable second-line drugs. Particularly, one super-resistant XDR TB has been found resistant to all tested drugs. The new and retreated cases were, respectively, 38.8% (19/49) and 61.2% (30/49) in MDR TB patients who all tested negative for HIV.

**Conclusions:** The MDR TB epidemics uncovered by the Genotype MTBDRs is of great importance and significant support to local MDR TB control, which indicates that improper and failed treatments are the
main potential causes of local MDR TB. The situation may be under-
estimated since the incomplete coverage of the molecular mechanisms
underlying anti-TB drug resistance by Genotype MTBDRs. Additionally,
we proved that Genotype MTBDRs are valuable complements in
establishing rapid laboratory diagnosis of MDR TB.

**P2036** Multidrug resistance profiles of pulmonary tuberculosis in
Georgia

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M. Akhalaia, R. Aspindzelashvili, M. Gegia (Tbilisi, GE)

**Background:** Multidrug-resistant tuberculosis (MDR TB) continues to
pose a significant threat to public health in Georgia. Understanding the
MDR patterns is of a great importance for advancing TB treatment
and prevention efforts in this region. We studied MDR profiles of
*Mycobacterium tuberculosis* (MTB) isolates from new and retreatment
cases of pulmonary TB registered in Georgia in 2006–2008.

**Design and Methods:** A frequency distribution of rifampin (RIF),
isoniazid (INH), streptomycin (STR) and Ethambutol (EMB) resistance
profiles with the 95% confidence interval (CI), in the MTB isolates from
new and retreatment MDR TB cases, was assessed using EpInfo version
3.4 (CDC, Atlanta, GA, USA).

**Results:** The MTB isolates with either INH-RIF-STR or INH-RIF-EMB-STR resistance profiles were predominant within both new and
retreatment MDR TB cases in 2006–2008. The frequencies of INH-
RIF-STR and INH-RIF-EMB-STR resistance profiles were respectively
40.3% (CI = 29.2–52.1)/46.5% (CI = 38.4–54.6) and 49.4% (CI = 37.8–
61.0)/49.7% (CI = 41.6–57.8) within new (n = 77)/retreatment (n = 155)
MDR TB cases in 2006. The frequency of INH-RIF-STR resistance
(being in a range of 26.4–28.0%) markedly decreased, even though MDR
TB infections continued to increase steadily in the following years. In
contrast, the occurrences of the INH-RIF-EMB-STR resistance were
73.60% (CI = 63.0–82.4) and 70.3% (CI = 63.1–76.9) correspondingly
in 87 new and 182 retreatment MDR TB cases identified in 2007. In
2008, the frequencies of the INH-RIF-EMB-STR resistance were 68.9%
(CI = 61.8–75.4) and 70.6% (CI = 65.0–75.8) respectively within 190 new
and 289 retreatment MDR TB cases.

**Conclusion and Recommendations:** The TB burden reflects a strong
tendency for an increased selection of MDR MTB strains that are
resistant to almost all first-line drugs, which, along with other factors,
can be suggestive of high transmission rates of such strains in Georgia.
More effective strategies are needed for TB control and prevention in
this region.

**P2037** Detection of gene mutations causing antibiotic resistance
in Mycobacterium tuberculosis complex by an array

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**Objectives:**This study aimed to develop an oligonucleotide array to
rapidly detect point mutations in seven genes that can cause antimicrobial
resistance in *Mycobacterium tuberculosis* complex (MTBC).

**Methods:** Antibiotic resistance in MTBC is associated with point
mutations in several genes including rpoB for rifampin (RIF), katG and
the mubA promoter for isoniazid (INH), embB for ethambutol (EMB),
rrsL and rrs for streptomycin (STR), and gyrA for fluoroquinolone
(FQs). Oligonucleotide probes were designed from the respective genes to
detect these point mutations. The method consisted of multiplex
PCR amplification of the seven genes, followed by hybridization of the
digoxigenin-labeled PCR products to oligonucleotide probes
immobilized on nylon membrane. A total of 195 clinical MTBC isolates
with known susceptibility patterns were tested. Among these strains, 50,
70, 52, 74, and 16 strains were resistant to RIF, INH, EMB, STR, and
OFX, respectively. After hybridization, alkaline phosphatase-conjugated
anti-digoxigenin antibodies were used to produce the hybridization
signals. The hybridized spots could be read by the naked eye.

**Results:** Single nucleotide mutations in genes of rpoB, katG, the mubA
promoter, embB, rpsL, rrs, and gyrA were successfully detected by the array.
The specificities were 97.1% (RIF), 98.2% (INH), 100% (EMB), 100% (SM)
and 100% (FQs), respectively; the positive predictive values were 92.5% (RIF),
96.9% (INH), 100% (EMB), 100% and 100% (FQs), respectively; and
the negative predictive values were 99.3% (RIF), 93.2% (INH), 82.6% (EMB),
73.7% (SM) and 97.7% (FQs), respectively.

**Conclusion:** The array could effectively detect point mutations in genes
associated with antibiotic resistance in MTBC. The test took about 5–6 h.

**P2038** Combinations of TNF α/IL-10 polymorphisms affect active
tuberculosis development in Tunisia

W. Ben Selma, A. Forjani, H. Harizi, M. Marzouk, I. Ben Kahla,
J. Boukadida* (Sousse, TN)

Tumor necrosis factor (TNF-α)/Interleukin 10 (IL-10) balance is
essential to control latent/reactivation stages of tuberculosis (TB).
In this study we focused on how association between combination of
cytokine gene single nucleotide polymorphism (SNP) linked to high
and low producer phenotypes [TNF-α −308Glw→Ahw and IL-10
(−1082Alow→Ghigh, −819Tlow→Chigh and −592Tlow→Chigh)] can
directly affect risk development of active TB in Tunisian population.
SNPs were investigated in population of 76 patients with active
pulmonary TB, 55 with active extrapulmonary TB and 95 healthy
subjects. We used Polymerase chain reaction-restriction fragment
length polymorphism method for genotyping study.

Mutant IL10 −1082G allele was statistically correlated with increased
risk development of active pulmonary TB and extrapulmonary TB
(P = 0.001, OR = 2.11; P = 0.0002, OR = 2.57). High producing IL10
−1082G/−819C/−592C (GCC) haplotype was over-represented among
pulmonary and extrapulmonary TB groups in comparison to control
group (P = 0.001, OR = 2.43).

Combinations of TNF-α −308 (A/A, A/G) (high TNF-α producer)/IL-10
(GCC/GCC, GCC/ACC, GCC/ATA) (high IL-10 producer) genotypes
were significantly associated with increased risk development of
pulmonary TB (P = 0.03, OR = 2.37; P = 0.007, OR = 3.13, respectively).
However, combinations of TNF-α −308 (GG) (low TNF-α producer)/
IL-10 (ACC/ACC, ACC/ATA, ATA/ATA) (low IL-10 producer) were
associated with resistance against development of pulmonary TB
and extrapulmonary TB (P = 0.01, OR = 0.44; P = 0.0007, OR = 0.26,
respectively).

Our results demonstrate that genetic polymorphisms of TNF-α and IL-10
association may affect the functional balance Th1/Th2 contributing to
the development of active TB in Tunisian populations.

**P2039** Host-pathogen relationship in tuberculosis: association
between the genetic diversity of *M. tuberculosis* strains and the
disease presentation in the State of Antioquia, Colombia

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**Objectives:** Genotyping *M. tuberculosis* is useful to understand the host-
pathogen relationship as well as the transmission dynamics of the disease.
The present work we analyzed the association between *M. tuberculosis*
genetic diversity and the clinical presentation of the disease in a cohort
of patients living in the state of Antioquia and who were all attended at
several health care institutions in the city of Medellín.

**Materials and Methods:** This prospective cohort study included 172 pa-
patients with pulmonary TB. All the patients were followed-up during their
treatment until the final clinical outcome assessment. The M. tuberculosis
isolates were genotyped using the RFLP IS6110 (Restriction Fragment
Length Polymorphism), Spoligotyping (Spacer Oligonucleotide Typing),
and MIRU (Mycobacterial Interspersed Units) methods. We identified
The presence of viable Mycobacterium tuberculosis in tissue specimens of lung surgery patients

N. Shubladze*, S. Vashakadze, S. Gogishvili, L. Kapreishvili, M. Pochkhua, I. Kalandadze (Tbilisi, GE)

Background: Multidrug- and extensively-resistant tuberculosis (MDR- and XDR-TB) is a serious health problem in Eastern European countries including Georgia. The aim of this study was to reveal the viable Mycobacterium tuberculosis (Mt) in sputum and pulmonary surgery specimens obtained from MDR- and XDR-TB patients at the National Center of TB and Lung Diseases of Georgia.

Design and Methods: Standard sputum microscopy and cultural analyses were performed for sputum and lung tissue samples from 15 MDR- and 7 XDR-TB patients treated with 2nd-line drugs according to DOTs+ standards prior to surgery from 8 to 16 months. 17 patients had cavitary forms of tuberculosis; in 5 were observed patients strongly pronounced residual morphological changes of lung tissue in the form of fibrosis and bronchiectasis. Lung tissue resections were taken during the surgery directly from tuberculous lesions. Susceptibility testing for first and second line drugs was performed before and after surgery.

Results: Out of 22 patients, 4 (18,1%) were sputum-smear and culture positive, and 18 (81,9%) were sputum-smear and culture negative immediately before the operation. A completely different picture was observed in the lung tissue specimens: smear microscopy was positive in 14 (63,6%) cases, and cultures showed the presence of M. tb in 12 cases (54,5%). Two patients had positive smear and negative culture. Growth of M.tb cultures was detected in lung tissue specimens from 88,2% patients with cavitary forms of tuberculosis.

Conclusion and Recommendations: Regardless of the long-term DOTs+ treatment, a large number of MDR- and XDR-TB patients caring viable MTB in their lung tissues, still reflect sputum smear and culture negativity prior to their surgery. This factor should be strongly considered when developing the TB treatment strategies.

Mycobacterium abscessus soft tissue infection in a murine model: fast infection clearance but long lasting pro-inflammatory cytokine production

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Soft tissue infections caused by rapidly growing mycobacteria, especially Mycobacterium abscessus, are being increasingly registered. This type of infection causes considerable tissue damage and because inflammatory processes last several weeks to months the infection is treated with antibiotics for 6 months or more.

Objectives: To characterize the immunological responses of M. abscessus soft tissue infection in a murine model.

Methods: Balb/C and C57BL/6 mice were intradermal inoculated in the footpads with approximately 250 thousand bacilli of several M. abscessus clinical isolates. Animals were euthanized at 3, 7, 14, 21, 28, 60 and 90 days after infection. Infected footpads were removed for histological studies, determination of the number of viable bacteria and RT-PCR analysis of cytokines expression.

Results: Bacteria were cleared from the footpads 14 days after the infection but during the whole study period of 3 months the pro-inflammatory cytokines IFN-γ and TNF-α were overproduced. A low expression of anti-inflammatory cytokines TGF-β and IL-4 was observed during the time course of this experiment, indicating an inefficient anti-inflammatory mechanism and consequently persistent inflammation, tissue damage and fibrosis.

Conclusion: Our model suggests that M. abscessus is easily eliminated from soft tissue after infection but that pro-inflammatory response continues because of a deficient anti-inflammatory response. Our experimental results suggest that treatment schemes based on only antibiotics should be revised. Possibly these schemes can be shortened and should include anti-inflammatory drugs to overcome continuing tissue damage after the infection has been cleared.

Cutaneous infections of Mycobacterium marinum in human aquarists and tracing of its possible sources

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Objectives: The low frequency of non tuberculous mycobacterial infections, non specific symptoms for individual mycobacteria and the...
Mycobacterium abscessus

Outcome of Port Health referrals for new entrant screening for tuberculosis in the United Kingdom

M. Healy, M.M. Raza* (Milton Keynes, UK)

Objectives: Screening of new entrants for tuberculosis (TB) in the United Kingdom (UK) has been re-endorsed as a prevention and control tool in the National Institute of Healthcare and Clinical Excellence (NICE) guidance on TB published in 2006. Port of arrival identification of new entrants for TB screening has been in place in UK since 1971.

Results: A total of 43 mycobacterial isolates grown on Lowneinstein-Jensen or Ogawa solid media originated from five human patients (n = 23), aquarium animals (n = 15) and aquarium environment (n = 5). Isolate identification was carried out using growth characteristics, biochemistry and sequence analysis of 16S rRNA and hsp65 genes. Results: A microbiology based approach, followed by sequence analysis was successfully used for detection of M. marinum in all five patients. Aquarium samples from patients' aquria were simultaneously examined and a total number of eight different mycobacterial species were isolated: M. chelonae, M. fortuitum, M. gordonae, M. haemophilum, M. kansasi, M. mantoni, M. marinum and M. peregrinum. The presence of M. marinum was proven in the aquarium environments of four patients.

Conclusions: Correct identification of the causative pathogen in the case of skin mycobacteriosis still remains a challenge for clinicians. A microbiology based approach, followed by the 16S rRNA and hsp65 sequence analysis is useful tool for identification of mycobacteria. The data, from sensitivity testing of M. marinum isolates were used for selection of targeted mycobacterial therapy with ciprofloxacin and ethambutol for one patient. The remaining patients were treated with clarithromycin. Fish-tank exposure is the source of most cases of cutaneous M. marinum infections and may be prevented by the use of waterproof gloves by persons with acute or chronic open skin lesions. Acknowledgement: This work was supported by grants Nos. MZ50002716202 and QH91240 from the Ministry of Agriculture and grant “AdmireVet” No. CZ.1.05/2.1.00/01.0006; ED0006/01/01 from the Ministry of Education, Youth and Sports of the Czech Republic.

P2046 Outcome of Port Health referrals for new entrant screening for tuberculosis in the United Kingdom

M. Healy, M.M. Raza* (Milton Keynes, UK)

Objectives: Screening of new entrants for tuberculosis (TB) in the United Kingdom (UK) has been re-endorsed as a prevention and control tool in the National Institute of Healthcare and Clinical Excellence (NICE) guidance on TB published in 2006. Port of arrival identification of new entrants for TB screening has been in place in UK since 1971.
Molecular diagnosis of mycobacterial infections

E. Mokaddas*, N. Al-Mutairi, S. Ahmad (Dasma, Jabrya, KW)

Objective: The extensively drug-resistant Mycobacterium tuberculosis (XDR-TB) strains are defined as multidrug-resistant M. tuberculosis (MDR-TB) strains additionally resistant to a fluoroquinolone and an injectable agent and have been detected in >55 countries. Infections with XDR-TB strains are extremely difficult to treat in developing countries. The aim of this study was to detect the occurrence of gyrA mutations associated with fluoroquinolone resistance among MDR-TB strains in Kuwait.

Methods: A total of 85 MDR-TB strains isolated from 55 TB patients and 25 pansusceptible M. tuberculosis strains were tested. Fluoroquinolone resistance-associated mutations were detected by direct DNA sequencing of quinolone resistance determining region of gyrA gene. For isolates exhibiting gyrA mutations, 3'-end of rrs (16S rRNA), three regions of rpoB, katG codon 315 and inhA regulatory region were also sequenced. Further fingerprinting of the isolates was achieved by polymorphisms at gyrA codon 95 and katG codon 463 and by double-repetitive element PCR.

Results: None of the pansusceptible but six of 85 MDR-TB strains contained gyrA mutations. Only gyrA codon 94 was mutated in all six (D94A in one and D94G in five) strains. Three of six mutant strains were recovered from the same patient while the other three strains represented individual patient isolates. Fingerprinting studies identified all individual patient isolates as epidemiologically distinct strains. All six strains with gyrA mutation contained wild-type rrS sequence.

Conclusion: Although fluoroquinolones are not generally used for the treatment of TB and drug susceptibility testing for second-line drugs is not routinely carried out in Kuwait, four of 55 (7%) individual patient MDR-TB strains contained fluoroquinolone resistance-associated mutations in gyrA gene. The data advocate routine drug susceptibility testing for this important second-line drug for proper management of MDR-TB in Kuwait. Lack of mutations in 3'-end of rrS gene that confer resistance to injectable agents reduce the likelihood of the occurrence, at least for now, of extensively drug-resistant tuberculosis in Kuwait.
Comparative evaluation of GeneXpert MTB/RIF assay Detection and differentiation of
Detection of dnaA and dnaN reagents for identification
Comparison of Advansure MDR-TB Genoblot assay kit

Results: Inhibitory substances were present in 4.1% of specimens (40/980). Among 40 inhibited specimens, inhibitory substances were removed in 12 (30%), 30 (75%), 27 (67.5%), 25 (62.5%) and 12 (30%) specimens with repeated run, dilution, addition of BSA, boiling and use of silica membrane, respectively.

Conclusions: The overall inhibition rate for COBAS TaqMan MTB MTB test was 4.1%. Dilution, boiling and addition of BSA were shown to be more effective than repeated run and use of silica membrane for removal of PCR inhibitors. Combination of two methods might be useful.

Comparative evaluation of GeneXpert MTB/RIF assay and Gen-Probe amplified Mycobacterium tuberculosis complex direct test for detection of Mycobacterium tuberculosis complex in smear-negative respiratory specimens

J.S. Lin*, C. Lin, R. Hsiao, L. Shih (Changhua, TW)

Background: The detection sensitivity of the Cepheid GeneXpert MTB/RIF assay (GX) for rapid detection of Mycobacterium tuberculosis complex (MTBC) in smear-negative respiratory specimens is rarely mentioned. An evaluation was thus conducted to compare GX and Gen-probe Amplified Mycobacterium tuberculosis Direct test (MTD) for detection sensitivity.

Methods: A total of 21 unrelated smear-negative but MTBC culture-positive respiratory specimens were submitted for GX assay and MTD test. After inoculation onto Lowenstein-Jensens slants and liquid culture media, all digested and decontaminated aliquots prepared from primary specimens were kept frozen at −70°C until molecular analysis.

Results: GX and MTD had been completed in each aliquot. Seven were positive and nine were negative by GX and MTD. Four and one were positive by GX only and MTD only, respectively. When using culture as a gold standard, the detection sensitivity was 52.4% (11/24) and 38.1% (8/21) for GX and MTD, respectively. The concordance rate between GX and MTD was 76.2% (kappa value, 0.53).

Conclusion: This preliminary study suggests that the sensitivity of GX in direct detection of MTBC in smear-negative respiratory samples is superior to MTD test.

Detection of dnaA and dnaN reagents for differentiation Beijing strains of Mycobacterium tuberculosis in pulmonary tuberculosis patients with culture-positive specimens

S. Jahani Shenas*, H. Goudarzi, E. Mirmamadi ( Tehran, IR)

Objective: The Beijing strain of Mycobacterium tuberculosis have attracted special attention due to association with multi drug resistance and rapid transmission. Spoligotyping is the gold standard for the identification and classification of Beijing strains of MTB. This technique however needs special equipment that are not in clinical laboratory. In this study we used of fast and cost-effective method for awareness to doctors, patient to prevent of spread of this genotype in community.

Methods: Using CTAB method for extract DNA from positive culture specimens in tuberculosis patients. Then with spoligotyping, we determined different strain of MTB, then using PCR with dna_A & dna_N primers.

Results: Total 200 MTB isolated we genotyped by both spoligotyping and PCR, by spoligotyping 19 isolates we determined to be Beijing strains and the remaining 181 isolated were non Beijing strains. Detection of dna_A & dna_N reagents show the same result, and all of Beijing strain had a insertion sequence in this reagent.

Conclusion: Considering the same detection power of two methods to distinguish Beijing strain, and higher cost effectiveness in comparison to spoligotyping, this method can be used in clinical laboratories settings.

Detection and differentiation of M. tuberculosis and non-tuberculous mycobacteria by real-time PCR on the BD MAX system targeting a region upstream of the 65 kDa heat-shock protein gene

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Objectives: To establish a real-time PCR assay for the differentiation of M. tuberculosis (MTB) and non-tuberculous mycobacteria (NTM) from positive liquid and solid cultures and to estimate its usefulness for the direct detection and differentiation of mycobacteria in clinical specimens using the BD MAX system (Becton Dickinson), a highly integrated, automated device for the extraction, amplification and detection of nucleic acids from up to 24 specimens within about 2.5 hours.

Methods: A real-time PCR system targeting a variable region upstream of the 65 kDa heat-shock protein gene (hop) was designed. The assay consists of a primer pair complementary to all mycobacteria and two different probes, one for the detection of all mycobacteria (FAM-BHQ1), the other specific for the MTB complex (Roxy-BHQ2). PCR conditions were optimized with regard to primer, probe and MgCl2 concentrations, denaturation and annealing/extension temperatures and times and the sensitivity was determined using a quantified MTB DNA standard. Cultures and (spiked) NALC-decontaminated sputum specimens were heat inactivated and mechanically disrupted. Sputa were processed both with and without a BD MAX extraction step, respectively. Amplification was performed with two different enzymes on the BD MAX system.

Results: The analytical sensitivity was approx. 10 target copies per PCR when using a hot start polymerase (Roche). All 10 MTB strains were PCR positive with both the genus and the MTB-specific probe whereas a genus signal only was observed for all 48 NTM representing 19 different species. No signal with either probe was observed for 7 Actinomyces, 5 Nocardia and 1 Gordonia species. However, the genus but not the MTB probe gave a positive result for Gordonia spuri and Rhodococcus equi. The assay detected MTB also in microscopy-negative spiked sputum specimens irrespective of the extraction procedure applied (mechanical disruption or full BD MAX extraction process). Similarly, 6/7 microscopy and culture negative sputum specimens were PCR-negative with the remaining specimen being very weakly positive with the genus probe only.

Conclusion: The target (upstream of the 65 kDa hop gene) in combination with the highly integrated analytical system (BD MAX) looks very promising for the easy and rapid detection and/or differentiation of MTB and NTM. Our results justify further validation.

Comparison of Advansure MDR-TB Genoblot assay kit with Genotype HMDRTBplus and conventional drug susceptibility test

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Introduction: Rifampin (RIF) and isoniazid (INH) are the most important drugs in the treatment of tuberculosis, and resistance to these antibiotics often results in incurable tuberculosis. Because of the prolonged turnaround time for conventional susceptibility testing, development of rapid methods for testing drug resistance is necessary. Recently, Advansure MDR-TB Genoblot assay kit (LG life science, Korea) using reverse hybridization line blot assay was developed. In this study, we compared the kit with Genotype MTBDRplus (HAIN lifescience, Germany) and conventional drug susceptibility test (DST).

Materials and Methods: Of the DNAs preserved after the DST with Genotype MTBDRplus, 150 samples with DST results by conventional methods were selected. The experiments with both kits were performed according to the manufacturer's instructions. For discrepant results,
Lyme borreliosis, toxoplasmosis

**Results:** 140 specimens showed identical results by the three methods. The discrepant results for 10 specimens are described in Table 1. For RIF, two molecular methods showed 100% agreement and the agreement between the conventional DST was 98.7% (148/150). One was very major error and the other was major error. For INH, the agreement between two molecular methods was 96.7% (145/150). The agreement with conventional methods was 96.0% (144/150) and 95.3% (143/150) for Advansure MDR-TB assay and Genotype MTBDRplus, respectively. Of the six samples showing discrepancy with conventional method and Advansure MDR-TB assay, five (no. 1, 2, 3, 4, 9) was very major error and one (no. 10) was major error. In one (no. 9) sample, resistance was detected by Genotype MTBDRplus due to loss of katG wild type. All the seven specimens (no. 1, 2, 3, 4, 6, 7, 8) which showed discrepancy between Genotype MTBDR plus and conventional DST, were very major error. Of the seven specimens, resistance was detected in three by Advansure MDR-TB assay, due to the mutation in ahpC. One sample showed resistance to INH by conventional method but showed susceptibility with both molecular methods. By sequencing, there was a 1027d mutations in katG gene.

**Conclusion:** Advansure MDR-TB Genoblot assay was comparable to Genotype MTBDRplus. It has the advantage in detecting INH resistance due to additional target (ahpC).

Table 1: Discrepant results among the three methods

| No. | Advansure MDR-TB Genoblot assay | Genotype MTBDRplus | Conventional DST |
|-----|--------------------------------|--------------------|------------------|
| 1   | S     | S       | S       | S     |
| 2   | S     | S       | S       | S     |
| 3   | S     | S       | r       | S     |
| 4   | S     | S       | r       | R     |
| 5   | S     | r       | r       | S     |
| 6   | r     | r       | r       | S     |
| 7   | r     | r       | r       | S     |
| 8   | r     | r       | r       | S     |
| 9   | S     | r       | r       | S     |
| 10  | r     | S       | r       | S     |

**Objective:** Primary toxoplasmosis in pregnancy is an immanent problem for pregnant women and their caregivers. Despite of a low index clinical manifestations can be severe and might lead to permanent disabilities in children affected. Incidences are ranging from 0.12–2 per 1000 pregnancies. Evidence-based recommendations for management or treatment are not available.

**Goal:** Prospective longitudinal evaluation of efficacy, safety and outcomes using short-term anti-protozoal treatment (21 to 28 days) for primary toxoplasmosis in pregnant women.

**Patients and Methods:** All women attending an infectious diseases outpatient clinic having serologically confirmed primary toxoplasmosis (documented seroconversion within four weeks, IgM antibody positivity with low or absent IgG antibody avidity or positive Toxoplasma gondii -PCR in plasma) between 2001 and 2009 were scheduled to treatment in a standardized manner using pyrimethamine and sulfadiazine with folinic rescue for three to four weeks. Postpartum newborn testing was performed. If available, follow-up interviews of mothers as well as children’s medical records were assessed at one to eight years after birth.

**Results:** Information was available for 125/133 (94%) mother-child pairs (mother’s median age 25 yrs, range 17–40 yrs). Diagnosis of toxoplasmosis was established in pregnancy week 21 (range 6–35). Treatment was either instituted immediately or week 12. In all pregnancies no toxoplasmosis-related complication occurred (child delivery in median week 38; child AGGAR score 0, range 6–10). In 66/134 children (49.3%), one sibling birth postnatal serologies were available: 5 out of 67 children (7.5%) showed evidence of infection: 4/5 Ig M antibody positive, one PCR positive (control PCR after one week negative). No newborn was given pre-emptive treatment. All five children are up to now without evidence for congenital toxoplasmosis (follow up 1, 2, 3, 4, and five years, respectively).

**Conclusions:** In this prospectively evaluated large case series short-term treatment of primary toxoplasmosis in pregnancy compares well to continuous or intermittent treatment regimen with regard to childrens and mothers outcomes. Short-term treatment is clearly superior regarding patient comfort in comparison to regimes which have to be taken permanently or intermittently during pregnancy.
Unusual case of early stage Lyme borreliosis presenting as acute anicteric hepatitis with splenomegaly

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Lyme borreliosis is one of the most common vector-borne illnesses in Eastern Europe, caused by Borrelia burgdorferi species transmitted via ticks. In the early stage it may present with skin manifestations (ECM) or a flu-like disease, but there can also be other unexpected clinical and biochemical findings.

This is a case report of a 29-year-old, previously healthy, male from urban surroundings, who presented at our Admission Ward with a history of 5-day low grade fever, malaise, sore throat and splenomegaly. Laboratory investigations revealed leucopenia (WBC 3.4) with neutropenia, lymphocytosis, and elevated liver enzymes (AST 98, ALT 98). During a 6 month-follow-up liver enzymes peaked on the 14th day of illness, reaching AST 609, ALT 1092, with normal bilirubine and preserved synthetic liver functions. Complete virology tests (hepatitis A, B, C, HIV, CMV, Adenovirus, EBV, Parvo B19, Coxackie B), PPD, test for Brucelae, M. pneumoniae, stool and urine cultures were negative, including an immunology tests panel (autoantibodies, protein electrophoresis, RF, AST), but ELISA for B. burgdorferi was positive, which was later confirmed by western blot. After a 21-day course treatment of doxycycline, patient was afebrile, with normal liver enzymes, blood count and spleen size. Repeated virology markers for hepatitis B, C were again negative, and also serology for B. burgdorferi became negative after a 6 month follow up. During this whole period, patient had no skin marks resembling ECM, was anicteric and without any signs of hemorrhagic manifestations. Few days after being diagnosed, he remembered a tick bite which occurred 12 days previous to his illness.

According to a few articles on this particular subject, most of the similar recorded cases were patients with ECM and only mild liver damage—which occurred in approximately 15–20% of patients with early stage Lyme borreliosis, and up to 66% of patients with disseminated disease. This case shows an unusual possibility of an early stage borreliosis, presenting as an acute anicteric hepatitis, with a complete recovery after a standard course treatment, and therefore can be considered as a diagnostic possibility in similar situations.

An unusual presentation of neuroborreliosis

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Introduction: Neurologic manifestations develop in approximately 15% of untreated patients with Lyme disease. Meningitis, cranial neuritis and radiculoneuritis are the classic triad of neurological abnormalities, emerging within several weeks or months.

Clinical case: The authors describe a clinical case of a previously healthy 25-year-old man that was admitted into our hospital due to three separate episodes of generalized seizures. After the first seizure he had started carbamazepine and levetiracetam. Epidemiological data was inconclusive. On admission’s day, neurological examination didn’t show abnormalities and there wasn’t nuchal rigidity. Funduscopy and Mini-Mental State Examination were normal. The levels of serum electrolytes, calcium, thyroid hormones, follic acid and B12 vitamin, renal and hepatic function and blood count were normal. Electroencephalogram showed irregular and slowed base rhythm and general paroxysmal activity.

The brain’s MRI revealed coronal T2-weighted and FLAIR high-signal, T1 low-signal in anterior and lateral pons, symmetrically, with upper extension and very thin leptomeningeal enhancement in anterior pons without root or ganglion enhancement. No white or gray matter involvement was found. The lumbar puncture revealed clear cerebrospinal fluid (CSF) containing 261 cells (98% mononuclear cells), with a protein level of 2.8 g/L and a glucose level of 1.9 mmol/L with positive oligoclonal bands. VDRL, Gram’s and fungal staining and PCR for Mycobacterium tuberculosis were negative; borrelia IgG >240. Serological tests for rickettsia and brucella were negative and borrelia IgG >240. ELISA and Western blot of Borrelia burgdorferi was performed in the blood and CSF and the result was positive. Ceftriaxone intravenous therapeutics was started and lumbar puncture performed 15 days later revealed improvement (50 cells mononuclear, protein level = 0.9 g/L and glucose level = 2.4 mmol/L). There was a complete resolution of symptoms.

Conclusion: In this case the authors didn’t find the classic triad of neuroborreliosis, not even the most typical MRI findings. When the characteristic prodrome of erythema migrans, exposure history, arthritis or MRI findings are absent, clinical suspicion and serological tests are fundamental in diagnosis. We alert for high prevalence of Lyme disease in Europe and the difficulties of diagnosis on the absence of this triad.

Correlation between clinical manifestation and reactive Borrelia antigens in patients with Lyme disease

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Objectives: The aim of this study is to investigate prevalence of antibodies against Borrelia-specific antigens in patients with early localized and early disseminated Lyme borreliosis in order to characterize the antibody patterns.

Methods: Serum samples from 47 patients with Lyme borreliosis (LB) were studied—9 patients with early localized LB and 38 patients with early disseminated LB, including 20 patients with neuroborreliosis, 8 patients with Lyme arthritis, one with ophthalmic disorders and 9 patients with multiple system disorders. Western blot tests (Mikrogen, Germany) coated with Borrelia specific antigens were used for IgM and IgG analysis.

Results: In patients with early localized LB, IgM class antibodies were most commonly detected against OspC in 6 (66,7%) cases, against internal fragment of p41 (p41i) of Borrelia afzelii in 8 (88,9%) cases and p41i of Borrelia garinii in 6 (66,7%) cases. Less frequently IgM antibodies in these patients were found against p100, VlsE and p41 – each in 2 (22,2%) cases. IgG class antibodies in these patients were most frequently reactive with VlsE and p1 in 9 (100%) cases, followed by p100 in 5 (55,6%) cases, BmpA, OspA, OspC and p41i of B. garinii in 3 (33,3%) cases and p41i of B. afzelii in 2 (22,2%) cases. Serum samples from patients with early disseminated LB showed much higher reactivity to all Borrelia antigens. IgM reactivity was shown against OspC in 30 (78,9%) cases, against p41 and p41i of B. garinii in 16 (42,1%) cases, p41i of B. afzelii in 10 (26,3%), VlsE in 4 (10,5%), p100 and p18 in 2 (5,3%) cases. Most frequently reactive with IgG antibodies were VlsE in 33 (86,8%) cases and p41 in 30 (78,9%) cases, followed by p100 in 20 (52,6%), BmpA in 16 (42,1%), OspC, p41i of B. afzelii and p18 in 14 (36,8%), p41i of B. garinii in 8 (21,1%), and OspA in 5 (13,2%).

Conclusions: Most commonly reactive antigens with IgM class antibodies in patients with early localized and early disseminated LB were OspC, p41i of B. garinii and p41i of B. afzelii. IgG class antibodies reacted most frequently with VlsE, p41, p100, BmpA, OspC, p18. Although infrequently, some IgM reactivity against VlsE and IgG reactivity against OspC could be detected.

Congenital toxoplasmosis in the health area of Pontevedra, Spain

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Objectives: Congenital toxoplasmosis continues to occur worldwide and its potentially severe consequences for the fetus and newborn remain one of the major problems of pregnancy. The aim of this study was to analyze serological patterns of toxoplasmosis in pregnant women and to document the occurrence of possible congenital toxoplasmosis during a three year period in the Health Area of Pontevedra (Spain).

Methods: 10,098 pregnancies were retrospectively studied from January 2008 to December 2010. 18,179 sera were analysed by ELISA IgG and
Susceptibility to Toxoplasma gondii infection in women of childbearing age: a two-year study (2009–2010)

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Background: Infection with T. gondii during pregnancy can cause severe illness in the developing fetus.

Objective: To evaluate the susceptibility to Toxoplasma gondii infection among women of childbearing age during a two-year period (2009–2010).

Materials and Methods: From January 2009 to November 2010, a total of 2609 women of childbearing age, who attended the department of Obstetrics and Gynecology of “Alexandria” General Hospital of Athens, were tested for anti-T. gondii IgG and IgM antibodies. Of these 2609 women, 1407 were Greek and 1202 were immigrants who came from Africa, the Middle East and Eastern Europe. Detection of anti-Toxoplasma IgG antibodies was performed in the serum by indirect immunofluorescence antibody test (bioMerieux, Marcy l’Etoile, France) and IgM by EIA (ABBOTT, AxSYM Toxo IgM Microparticle Enzyme Immunoassay) according to the manufacturers’ instructions.

Results: Anti-toxoplasma IgG and IgM antibodies were detected in 210 (14.92%) and 8 (0.57%) Greek women respectively out of 1407 examined, whereas IgG and IgM antibodies were detected in 358 (29.78%) and 7 (0.58%) out of 1202 immigrant women respectively. The susceptibility to Toxoplasma gondii infection of Greek women of childbearing age was estimated to 85.075%, while the susceptibility of the immigrant women to 70.22% (P < 0.0001, odds ratio: 2.418, 95% confidence interval: 1.99–2.94).

Conclusions: (a) The prevalence of specific antibodies to T. gondii in Greek women of childbearing age is low. (b) The seroprevalence in immigrant women of childbearing age is higher (almost twice as more) than that of Greek women, while the incidence of acute or recently acquired infection remains very low in both groups. (c) The high proportion of susceptibility to toxoplasma infection, especially in Greek women of childbearing age requires raising awareness about prevention of infection during pregnancy.

Multiple serological diagnosis in congenital toxoplasmosis

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Toxoplasma gondii shows the highest incidence of any human pathogenic parasite in central Europe. As a rule, the course of the infection is asymptomatic or mild and confers lifelong immunity to immunocompetent people. Initial contact with the pathogen during pregnancy can result in the transmission of the pathogens to the foetus, resulting in severe damage. In order to obtain an early diagnosis of congenital toxoplasmosis, the performance of the following were retrospectively evaluated: i) IgG and IgM ELFA tests, ii) Western Blot assay, by comparison of the mother’s and the newborn’s pattern of antibody reactivity to Toxoplasma gondii IgG and IgM, and iii) ISAGA IgM test. Fifty-seven serum specimens serially collected from 6 infants born to mothers in our centre and 61 sera from 4 congenitally infected newborns born to mothers not in our centre, were tested. All mothers suffered, or were strongly suspected of suffering, from primary toxoplasmosis acquired during pregnancy. Results demonstrate that the combination of Western Blot and ISAGA IgM assays represents a useful tool for early postnatal diagnosis of congenital toxoplasmosis. The gold standard still remains the follow-up of the newborn based on the evaluation of the IgG decreasing up to negativity within 9–12 months of life. Our results indicate that infants born to mothers who acquired the primary infection early in pregnancy and who were adequately treated did not present congenital infection. On the contrary, those born to mothers who were not treated early or who acquired the infection after the 24th week of pregnancy were congenitally infected but not symptomatic.

In conclusion, the use of comparative IgG and IgM Western Blot together with ISAGA IgM test seems to be extremely reliable as a diagnostic tool of early diagnosis of congenital toxoplasmosis that leads to a prompt specific therapy.
Epidemiological study of occurrence of urogenital Chlamydia trachomatis independently associated with STD that included syphilis, amoebiasis, and gonorrhea. Among those VCT clients, male homosexuality was consistently associated with chlamydia was oral-anal sex practices (OR, 1.95; 95%CI, 1.19–2.91). The factors independently associated with amoebiasis were MSM (OR, 7.20; 95%CI, 2.96–17.51) and recreational drug use (OR, 2.11; 95%CI, 1.47–3.03). Independent factors associated with gonorrhea were being under 20 years of age (OR, 1.5; 95%CI, 1.1–1.9), and having visited sex workers in the previous 3 months (yes vs. no, OR 1.5; 95%CI 1.1–1.9).

Conclusions: HIV infection, chlamydia, and syphilis were the leading STD among VCT clients seeking HIV anonymous testing. Among those VCT clients, male homosexuality was consistently independently associated with STD that included syphilis, amoebiasis, and HIV infection.

Epidemiological study of occurrence of urogenital chlamydial infection in women from Slovakia

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According to WHO estimate more than 340 million new cases of curable, sexually transmitted infections, namely those due to Neisseria gonorrhoeae, Chlamydia trachomatis, and Trichomonas vaginalis, occur throughout the world every year in men and women aged 15–49 years. From this number about 92 million infections are caused by Chlamydia trachomatis with the largest proportion in developing countries. In Slovakia, there is no specific law establishing systematic screening of this infection and the disease is not included in the category of notifiable diseases. Therefore the data about prevalence of urogenital chlamydial infections differ significantly depending mainly on the diagnostic method used.

A total of 796 women were examined for the presence of bacterium Ch. trachomatis by the method PCR. The women were tested at the 2nd Department of Gynecology and Obstetrics of Medical Faculty of P. J. Šafárik University in Košice and Gyncentrum, Ltd., in Košice.

Out of 796 examined women, 723 women had various clinical symptoms of inflammatory of urogenital tract. The control group consisted of 73 women before abortion without clinical signs of infection. The patients were divided into 6 groups according to the age. In the first group were women between 15–20 years, in second group were 21–25 years old women, in the third group women 26–30 years old, in fourth group women aged 31–40 years, in fifth group women between 41–50 years, and in the last, sixth group were women older than 51 years.

Out of 723 examined women was positive result detected in 17 cases, which represented 2.35% positivity. From 17 positive women were 15 with clinical signs and 2 coming from the control group. In examination in relation to the age group the highest positivity was observed in the group of 21–25 years old women.

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Chlamydia trachomatis infection among women in Italy: data from a laboratory-based sentinel surveillance system

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Objective: To assess the prevalence and risk factors associated with Chlamydia trachomatis (Ct) infection among Italian sexually active women.

Methods: In 2009, the Istituto Superiore di Sanità (Italy’s National Institute of Health), in collaboration with the Association of Italian Clinical Microbiologists (AMCLI), launched a programme for the surveillance of Ct infection, gonorrhoea and Trichomonas vaginalis infection. A sentinel network including 13 large clinical microbiology laboratories, located in the main Italian cities throughout Italy, provide monthly reporting of 2009. Routine MIC of cefixime was determined based on nucleic acid amplification tests on samples from the genital or anal area; individual socio-demographic, clinical, and behavioural information are also collected.

Univariate and multivariate analyses (odds ratio, OR) were conducted to identify associations between potential risk factors and Ct infection.

Results: Overall 19,106 women were tested for Ct, and 2.4% were positive. About 40.0% of Ct positive women were asymptomatic. At the multivariate analysis, the positivity for Ct was significantly associated with young age [15–24 years vs. >24 years of age, OR 3.3; 95%CI 1.1–10.1], presence of genito-urinary symptoms (yes vs. no, OR 1.4; 95%CI 1.1–1.8), having had two or more partners in the previous six months (≥2 vs. 0–1 partners, OR 4.7; 95%CI 3.3–6.7), and having used oral contraceptives in the previous six months (yes vs. no, OR 1.5; 95%CI 1.1–1.9).

Conclusions: In Italy there do not exist screening programmes nor guidelines for Ct testing. Our results suggest that testing for Ct should be primarily offered to young women, women with multiple partners, and women who use oral contraceptives, even if asymptomatic.

Remarkable increase of Neisseria gonorrhoeae with decreased susceptibility to azithromycin in Japan

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Objective: We examined clinical strains of N. gonorrhoeae isolated from men with gonococcal urethritis from 2000 to 2009 in Japan for susceptibilities to antimicrobial agents.

Methods: A total of 1,034 clinical isolates of N. gonorrhoeae were collected from 2000 to 2009. The CLSI agar dilution method was used to measure their susceptibilities to penicillin G, tetracycline, cefixime, ceftriaxone, spectinomycin, azithromycin (measured from 2002), and levofloxacin.

Results: The MIC90s of penicillin G were 4 mg/L from 2000 to 2001 and 8 mg/L from 2002 to 2009, respectively. The MIC90s of tetracycline were 2 mg/L in 2000 and 2009. The MIC90s of cefixime were 0.05 mg/L in 2000 and 2009. The MIC90s of ceftriaxone were 0.125 mg/L in 2000 and 2009. The MIC90s of spectinomycin 32 mg/L in 2000 and 2009. The MIC90s of azithromycin were 0.25 mg/L in 2002 and 1 mg/L from 2004 to 2009. The MIC90s of levofloxacin were 8 mg/L in 2000 and 16 mg/L from 2001 to 2009.

Conclusions: Fluoroquinolones have not been recommended to treat gonococcal infections anymore, because about 80–90% of N. gonorrhoeae isolates from patients with gonococcal urethritis are quinolone-resistant strain in Japan. Therefore the majority of clinical N. gonorrhoeae isolates were resistant to penicillin G, tetracycline and levofloxacin. In place of fluoroquinolones, oral cephalosporins had been commonly used for the treatment of gonococcal infections. However, oral cephalosporin treatment failure for gonococcal infection was reported. Indeed, about 20–30% of the N. gonorrhoeae isolates exhibited the decreased susceptibility to cefixime in Japan. However, all the clinical isolates were still sensitive to ceftriaxone and spectinomycin. Therefore, only three drugs, spectinomycin, cefodizime and ceftriaxone,
Factors determining serological response to syphilis treatment

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Objectives: Serology is the mainstay for diagnosis of syphilis and monitoring of treatment. There have been unusual reports in serologic testing in HIV-positive patients, such as increased rate of treatment failures for non-treponemal antibody tests and higher rate of seroconversion to negative for specific treponemal test after therapy. We aimed to compare the serological response to treatment between (1) HIV-negative and HIV-positive persons, and (2) different clinical stages of syphilis.

Methods: Data from syphilis patients who were treated and followed at the University Hospital of Zurich and at the City Hospital Triemli in Zurich between January 1999 and December 2008, and who had at least one follow-up serology 20 to 375 days after treatment were analyzed retrospectively. A total of 304 patients were included (98 primary, 150 secondary, 47 latent, and 9 tertiary cases of syphilis). The mean age was 38 years: 92% of patients were men, and 45% were known to be HIV-positive. The response of the Venereal Disease Research Laboratory (VDRL) test and a specific IgM capture ELISA test (Pathozone syphilis M capture, Omega Diagnostics Ltd.) to treatment was assessed with a Cox regression analysis. Endpoints were defined as fourfold decrease in VDRL titres or reversion to nonreactive and a drop of the Pathozone-IgM index below 0.9.

Results: The serological response to treatment was not influenced by HIV co-infection (p = 0.26 for VDRL, p = 0.12 for Pathozone-IgM) or by the gender of the patient (p = 0.71 for VDRL, p = 0.82 for Pathozone-IgM). However, the clinical stage of syphilis and re-infection influenced serological outcome: Compared with primary syphilis, secondary, tertiary, and latent syphilis showed a slower serological response to treatment; the hazard ratios (95% confidence intervals) of VDRL response in secondary, tertiary, and latent syphilis were 0.76 (0.55, 1.0), 0.46 (0.22, 0.94), and 0.45 (0.29, 0.68); and of Pathozone-IgM response 0.51 (0.36, 0.70), 0.13 (0.02, 0.97), and 0.46 (0.27, 0.79), respectively. Reinfected individuals also showed a slower response of the VDRL titre, the hazard ratio was 0.69 (0.49, 0.97).

Conclusions: Serological treatment response in patients with syphilis is not influenced by HIV co-infection. However, patients with secondary, tertiary or latent syphilis respond slower to therapy than patients with primary syphilis.

Inflammation on the cervical Papanikolaou smear: evidence for infection in asymptomatic women?

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Objectives: Most laboratories reporting the results of cervical Papanikolaou (Pap) smears tests comment on the possible presence of infection based on cytological criteria. The clinical importance of these findings is unknown especially in asymptomatic women. This study assessed the possible association between inflammation on Pap smear with the presence of pathogens in the genital tract.

Methods: Asymptomatic women with inflammatory changes on routinely performed Pap smear and recalled for cultures in the last two years were included in the study. Genital tract samples (vaginal and cervical) were available for analysis. Clinical specimens collected from patients were inoculated onto appropriate plates for standard aerobic and anaerobic cultures and incubated at 37°C for 24h and 48h, respectively. A wet mount as well as a Gram-stained smear were examined under microscope to obtain valuable information about the microorganisms present. The isolated pathogens were identified using the automated system VITEK 2 (BioMérieux, Marcy l’Etoile, France). Furthermore, the presence of Chlamydia trachomatis as well as Ureaplasma urealyticum and Mycoplasma hominis, in the specimens studied, was determined using the COBAS AMPLICOR Chlamydia trachomatis test (Roche Diagnostics, USA) and Mycoplasma IST2 (BioMérieux, Marcy l’Etoile, France), respectively.

Results: Out of the 280 women studied, 109 (38.9%) had negative cultures (normal flora present), in one case no microorganisms were observed, while 170 (60.7%) women had positive cultures with different pathogens. Gardnerella vaginalis and various anaerobes were isolated from 87 (51.2%) women (in 46 G. vaginalis only, in 21 anaerobes only and in 20, both). Ureaplasma urealyticum was isolated in 82 (48.2%) women, Mycoplasma hominis in 7 (4.1%) and only in one case (0.6%) Chlamydia trachomatis was detected. Anaerobes together with mycoplasmas were isolated in 33 (19.4%) cases, Candida species in 18 (10.6%), Streptococcus agalactiae in 15 (8.8%), Gram-negative rods in 6 (3.5%) and Staphylococcus aureus in 2 (1.2%) cases.

Conclusions: The results of our study suggest that a report of inflammatory changes on the cervical Pap smear cannot be used to reliably predict the presence of a genital tract infection, especially in asymptomatic women. Nevertheless, the isolation of different pathogens in about 60% of the women studied, in the absence of Lactobacillus species, cannot be overlooked and must be regarded with concern.

Acute diarrhea

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Objectives: Acute diarrheal illness is a significant clinical problem. More than 90% of cases of acute diarrhea are caused by infectious agents and the cornerstone of diagnosis is microbiologic analysis of the stool. However, stool cultures have been shown to have poor yield and incur high costs. Though several guidelines recommended the indications for stool culture, it have been still inappropriately used for patients with diarrhea. The aims of this study were to investigate the positive rates of stool cultures and to determine the predictor of positive stool cultures in patients with acute diarrhea.

Methods: From December 2004 to July 2009, we conducted a retrospective study of patients who underwent stool culture in Chung-Ang university hospital in Seoul, Korea. The patients who visited the emergency department complaining of acute diarrhea and had acute diarrhea among patients hospitalized with another illness were included in the study. Stool samples were routinely examined for Salmonella spp., Shigella spp., and Vibrio spp. To investigate the predictors for positive stool cultures, we compared the characteristics between the patients with positive culture and those without.

Results: Of the patients who presented with acute diarrhea in the setting of emergency department, and inpatient during the study period, 5647 patients underwent stool culture. Among these patients, 115 patients had positive results (2.0%). Salmonella spp. (73.0%) was detected most frequently, followed by Vibrio spp. (27.0%). Out of the clinical features, the frequency of diarrhea and fever were statistically associated with positive cultures. Of the laboratory datas, C-reactive protein had a significant association with positive cultures. Multivariate logistic regression analysis found that frequency of diarrhea and CRP were the independent factors related with positive stool cultures. If the CRP was more than 50 mg/L, the odds ratio was 3.3 (95%CI, 2.12-5.08) for positive stool cultures.
Conclusion: Our study has suggested the frequency of diarrhea and the high value of CRP may be the independent predictors of patient with positive stool cultures in emergency and inpatient setting. These findings will likely lead to more discerning and cost-effective utilization of stool culture testing by clinicians.

Methods: In this ongoing study we have enrolled 140 children from 24 daycare centers. The children are asked to deliver a faecal sample every second month during a ten month period. When the sample was, taken an online questionnaire were filled out and send to us. The faecal samples were cultured at Statens Serum Institut by standard operating procedures for detection of enteric pathogens.

Results: So far we have received 276 samples, 5 of these were EAEC, 30 were Attaching and Effacing E. coli (A/EEC), 5 Enterotoxigenic E. coli (EIEC), 1 Entero Invasive E. coli (EIEC) and 1 Salmonella (S. muenchen).

None of the EAEC were collected from the same child or from children in the same daycare center. However both EPEC and A/EEC were isolated from child attending the same daycare center and in two cases the same types were found in siblings attending the same center. These types were also isolated from the same children at different time points.

Conclusion: Preliminary results suggest that the children are mainly colonised by E. coli strains containing the eae gene and these strains seem to be transmitted from child to child. Only few children were found to be colonised with EAEC strains.

P2069 Outcomes of and predictors for colectomy in patients with Clostridium difficile associated diarrhoea

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Background: Clostridium difficile associated diarrhoea (CDAD) has been a major problem in the last 5 years. A small minority of patients with CDAD have fulminant colitis requiring colectomy in addition to anti-microbials and general support.

Objectives: To review the prognostic factors for and outcomes of colectomy for CDAD in a large British Hospital.

Methods: A retrospective case note review of all patients with CDAD-related colectomy in 2008–2009 inclusive. This was a case control study using randomly selected CDAD patients admitted in the same period, with roughly 4 controls:1 case.

Results: There were 654 CDAD cases in 2008–2009 at the Royal Liverpool University Hospital. In total, 19 required colectomy. The majority of these patients (13/19; 68%) developed CDAD in the community. The median ages of colectomy and control groups were 72 and 74 years respectively. Seventy three percent of the colectomy group and 64% of the non-colectomy group were female. All but one of the surgical group had a sub-total colectomy. Median (range) time to surgery was 2 (0–8) days, the primary indications being systemic toxicity (N = 6), peritonitis (4), toxic megacolon (3), failure of medical treatment (2), worsening symptoms (2) and pancolitis identified on CT (2). Mortality in the colectomy group was 7/19 (36%) compared to 4/80 (5%) in the controls. Radiological abdominal imaging revealed features of severe CDAD in 13/19 (72%) of the colectomy group compared to 7/80 (7%) of the control group. The colectomy group had an increased occurrence of acute renal failure (9/19) compared to the controls (4/80), which was not not significantly associated with increased mortality within the colectomy group (4/9; 44% mortality). Individuals in the colectomy group also had considerably raised crp and peripheral white counts at diagnosis compared to the control group. No differences were found in liver function tests between the colectomy and non-colectomy group.

Summary: In this large series of patients with CDAD, 3% of patients required urgent colectomy, with favourable mortality compared to other published series. In addition to clinical & radiological indications, the main associations with need for colectomy were ARF, and markedly raised white cell count and CRP. Poor outcome following surgery related to delay of presentation to hospital following development of CDAD within the community, failure to review patients regularly within hospital and delay between surgical review and surgery.

P2070 Enterotoaggregative Escherichia coli in Danish children attending daycare centres in greater Copenhagen

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Objectives: Enterotoaggregative Escherichia coli (EAEC) are suspected to be a cause of child-hood diarrhoea. – Yet, epidemiology and severity of disease is not assessed. Therefore a large cohort study was established in order to investigate the pre-ence of enteropathogenic bacteria in faecal samples from children aged 1–6 years, who are attending daycare centers in greater Copenhagen. The aim of the study is to determine to which extend EAEC is present in the gas-tro intestinal tract of Danish children and whether it has any influence on their general health.

Methods: A epidemiological retrospective investigation was conducted, and 28 stool samples were analysed from a total of 698 revised cases. RV and NoV were detected using a commercial immunoenzymatic assay. RV-positive stools samples were typed after reverse transcription followed by nested PCR and sequenced. Results: NoV and RV infection were detected in 35.7% (10/28) and 17.9% (5/28), respectively. RT-PCR assay identified G2P[4] genotype in 4 samples, and a P mixed infection G2P[4]-P[6] in one sample. Comparison of the SP G2 sequences showed 99.9% to 100% of similarity among them, and a genetic relationship with RV strains from India. Comparison of the SP P[4] sequences showed 99.9% to 100% of similarity among them, and a genetic relationship with RV strains from Russia.

Conclusion: RV G2P[4] was recognized as the etiological agent of this confined gastroenteritis outbreak occurred during the summer. RV outbreaks during the summer are very uncommon, and it seems that is not an absolute temperature or humidity level that favors RV transmission, but rather a relative change in climatic conditions. The isolation of a Brazilian G2 strain exhibiting double P[4] and P[6] specificity was not unexpected. The inclusion of alternative P types in serotype G2 strain gene may provide a means of escaping protective immunity, and an increase in RV diversity. Moreover, these results suggest that was not the same strain circulating during this period, and there was not a common source of RV contamination.

P2071 Rotavirus G2P[4] and G2P[4]-[6] infections during norovirus gastroenteritis outbreak in 2010 summer season in the coastline area of São Paulo State, Brazil

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Objective: A key characteristic on rotavirus (RV) epidemiology is the distinct seasonal pattern. In Brazil, RV detection peaks during the winter or dry season (from May to September) in central and southern states who exhibit a temperate-like climate. During summer 2010 (December 2009 to February 2010), São Paulo State (SP), southern Brazil, experienced a large gastroenteritis outbreak due to Norovirus (NoV). A particular event was observed in Guarujá, a popular seashore city, where occurred atypical RV infections by the side of the large NoV outbreak. The aim of this study was to contribute to knowledge of seasonality of RV infection in tropical countries, performed molecular characterization of RV positive samples, and carried out sequence analyses in order to provide additional sequence data and to gain insight into the variability of Brazilian strains.

Methods: An epidemiological retrospective investigation was conducted, and 28 stool samples were analysed from a total of 698 revised cases. RV and NoV were detected using a commercial immunoenzymatic assay. RV-positive stools samples were typed after reverse transcription followed by nested PCR and sequenced. Results: NoV and RV infection were detected in 35.7% (10/28) and 17.9% (5/28), respectively. RT-PCR assay identified G2P[4] genotype in 4 samples, and a P mixed infection G2P[4]-P[6] in one sample. Comparison of the SP G2 sequences showed 99.9% to 100% of similarity among them, and a genetic relationship with RV strains from India. Comparison of the SP P[4] sequences showed 99.9% to 100% of similarity among them, and a genetic relationship with RV strains from Russia.

Conclusion: RV G2P[4] was recognized as the etiological agent of this confined gastroenteritis outbreak occurred during the summer. RV outbreaks during the summer are very uncommon, and it seems that is not an absolute temperature or humidity level that favors RV transmission, but rather a relative change in climatic conditions. The isolation of a Brazilian G2 strain exhibiting double P[4] and P[6] specificity was not unexpected. The inclusion of alternative P types in serotype G2 strain gene may provide a means of escaping protective immunity, and an increase in RV diversity. Moreover, these results suggest that was not the same strain circulating during this period, and there was not a common source of RV contamination.
Solid organ transplant infections

**P2073** Optimal duration of selective antifungal prophylaxis in heart transplant recipients

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**Background:** Invasive aspergillosis (IA) is a major cause of infection in heart transplant (HT) patients (pts) and universal prophylaxis with 3 months of itraconazole is effective and reduces mortality (Am J Transpl 2004; 4:636–643). However tolerance and interactions of itraconazole are a limitation. Newer antifungals are tolerated better but long term use may result in toxicity and they are expensive. In a previous study we demonstrated that the time period of risk for developing IA is from the onset of one or more risk factors (RF) – re-operation, CMV disease and post-HT hemodialysis – until 30 days after their disappearance. Targeted antifungal prophylaxis (TP) in this setting is feasible.

**Objective:** To determine the efficacy of TP in HT recipients given during the risk period of developing IA (onset of RF/s until 30 days after their disappearance).

**Methods:** From Jan 2003 to Nov 2010, TP was only administered to pts with at least one of the mentioned risk factors (RF). The antifungal agent was selected by the attending physician based on patient characteristics, toxicity and interactions. Endpoints were: cases of IFI during the first year after HT and adverse effects related to TP.

**Results:** During the study, 133 pts received a HT and TP was only administered to 15 (11.2% of the Tx population): caspofungin (10), anidulafungin (3), micafungin (1), and voriconazole (1). TP failed in 1 patient who was receiving a low dose of caspofungin due to liver failure (35 mg/qd despite a 35 kg/m² BMI). He developed pulmonary aspergillosis (IPA) on day +16 after HT and died. All the other high-risk HT pts were successfully protected. All pts tolerated TP, except one who developed leukopenia while receiving caspofungin.

Of the remaining 118 pts who did not receive TP, 3 (2.5%) developed IA during an outbreak in our cardiac surgical ICU caused by an extremely high environmental load of *Aspergillus* spores. The first patient had one RF (hemodialysis) and developed *Aspergillus* mediastinitis on day +44 after stopping hemodialysis. The other 2 pts did not have any RFs and developed mediastinitis and IPA on days +31 and +4 postHT, respectively.

**Conclusion:** Prophylaxis for IA in HT patients should be provided only to patients with one or more risk factors and should be maintained 30 days after their disappearance. In addition to this a high environmental load of *Aspergillus* spores should be included as a risk factor and prophylaxis should also be offered when present.

**P2074** Histoplasmosis and blastomycosis in transplant patients in Chicago

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**Background:** Blastomycosis and histoplasmosis are rare complications of solid organ and stem cell transplantations in areas surrounding the Mississippi and Ohio Rivers (USA).

**Objective:** We collected all cases of histoplasmosis and blastomycosis in transplant recipients from bill records in Northwestern hospital, Chicago, Illinois in order to describe the epidemiology and clinical course of these endemic mycoses between 2000 and 2010. We describe immunosuppressive agents used in patients, the incidence in different transplant populations, clinical and radiological presentation, time for diagnosis, diagnostic tools, patient and graft outcome, antifungal treatment and maintenance therapy.

**Results:** See the table. 10 (71%) patients had a positive culture, 4 for blastomycosis and 6 for histoplasmosis. Five patients had histological confirmation of diagnosis 52 for blastomycosis and 3 for histoplasmosis).

For patients with blastomycosis 3 out of 3 patients tested had positive blastomyecite antigen (rate 50±20) and 1 out of 2 tested had also a positive histoplasmosis antigen (rate 3), blastomycosis diagnosis was confirmed with culture. For patients with histoplasmosis 3 out of 3 patients tested had positive blastomyecite antigen (rate 61±34) and 6 out of 9 tested had a positive histoplasmosis antigen (23±20), one patient had a negative histoplasmosis antigen and a positive blastomyecite antigen, but diagnosis was confirmed with Histoplasma growing from bronchoalveolar lavage and blood culture.

| Sex ratio, male (%) | All (n=34) | Blastomycosis (n=3) | Histoplasmosis (n=9) |
|--------------------|-----------|---------------------|---------------------|
| Male               | 53±13     | 53±10               | 53±13               |
| Mean age (years)   | 14        | 5                   | 9                   |
| Solid organ transplant | 11       | 4                   | 7                   |
| Stem cell          | 3         | 1                   | 2                   |
| Time post transplant (years) | 3.8±4.5 | 5.3±6.8            | 3.0±2.8             |
| Sputum, BAL, lung biopsy | 10 (71%) | 4 (60%)            | 6 (67%)             |
| Blood culture      | 5 (31%)   | 0                   | 5 (55%)             |
| Treatment duration (months) | 20±18     | 21±24               | 19±17               |
| Hospitalisation    | 12        | 4                   | 8                   |
| Mean duration      | 19±15     | 34±14               | 21±16               |
| Hospitalisation in Intensive Care Unit | 4 | 1 | 3 |
| Mean duration (months) | 22±16   | 28                  | 19±19               |
Conclusion: Blastomycosis and histoplasmosis are rare but severe infections in the transplant populations with no reliable serologic markers.

**Primary HHV8 infection with systemic inflammatory syndrome and visceral Kaposi sarcoma after OLTx, successfully treated with cidofovir, low-dose of steroids and liposomal-doxorubicin: case report**

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**Background:** Primary post-transplant HHV8 infection has extremely high mortality. A 29-year-old woman with hemangio-endoteliolemia underwent liver transplantation. Six months later she developed fever, weakness, severe sinus tachycardia, maculo-papular skin rash. Bacterial cultures and routine viral PCRs were negative. Donor/recipient HHV8 serology was mismatched and HHV8-DNA, searched for on patient’s blood, came back positive 1,500,000 copies/ml. On post-admission day 1 she was started on cidofovir. Total body CT scan showed multiple lymphoadenopathies on neck, chest and abdomen; no other abnormalities. On day 5, she had continuous fever (40.3°C), severe sinus tachycardia (160/min), ARDS, oligo-anuria, severe anemia and thrombocytopenia (5,000/ml); skin rash turned violet. Blood pressure, WBC and graft function tests surprisingly normal. HHV8-viral load was 19,000,000 copies/ml; 17,000,000/ml on day 7. She had a second dose of cidofovir. Cervical lymph node biopsy showed Kaposi’s sarcoma (KS) and positive latent HHV8-Ag immunohistochemistry. Liposomal-doxorubicin (20 mg/m2 q2weeks) was then administered. With clinical status approaching MOF, low dose of steroid (methyl-prednisolone 0.5 mg/Kg bid) was added. After 72 hrs (day 11), patient had no fever, HR below 100/min, respiratory status and urine output back to normal. Meanwhile, HHV8-viral load was 600,000/ml (day 11), 2,000/ml on day 13, undetectable on day 18. Patient had two additional cidofovir and five liposomal-doxorubicin doses. In the last 15 months of 18-month follow-up, viral load was persistently below 2,500 copies/ml; undetectable in the last 6 months. CT scan (days 22 and 62) showed progressive decreasing of lymph node size and was totally normal on month 18. EGDS negative; colonoscopy refused. Data from the acute phase were analyzed with Pearson’s test. We found significant correlation between viral load and both heart rate (p = 0.0246, r = 0.7725) and platelet count (p = 0.0158, r = -0.8054); and between heart rate and platelet count (p = 0.0003, r = -0.9515).

**Conclusion:** Primary HHV8 infection after liver transplantation was associated with a systemic non-neoplastic life-threatening inflammatory illness, in addition to KS. Sinus tachycardia and thrombocytopenia correlated with viral load. Cidofovir and liposomal-doxorubicin controlled virus replication and neoplastic disease (KS) while low dose of steroids seemed to have been crucial during the acute phase in controlling inflammatory response.

**Monitoring of cell-mediated immune parameters identifies kidney transplant recipients at high risk of severe infection**

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**Objectives:** Infection represents a significant source of morbidity and mortality after kidney transplantation (KT). End-stage renal disease (ESRD) is associated with immunosuppression, as measured by low absolute lymphocyte count and peripheral blood lymphocyte populations (PBLPs). We aimed to assess if monitoring of these simple, broadly available, surrogate parameters of the cell-mediated immune response might predict the occurrence of infection in KT recipients.

**Methods:** ATALANTA is an ongoing, observational study in which ESRD patients undergoing KT at our institution are enrolled in a protocol including the evaluation of total lymphocyte count and PBLPs (assessed by flow cytometry) at baseline and at months 1 and 6 post-transplant, with prospective surveillance of infectious and non-infectious complications. For the present study we analyzed data from patients included between November 2008 and November 2009. Risk factors for severe infection occurring during the first year post-transplant (defined as blood stream infection, pneumonia, cytomegalovirus [CMV] infection, or invasive fungal infection) were analyzed by logistic regression.

**Results:** We included 143 KT recipients (89 males; mean age: 54.9±14.9 years). During the follow-up period (median: 349 days), 32 patients (22.4%) had at least one episode of severe infection (including 24 episodes of blood stream infection, 12 episodes of pneumonia, 42 episodes of CMV infection, and 3 episodes of invasive fungal infection). Recipients with low total CD3 (<1.125×10^5/μL) and CD8 T-cell counts (<0.375×10^5/μL) at baseline developed a higher incidence of severe infections (P = 0.037 and 0.008, respectively). Recipients with low CD8 T-cell count (<0.165×10^5/μL) at month 1 were at higher risk for subsequently developing severe infection between months 2 and 6 post-transplant (P = 0.002). After adjustment for other factors (including type of induction therapy, long-term graft dysfunction and acute rejection), low CD8 T-cell count at baseline emerged as an independent risk factor for severe infection (OR = 6.01; 95% CI = 1.69–21.35; P = 0.006).

**Conclusion:** Low CD8 T-cell counts at baseline and at month 1 after KT are associated with increased risk of severe infection. In our
Identification of patients at high risk for infections after liver transplantation

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Objectives: Liver transplantation (LTx) is a successful therapy for patients with end-stage liver disease. Infections are an important cause of morbidity and mortality in the first three months after transplantation with a reported incidence up to 60%. Thus, we aimed to conduct a study to develop a model for predicting both bacterial and fungal infections.

Methods: Medical records of 200 LTx recipients transplanted between 2005 and 2009 at the Erasmus MC Rotterdam were reviewed. We registered all infections according to CDC definitions during the first three months after LTx and noted potential risk factors for developing infections. Multivariate logistic regression was used to create a predictive model for infections in general. Subsequently, we validated this model in another cohort of 94 LTx recipients transplanted between 2007 and 2009 at the Ghent University Hospital.

Results: The model consists of the following parameters: (1) preoperative haemodialysis; (2) preoperative Candida colonization; (3) history of abdominal surgery; (4) Sodium <130mmol/l on day of LTx; (5) intubation >3 days after LTx; (6) relaparotomy within 5 days for intra-abdominal bleeding, anastomosis leakage, vascular insufficiency or (7) retransplantation.

In high risk patients 2 or more conditions were present. In the Rotterdam cohort 81% of high risk patients developed an infection versus 50% of low risk patients [OR 4.273 (2.1–8.9)]. Surgical site infections were the most frequent and 41 out of 109 bacterial infections (38%) were caused by 41 Enterococcus species. In the Ghent cohort 80% of the high-risk patients developed an infection compared to 48% of the low-risk patients [OR 4.3 (CI 1.3–14.2)].

Conclusion: This model based on 7 clinical parameters identifies patients at high risk for bacterial and fungal infections in the first 3 months after liver transplantation. Close monitoring, starting anti-bacterial and fungal prophylactic treatment and minimization of immunosuppressive therapy should be considered in these high risk patients.

Infections in patients with pancreas transplantation

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Background: Pancreas transplantation has a high incidence of nosocomial and opportunistic infectious complications. The purpose of this study was to analyze an analysis of all pancreas transplant recipients from July 2003 to December 2009 with at least one year of follow-up. Multivariate logistic regression analysis was performed to identify independent variables associated with post-transplant infections.

Results: We included 147 patients (18 isolated pancreas and 129 simultaneous kidney pancreas) with a median follow-up of 729.5 days. All patients had enteric pancreas drainage. We diagnosed 151 episodes of infection in 72 patients (49%). Out of 151 episodes, bacterial were the most frequent (129, 85%), followed by viral (18, 12%), fungal (3, 2%) and parasitic (1, 0.7%). By site, the most frequent locations were: urinary tract (70, 46%), lung (17, 11%), surgical wound (15, 10%), intra-abdominal (12, 8%), catheter (9, 6%), gastrointestinal (6, 4%), primary bacteraemia (6, 4%), bone and joint infections (2, 1%) and others (14, 9%). Isolated species were: Gram negative bacteria 62% (E. coli 19%, P aeruginosa 12%, Enterobacter sp. 11%, Klebsiella pneumoniae 10%, Citrobacter 5%) and Gram positive 22% (Enterococcus sp. 8%, coagulase-negative staphylococci 5%, S. aureus 2%). Multidrug resistant bacteria were found in 33% of the episodes of bacterial infection. Nineteen episodes (15%) of bacterial infection episodes coursed with bacteraemia. There were no differences in the incidence of infections between isolated pancreas and simultaneous kidney-pancreas transplantation. Forty-eight percent of patients developed infection during the first month after SOT (65 bacterial infection, 3 CMV, 1 pneumonia by influenza, 1 surgical wound infection and 1 intra-abdominal infection by Candida sp., 1 Cryptosporidium-associated diarrhoea). Independent variables associated with infection after pancreas transplantation were: Post-transplant dialysis (OR 15.7, 95% CI 1.9–128.2), post-transplant surgery requirement (OR 3.5, 95% CI 1.6–7.7) and previous CMV disease (OR 23.8, 95% CI 2.9–193.0). Three patients died during the follow up of which 2 were deaths related to infection.

Conclusions: Infections are frequent after pancreas transplantation but are accompanied with low mortality rates (<5%). More complicated course of the transplant procedure is associated with higher risk of infections.

Descriptive analysis of infections produced by respiratory viruses in solid organ transplant recipients

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Objectives: Respiratory infections constitute a major cause of morbidity and mortality in solid organ transplant recipients (SOTR). Published data regarding infections by respiratory virus in these patients are scarce, and their clinical features may be noticeably different from those observed in general population. This study aims to describe the etiology, epidemiology, clinical features and prognosis of viral respiratory infections in non-pulmonary SOTR.

Methods: All patients receiving liver, kidney and/or heart transplant in our center between January 1st and September 30th were prospectively included. Nasopharyngeal swabs were collected from each patient at inclusion and when respiratory symptoms presented. Systematic follow-up was programmed with one-month periodicity, and additional clinical evaluation was performed whenever respiratory symptoms were detected. Multiplex PCR was carried out on nasopharyngeal swabs from symptomatic patients.

Results: 97 cases were included. The nasopharyngeal swab performed at inclusion revealed positive PCR results in 23 patients (23.7%), 21 of which were asymptomatic. Up to 84.5% of patients had received influenza vaccination by the time they were included. 20.6% cohabited with children with ages below 3-years-old attending to nursery schools. 23 episodes of respiratory infections in 18 patients were included. The most common symptoms were rhinorrhea (95.7%), cough (73.9%), sore throat (73.9%) and fever (17.4%). The etiologic agent most frequently isolated were influenza A (n = 8, 34.8%) – 3 of which corresponded to H1N1 serotype, coronavirus (n = 3), and rhinovirus (n = 2). PCR resulted negative in 5 patients. None of these episodes of infection presented signs of severity nor required hospital admission. In up to 30.4% of cases, symptoms were not completely solved after 14 days from the beginning of the clinical disease, although only 4.3% presented secondary complications (bacterial sinusitis).

Conclusions: Infections produced by respiratory viruses is a frequent, usually non-severe entity in SOTR, though the clinical course may be more prolonged in this population. The influence of epidemiologic factors like cohabitation with children attending to nursery schools, or the immunization of close contacts constitute issues still not resolved. Asymptomatic carriers of respiratory viruses detected by multiplex-PCR techniques were prevalent in our sample, and the clinical value of this finding needs to be established.
Prevalence and clinical characteristics of mycobacterial infection in a cohort of solid organ transplant recipients over a 13-year period

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Objectives: Solid-organ transplant (SOT) recipients have an increased susceptibility to mycobacteria, but the epidemiology of mycobacterial infection in this population is not well characterized. We assessed the prevalence and described the clinical characteristics of mycobacterial infection in a large cohort of SOT recipients.

Methods: We included all consecutive patients who received a solid organ transplant at our institution between 1997 and 2010. All positive isolates for mycobacteria (either by culture or PCR) were identified from the database of the microbiology laboratory. Medical records of patients with a positive mycobacterial isolate were reviewed. Standard ATS criteria were applied for the diagnosis of mycobacterial colonization or disease.

Results: Overall 852 patients received a solid organ transplantation between 1997–2010. 46% received a kidney, 25% a lung, 16% a heart and 13% a liver transplant. 65% of patients were male and mean age at the time of transplantation was 48.8 years. Two patients (1 heart and 1 kidney transplant recipients) presented with post transplant pulmonary tuberculosis. Mycobacteria other than tuberculosis (MOTT) were isolated in 9 patients: 6 were considered as having bronchial colonization (2 cases of M. gordonae, and 1 case each of M. szulgai, M. hassiacum, M. xenopi, and one non-identified rapidly-growing mycobacteria) and 3 patients as having disease. Median time from transplantation was 24 months (3.5, 45) for TB, 15 months (0–55) for MOTT colonization, and 0.2 months (0–5) for MOTT disease. The prevalence of tuberculosis, MOTT colonization and MOTT disease was 0.2%, 1% and 0.4%, respectively. Of the 3 patients with MOTT disease, 1 kidney transplant recipient had urinary tract infection due to M. kansasi, and 2 lung transplant recipients had pulmonary infection with M. avapense and M. avium intracellulare, respectively. All 3 patients received antymycobacteral treatment with azithromycin and ethambutol plus a third drug (either isoniazide, ciprofloxacin or rifabutin). Median duration of treatment was 8.5 months (3.5–12). No relapse or infection-associated mortality was observed.

Conclusions: In this cohort of SOT recipients, the prevalence of mycobacterial infection after transplantation was very low. Most patients with a positive isolate of MOTT were considered to be colonized rather than infected. Larger cohorts are needed to better characterize mycobacterial infection after organ transplantation.

Tuberculosis in solid organ transplant recipients: risk factors and treatment outcome

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Objectives: Solid organ transplant (SOT) recipients are at increased risk of tuberculosis compared to the general population with well-recognized risk factors established. Diagnosis and treatment of TB are further complicated by immunosuppression, drug toxicity, interaction between immunosuppressive agents and anti-TB drugs. Purpose of this study was to investigate incidence, risk factors, and treatment outcome with rifampicin of TB in SOT recipients.

Methods: By a retrospective cohort study, incidence density of TB was calculated. Risk factors for TB were analyzed by a nested case-control study among the 2144 SOT recipients. Treatment outcome and effect on immunosuppressants and allograft were compared between patients whose initial 2-month intensive regimen included rifampicin or not. Four patients who received rifabutin were excluded from the analysis.

Results: From Feb 1995 to June 2010, a total of 2144 patients received solid organ transplantation. The median duration of follow-up in these patients was 1522 days (range, 1–5693 days). During the follow-up of 2144 SOT recipients, 40 cases of post-transplantation TB were found (1.7%). The incidence density was 372 cases per 105 patient years (95% CI, 270–503), which was higher than that for the general Korean population (90 cases per 105 person years). The median time to the development of TB was 234 days (range, 33–3940 days). TB developed within 1 year of transplantation in 24 (60%) recipients. In a nested case-control study, use of tacrolimus (OR 4.90; 95% CI, 1.74–13.80; P = 0.003) and CMV infection within the prior 3 months (OR 4.62; 95% CI, 1.44–14.87; P = 0.01) were found to be risk factors for TB. Patients whose intensive regimen included rifampicin were more likely to increase the dose of calcineurin inhibitors than patients whose intensive regimen spared rifampicin (13/15 (86.7%) vs. 3/14 (21.4%), P = 0.001). However, graft rejection and mortality were not different between the two groups.

Conclusions: Use of tacrolimus and CMV infection as risk factors for TB in SOT recipients from this comparative study are novel findings to our knowledge. Although patients with rifampicin needed to increase the dose of immunosuppressants more frequently, either rifampicin-using regimen or rifampicin-sparing regimen were used with similar efficacy and safety in terms of graft outcome and mortality.

Validation of a fungal infection prediction model in 2 European liver transplant centres

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Objectives: Liver transplantation (LTx) is a successful therapy for patients with end-stage liver disease. Invasive fungal infections (IFI) are an important cause of morbidity and mortality in the first three months after transplantation with a reported incidence up to 47%. A recent described prediction model identified LTx recipients who were high risk for developing IFI. This model was based on the presence of >1 of six following conditions: hepatoenjumostomy biliary anastomosis; retransplantation; intra-operative administration of >40 units blood products; return to the operating room for intra-abdominal bleeding, anastomotic leak or vascular insufficiency; preoperative serum creatinine of >=177µmol/l and perioperative Candida colonization (Pappas et al., Am J Transplant. 2006;6:386–91). This retrospective study was conducted to validate this American risk model in two European transplant centers.

Methods: Medical records of 200 LTx recipients transplanted between 2005 and 2009 at the Erasmus MC Rotterdam and 94 LTx recipients transplanted between 2007 and 2009 at the Ghent University Hospital were reviewed. We registered invasive fungal infections for a period of three months after LTx. All six risk factors were recorded from the patient files.

Results: In Rotterdam 37 patients were defined as high risk of which 14 (37.8%) developed IFI; 163 patients were at low risk, 17 (10.4%) developed IFI (p < 0.001). Two (6.5%) IFI were due to A. fumigatus, 29 (93.5%) due to Candida spp. In the Ghent cohort, 25% of high risk and 2.9% of low risk patients developed IFI (p = 0.021).

Conclusion: This study in two European transplant centres confirmed the value of the so called Pappas risk model identifying ‘high risk’ patients for developing IFI. As the numbers of IFI due to Aspergillus spp are very low, fluconazol as perioperative prophylaxis in these patients is recommended.

Infections in the immunocompromised host

Risk factors for development of Pseudomonas aeruginosa in non-neutropenic cancer patients with Gram-negative bacteremia: a case-control study

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Objective: Even though febrile neutropenia occurring after chemotherapy in cancer patients has been identified as a strong risk factor for
Vancomycin-resistant enterococci control measures in a hematologic and bone marrow transplant unit

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Introduction and Objectives: It’s known that healthcare associated infections (HCAI) increases morality, costs and hospitalization length, and can be prevented by measures focused on patients care. Vancomycin-resistant enterococci (VRE) is a nosocomial pathogen with increasing concern in hematologic units. Our aim was to assess the VRE incidence in a hematologic and bone marrow unit, featuring outbreaks and analyzing employed control measures.

Methods: We analyzed oncohematologic and stem-cell transplanted patients admitted on HC-FMUSP hematologic unit from 2002 to 2010. VRE data was collected by active surveillance, with cultures obtained weekly on all patients, except those known to be VRE positive. Incidence density (cases/1000 patient-days) rates were calculated for colonization (cID) and infection (iID), and graphical representations were built to detect outbreaks. For outbreak periods a data ase was built and used for assessment of several markers: M-ficolin and H-ficolin, members of the human innate immune system, bind surface structures of different micro-organisms and activate the lectin pathway of complement. We hypothesized that ficolin deficiency could be responsible for infections in hematologic malignancies.

Results: We analyzed 4413 patients, 48470 patient-days and 775 HCAI. VRE colonization and infection were diagnosed in 442 and 46 patients respectively. There were two distinct periods, from 2002 to Oct-2005, where E. faecium cases occurred, and from Nov-2005 to 2010, where E. faecium was predominant. Only two outbreaks were identified during the study period, caused one by E. faecalis and other by E. faecium. The iID was significant greater during outbreak period. The most common infection was bloodstream infection (BSI) in both outbreaks. The intervention measures used was staff education, hand hygiene and contact precaution measures reinforcement. Since 2005 linezolid is used pre-emptively on VRE colonized febrile patients during empirical antimicrobial coverage, and since 2009 all patients received daily chlorhexidine bathing.

Discussion: VRE has become a true challenge to haematological patients. As showed above and in other reports infection represents only 10% of real VRE magnitude, and it control requires particular techniques. Pre-emptive use of Linezolid is still a controversial issue.

Chlorhexidine bathing has shown helpful preventing BSI on critical patients, and although more empowered assays are needed, it seems to provide beneficial effect in haematological patients too.
chemotherapy cycles) could be used resulting in 117 samples. The endpoints were febrile neutropenia, documented infections, bacteremia or severe infections during the chemotherapy cycle and the neutropenic period.

**Results:** The median age of the patients was 54 years. 33% of the patients had acute leukemia; 35% had lymphoma/HD. Patients developed a severe infection during chemotherapy in 34 of the 117 chemotherapy cycles. Lower M-ficolin concentration was found in patients who developed a severe infection: median 267 ng/ml compared to 473 ng/ml in patients who did not develop a severe infection (P < 0.01). Significant association was also found for low M-ficolin and the three other endpoints.

Thirty-two of the 105 patients developed a severe infection. Considering M-ficolin <351 ng/ml as deficient, the time to development of severe infection was shorter in the M-ficolin deficient group: hazard ratio of 2.60 (95% CI, 1.23 to 5.49).

No statistically significant associations between severe infection and H-ficolin or MASP-3 concentration were found. Higher MASP-2 concentration was present in patients who developed a severe infection: median 355 ng/ml compared to 367 ng/ml in patients who did not develop a severe infection (P < 0.01).

**Conclusion:** Patients with M-ficolin deficiency who receives chemotherapy are more likely to develop severe infection. This result needs to be prospectively confirmed with adjustment for other risk factors for the development of severe infection.

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**Methods:** We determined immunoreponses to two doses of the AS03-adjuvanted pandemic influenza vaccine in SOL recipients.

**Objectives:** Solid organ transplant (SOT) recipients are considered a priority group for influenza vaccination. However, immune responses have been unsatisfactory and strategies enhancing immunogenicity, including adjuvants, are needed. The objectives of our study were to determine humoral immune responses to adjuvanted 2009-pandemic influenza vaccines in SOT recipients compared to healthy controls.

**Results:** Twenty-eight patients (95 males, mean age of 61 years) were included. Nineteen patients received the AS03-adjuvanted influenza vaccine, while nine received the non-adjuvanted vaccine. The geometric mean titre (GMT) for 1:40, 1:80, 1:160, 1:320, and 1:640 were 21, 26, 53, 67, and 76, respectively, in the AS03 group, and 18, 20, 32, 38, and 44, respectively, in the non-adjuvanted group. The GMT was significantly higher in the AS03 group for all dilutions except 1:320. The GMT for the 1:40, 1:80, 1:160, 1:320, and 1:640 were 21, 26, 53, 67, and 76, respectively, in the AS03 group, and 18, 20, 32, 38, and 44, respectively, in the non-adjuvanted group.

**Conclusion:** Patients with M-ficolin deficiency who receives chemotherapy are more likely to develop severe infection. This result needs to be prospectively confirmed with adjustment for other risk factors for the development of severe infection.

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**Methods:** Of 196 consecutive adult liver transplant patients 32 belonging to the CMV high risk group received antiviral prophylaxis (valganciclovir 900 mg daily and/or i.v. ganciclovir 5mg/kg/d) up to 3 months after transplant-tation. The basic immunosuppression consisted of CN inhibitors, azathioprine/MMF plus steroids. The patients were frequently monitored for CMV by real-time quantitative plasma PCR. Tissue invasive CMV infections were demonstrated in biopsy material by immunohistochemistry.

**Results:** During antiviral prophylaxis, no break-through CMV infections were recorded. After cessation of valgan-ciclovir prophylaxis 12/32 (37%) patients developed primary CMV infection mean 181 days (range 95–365 days) post transplantation. Two low level CMV infections were asymptomatic and were not treated with antivirals, 6 primary infections were successfully treated with valganciclovir, and 4 severe cases of CMV disease with intrave-nous ganciclovir and secondary valganciclovir prophylaxis. No intragaft CMV infection was found in these pa-tients, but one developed gastrointestinal complication with a positive CMV finding in ileum biopsy material. No patient or graft was lost due to CMV.

**Conclusions:** No break-through CMV infections were recorded during valganciclovir prophylaxis. Primary CMV infections were common after cessation of prophylaxis. However, of these late-onset infections only a few were severe, and all infections successfully treated with valganciclovir or intravenous ganciclovir.
Safety and clinical outcomes of high-dose daptomycin for enterococcal infections

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Objective: Few optimal agents are available to treat enterococcal infection, including vancomycin-resistant enterococci (VRE). Daptomycin (DAP), approved at dosages up to 6 mg/kg/day for many clinically significant Gram-positive infections, may be beneficial at higher dosages due to its concentration-dependent activity. We evaluated the safety and clinical outcomes of high-dose (HD) DAP (>6 mg/kg/day) for enterococcal infections.

Methods: A retrospective case series from 8 academic medical centres. Consecutive patients (pts) treated for non-urinary enterococcal infection with HD DAP for >72 h, excluding dialysis pts, were collected from 2005–2010. Charts were reviewed for demographics, comorbidities, antimicrobial therapy, microbiologic cultures, clinical outcomes, and adverse events.

Results: 102 eligible pts were identified. Baseline characteristics: Median (interquartile range (IQR)) age 57 yrs (46–66 yrs) and APACHE II 10 (6–16), 87.3% prior hospitalization within 1 yr, 39.2% diabetes, 9% <3 and 37% >4. Most common DAP dose: 8.0 (8–10) mg/kg/day, duration of HD DAP: 12 days (6–16), length of hospital stay: 26 days (14–48), duration of bacteremia: 3 days (1–8). 85.9% had a favourable clinical outcome, defined as clinically improved or cured, and 91.8% microbiological eradication. Safety: 81.4% pts had peak creatine phosphokinase (CPK) levels <200 IU/L (29.8–121.3), and 89.1% had end-of-therapy CPK levels <200 IU/L (22.5–82.3). No discontinuation of HD DAP due to adverse events was observed.

Conclusion: HD DAP may prove to be a therapeutic option in pts with VRE infections. Efficacy and safety rates were favourable in these pts, with no pts discontinued from therapy due to adverse events. Further studies with a larger cohort are warranted in pts with VRE infections.

P2090 Results from the European Cubicin® outcomes registry and experience: daptomycin is effective and safe in patients with neutropenia and Gram-positive infections

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Objectives: The use of bactericidal agents is preferable in neutropenic patients (pts). In comparison to other anti-MRSA agents, e.g. linezolid, tigecycline, vancomycin, and teicoplanin, daptomycin (DAP) has the most potent in-vitro and bactericidal activity against Gram positive (G+) pathogens (Fuchs, 2002; Huang, 2008). The objective of this analysis was to evaluate the efficacy and safety of DAP in neutropenic pts with G+ infections.

Methods: The European Cubicin® Outcomes Registry and Experience (EU-CORE) is a retrospective, non-interventional, multicenter study describing characteristics and outcomes of pts treated with DAP. Outcomes (cured and improved = success, failure, non-evaluable) were assessed at the end of DAP therapy by the investigators and safety data collected up to 30 days after end of therapy. Among 3621 pts enrolled from 2006 to 2010 all pts with neutropenia at baseline or during DAP therapy were eligible. Subgroups were specified: Lowest neutrophil count ≤100, 100–499 and 500–1000 cells/mm³.

Results: 259 neutropenic pts were identified mostly with hematologic malignancies or transplantation. 114 pts (46%) had severe neutropenia (neutrophil ≤100 cells/mm³), 60% were male, 26% pts were ≥65 yrs old, and 6% pts had CrCl <30 mL/min. The most frequent reasons for prior antibiotic discontinuation were clinical failure or resistance. Bacteremia was the most common diagnosis. Positive cultures were reported in 159 pts (61%). Coagulase-negative staphylococci was the most common pathogen (72/159, 45%), mainly S. epidermidis. S. aureus was isolated in 21% of cases (49% MRSA rate). Overall clinical success was 76% (196/259) (Figure 1). Success rates were 86% for coagulase-negative staphylococci, 73% for S. aureus (75% against MRSA) and 63% for enterococci. DAP was well tolerated. CPK elevation of >5 to 10xULN was reported in only one patient. DAP was discontinued in 6% of pts due to adverse events, mostly infections.

Conclusion: Daptomycin was effective and safe in the treatment of Gram-positive infections in pts with neutropenia regardless of severity.

P2092 Nosocomial Pneumocystis jirovecii pneumonia: lessons learned from a case cluster in kidney transplant recipients

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Objectives: Pneumocystis jirovecii pneumonia (PJP) is an important infectious complication in kidney transplant recipients with an associated mortality of approx. 48%. The risks for, and route of transmission of infection in an outbreak setting are incompletely defined. We describe the epidemiology and risk factors during a case cluster of PJP.

Methods: PJP cases from March-October 2010 in renal transplant patients, Westmead Hospital, Sydney, were reviewed for clinical, microbiological and radiological data, and compared with those from unaffected patients in a case control study. Investigations to determine contact between patients identified a common clinic area where patients had co-localized. Diagnosis was based on a combination of clinical assessment, X Ray changes and positive PCR, targeting β-tubulin gene, of induced sputum/BAL fluid. Patients with typical clinical/X Ray features where BAL fluid/induced sputum specimens were not tested by PCR but where there was no alternate cause of infection had probable PJP. Oropharyngeal rinses from staff and samples of ambient air were tested for P. jirovecii. P. jirovecii genotypes were determined by sequencing of the ITS, DHPS, β-tubulin and mtLSU genes.

Results: 10 definite, and 2 probable, cases of PJP occurred (expected rate 0.2±0.42 cases/yr). Of the PJP cohort, 9 (75%) were male (mean age 46 yr [range 20–64]). The median time from transplant was 4 years. Multivariate analysis identified preexisting lung disease (OR 15.5; p < 0.001), prior/current CMV infection (OR 92.3, p < 0.001) and impaired eGFR (OR 9.6±10mL/min, p < 0.05) as independent predictors of PJP. 9 patients tested unaffected patients. Prednisone use or greater number of outpatient visits were not associated with increased PJP risk. Identification of a common source prompted extension of PJP prophylaxis (Bactrim) till 12 mo. in recent recipients and reintroduction
of BacTrin to all exposed patients. B-tubulin, DHPS and ITS genotypes were identical for 8 patients tested but mtLSU genotypes differed in 2 patients. P. jirovecii was not detected in staff samples.

Conclusions: PJP clusters in kidney transplantation may result from interhuman transmission. Underlying lung disease, CMV infection and impaired graft function were risk factors. Early recognition of an outbreak and prompt pre-emptive prophylaxis to exposed patients may limit its escalation.

**P2093** Orthotopic liver transplantation in HIV-infected patients: experience in a cohort of 27 patients

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**Objective:** We evaluated clinical, biological and immunovirological outcomes of a prospective cohort of HIV infected liver transplant recipients.

**Methods:** We evaluated 27 HIV positive patients who underwent 29 liver transplantations between September 2003 and October 2010 at our center.

**Results:** Participants’ characteristics are described in Table 1. Causes of end stage liver disease were HCV related liver cirrhosis (n = 22), HBV related cirrhosis (n = 4), and cryptogenic liver cirrhosis (n = 1). Ten patients had hepatocellular carcinoma. At time of waiting list enrollment all but one patient had a CD4 T cell count greater than 100 cells/mmc, median CD4 count was 303 cells/mmc (range 49–836). Twenty-two patients had undetectable viral load, the 5 patients with detectable viral load had genotype predictive of viral suppression with HAART. Post transplant HAART consisted of 2 NRTIs plus a third agent that was NNRTI for 19 patients, PI for one patient, enfuvirtide for 2 patients and raltegravir for 5 patients. The average CD4 count fell to 218 cells/mmc at 1 month post-transplant but then rose to 291 and to 316 cells/mmc at 6 and 12 months post transplant, respectively. At 1 month after transplantation, six patients experienced an increase in HIV viral load but all the 19 patients who reached one year follow up had HIV-RNA below 50 copies/ml. After transplantation 20 patients presented detectable HCV-RNA. Two-third of patients (12/20) received therapy for HCV infection and the median viral load CMV at onset the treatment was 6620 copies/ml (range 2755, 20800). After assessing the different cutoff points by ROC curves, 2870 copies/ml was defined as the best cutoff point for early therapy initiation, with specificity 96%, sensitivity 85.7%, negative predictive value (NPV) 99.9% and positive predictive value (PPV) 9.8%. The area under the ROC curve was 97.9%. In the validation cohort 123 patients were included (51 kidney, 23 liver, 1 liver-kidney, 1 heart) and a total of 1381 CMV RT-PCR were performed, of whom 346 (25.1%) were positive. Seven patients (8.1%) of CMV disease were diagnosed: 4 viral syndrome and 3 CMV organ-disease. In the remaining, 79 patients (94.2%), the diagnosis was asymptomatic viremia. Twenty-nine patients (32.6%) received therapy for CMV infection and the median viral load CMV at onset the treatment was 6620 copies/ml (range 2755, 20800). After assessing the different cutoff points by ROC curves, 2870 copies/ml was defined as the best cutoff point for early therapy initiation, with specificity 96%, sensitivity 85.7%, negative predictive value (NPV) 99.9% and positive predictive value (PPV) 9.8%. The area under the ROC curve was 97.9%. In the validation cohort 123 patients were included (51 kidney, 41 liver, 10 heart) and a total of 2508 CMV RT-PCR were performed, of whom 392 (15.6%) were positive. Were diagnosed 11 patients (8.9%) of CMV organ-disease, no cases of viral syndrome. Fifty-six patients (45.5%) received therapy for CMV infection and the median viral load CMV at onset the treatment was 3620 copies/ml (2293, 5570). In plotting the ROC curve in the validation cohort, 2870 copies/ml was associated with a sensitivity, specificity, NPV and PPV of 70.0%, 96.6%, 99.8% and 12.3% respectively.

**Conclusions:** 1. Real time PCR CMV is an effective predictive diagnostic test to guide preemptive therapy. 2. A value of 2870 copies/ml is defined and validated as the best cutoff point for starting preemptive therapy in low risk SOTR.

**Molecular diagnosis of invasive fungal infections: has the era of empirical antifungals come to an end?**

**P2094** Evaluation of real-time PCR CMV, clinic correlation, determination and validation of cut-off point for CMV infection in low-risk CMV solid organ transplant recipients

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**Objectives:** The aims of this study were: 1. To study the correlationship between CMV viral load determined by RT-PCR and signs or symptoms of CMV infection in low risk patients SOTR. 2. To set up a cutoff point of CMV viral load to start preemptive therapy. 3. To validate this cutoff point in a external cohort of these patients.

**Methods:** A prospective cohort study was performed (10/08–08/10). All low risk CMV infection SOTR were included. CMV infection and outcome was evaluated. A descriptive analysis was done. In the derivation cohort (10/08–05/09), a cutoff point was determined using G-Stat 2.0 for analysis by ROC curves of sensitivity and specificity in the full spectrum of cutoff points in the over range of observed results. Validation of the cutoff point was carried out with cases included from 06/09–08/10.

**Results:** In the derivation cohort 86 patients were included (51 kidney, 23 liver, 1 liver-kidney, 11 heart) and a total of 1381 CMV RT-PCR were performed, of whom 346 (25.1%) were positive. Seven patients (8.1%) of CMV disease were diagnosed: 4 viral syndrome and 3 CMV organ-disease. In the remaining, 79 patients (94.2%), the diagnosis was asymptomatic viremia. Twenty-nine patients (32.6%) received therapy for CMV infection and the median viral load CMV at onset the treatment was 6620 copies/ml (range 2755, 20800). After assessing the different cutoff points by ROC curves, 2870 copies/ml was defined as the best cutoff point for early therapy initiation, with specificity 96%, sensitivity 85.7%, negative predictive value (NPV) 99.9% and positive predictive value (PPV) 9.8%. The area under the ROC curve was 97.9%. In the validation cohort 123 patients were included (72 kidney, 41 liver, 10 heart) and a total of 2508 CMV RT-PCR were performed, of whom 392 (15.6%) were positive. Were diagnosed 11 patients (8.9%) of CMV organ-disease, no cases of viral syndrome. Fifty-six patients (45.5%) received therapy for CMV infection and the median viral load CMV at onset the treatment was 3620 copies/ml (2293, 5570). In plotting the ROC curve in the validation cohort, 2870 copies/ml was associated with a sensitivity, specificity, NPV and PPV of 70.0%, 96.6%, 99.8% and 12.3% respectively.

**Conclusions:** 1. Real time PCR CMV is an effective predictive diagnostic test to guide preemptive therapy. 2. A value of 2870 copies/ml is defined and validated as the best cutoff point for starting preemptive therapy in low risk SOTR.

**P2095** Clinical validation of a marketed real-time PCR assay for diagnosis of invasive aspergillosis in patients without haematological cancer

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**Background:** Methods based on real-time PCR may anticipate the diagnosis of invasive aspergillosis (IA) but are still limited by the lack of standardization. We validated the standardized MycAssay™ Aspergillus (Myconostica, Ltd) for the IA diagnosis in patients without haematological cancer, an emerging population at risk of IA.

**Methods:** We prospectively collected 394 samples (November 2009–May 2010) from 207 patients with these IA predisposing conditions: solid cancer (19%), cirrhosis (15%), corticosteroids use (60.4%), HIV (12.6%), COPD (50%), solid organ transplantation (11%), or none (8.2%). Specimens were obtained when clinically indicated and
sourced mostly from the lower respiratory tract (97%). Specimens were processed for microbiological culture; *Aspergillus* DNA was extracted and amplified by means of MycXtra™ and MycAssay™.

**Results:** According to the EORTC and Bulpas’s (for patients with COPD) criteria, patients had proven/probable/possible IA (n=2/11/5), probable scedosporiosis (n=1), or no invasive mold infection. The 13 patients with proven/probable IA had solid cancer (23%), cirrhosis (23%), corticosteroids use (85%), HIV (15%), COPD (38%), or heart transplantation (8%). *Aspergillus* spp. was isolated from 63 samples (44 patients). The mean number of days from sample culture until fungal growth visualization was 5 (range, 1–21; SD, 5). PCR results (>4 hrs sample to result) were negative (n=330), positive (n=45), or indeterminate (n=18). Sensitivity (S), specificity (E), positive and negative predictive values (PPV and NPV) of the assay for the diagnosis of IA (first sample/any sample) were: S (77/92), E (86/85), PPV (35/32), NPV (98/99).

**Conclusions:** MycAssay™ showed a high sensitivity for the diagnosis of IA in patients without hematological cancer, which increased when multiple samples were used. PCR significantly reduced the time to diagnosis compared to fungal culture. Maria Alonso (UCAM) was involved in the RT-PCR analysis.

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**P2096** A novel clinical diagnostic assay for the identification of multiple human fungal pathogens in a single sample

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**Objectives:** Surface-Enhanced Resonance Raman Scattering (SERRS) is a highly sensitive and molecular-specific detection system which can be used to detect multiple human pathogens in a single sample. The objective of this study was to develop a screening assay for the multiplex detection of fungal targets using PCR and SERRS.

**Methods:** The assay was designed to detect 23 species of fungi known to cause invasive fungal disease (IFD). As *Candida* and *Aspergillus* species account for >80% of all IFDs, one well of this assay was specifically designed to detect a broad range of both genera and specifically identified *C. glabrata* and *C. krusei* as these organisms may require alternative antifungal therapy. A second well of this assay is being developed to detect other causes of IFD.

A multiplex diagnostic assay was developed to detect targets at low copy number in clinically relevant samples. Universal primers and specific probes were designed to amplify and detect each target. One primer from each primer set was biotinylated to allow capture of the amplified DNA by streptavidin-coated beads. A labelled probe sequence specific for each target was hybridised to the PCR products and captured on the beads. The DNA/probe complex was washed, the probe released then analysed by SERRS.

**Results:** Multiplex PCR for both genera amplified fungal species from a single sample. Each was successfully identified by SERRS using the probe’s unique molecular fingerprint, confirming the presence or absence of each target. The function of this multiplex assay is as diagnostic screening test to exclude the presence of invasive fungal disease and requires excellent analytical sensitivity. The analytical thresholds for the detection of *Candida* and *Aspergillus* species are equivalent to real-time PCR assays. The reproducible 100% detection limit for the targets of this assay is 20 input copies, which correlates to less than 1 genome per reaction (Table 1).

**Conclusions:** This clinically relevant assay demonstrates excellent sensitivity and specificity for the range of fungal species tested. The reliably detection of common fungal species at 20 copies was clearly demonstrated.

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**P2097** Evaluation of a panfungal PCR assay to detect and identify fungal DNA in up to 7-year-old formalin-fixed paraffin-embedded tissue specimens

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**Objectives:** Fungal cultures from intraoperatively resected biopsies of patients with invasive fungal infections (IFI) often remain negative. DNA-based test approaches might be promising alternatives for the detection of the etiologic fungal organisms. In a retrospective study, we applied a panfungal PCR assay on formalin-fixed paraffin-embedded (FFPE) tissue samples from patients with IFI.

**Methods:** We used a 45-cycle panfungal PCR test targeting the ITS1-ITS2 and ITS2 rDNA region. Detection and identification of the amplified DNA was determined by agarose gel electrophoresis, sequencing and the basic local alignment search tool (BLAST). In addition we tested each extract using an inhibition control and the quality of the DNA was evaluated with multiplex β-globin PCR analysis. Results were compared with culture, histology, and/or panfungal PCR from fresh tissue.

**Results:** We investigated a total of 28 archived FFPE specimens stored up to 7 years from 28 patients with IFI. The panfungal PCR identified the correct fungal pathogen in 61% of culture-proven and in 33% of histologically-proven or PCR-proven cases. The ITS2 PCR had a higher sensitivity compared to ITS1-ITS2 PCR in 4 from 14 samples. The fungal organisms found were: *Aspergillus* spp. (n=9), *Hormonomgiella aspergillata* (n=3), *Alternaria* sp. (n=1), and *Rhizopus* sp. (n=1). Ten FFPE control samples with no suspicion of IFI were included. All 10 samples were PCR-negative. A high amount of the extracted DNA from FFPE samples showed degradation and partial inhibition.

**Conclusions:** The results support the complementary use of the panfungal PCR approach applied on FFPE specimens in combination with conventional laboratory test methods. Despite a reduced sensitivity, our method provides accurate identification of fungi on species level in partially degraded tissue samples. Correct identification can be accomplished in samples stored up to 7 years.

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**P2098** Rapid identification of yeast species by the PCR- and microarray-based Prove-it™ Sepsis assay

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**Objective:** Prove-it™ Sepsis is a rapid PCR- and microarray-based assay platform with proven excellent diagnostic performance for most bacterial pathogens causing sepsis. We have extended this platform’s diagnostic range to include 13 yeast species and evaluated its performance against a large number of fungal isolates.

**Methods:** 159 clinically relevant (Germ tube, growth on Corn Meal Tween 80, API 20 AUX and API 32C) as well as molecular after all failed) clinical fungal isolates were tested. The isolates were cultured on Sabouraud dextrose agar with penicillin for 48 h aerobically at 35 °C and blindly tested using the Prove-it™ Sepsis assay after DNA extracted with an easyMAG (bioMérieux). Original routine identifications of the clinical samples were revealed after the analysis.

**Results:** 151 out of 159 samples yielded a microarray-based result, all of which were correct; 8 were negative. The microarray correctly identified 30% as *Candida albicans*, 19% as *C. glabrata*, 14% as *C. parapsilosis*, 9% as *C. tropicalis*, 4% as *C. krusei*, 3% as *C. guilliermondii* and 1% as *C. lusitaniae*. 12% (19/159) of the organism panel were correctly assigned to a pan-yeast group, designed to identify *C. kelyr, C. haemulonii, C. neoformans, C. dubliniensis, and Saccharomyces cerevisiae*. Cryptococcus albidus, *C. neoformans*, *Trichosporon asahii*, *T. mycotoriniconoris*, *T. mucoides*, and *T. inkin* were not identified by this system.
Conclusions: We have modified and extended the Prove-it Sepsis array platform to detect almost all clinically relevant fungi. As previously reported for over 50 bacterial species, the addition of rapid and accurate yeast identification to this diagnostic platform will now allow faster, more evidence-based choice of antifungal agent and better patient outcomes.

P2099 DNA fingerprinting for genotype assignment and molecular genetic basis of 5-flucytosine resistance among clinical Candida dubliniensis isolates in Kuwait
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Objective: Clinical Candida dubliniensis isolates have previously been classified into four genotypes based on nucleotide sequences in internal transcribed spacer (ITS1) and ITS2 regions of rDNA. All genotype 3 and genotype 4 isolates from the Middle East were found to be resistant to 5-flucytosine (5FC). This study determined the signature nucleotides within rDNA to define various genotypes and their association with 5FC resistance among clinical C. dubliniensis isolates in Kuwait. The 5FC-resistant status of selected isolates was also explored by direct sequencing of DNA region encompassing codon 29 of cytosine deaminase.

Methods: A total of 103 C. dubliniensis isolates were studied. The susceptibility of the isolates to anti-fungal agents including 5FC was performed by Etest. Genotype assignment was carried out by rDNA based PCR of extracted DNA was made using the DiversiLab system as a genotyping method for differentiation of Candida glabrata isolates heteroresistant to fluconazole.

P2100 DiversiLab system, automated repetitive sequence-based PCR, as typing method for Candida glabrata
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Objectives: The aim of this study was to explore the DiversiLab system (bioMérieux, Marcy l’Etoile, France) system as a genotyping method for differentiation of Candida glabrata isolates heteroresistant to fluconazole.

Methods: Forty C. glabrata isolates, isolated from patients from different departments of a tertiary hospital, were cultured on Sabouraud agar (SABA) for 24–48 h at 35°C. DNA was made with the UltraClean microbial DNA isolaton kit (Mo Bio Laboratories, Inc., Carlsbad, CA), quantified spectrophotometrically and diluted to 35 ng/µl with molecular grade water. Repetitive sequence-based PCR of extracted DNA was made using the DiversiLab system.
Comparison of two Aspergillus real-time PCR methods and clinical significance of Aspergillus DNA detection in BAL
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Objectives: Aspergillus DNA detection in bronchoalveolar lavage (BAL) could be useful for invasive pulmonary aspergillosis (IPA) diagnosis. Many PCR methods are available, but no comparative studies have been done. Here, we evaluated two home-made real-time PCR methods in order to (i) determine their limit of detection (LOD) (ii) evaluate their clinical utility for the diagnosis of aspergillosis.

Methods: First, we determined LOD using serial dilutions of Aspergillus fumigatus DNA (10^5 to 10^0 fg/µL). Then, we selected PCR and/or GM positive BAL fluids sampled at Lille University Hospital in 2009 (1st January to 31st December) using the results of galactomannan (GM) detection and of the mitochondrial DNA targeting PCR we perform routinely in our laboratory. DNA extracts which had been obtained using the QIAamp DNA minikit (QIAGEN) were then analyzed with a 28S targeting method. Finally, we collected mycological data (microscopic examination and culture of BAL, serum GM, anti-A. FUMIGATUS precipitins, results of respiratory samples), clinical and radiological features.

Results: The mitochondrial DNA and 28S targeting methods LOD reached 1–10 fg/µL and 1 fg/µL, respectively. Fifty-two PCR and/or GM positive BAL fluid samples were collected. Among them, 16 were both PCR and GM positive (Group 1), 29 were PCR positive but GM negative (Group 2) and 7 were GM positive but PCR negative (Group 3). In Group 1, 15 out of 16 BAL were positive using the 28S method. IPA or semi-invasive aspergillosis (SIA) was diagnosed in 5 haematology (6 BAL), 1 heart transplant, and 6 intensive care patients. Three patients were considered as colonized. In group 2, which mainly included BAL with low amounts of DNA (Cp < 40), only 6 out of 29 BAL were positive with the 28S method. In group 3, all the BAL were negative with both methods. Clinical significance of Aspergillus DNA or GM detection is discussed in these two groups.

Conclusion: Both Aspergillus PCR methods were useful to confirm IPA or SIA diagnosis in immunocompromised or intensive care patients. Low amounts of DNA failed to be detected in some BAL by the 28S method (Group 2). Regarding the low LOD of the 28S method, this result could be related to freeze-thaw DNA damage, but a higher specificity of the 28S method could also be hypothesized. In fact, further prospective studies are needed to assess clinical significance of low amounts of DNA when GM is negative.

Molecular identification and antifungal susceptibility of clinical Aspergillus terreus complex isolates
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Background: Although Aspergillus fumigatus is still the most frequent species causing invasive aspergillosis (IA), other species of Aspergillus are emerging. Molecular techniques are necessary to identify clinical Aspergillus spp. isolates to species level and to provide a better understanding of the epidemiology of IA. We identified a collection of recent clinical Aspergillus terreus complex isolates using molecular techniques. We also obtained their antifungal susceptibility to triazoles and amphotericin B (AMB).

Methods: We studied 89 clinical A. terreus complex (morphological identification) isolates collected from October 2005 to March 2010. Isolates were from 72 patients, 12 of whom had proven (n=3) or probable IA, according to EORTC criteria. Only 1 isolate per sample was selected and subsequently identified by amplification and sequencing of the β-tubulin, and calmodulin genes. A BLAST search was performed to identify the isolates. To avoid the presence of cryptic species initially identified as A. terreus, a phylogenetic tree was obtained using the two regions sequenced, including the reference sequences. Antifungal susceptibility to iraconazole (ITRA), voriconazole (VORI), posaconazole (POSA), and AMB was determined using the CLSI M38-A procedure. The antifungal activity of AMB was also obtained using the Etest.

Results: Molecular identification proved that all isolates were A. terreus sensu stricto. The antifungal susceptibility of the isolates (range of MICs, MIC90, and geometric mean), in µg/ml, was as follows: ITRA (0.25–2/2/1.097), VORI (0.125–2/2/1.176), POSA (0.25–1/1/0.836), AMB CLSI (4–32/16/9.689), and AMB Etest (0.75–64/6/3.106). None of the isolates showed an MIC for ITRA, VORI, or POSA greater than 2 µg/ml. In contrast, the MICs for AMB were significantly higher than those found for the 3 triazoles (P < 0.001), regardless of the method chosen.

Conclusions: No cryptic species of the A. terreus complex causing IA or colonization were found in the isolates studied. A. terreus sensu stricto, a species with a known lack of susceptibility to AMB, remains fully susceptible to the triazoles.
**Fungal infections: epidemiology, molecular bases and management**

SNPs associated with a high IL-6, low IL-10 cytokine pattern predispose lung transplant recipients to pneumonia due to moulds

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**Background:** Host immunity is an important outcome determinant for transplant patients (pts) with invasive fungal infections (IFI). In general, Th1 responses are felt to be protective against IFI, and Th2 responses deleterious. We determined if single nucleotide polymorphisms (SNPs) associated with altered cytokine levels predisposed lung transplant (LT) pts to invasive fungal pneumonia caused by moulds.

**Methods:** We retrospectively reviewed the records of 155 LT pts between 2003–2007 who received alemtuzumab induction and consented to genetic studies. Only pts with proven or probable pneumonia due to mould were included in the disease group. Genotypic analysis (SSP-PCR) detected the presence of SNPs in genes encoding TNF-a, TGF-B1, IL-4, IL-6, IL-10 and IFN-g.

**Results:** 25 had pneumonia due to moulds, and 130 pts did not develop any fungal infection (controls). All were Caucasians. We excluded 5 patients with mould pneumonia who were treated for acute rejection within 3 months of the diagnosis. No pts with mould pneumonia had CMV infection within 3 months. 60% of pneumonias were caused by Aspergillus, 15% Rhizopus, 15% dematiaceous fungi and 10% other moulds. 55% were men. Mean and median ages were 53 and 56 yrs. 75% were double-LT, 20% single-LT and 5% heart-LT. 40% had COPD, 20% IPF and 10% CF. The time to pneumonia post-LT was <6 mo in 10%, 6–12 mo in 10%, 12–18 mo in 25%, 18–24 mo in 25%, 24–36 mo in 15% and >36 mo in 15%. Genotypes were in HW equilibrium. There was no significant association between SNPs for individual genes and fungal pneumonia. However, the combination of SNPs associated with high level IL-6 and low level IL-10 was significantly more common among patients with pneumonia than controls (40% vs 14%, P < 0.01).

**Conclusions:** Surprisingly, LT pts with SNPs associated with a pro-inflammatory cytokine profile of high IL-6 and low IL-10 were at increased risk of pneumonia due to moulds, independent of acute rejection. Over-exuberant inflammatory responses might contribute to the pathogenesis of late onset fungal pneumonia in pts receiving alemtuzumab.

**P2105**

Cytokine profile of patients with invasive aspergillosis: initial results from the first 100 patients recruited into the Aspergillosis Study

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**Objectives:** Invasive Aspergillosis (IA) is an important cause of morbidity and mortality in haematology patients undergoing haematopoietic stem cell transplantation (HSCT) or high-dose chemotherapy. We set up an observational prospective cohort study in order to improve our diagnostic and management strategies using the EORTC/MSG criteria as a diagnostic tool. Here we assess the serial cytokine profile of patients to evaluate their potential role in the diagnosis and management of IA.

**Methods:** All study patients were retrospectively recruited and followed up for at least 4 months after chemotherapy or HSCT and had baseline and fortnightly follow-up serum samples profiled for 30 inflammatory cytokines using multiplex bead immunoassays by Luminex 100TM instrument. The cytokines measured were: EGF, Eotaxin, FGF, G-CSF, GM-CSF, HGF, IFN-a, IFN-g, IL-1α, IL-1β, IL-2, IL-8, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12/20/70, IL-13, IL-15, IL-17, IP-10, MCP-1, MIG, MIP-1α, MIP-1β, Rantes, TNF-a and VEGF. The first hundred patients enrolled with sufficient follow up data were included in this initial analysis. We used generalised logistic regression model for binary outcome of proven/probable and no evidence of IA (R2 = 0.4355, P < 0.001) adjusting for clustering, age, sex, underlying diagnosis and treatment.

**Results:** The median (range) age was 52.5 (19–73) years and M:F ratio was 88:42. The main diagnosis were AML/MDS (40), Myeloma (23), NHL (17) and aplastic anaemia (9) treated by allogeneic HSCT (43), autologous HSCT (32), chemotherapy (20), and immunosuppressive therapy (5). The diagnosis of invasive fungal infection was based on the revised 2008 EORTC/MSG criteria. The incidence of proven and probable infection was 17% and possible infection accounted for 12%. Eight patients were excluded from analysis because of lack of proper baseline sample. Ten cytokines were found to be significantly different. Patients with IA were found to have lower IL-1b (P = 0.003), IL-10 (P < 0.001), IL-12 (P = 0.05), IL-17 (P = 0.016), IL-15 (P = 0.027), INF-a (P = 0.05), and GMCSF (P = 0.023) but higher IL-6 (P = 0.08) and IP-10 (P = 0.022). Older age correlated with IA (P = 0.014).

**Conclusion:** Overall patients with invasive fungal infection have significantly lower pro-inflammatory cytokine profile in the Th1 and Th17 axis and therefore unable to effectively deal with infection. If this profile is validated in the larger cohort it may be used as a predictive model for targeted anti-fungal prophylaxis.
the healthy group but remained similar in the CCPA group. In some, expression remained stable until 9hr, and in others expression increased in both groups until 9hr. By 9hr most of the fungus present is in the hyphal form, and differences at this timepoint may indicate a differing response to the hyphal form of the fungus.

**Conclusion:** Differences are seen in both the baseline expression of immune genes, in the change in expression over time, and in response to different forms of the fungus. These differences demonstrate the response of immune cells to *A. fumigatus* and may help to explain the genes and pathways involved in susceptibility to CCPA.

**P2107** Fungal pathogens secrete soluble factors that activate thrombocytes

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**Objective:** The pathogenesis of invasive aspergillosis involves activation of thrombocytes. Putative consequences are inflammation and thrombosis, but also antifungal defense. Since the mechanism of platelet activation by *Aspergillus* is unknown we investigated the hypothesis that fungal secretion products might be responsible for the stimulation of the thrombocytes.

**Methods:** Human platelets were incubated with culture supernatant derived from *Aspergillus fumigatus*. Activation of the thrombocytes was quantified by FACS analysis using CD62P and microparticle formation as markers. To identify the active components in the supernatant the incubation was performed in some samples in the presence of protease inhibitors or inhibitors of signal transduction molecules. Furthermore an isolate of *A. fumigatus* which was defective for gliotoxin production was included in the experiments.

**Results:** The culture supernatant of *Aspergillus fumigatus* was highly active to induce activation of thrombocytes and fusion of secretory vesicles with the plasma membrane. Even minimal amounts of the fungal supernatant was able to upregulate the activation marker CD62P on the platelet surface and to stimulate formation of platelet-derived microparticles. Co-incubation experiments of thrombocytes and fungal secretory products in the presence of different protease inhibitors showed that a serine protease might be one relevant component for the activation. Furthermore the AglI/P mutant isolate of *A. fumigatus* which is defective for the production of the mycotoxin gliotoxin was unable for secrete molecules which stimulate platelets.

**Conclusions:** Fungal metabolites were identified as putatively potent stimulators of thrombocytes. This mechanism might be responsible for thrombosis and thrombocytopenia as visible in the course of invasive aspergillosis. The secretion of the factors imply that this effect can occur all through the body and not only in close vicinity to the hyphae. A serine protease and the mycotoxin gliotoxin are amongst the secreted compounds which efficiently stimulate the platelets and thus might contribute to the pathogenesis of invasive aspergillosis.

**P2108** Economic evaluation of voriconazole versus itraconazole for primary prophylaxis of invasive fungal infection in allogeneic haematopoietic stem cell transplantation

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**Objective:** Voriconazole (VOR) has demonstrated better tolerability with a longer treatment duration compared to itraconazole (ITR) after allogeneic hematopoietic stem cell transplant (HSCT). The aim of our prospective study was to measure the PPCs in prophylactically treated patients to evaluate the impact of different factors on these concentrations.

**Methods:** All consenting patients hospitalised between February 2009 and June 2010 in Institute J. Bordet’s or Erasme hospitals’ Hematology department for acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) and treated prophylactically with posaconazole were included in the study. PPCs were measured after 7 days of treatment and then twice weekly. The following information was collected and evaluated: demographic data, clinical data (including gastrointestinal disorders, co-medications, and treatment compliance), caloric and fat intake, and biological data.

**Results:** 37 patients were included in the study. We obtained 259 measures of posaconazole with a median PPC of 520 ng/ml (range 0–4500 ng/ml). PPCs were significantly lower in patients with mucositis (p < 0.001) and nausea (p = 0.02). PPCs were also lower in patients with diarrhea (p = 0.05) or vomiting (p = 0.04). PPCs were higher in patients with a higher caloric intake (p = 0.02) while the proportion of fat intake had no influence on PPCs (p = 0.69). The concomitant use of proton pump inhibitors decreased the PPCs (p = 0.007) while the use of tacrolimus increased the PPCs (p = 0.04). We observed no correlation between PPCs and liver enzymes, albumine or prealbumine.

The multivariate analysis revealed that the factors influencing the PPCs independently were the concomitant use of proton pump inhibitors (p = 0.03), the use of tacrolimus (p < 0.0001) and the food intake (p = 0.026). 3% of patients interrupted their treatment for hepatotoxicity and 10% for digestive intolerance. Only one patient developed probable aspergillosis and his median PPC was 140 ng/ml (range 110–170 ng/ml).
**Conclusion:** Our study confirmed the high variability of posaconazole bioavailability and showed the significant influence of gastrointestinal disorders, food intake and concomitant medication on the PPCs. Therapeutic drug monitoring of posaconazole should be recommended in the patient population at risk for low PPCs. The impact of intervention measures could thus be evaluated.

**[P2110] Therapeutic drug monitoring among lung transplant recipients receiving voriconazole prophylaxis: identification of preventive breakpoints**

D. Mitsani, M. Nguyen, R. Shields, Y. Toyoda, C. Clancy* (Pittsburgh, US)

**Background:** Voriconazole therapeutic drug monitoring (VOR TDM) is advocated in patients (pts) treated for invasive fungal infections (IFI). Associations between VOR levels and outcomes among lung transplant (LTx) recipients receiving VOR prophylaxis (px) are unclear.

**Methods:** Prospective, observational study of LTx pts receiving VOR px from Jan-Dec 2009 (2 doses IV (6 mg/kg) then 200 mg po BID). TDM was performed by HPLC, beginning after >7 d of px.

**Results:** 93 LTx pts underwent TDM. Median age was 60 yrs (range: 20–74), 54% (50/93) were men, 91% (85/93) were white. Underlying causes were COPD 39% (36/93), IPF 32% (30/93), CF 13% (12/93), others 16% (15/93). 438 VOR serum troughs were measured. 35% of pts (33/93) had initial levels <1 μg/mL (20 ± 0.5, 13 between 0.5–1), 19% (18/93) were 1–2, 30% (28/93) were 2–4 and 15% (14/93) were >4. 81% (72/89) of pts had significant variation (>0.5) in levels upon serial testing. Pts >60 yrs were old more likely to have significant variation in levels and levels >4 (both p = 0.02). CF pts were more likely to have levels <1 and less likely to have levels >4 (p = 0.06 and 0.03, respectively). IPF pts were more likely to have levels >4 (p = 0.01). During VOR px, 12% (10/93) and 27% (25/93) of pts developed IFI (anastomotic tracheobronchitis) or were colonized by fungi, respectively. 152 levels were drawn at time of respiratory cultures. 60% (6/10), 50% (19/38) and 31% (32/104) of VOR, colonization and negative cultures, respectively, were associated with levels <1 (p = 0.03). 56% (14/25) and 48% (10/21) of cultures for yeasts and moulds, respectively, occurred at levels <1. GI symptoms, hepato- or neurotoxicity occurred in 29% (27/93) of pts, but there were no significant associations with levels.

**Conclusions:** Our data support target VOR serum troughs >1 μg/mL for LTx pts receiving px. VOR TDM may be particularly useful for pts >60 and those with IFI who are at increased risk for higher levels, and CF pts who are at increased risk of lower levels. Studies of dose adjustments in response to VOR TDM are underway.

**[P2111] Risk factors for candidaemia-related death in non-neutropenic critically ill patients**

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**Objective:** To identify risk factors for death in non-neutropenic patients admitted in ICU departments who developed candidemia during hospitalization.

**Material and Methods:** A retrospective study based on patient records for all non-neutropenic patients admitted in 6 ICU departments (4 surgery ICU, 1 medical ICU and 1 PICU) in our city between Jan 2007 and Oct 2010. We performed a statistical analysis using Kaplan-Meier survival curves, ROC curves and univariate analysis regarding: age, sex, morbidities (trauma, sepsis, recent surgery, gastrointestinal surgery, solid cancer, acute renal failure), days of CVC, days of antibiotics, days of corticotherapy, days of mechanical ventilation, use of parenteral nutrition, days of fever before candidemia, etiology, fungal prophylaxis, as risk factors for death in non-neutropenic patients with candidemia.

**Results:** 60 non-neutropenic patients with candidemia, 29 women, median age 53 years (min 1 week, max 82 years, average 43.5 years), 75% with recent surgery (60% with gastrointestinal surgery), 22% with solid cancers, 52% with bacterial sepsis, 8% with trauma. The etiology was represented by Candida albicans in 27 cases (45%) and by non-albicans Candida spp in 33 cases. The survival rate was 68% at 30 days and 40% at 120 days (CI 29–53). The risk factors associated with death were: age over 69, mechanical ventilation, more than 14 days of antibiotics and less than 3 days of fever before candidemia onset.

**Conclusions:** The incidence of candidemia in our ICU departments is low in non-neutropenic critically ill patients. More than half of cases are produced by non-albicans Candida spp. Old age, prolonged antibiotic treatment, mechanical ventilation and short period of fever before candidemia onset are associated with poor outcome.

**[P2112] A retrospective trial to evaluate the safety and efficacy of anidulafungin in individuals with hepatic dysfunction including liver transplant recipients**

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**Objectives:** Anidulafungin does not undergo hepatic metabolism but is instead eliminated by slow, non-enzymatic degradation to inactive moieties. Its levels may be more predictable compared to caspofungin in patients with liver dysfunction and may be better tolerated; however, this has not been formally evaluated. We evaluated the safety of anidulafungin as empiric or directed treatment of invasive candidiasis (IC) in patients with hepatic insufficiency and/or liver transplantation.

**Methods:** A retrospective chart review of 50 patients with hepatic dysfunction (Child’s Class B or C) or liver transplantation who received anidulafungin for empiric or directed treatment of IC from July 1, 2006 through January 31, 2010.

**Results:** 28 males and 22 females were enrolled with a median age of 56 years. 96% of the subjects were Caucasian with 10% Hispanics. 50% of subjects were liver transplant recipients, 14% were other organ transplant recipients, and 8% had hematologic malignancies. The median hospital stay was 28 days. Hepatitis C was the most common cause of initial liver dysfunction. The median MELD score was 20 (the mean was 21) and 76% of subjects were Child Class B. Risk factors for IC included: antibiotics >3 days (92%), vascular access device (82%), immunosuppression (80%), steroids (68%), ICU stay >4 days (60%), TPN (50%), renal failure (40%), and hemodialysis (24%). 33 subjects (66%) received empiric therapy and 17 subjects (34%) received directed therapy based on culture data. Intra-abdominal infection (peritonitis and abdominal abscess) was the most common site of infection followed by candidemia. C. albicans and C. glabrata were the most common yeast species isolated. AST did not change from baseline in 28%, improved or normalized in 42%, and worsened in 30% of subjects. ALT did not change from baseline in 32%, improved or normalized in 46%, and worsened in 28% of subjects. Clinical symptoms (fever, elevated white blood cell count, positive cultures) did not change in 8%, improved or resolved in 70%, and worsened in 22% of subjects. 9 subjects (38%) died during their hospitalization with 3 deaths attributable to IC despite treatment with multiple antifungals.

**Conclusion:** Anidulafungin was well tolerated in patients with liver dysfunction and/or liver transplantation with stable or improved liver function tests in the majority of patients.

**[P2113] Efficacy of intravenous itraconazole for the treatment of invasive candidiasis in surgical department and acute care medicine – a multicentre clinical study**

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**Background:** Although itraconazole (ITCZ) has potent activity against Candida species, there are few data that examine the use of intravenous (iv) ITCZ in the treatment of invasive candidiasis (IC). A nationwide multi-center clinical study was conducted to evaluate the efficacy and safety of iv itraconazole in the management of IC including non-albicans species.
Candida species in non-neutropenic patients of surgical department and acute care medicine.

**Methods:** Between September 2007 and August 2009, patients with proven and presumed IC were enrolled at 22 participating institutions. Patients with presumed IC had a deep-body temperature of 37.8°C or higher, and were positive for serum β-D glucan or two or more colonization sites of Candida species. Main exclusion criterion was severe renal impairment (creatinine clearance <30 mL/min). The primary efficacy analysis was based on clinical and microbiologic response 7–10 days after the end of treatment, assessed by an independent data-review committee using the AKOTT algorithm (Aikawa N et al.: J Infect Chemother 2009).

**Results:** Of a total of 60 patients enrolled, 49 were included in the modified intention-to-treat population. Twenty-five patients received a definite diagnosis and 24 patients a presumed diagnosis. Thirty-nine patients were treated with iv ITCZ as the first-line therapy and 10 patients as the second-line. The isolated species included C. albicans (25 strains with definite diagnosis and 17 with presumed diagnosis) and non-albicans species (16 and 10 respectively). Treatment was successful in 61.5% of patients (65.5% in first-line therapy and 50.5% in second-line therapy). Sixty percent of proven IC patients were judged as a success compared with 63.2% in presumed IC patients. The eradication rate was 63.6% for C. albicans and 71.4% for C. glabrata. Adverse effects occurred in 9 of 60 patients (15.0%), and impaired liver function was common among them.

**Conclusion:** The clinical efficacy and safety of iv ITCCZ was suggested in the management of proven and presumed IC, including C. glabrata in non-neutropenic patients. The position of iv ITCCZ in the Japanese guidelines warrants reconsideration.

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**P2114 Economic evaluation of micafungin versus liposomal amphotericin B in the treatment of candidaemia and invasive candidiasis**

**C.F. Neoh**, D. Liew, M. Slavin, D. Marriott, S.C. Chen, O. Morrissey, K. Stewart, D. Kong (Melbourne, Sydney, AU)

**Objective:** Micafungin was as efficacious as liposomal amphotericin B (LAmB) for the treatment of candidaemia and invasive candidiasis in a major randomised clinical trial. We performed an economic evaluation of micafungin compared with LAmB for the treatment of candidaemia and invasive candidiasis in the Australian setting.

**Methods:** A decision analytic model was constructed to capture downstream consequences of using either micafungin or LAmB. The main outcomes were treatment success and treatment failure due to mycological persistence or death. Outcome probabilities and treatment pathways were derived from a published randomised clinical trial (Lancet 2007;369:1519–1527) and literature. Resource use was estimated by an expert panel and cost inputs were from the latest Australian resources. The analysis was from an Australian hospital perspective. Sensitivity analyses using Monte Carlo simulation were conducted.

**Results:** Micafungin (AUD$61,425) had a lower total cost than LAmB (AUD$10,957) per successfully treated patient. This was primarily due to the lower cost associated with initial antifungal treatment and shorter length of stay in the micafungin arm. Hospitalisation was the main cost driver for both arms, followed by drug acquisition costs. Results were robust over a wide range of variables (±100.0% from the base case value). Uncertainty analysis demonstrated micafungin had a 99.9% chance of being more cost-saving than LAmB.

**Conclusion:** Micafungin was associated with cost-saving relative to LAmB in the treatment of candidaemia and invasive candidiasis in the Australian hospital setting.

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**P2115 Fungiscope – a global rare fungal infection registry**

M. Vehreschild, W. Heintz, A. Hamprecht, G. Fischer, S. De Hoog, J. Vehreschild, O.A. Cornely on behalf of the Fungiscope Working Group

**Background:** We are coordinating a global registry for cases of rare invasive fungal diseases (IFD). Our objective is to broaden the knowledge on epidemiology, to determine the clinical pattern of disease, to describe and improve diagnostic procedures and therapeutic regimens, as well as to facilitate exchange of clinical isolates among the contributors.

**Methods:** Fungiscope™ – A Global Rare Fungal Infection Registry uses a web-based electronic case form accessible via www.fungiscope.net. For inclusion in the registry we require positive cultures or histopathological, antigen or molecular genetic evidence of IFD and the associated clinical symptoms and signs of invasive infection. The data entered onto the registry include demographics, underlying conditions, neutrophil count, concomitant immunosuppressive medications, clinical signs and symptoms of IFD, site of infection, diagnostic tests performed, pathogen identification, antifungal treatment, surgical procedures performed, response to treatment, overall survival and attributable mortality.

**Results:** 209 cases have been registered. Zygomycetes (n = 71; 34%), Fusarium spp. (n = 35; 17%), yeasts (n = 33; 16%) and Dematiaceae (n = 28; 13%) were the most frequently registered pathogens. Chemotherapy or agnostic stem cell transplantation for a haematological malignancy was the most predominant risk factor (n = 83; 40%), as well as diabetes mellitus (n = 56; 27%), stay at an ICU (n = 43; 21%) and chronic pulmonary disease (n = 26; 12%). In 77 patients (37%), the lung was the organ of first diagnosis, followed by blood stream infections (n = 40; 19%), the sino-nasal region (n = 31, 15%) and deep soft tissues (n = 23; 12%). For 110 (53%) patients, a favourable outcome was defined as a complete or partial response to treatment of IFD was documented. Overall mortality and mortality attributable to IFD was 38% (n = 79) and 26% (n = 55), respectively.

**Conclusion:** The clinical relevance of rare IFD is increasing steadily. In a short period of time, a wide variety of cases from Europe, Asia and South America could be documented. Further investigators are cordially invited to contribute to Fungiscope.

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**P2116 Epidemiology of fungaemia in Spain: preliminary results of 1,177 episodes from a multicentre study (FUNGEMYCA) in 2009**

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**Objectives:** Candida albicans is the most common cause of nosocomial fungal bloodstream infections (fungaemia). However, several epidemiological surveillance studies have recently reported an increase in fungaemia caused by non-C. albicans species in different proportions, depending on the geographical area evaluated. The aim of this study was to assess the changes in the epidemiology of fungaemia in Spain comparing data from a new surveillance epidemiological study conducted in 2009 with a previous study carried out from 1997–1999 (Pemán J, et al. Eur J Clin Microbiol Infect Dis, 2005).

**Methods:** From January 2009 to December 2009, 43 Spanish hospitals participated in the prospective multicenter fungaemia surveillance study. Demographic and clinical data and the first isolate of each episode were gathered. Isolates were identified in the participating institution by routine methods and sent to the central laboratory for posterior studies.

**Results:** A total of 1,177 fungaemia episodes were collected, 85% from adult patients. The hospitalization Unit distribution (%) of patients was: ICU 36.8; Surgery 16.6; Internal Medicine 12.8; Hematology 6.6; Oncology 6.5; Neonatology 4.8 and Pediatrics 3.4%. C. albicans was the most frequent species isolated (43%), followed by C. parapsilosis (30%), C. glabrata (11%), C. tropicalis (8%), C. krusei (2%) and other species (6%). In ICU settings, a reduction in C. parapsilosis frequency was observed with increasing age (47% in children vs. 25% in adults) and a parallel increase in C. glabrata and C. tropicalis (from 5% to 11% and from 2% to 10%, respectively). The most frequent risk factors were intravascular catheter (61%), surgery (37%) and ICU stay (33%). The species distribution of fungaemia is not regular for all hospitals and depends on the hospital characteristics.

**Conclusion:** There has been an increase both in the number of participating centres and the total number of isolates. The incidence of fungaemia has not been yet analyzed. Fungaemia due to non-C. albicans
P2117 Hospital contacts with Candida infections and risk of cancer
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Objectives: To examine whether an inpatient or outpatient hospital contact with a candida infection is a marker of increased cancer risk.

Methods: We conducted a nationwide population-based study using linked data from the Danish National Registry of Patients and the Danish Cancer Registry for the 1977–2003 period. We identified all patients with a first-time hospital contact with a candida infection (ICD-8 code: 112 candidiasis; ICD-10 codes: B37 (except B37.2 candida of skin/nails) and G021A candidiasis), who had no previous cancer history. To assess the data quality of candida diagnoses, we reviewed a random sample of 159 hospital records for presence of clinically and microbiologically confirmed candida infection. We followed all patients with candida infection until a diagnosis of cancer, death, or 1 January 2004. We computed the overall incidence of cancer and computed standardized incidence ratios (SIRs) using Danish national cancer rates for the study period. We stratified the SIRs by cancer type and follow-up interval (first year, more than one year).

Results: We identified 17,390 patients (11,199 women and 6,191 men) with a first-time hospital contact with candida infection. In the sample of 159 cases for which we reviewed hospital records, 139 (87%) fulfilled criteria for confirmed candida infection. Among the 17,390 patients with a diagnosis of candida, we identified 966 cancers during 138,364 person-years of follow-up (SIR= 1.5; 95% confidence interval (CI): 1.4–1.6). Within the first year following a candida diagnosis, the SIR for all cancers was 3.1 (95% CI: 2.8–3.5). We found particularly high SIRs during the first year for cancer of the mouth [32.8 (95% CI: 19.1–52.5)], tongue [17.4 (95% CI: 4.7–44.6)], and oesophagus [12.3 (95% CI: 6.6–21.1)]. SIRs were also high for lymphomas and leukaemias.

Compared with the general population, the overall cancer risk after one year of follow-up remained 20% higher than expected (SIR = 1.2; 95% CI: 1.1–1.3), while risk of cancer of the mouth, tongue, and pharynx remained more than threefold higher than expected.

Conclusions: In the first year after a hospital contact with candida, the risk of any cancer is more than threefold higher than expected in the general population. In subsequent years, a persistent 20% increase in cancer risk remains.

P2118 Candidaemia after liver transplantation in the era of fluconazole prophylaxis: a prospective survey at one centre over 10 years
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Objectives: Fluconazole (F) prophylaxis (P) in high risk liver transplant recipients has been recommended to prevent invasive candidiasis. We assessed the incidence, microbiological spectrum, characteristics and outcome of candidemia (C) after liver transplantation (LTx) under a prophylaxis protocol.

Methods: During a 10-year period (2000–2010), 845 patients underwent 914 LTx at Rennes Teaching Hospital. Over this period, antifungal P in high risk patients with F 400mg/day during 6 weeks was in use and remained unchanged. Immunosuppression was mostly based on tacrolimus low dose, mycophenolate and steroid.

Results: C was observed in 12 pts (incidence rates of 1.4 per 100 pts, 3.7 per 1000 pt-year). Median (IQR) delay after LTx was 15 days (1–42). C occurred during P for 9 patients (6/9 during the first week), very late after P for 1 patient or without P for 2 patients considered not at risk. Reduced/non susceptibility to F was observed for 8 strains (67%) (C. parapsilosis [5], C. krusei [2], C. valida [1]). Four strains (33%) were F-susceptible (C. albicans [2], C. glabrata [1], C. dubliniensis [1]). Three C. parapsilosis C were diagnosed over a 2-month period. Six pts (50%) had septic shock. Other sites involved were: catheter (5); a central venous catheter was the source of C in 4); bile (4) ascites (2) urine (2), graft preservaton solution (1) retina (1). No pt had endocarditis. In 5 pts, death was either directly (3) or possibly (2) related to C. Mortality within 30 days of C was 63% (5/8) during F-resistant infections versus 0/4 during F-susceptible episodes. When compared to the 833 non C pts, there was a difference in primary diagnosis (less non viral cirrhosis, more acute liver failure in the C group). Cumulative mortality rate at all time points were higher in the C group (Table).

Conclusion: With targeted F prophylaxis, the incidence of C remains low after LTx, mostly involving F-nonS species (67%). However, C remains associated with significant direct or indirect mortality. Studies investigating new agents for the prophylaxis of invasive fungal infections but also for empirical therapy in the setting of LTx are warranted.

Table
| Variable | Candidemia (n=12) | No Candidemia (n=833) | P value |
|----------|------------------|-----------------------|---------|
| Age (median, years) | 60 (24-92) | 62.5 (18-92) | 0.65 |
| C. parapsilosis | 6 (50%) | 0 | <0.001 |
| C. krusei | 3 (25%) | 0 | 0.009 |
| C. valida | 1 (8%) | 0 | 0.002 |
| C. dubliniensis | 2 (17%) | 0 | 0.003 |

P2119 The value of the Candida Colonisation Index for predicting Candida infections in a mixed intensive care unit
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Objective: The aim of the investigation was to assess the usefulness of "Candida colonisation index" (CCI) as an early microbiological documentation for predicting candida infections.

Methods: A retrospective, cohort, observational study was performed between January 2006 and July 2007 in Neurological and Reanimation ICU’s of total 18 beds for medical and surgical patients. A total of 82 non-neutropenic adult intensive care unit patients admitted for at least 7 days are enrolled in the study. Clinical and demographic data of the patients, APACHE II score, hospitalization during the last 6 months, history of infection, antimicrobial treatment and invasive procedures were recorded. Oral mucosa, axillae, rectal swab, enteral nutrition catheter and urine samples were obtained for culture on admission and three weekly. The colonisation index was considered as positive if candida isolated sites over sampled sites rate was ≥0.5. The patients were observed for candidemia, urinary candidiasis or other invasive candidiasis lateron. Statistical analysis was performed by chi-square test, Spearman’s correlation test and logistic regression analysis.

Results: Candida infections were diagnosed in 29 (35.4%) patients. CCI was ≥0.5 in 27/29 (93.1%). CCI and candida infections are shown in table 1. Among the advanced age, prolonged hospitalization prior to ICU, extended spectrum cephalosporin use, renal insufficiency, unconsciousness, preceding bacterial urinary infection, central venous catheter, peripheral catheter use or intubation as risk factors for candidiasis, CCI was the only independent risk factor (OR:701,553;
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95% CI; 28.310–173.852.22; p < 0.001) in a multivariate logistic regression analysis. Positive predictive value of CCI was 93.1%, susceptibility 93.1%, and specificity 96.2% for candida infections.

**Conclusions:** Our results documented that CCI may be a good predictor for candidiasis in medical ICU patients. With limited patient population and lack of investigation for value of preemptive treatment, our results are only promising. Prospective trials with a large patient population on preemptive treatment based on CCI would be useful.

**P2120** Candida peritonitis: analysis of 81 episodes

A. Loza*, J. Gonzalez, A. Ubeda, C. León, C. Castro, P. Saura, S. Ruiz on behalf of the Cava II study group

**Objective:** To describe the epidemiology and prognosis of Candida peritonitis in non-neutropenic critically ill adult patients with complicated abdominal surgery.

**Material and Methods:** In a prospective, observational and multicenter study, the following cohorts were included: 1699 patients admitted to 73 ICUs in 1998/1999 (EPCAN database), 1107 patients admitted to 36 ICUs in 2006/2007 (CAVA I project), and 254 ICU patients admitted in 2009/2010 (CAVA II project). Length of ICU stay for at least 7 days was an inclusion criterion. Patients with complicated abdominal surgery were selected for the present analysis. Candida peritonitis was defined as isolation of Candida spp. in a peritoneal sample obtained by laparotomy or percutaneous puncture in patients with associated clinical findings. Variables independently associated with Candida peritonitis were analyzed in a logistic regression model and the discriminating capacity of the predictive variable selected was assessed with the area under the ROC curve.

**Results:** There were 81 episodes of Candida peritonitis among 1120 patients undergoing abdominal surgery (incidence 72.32 episodes/1000 patients). Risk factors for Candida peritonitis were multifocal colonization by Candida spp. (OR = 4.74, 95% CI 2.21–10.17), severe sepsis or septic shock (OR = 3.54, 95% CI 1.77–7.07), extrarenal depuration procedures (OR = 2.31, 95% CI 1.19–4.52), and days of ICU stay (OR = 1.03, 95% CI 1.01–1.04). Pre-emptive antifungal treatment was a protective factor (OR = 1.03, 95% CI 0.05–0.34) and showed a high discriminating capacity (AUC = 0.842, P < 0.001). Crude mortality in patients with and without Candida peritonitis was 39.5% and 25.4% (P = 0.006).

**Conclusions:** Multifocal colonization, degree of sepsis, extrarenal depuration procedures, and length of ICU stay all identifying patients with complicated abdominal surgery at risk for Candida peritonitis. Candida peritonitis is associated with a high mortality. Pre-emptive fungal therapy prevents intraabdominal Candida spp. invasion during the postoperative period in ICU patients after complicated abdominal surgery.

**P2121** Trends in species distribution and antifungal susceptibility in yeasts isolated from peritoneal fluid in a general hospital: an overview of a 16-year period

T. Peláez*, B. Gama, A. Luis, E. Reigadas, R. Flores, J. Guinea, P. Muñoz, E. Bouza (Madrid, ES)

**Background:** Yeasts may cause human peritonitis both alone or as part of a polymicrobial infection. We assessed the evolution of peritoneal fluids (PF) with the isolation of yeasts over a 16 year period and the activity of several antifungals against the available isolates.

**Methods:** From 1994 to 2009, the yeasts isolated from PF were considered. The in vitro activities of amphotericin B (AB), fluconazole (FZ), itraconazole (IZ), voriconazole (VZ), posaconazole (POS), caspofungin (CAS), anidulafungin (AND) and micafungin (MF) were determined by the broth microdilution method following CLSI criteria.

**Results:** During the study period, a total of 394 peritoneal fluids, from 386 patients, showed positive yeast growth (425 isolates) and the incidence of yeast peritonitis was 0.45 episodes/1000 admissions. Overall Candida albicans was the species more frequently isolated (250; 58.8%) followed by C. glabrata (65; 15.3%), C. tropicalis (43; 10.1%), C. parapsilosis (37; 8.7%), C. krusei (12; 2.8%) and other (18; 4.2%). The MICs ranges and MIC50 of the 317 available isolates were as follows: AMB (0.06–4/2), FZ (0.125–256/16), IZ (0.015–32/0.5), VZ (0.015–32/0.25), POS (0.015–32/0.5), CAS (0.03–4/0.5), AND (0.007–4/2) and MF (0.007–2/0.5). The geometric mean, expressed in μg/ml were: AMB (0.353), FZ (4), IZ (0.088), VZ (0.043), POS (0.062), CAS (0.25), AND (0.015) and MF (0.007).

**Conclusions:** In our hospital, the incidence of fungal peritonitis has remained stable during the study period. However, the species distribution was different between adult and paediatric population; and the percentage of Candida non-albicans increased significantly during the last years. Candins exhibited an excellent in vitro activity against the majority of isolates causing fungal peritonitis in adults and children.

**P2122** Trends of candidaemia in Slovakia – a comparison of two consecutive, prospective studies

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**Objectives:** To show and analyze the trends in epidemiology of candidaemia in Slovakia during the recent period (2005–2009).

**Methods:** The results of two prospective, national studies were compared. The 1. study (S1) was performed in 2005–7 (1) and the 2. study in 2008–9 (S2). Both studies had the same design. All bloodstream fungal isolates from participating sites (teaching and regional hospitals) were collected for identification and for sensitivity testing in central study laboratory. Germ-tube tests, chromogenic media (Chromagar, BBL), API 20C and YBC Vitek (bioMérieux) were used for identification of yeasts. The CLSI M44-A method was used to test all yeast isolates. Quality control strains C. albicans ATCC 90028 and C. parapsilosis ATCC 22019 were used. Breakpoints for fluconazole and voriconazole were based on CLSI-S2 standard. Basic clinical data in both studies were obtained and compared.

**Results:** 171 (S1) and 239 (S2) episodes of candidaemia were documented, analyzed and compared. The incidence of candidaemia in Slovakia is increasing – from 2.2 to 3.0 (cases/100 000/year) in 2005–7 and 2008–9, respectively. Majority of patients with candidaemia were from ICUs (38% in S1 and 64% in S2) and from hematology/oncology departments and the most common pathogen was C. albicans in 38–50% of all episodes followed by C. parapsilosis in 26–29%. The resistance to fluconazole and voriconazole was 21.6% (S1) and 24.3% (S2). Voriconazole resistance was 11.3% in S1 and 14.2% in S2. Additional testing showed that 15 non-albicans strains, mainly C. parapsilosis, were non-sensitive to anidulafungin.

**Conclusion:** Our consecutive, prospective studies documented the increasing incidence of candidaemia in Slovakia during the recent 5 years. The majority of population at risk were patients at ICUs and hematology/oncology departments and the most common pathogen was C. albicans in 38–50% of all episodes followed by C. parapsilosis in 26–29%. The resistance to fluconazole and voriconazole is slightly increasing and we detected strains non sensitive to echinocandins. Continual survey can help to identify the patients at risk and resistant strains and should have the impact on therapy of candidaemia.

**P2123** Nosocomial bloodstream infections due to Candida spp. in the US – species distribution, clinical features and susceptibilities

H. Wisplinghoff*, J. Ebbers, L. Geurtz, D. Stefanik, Y. Major, M.B. Edmond, R.P. Wenzel, H. Seifert (Cologne, DE; Richmond, US)

**Background:** Candida species are among the most prevalent nosocomial pathogens contributing significantly to morbidity and mortality, mainly among patients in the ICU setting. Even though several studies have
analyzed the susceptibility of Candida spp. to newer agents and over time in single institutions, comprehensive clinical and microbiological data are still limited.

Methods: A total of 1338 episodes of Candida bloodstream infection (BSI) prospectively collected from patients in 52 hospitals in the United States between 1997 and 2005 were analyzed. Susceptibilities to amphotericin B (AMB), fluconazole (FLC), fluconazole (FLU), posaconazole (POS), voriconazole (VOR), anidulafungin (ANI), caspofungin (CAS), and micafungin (MIC) were determined for a subset of 1062 Candida isolates by the CLSI reference broth microdilution method.

Results: C. albicans was the most prevalent species accounting for 524 (45%) of all isolates, followed by C. parapsilosis (19%), C. glabrata (18%), and C. tropicalis (10%). There was no trend in favour of non-Candida albicans species over time. Patients had a mean age of 51 years (range 0–94 years) and their length of stay prior to BSI averaged 22 days. The main underlying conditions were gastrointestinal (20%), pulmonary (13%) and malignant (12%) diseases. IV catheters (20%) and the urinary tract (9%) were the most frequently determined sources of BSI, while in the majority of patients (59%) no source could be identified. Overall mortality was 37%. MIC50/MIC90 values are shown below. Resistance to fluconazole occurred in <1% of C. albicans isolates, 10% of C. glabrata isolates, and 3% of C. tropicalis isolates. Resistance to fluconazole was associated with resistance to voriconazole and posaconazole. Non-susceptibility to echinocandins was only found in C. parapsilosis.

Conclusions: C. albicans and C. parapsilosis were the most prevalent Candida species causing BSI in patients from US hospitals. Even though all tested agents displayed good activity against most species decreased activity of all azoles against C. glabrata and of echinocandins against C. parapsilosis are important to consider in empirical therapy.

| C. albicans (n=477) | C. glabrata (n=200) | C. krusei (n=20) | C. parapsilosis (n=204) | C. tropicalis (n=123) |
|---------------------|--------------------|------------------|------------------------|----------------------|
| AMB 0.5/0.5 | 0.5/0.5 | 0.5/0.5 | 0.5/0.5 | 0.5/0.5 |
| FLC 0.125/0.25 | 0.065/0.125 | 0.065/0.125 | 0.065/0.125 | 0.065/0.125 |
| FLU 0.25/0.5 | 0.065/0.125 | 0.065/0.125 | 0.065/0.125 | 0.065/0.125 |
| POS 0.065/0.125 | 0.065/0.125 | 0.065/0.125 | 0.065/0.125 | 0.065/0.125 |
| VOR 0.065/0.125 | 0.065/0.125 | 0.065/0.125 | 0.065/0.125 | 0.065/0.125 |
| JAK 0.065/0.125 | 0.065/0.125 | 0.065/0.125 | 0.065/0.125 | 0.065/0.125 |
| CAS 0.065/0.125 | 0.065/0.125 | 0.065/0.125 | 0.065/0.125 | 0.065/0.125 |
| MIC 0.065/0.125 | 0.065/0.125 | 0.065/0.125 | 0.065/0.125 | 0.065/0.125 |

Candida species distribution at UHC and UHF 2001-2010

C. albicans UHC | C. albicans UHF | C. glabrata UHC | C. glabrata UHF | C. parapsilosis UHC | C. parapsilosis UHF

Epidemiology of candidaemia in two tertiary-care university hospitals in Germany

A. Hamprecht* S. Göttig, H. Seifert, F. Knapp, H. Wisplinghoff, G. Plum (Cologne, Frankfurt, DE)

Objectives: This study was initiated to assess the impact of candidemia and to compare differences in species distribution and resistance at two large University Hospitals in Germany. Currently, there is a lack of studies about the epidemiology of candidemia and resistance rates in Germany.

Methods: A retrospective analysis of all cases of candidemia from the University Hospital Cologne (UHC) and University Hospital Frankfurt (UHF) was carried out (UHC 2001–11/2010, UHF 2005–11/2010).

Results: A total of 542 cases of candidemia were identified at the two centers, with an increasing number of cases over the study period (from 22 in 2001 to 50 until 11/2010 at UHC). C. albicans was the most frequent species at UHC and UHF (62.4%), C. glabrata the most frequent non-albicans species (18.6%), followed by C. tropicalis (7.0%) and C. parapsilosis (6.6%). Non-albicans species increased at both centers, from 35.6% in 2005 to 47.3% in 2010. This increase could be observed earlier and more markedly at UHF with an almost even distribution of C. albicans and non-albicans cases in 2007–2010. 155 isolates were susceptibility tested by E’test or Vitek-2 YST-01 card during the study period. C. albicans strains were susceptible to fluconazole in 100% at UHC and 97.6% at UHF. Amphotericin B resistance was not reported at UHF (7.1% of all Candida species). Susceptibility of C. glabrata isolates to fluconazole was 66.7% (UHC) and 62.9% (UHF). Echinocandin resistance was rare for species other than C. parapsilosis. One case of clinical failure to caspofungin treatment and in vitro caspofungin resistance (minimal inhibitory concentration (MIC) >8μg/ml) was observed in 2010 at UHC.

Conclusion: The incidence of candidemia has markedly risen in the study period at both study sites, with an increase of infections due to non-albicans species. This change in the frequency of the different species may be due to an increased use of antifungals. Use of azoles is known to select for C. glabrata. Echinocandins select for C. parapsilosis, which exhibits intrinsically high MIC to this drug class. Resistance to echinocandins in other species is so far rarely reported in Europe. Currently, susceptibility testing of echinocandins is therefore not routinely performed in most laboratories. However, the case of candidemia due to a caspofungin resistant C. glabrata strain with clinical failure warrants a close monitoring of echinocandin resistance rates.

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Epidemiology of candidaemia in two tertiary-care university hospitals in Germany

A. Hamprecht*, S. Göttig, H. Seifert, F. Knapp, H. Wisplinghoff, G. Plum (Cologne, Frankfurt, DE)

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Results: A total of 542 cases of candidemia were identified at the two centers, with an increasing number of cases over the study period (from 22 in 2001 to 50 until 11/2010 at UHC). C. albicans was the most frequent species at UHC and UHF (62.4%), C. glabrata the most frequent non-albicans species (18.6%), followed by C. tropicalis (7.0%) and C. parapsilosis (6.6%). Non-albicans species increased at both centers, from 35.6% in 2005 to 47.3% in 2010. This increase could be observed earlier and more markedly at UHF with an almost even distribution of C. albicans and non-albicans cases in 2007–2010. 155 isolates were susceptibility tested by E’test or Vitek-2 YST-01 card during the study period. C. albicans strains were susceptible to fluconazole in 100% at UHC and 97.6% at UHF. Amphotericin B resistance was not reported at UHF (7.1% of all Candida species). Susceptibility of C. glabrata isolates to fluconazole was 66.7% (UHC) and 62.9% (UHF). Echinocandin resistance was rare for species other than C. parapsilosis. One case of clinical failure to caspofungin treatment and in vitro caspofungin resistance (minimal inhibitory concentration (MIC) >8μg/ml) was observed in 2010 at UHC.

Conclusion: The incidence of candidemia has markedly risen in the study period at both study sites, with an increase of infections due to non-albicans species. This change in the frequency of the different species may be due to an increased use of antifungals. Use of azoles is known to select for C. glabrata. Echinocandins select for C. parapsilosis, which exhibits intrinsically high MIC to this drug class. Resistance to echinocandins in other species is so far rarely reported in Europe. Currently, susceptibility testing of echinocandins is therefore not routinely performed in most laboratories. However, the case of candidemia due to a caspofungin resistant C. glabrata strain with clinical failure warrants a close monitoring of echinocandin resistance rates.
Conclusions: The safety of ANID in critically ill patients was comparable to the known profile of ANID. Initial results from this small study suggest that short-course ANID, followed by optional oral VORI, might be effective for the treatment of CIC, including the reduction of CIC-associated mortality, in Latin America. Further studies are warranted.

Clinical, microbiological, and global response at secondary time point (MITT population)

| Subjects with response | Clinical response | Microbiological response | Global response |
|------------------------|------------------|--------------------------|----------------|
| Clinical response      | 32/41 (78.0%)    | 33/41 (80.5%)            | 33/41 (80.5%)  |
| Microbiological response| 32/41 (78.0%)   | 33/41 (80.5%)            | 33/41 (80.5%)  |
| Global response        | 32/41 (78.0%)    | 33/41 (80.5%)            | 33/41 (80.5%)  |

Study of invasive aspergillosis in haematologic patients: description of 127 cases enrolled in a single-centre prospective surveillance system, 2004–2009

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Objectives: Invasive aspergillosis (IA) is a major concern in hematology. Incidence monitoring and clinical course of IA can enhance medical support. The objectives were 1) to report a description of patients with IA in a hematologic department, and 2) to estimate the incidence by hematological diagnosis.

Methods: A prospective surveillance of IA cases has been implemented between January, 1st 2004 and December, 31st 2009 in the Hematology Department (42 beds) of Edouard Herriot hospital (Lyon, France). Multidisciplinary meetings with clinician, mycologist and infection control practitioner were organized every 2 months to confirm suspected IA cases. Demographic characteristics, clinical and complementary exams were prospectively collected. Hospital stays were extracted from the hospital information system. IA diagnosis followed the European Organization for Research and Treatment of Cancer criteria, proven and probable IA were retained. A descriptive analysis was done; temporal trends of IA incidence were assessed by Poisson regression.

Results: Overall, 4,073 hospitalized patients counting for 7,836 patient-days were included. Totally, 127 (3.1%) patients had IA, the global IA incidence was 1.6 per 1,000 patient-days (95% CI 1.4–1.9 per 1,000 patient-days). In patients with acute myeloid leukaemia (AML) the incidence was 1.9 per 1,000 patient-days (95% CI 1.5–2.3 per 1,000 patient-days), in patients with acute lymphoblastic leukaemia (ALL) the incidence was 1.3% per 1,000 patient-days (95% CI 0.8–2.0 per 1,000 patient-days). The IA incidence decrease was 16% per year (95% CI 1%–28%) between 2004 and 2009. The IA incidence decrease in AML was 20% (95% CI 6%–36%). Serum Aspergillus antigen was detected for 89 (71%) patients, 29 (23%) patients had positive culture, 118 (93%) had abnormal lung CT-scan. Three-month mortality was 42%. Between 2004 and 2009, there was a trend for a decrease of 1-month mortality (22% in 2004 vs. 14% in 2009) and 3-month mortality (50% in 2004 vs. 29% in 2009) (P = 0.02 and P = 0.8 respectively).

Conclusions: A decrease of IA incidence and mortality was observed between 2004 and 2009. The knowledge of characteristics and clinical course of patients with IA should permit to improve the management of these patients with severe disease. Observational data might complement experimental studies for reporting effect of improvement of care.

The epidemiology of neutropenic patients with invasive candidiasis using electronic data

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Objective: Because of limited data, current treatment regimens for invasive candidiasis (IC) in neutropenic patients are mainly based on data from non-neutropenic patients. The objective of this study was to identify and describe the epidemiology of neutropenic patients with IC using an electronic clinical database.

Methods: Patients admitted to Barnes-Jewish Hospital (BJH), a 1,250-bed tertiary care center in the US, with either a positive candida culture from blood or a normally sterile site and an ANC < 500 at the time of positive culture were included in the analyses. Electronic medical data was collected from the Washington University and BJC Healthcare Medical Informatics Database between the periods of 10/1/2004 and 12/31/2009.

Results: There were 81 patients included in the analysis. Mean age was 53 ± 16 years (0–67 years). Median modified APACHE II score was 13 (0–28) and median Charlson Index of Co-morbidity was 4 (0–10). 29/81 (36%) were admitted to the ICU. 25/81 (31%) were treated with anidulafungin, 22/81 (27%) with caspofungin, 17/81 (21%) with fluconazole, and 17/81 (21%) with other systemic antifungals. Crude in-hospital mortality was 44% (36/81). The mean length of stay was 32 ± 21 days (0–85 days) and the mean length of stay until the first positive culture was 18 ± 16 days (0–74). Co-morbid illnesses included heart disease (20%; 16/81), diabetes (17%; 14/81), and chronic lung disease (10%; 8/81). The most common species isolated was C. albicans (42%) followed by C. glabrata (16%), C. krusei (14%), C. parapsilosis (11%), C. tropicalis (6%), and C. dubliniensis (6%).

Conclusions: The retrospective review of electronic clinical data identified a considerable number of neutropenic patients with IC. Over half of the study population were treated with an echinocandin, but a significant number of patients received fluconazole and other systemic antifungals. The in-hospital mortality rate and distribution of species were consistent with previously reported studies in non-neutropenic patients.

Respiratory aspergillosis in non-transplant, non-neutropenic patients

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Objective: To analyse the significance of lower respiratory tract cultures yielding Aspergillus spp. growth in non-transplant/non-neutropenic patients.

Methods: A multicenter (24 hospitals) retrospective review (2003–2010) of clinical records corresponding to non-transplant/non-neutropenic patients with at least 2 respiratory samples yielding Aspergillus spp. was performed. Patients were assigned to 3 categories based on the criteria by Multha et al. for patients with chronic obstructive pulmonary disease (COPD): colonized, probable or proven aspergillosis.

Results: 200 patients were identified (mean age: 68 ± 15.2 years; 72.0% males). Most patients were admitted in Pneumology (50.5%), Internal Medicine (20.5%) and Intensive Care Unit (11.0%), with 7.0% non-hospitalized patients. COPD was present in 69.5% patients (of them 28.0% Gold IV; 36.9% Gold III and 24.5% Gold II), diabetes in 20.5%, malignancies in 16.5% and chronic renal impairment in 8.0% patients. Hospital admission within the previous 3 months was recorded in 26.0% patients. In the Rxs performed, 56.8% patients presented infiltrates, 17.6% nodules, 10.0% pleural effusion and 9.6% cavitations. Thoracic CT scan showed halo and air-crescent signs in 3.6% and 2.7%, respectively, of 112 (56.0%) patients with scan performed. Prior to admission 41.0% patients had received corticosteroids (61.0% of them with ≥ 20 mg/day prednisone equivalent) and 35.5% antibiotics, and during hospitalization, rates were 74.0% (89.2% patients with
Chronic invasive pulmonary aspergillosis: diagnosis and treatment

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Methods: Diagnosis of invasive pulmonary aspergillosis (IPA) was based on EORTC/MSG 2008 criteria. Cases with a duration of the disease longer than 3 months as well as cases of relapse within 6 months from the onset of the disease were considered as CIPAP.

Clinical manifestations of CIPA were: fever (91%), dyspnoea (57%), cough (77%), haemoptysis (18%), broncho-obstructive syndrome (16%) and chest pain (17%). On CT scan bilateral lung damage (85%) and consolidation (54%). There was a significant association between a finding of nodular infiltrates or consolidation and positive BAL GM index (p = 0.02). Indeed, 100% (6/6) of patients with nodules had BAL GM >1.0, whereas 78% (18/23) of patients with consolidation had BAL GM >1.0. Moreover, BAL GM indices were significantly higher among patients with consolidation or nodular opacification (median: 3.6±1.2) than among those with nodules (median: 0.5±1.3; p = 0.02). Overall, 56% (9/16) of HSCT patients and 69% (9/13) of LuTx recipients died. There was no association between BAL GM and outcome of patients.

Conclusions: Consolidation on chest CT and positive BAL GM reflect high burdens of Aspergillus. Lesions associated with earlier or localized stages of IPA, such as nodules or nodular infiltrates, are likely to cause lower burdens of Aspergillus and lower BAL GM indices. Our findings are consistent with histopathologic studies of IPA demonstrating that consolidation is airway-vasive, with fungi found deep in the vicinity of the airway basement membrane.

Consolidation and nodular infiltrates on CT scans of patients with invasive pulmonary aspergillosis correlate with elevated BAL galactomannan levels

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Background: Invasive pulmonary aspergillosis (IPA) causes significant mortality and morbidity in hematopoietic stem cell (HSCT) and lung transplant (LuTx) recipients. Galactomannan (GM) levels correlate well with fungal burden in animal models. We studied the relationship between BAL GM levels and CT scan findings among HSCT and LuTx recipients with IPA.

Methods: Retrospective study of HSCT and LuTx recipients at Universities of Florida and Pittsburgh, respectively, for whom BAL GM and CT scans were performed within one day. CT scans were read by two investigators who were blinded to clinical data.

Results: CT findings among the 16 HSCT patients were nodules (25%), nodular infiltrates (50%) and consolidation (25%). CT findings among the 13 lung transplant recipients were nodules (31%), nodular infiltrates (15%) and consolidation (54%). There was a significant association between a finding of nodular infiltrates or consolidation and positive BAL GM index (p = 0.02). Indeed, 100% (6/6) of patients with nodules had BAL GM >1.0, whereas 78% (18/23) of patients with consolidation had BAL GM >1.0. Moreover, BAL GM indices were significantly higher among patients with consolidation or nodular opacification (median: 3.6±1.2) than among those with nodules (median: 0.5±1.3; p = 0.02). Overall, 56% (9/16) of HSCT patients and 69% (9/13) of LuTx recipients died. There was no association between BAL GM and outcome of patients.

Conclusions: Consolidation on chest CT and positive BAL GM reflect high burdens of Aspergillus. Lesions associated with earlier or localized stages of IPA, such as nodules or nodular infiltrates, are likely to cause lower burdens of Aspergillus and lower BAL GM indices. Our findings are consistent with histopathologic studies of IPA demonstrating that consolidation is airway-vasive, with fungi found deep in the vicinity of the airway basement membrane.

occurrences were 303, 250, 217 spores/day and were significant different compared to 124 (p < 0.001), 124 (p < 0.01) and 123 (p < 0.01) spore/day for the 848, 768 and 692 other days, respectively (table 1).

Conclusion: The level of outdoor FF spore load was higher before community-acquired IA than during the periods without infections and could potentially favor the Aspergillus contamination of immunocompromised patients at high risk for IA. This observation underlines the importance of preventive measures to protect high-risk patients outside the hospital: air control measures in a patient’s residence is not realistic contrary to the common sense measures (using mask, avoiding places with high-humidity and dust ...).

| Table 1. The outdoor fungal spore levels during pre periods for the residents who acquired amphotericin B (L-AmB) during pre periods |
|-----------------|----------------|----------------|----------------|----------------|
|                | 1st period | 2nd period | 3rd period | 4th period |
| Pre-infection Spore load | 13.8 (0.007-23) | 9.7 (3.8-25.7) | 17.0 (12.1-34) | 26.1 (21.1-49) |
| Median Cr level | 1.3 (1.0-1.7) | 1.4 (1.1-1.7) | 1.4 (1.1-1.7) | 1.6 (1.2-1.9) |

Methods: A comparative retrospective and multicenter study was conducted in two groups of critically ill patients according to serum Cr values < or ≥1.5 mg/dL at baseline and treated with L-AmB for at least 3 days. Patients with renal replacement therapy before or within 48 h after starting L-AmB were excluded. The primary endpoint was the difference in the mean Cr level at the end of L-AmB treatment as compared with baseline. Secondary endpoints were risk factors for nephrotoxicity and treatment-related withdrawals. Demographics, underlying illness, APACHE II score, fungal infectious disease, risk factors for nephrotoxicity, use of antifungals, and vital status at ICU and hospital discharge were collected.

Results: A total of 122 patients recruited in 27 ICUs were included (increased Cr, n = 16; normal Cr, n = 106). L-AmB was mainly selected because of broad spectrum of the drug and hemodynamic instability of the patients, and was given as first-line antifungal treatment in 68.8% and 52.8% of patients with elevated and normal Cr values, respectively. The mean APACHE II score was 25 in patients with increased Cr and 17 in those with normal Cr (P < 0.001). Median duration of L-AmB treatment was 16 and 12 days in patients with elevated and normal Cr values, respectively, and mean dose 3.5 vs 3.9 mg/kg/day. Concomitant use of other nephrotoxic drugs, mortality rate, and length of ICU and hospital stay were similar for both groups. Difference in the mean serum Cr level at the end of L-AmB treatment as compared with baseline was a reduction of 1.1 mg/dL (P < 0.001). In none of the patients (0.0%), L-AmB had to be stopped due to nephrotoxicity. In the multivariable analysis, neuropaenia was independently associated with nephrotoxicity (odds ratio 8.6) and L-AmB doses <4 mg/kg/day were inversely associated with nephrotoxicity (odds ratio 0.5). Low number of neutropenic patients (n = 16) make it difficult to accurately estimate risk factors of nephrotoxicity.

Conclusions: Treatment with L-AmB in critically ill patients with impaired renal function had minimal impact on renal function as measured by serum Cr concentration. Low doses of L-AmB (<4 mg/kg/day) showed renal-protective effects.

Impact of liposomal amphotericin B on renal function in ICU patients with increased serum creatinine level

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Objective: To assess the effect of liposomal amphotericin B (L-AmB) in ICU patients with increased serum creatinine (Cr) concentrations (≥1.5 mg/dL) at the beginning of L-AmB treatment.

Methods: A comparative retrospective and multicenter study was conducted in two groups of critically ill patients according to serum Cr values < or ≥1.5 mg/dL at baseline and treated with L-AmB for at least 3 days. Patients with renal replacement therapy before or within 48 h after starting L-AmB were excluded. The primary endpoint was the difference in the mean Cr level at the end of L-AmB treatment as compared with baseline. Secondary endpoints were risk factors for nephrotoxicity and treatment-related withdrawals. Demographics, underlying illness, APACHE II score, fungal infectious disease, risk factors for nephrotoxicity, use of antifungals, and vital status at ICU and hospital discharge were collected.

Results: A total of 122 patients recruited in 27 ICUs were included (increased Cr, n = 16; normal Cr, n = 106). L-AmB was mainly selected because of broad spectrum of the drug and hemodynamic instability of the patients, and was given as first-line antifungal treatment in 68.8% and 52.8% of patients with elevated and normal Cr values, respectively. The mean APACHE II score was 25 in patients with increased Cr and 17 in those with normal Cr (P < 0.001). Median duration of L-AmB treatment was 16 and 12 days in patients with elevated and normal Cr values, respectively, and mean dose 3.5 vs 3.9 mg/kg/day. Concomitant use of other nephrotoxic drugs, mortality rate, and length of ICU and hospital stay were similar for both groups. Difference in the mean serum Cr level at the end of L-AmB treatment as compared with baseline was a reduction of 1.1 mg/dL (P < 0.001). In none of the patients (0.0%), L-AmB had to be stopped due to nephrotoxicity. In the multivariable analysis, neuropaenia was independently associated with nephrotoxicity (odds ratio 8.6) and L-AmB doses <4 mg/kg/day were inversely associated with nephrotoxicity (odds ratio 0.5). Low number of neutropenic patients (n = 16) make it difficult to accurately estimate risk factors of nephrotoxicity.

Conclusions: Treatment with L-AmB in critically ill patients with impaired renal function had minimal impact on renal function as measured by serum Cr concentration. Low doses of L-AmB (<4 mg/kg/day) showed renal-protective effects.
Diagnostic value of the 1,3-β-D-glucan test for detecting candidaemia

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Objectives: Candidaemia is a life-threatening disease, requiring early diagnosis and treatment. Blood cultures are the gold standard for diagnosis but may lack sensitivity. Therefore, we evaluated the diagnostic value of the 1,3-β-D-glucan test (BG) in patients with proven candidemia.

Methods: 144 serum samples of 63 hospitalized patients with candidaemia (July 2007 till July 2009) were collected. As controls we selected 25 patients with multisite colonization, 25 patients with Gram-positive bacteremia, 25 patients with Gram-negative bacteremia and 15 healthy donors. BG measurement was performed in duplo with the Fungitell® kit (Associates of Cape Cod).

Results:
- Sensitivity of BG for detecting candidaemia was 81% for cut-off 80 pg/ml and 85.7% for cut-off 60 pg/ml. 8 patients with C. albicans and 1 patient with C. glabrata infection had negative BG values. The specificity was low with false-positive results ranging from 20 to 40% in our control populations. Only in healthy donors no false-positive results were observed.
- Considering the average epidemiologic proportion of nosocomial Candida colonization, Gram-negative bacteremia and candidemia, PPV of BG for detecting candidaemia was 21.9% and NPV 97.0% with cut-off 80 pg/ml.
- In 11 patients with negative blood cultures prior to developing candidaemia, BG positivity (>80 pg/ml) preceded blood culture positivity.

Conclusion: Due to its low specificity BG lacks power to discriminate between candidemia and bacteremia. Therefore its use cannot be recommended in routine practice.

The study was supported by an unrestricted grant from MSD Belgium.

| Population                  | n  | Cut-off 80 pg/ml | Cut-off 60 pg/ml |
|-----------------------------|----|-----------------|-----------------|
| Candidaemia                 | 63 | 81%             | 85.7%           |
| Candida colonization        | 25 | 24%             | 32%             |
| Gram-positive bacteremia    | 25 | 40%             | 44%             |
| Gram-negative bacteremia    | 25 | 20%             | 28%             |
| Healthy donors              | 15 | 0%              | 0%              |

* C. albicans (n=43), C. glabrata (n=10), C. krusei (n=4), C. parapsilosis (n=6), C. tropicalis (n=3) and C. lusitaniae (n=1)

P2137 The use of galactomannan detection in urine samples from haemato-oncology patients at high risk of invasive aspergillosis

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Objectives: Aspergillus fumigatus is a ubiquitous saprophytic fungus capable of causing life-threatening lung infection in the immunocompromised, including recipients of haematopoietic stem cell or solid organ transplants, and those with advanced HIV infection. Diagnosis of invasive aspergillosis (IA) is problematic; delay is associated with poor outcome. Symptoms of IA are non-specific, blood cultures usually negative, and sputum culture has poor sensitivity and specificity; the diagnostic yield of broncho-alveolar lavage is <30%. Galactomannan is a cell wall component of Aspergillus spp. released during growth. This antigen is detected in blood using the Platelia™ ELISA. It has been proposed that screening for GM in urine may be clinically useful, although there is little data addressing this. We studied the role of GM testing of urine samples in a prospective cohort of haematology-oncology patients at high risk of IA.

Methods: 405 urine samples from 49 haematology-oncology patients were screened for the presence of GM, a mean of 8.3 samples were tested per patient (range 1–35). Urine was tested using the Platelia ELISA according to manufacturer’s instructions with amendments made to the
pre-treatment stage: there were four pre-treatment conditions: 1) no pre-treatment; 2) centrifuged at 3000rpm for 10m, followed by no pre-treatment; 3) centrifuged and then treated as per the manufacturer's instructions for serum; 4) treated exactly as per manufacturer's instructions for serum.

**Results:** Pre-treatment conditions 1 and 2 gave results with higher indices than methods 3 and 4. Applying EORTC/MSG criteria: 6, 10 and 5 patients had proven, probable and possible fungal disease, respectively, and 28 had no evidence of fungal infection. Of 6 proven patients 2 had mould on histology but were culture-negative, 2 had invasive aspergillosis, 1 had candidiasis and 1 fusariosis, these last 2 patients were excluded from the analysis. From the 14 patients classified as having proven or probable fungal disease only 5/14 (35.7%) tested positive for the presence of GM in urine. Of the 28 patients that had no evidence of fungal infection, 2/28 (7%) tested positive for the presence of GM. This gives a sensitivity of 35.7% and specificity of 92.9%.

**Conclusions:** Although GM can be detected in urine this assay lacks the sensitivity required to replace serum as the sample of choice for GM detection.

**P2138** Study of receptor expression and anti-Aspergillus functions on phagocytes from healthy donors before monitoring transplant patients

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Invasive aspergillosis is an infection for which the mortality rate is extremely high in transplant patients (50–100%). The diagnosis is difficult and late, which often leads to delayed initiation of antifungal treatment.

**Objectives:** For this reason, our objective is to follow innate immune system rebuilding, targeted on key factors in anti-aspergillosis response, in order to research one or more markers that could assist earlier diagnoses of invasive aspergillosis and allow for rapid initiation of antifungal treatment.

**Phagocytes are the first line of defense against Aspergillus. So, the quality of phagocytic cells is important to evaluate the risk of invasive aspergillosis in immunocompromised patients. However, the variability in human phagocytic cell function or receptor expression which occurs with age, gender or time is currently poorly characterized. A better characterization of these parameters in healthy donors is essential before a longitudinal study in patients.**

This study determined the expression level of nine receptors involved in *Aspergillus* immune response as well as the values of phagocytosis and production of radical oxygen species after *Aspergillus* stimulation, in a healthy adult population.

**Materials:** The study was conducted on 50 healthy subjects, five of whom were followed for 1 year. For these donors, samples were collected 15 days and then 1, 2, 3, 6, 9, and 12 months after their first sample was taken. None of the donors had known disease or fever at the time of the study. Thirty females and 20 males were included. Their age varied from 22 to 64 years. This study was approved by the Ethics Committee (CCPPRB).

**Results:** The expression values of the CD11b, CD11c, CD14, CD18, CD35, CD181, CD182 and CD282 and CD284 receptors on peripheral human monocytes and granulocytes was established (figure 1). A heterogeneous expression of the CD282 on granulocytes was observed as CD181, CD182, CD282 on monocytes. Similarly, we observed considerable variation in the expression of these receptors over time. Only CD282 on granulocytes varied with sex. No variation with age was observed. Consequently, the study of CD282 expression is delicate for disease follow-up. Adherence of *Aspergillus* conidia to phagocytes was dependent of individual, sex, age and time.

**Conclusion:** A better characterization of these innate immunity parameters was necessary to develop in the future an immunologic surveillance strategy for transplant recipients.

**P2139** Cost-effectiveness analysis of two recommendations for antifungal therapy indication in patients with persistent febrile neutropenia

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**Objective:** To evaluate cost-effectiveness of antifungal therapy (AT) indication in persistent febrile neutropenia (PFN) episodes in haematological patients, selected by invasive fungal infection (IFI) risk profile and clinical criteria, recommended by Andalusian Society of Infectious Diseases (ASID), Cisneros et al. EIMC 2005, vs. Infectious Diseases Society of America (IDSA) recommendation, Hughes et al. CID 2002, of universal empirical AT in all PFN episodes.

**Methods:** A cost-effectiveness analysis based on a decision making tree with two different branches, each of them for a recommendation, was performed through TreeAge Pro Suite 2009 program. In branch 1 (B1), IDSA recommendation, probability data of indicating each AT were taken from Lafaurie et al. CMI 2010, effectiveness and side effects (SE) probability data for each AT were taken from clinical trial, Walsh et al. NEJM 2004 (a). In branch 2 (B2), ASID recommendation, probability of indicating each AT and effectiveness data, were taken from a prospective patients serie Martin-Peña et al. EBMT 2010. SE data was same as in B1. The perspective of economic analysis was of Virgen del Rocio Hospital (VRH) and time horizon was seven days after end of AT. Costs considered were: purchase costs of AT for VRH, costs of SE treatment Carnerero-Gomez et al. REES 2005. AT was considered effective when fulfilled the criteria considered in clinical trial (a).

**Results:** In B1, 56 PFN episodes included, in all of them AT was indicated. The effectiveness and average cost for each AT was: 0.049 and 7660€ amphotericin B deoxycholate (ABD), 0.05 and 3211€ amphotericin B liposomal (ABL), 0.34 and 5613€ caspofungin (C). In B2, 85 NFP episodes were included, AT was indicated in 52 (61.2%). The effectiveness and average cost of each AT was: 1 and 7660€ for ABD, 1 and 3117€ ABL, 0.5 and 4844€ for C; 0.87 and 8073€ voriconazole, 1 and 217€ fluconazole. The average cost per PFN episode treated in B1 was 5430€ with a effectiveness of 38% vs. and 3723€ and 82% in B2. Cost per unit of effectiveness in B1 was 14049€ vs. 4521€ in B2.

**Conclusion:** The indication of AT only in selected patients, ASID recommendation is better cost-effective than the IDSA recommendation, universal empirical AT in PFN episodes, avoiding over AT, saving direct costs of AT and indirect costs of SE treatment, without a worse prognosis in non treated patients.

**Systemic antifungals: in vitro and in vivo data**

**P2140** Functional characterisation of endogenous regulators of calcineurin in *Candida glabrata*

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**Objectives:** Infections caused by the opportunistic fungal pathogen *Candida glabrata* are often difficult to treat due in part to its intrinsic or rapidly acquired resistance to azole antifungals. We have previously demonstrated that a *C. glabrata* mutant lacking the serine-threonine-specific protein phosphatase calcineurin displays increased azole susceptibility and reduced virulence, and these phenotypes are
Functional characterisation of the Slt2-regulated Flow cytometry: a faster method to assess the in vitro antifungal tolerance in *C. glabrata*.

**Results:** *S. cerevisiae* pmc1 mutant is unable to grow under high-calcium conditions unless calcium function is abolished. Overexpression of CgRCN1, CgRCN2, ScRCN1 or ScRCN2 rescued growth of the *S. cerevisiae* pmc1 mutant in the presence of 0.3 M calcium chloride, suggesting that they have retained functional similarity regarding the inhibitory activity on calcineurin signaling. While the *C. glabrata* ren1 mutant exhibited decreased tolerance to micafungin and fluconazole, the ren2 mutant did not display these phenotypes. In the presence of micafungin, the expression level of YPS1 was increased approximately fourfold in the wild-type and ren2 strains but no induction was observed in the ren1 strain.

**Conclusion:** Ren1 exerted both inhibitory and stimulatory effects on calcineurin signaling, but Ren2 displayed only inhibitory activity. Phenotypic analyses of single and double mutants of calcium and RCNs revealed that calcium requires Ren1, but not Ren2, for antifungal tolerance in *C. glabrata*. Further elucidation of this important signaling pathway will aid in the development of novel strategies for antifungal therapy.

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**Objective:** To assess the incubation time to determine the minimum inhibitory concentration (MIC) of amphotericin B (AMB) for *Candida* species by flow cytometry (FC) using FUN-1 as a metabolic marker.

**Material and Methods:** A total of 11 *C. albicans*, 10 *C. glabrata* and 9 *C. parapsilosis* strains from blood cultures were tested. *C. parapsilosis* ATCC 22019 was used as control. Dilution method (DM) was performed according to M27-A3 document. The FC method was based on a 4-h incubation of yeasts with two-fold dilutions of AMB. Metabolic activity was measured with FUN-1, which binds to nucleic acids and has a maximum fluorescent emission after 30 minutes. While dead cells express diffuse yellow-green fluorescence, metabolically active cells (which incorporate FUN-1) have circular intravacuolar structures that emit fluorescence between 470 and 590 nm (orange-red fluorescence). Measures were performed every 30 or 60 min depending on the strain analyzed.

**Conclusion:** The CLSI method, while the FC study was done with comparable AMB concentrations and times. Percentage of metabolically active cells obtained by FC was plotted versus time. The 48-h MIC value of CLSI was interpolated in the graphic. This allowed to estimate the time-point at the MIC value which diminished metabolic activity at least 90% for each strain. The average times for each species were determined; these calculated times allowed to estimate for each strain the value of minimum concentration of AMB that reduced metabolic activity at least 90%. Finally, we compared this minimum concentration with CLSI MIC.

**Results:** Average times were 64 (CI 95% [52–76]), 117 (CI 95% [98–136]) and 97 (CI 95% [79–115]) minutes for *C. albicans*, *C. glabrata* and *C. parapsilosis*, respectively. The same procedure was applied for ATCC-22019 control in triplicate, providing MIC values within the range of CLSI. All isolates were AMB susceptible by both methods. At most, a difference in two dilutions was observed when CLSI and FC results were compared.

**Conclusions:** 1) Calculated flow cytometry average times allow a proper classification of the *Candida* strains as susceptible or resistant to AMB in a shorter time compared with the CLSI method. 2) Different average times were observed in a species-dependent pattern. 3) This study could be the beginning to establish the flow cytometry reading times, but larger studies are warranted to assure its usefulness.

**Figure 1.** Flow cytometry report showing *Candida glabrata* CG-0411 metabolically active cells versus time.
Antifungal activity of echinocandins against isolates of Candida parapsilosis, Candida metapsilosis and Candida orthopsilosis

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Candida parapsilosis forms a complex composed of three genetically distinct groups that have been recognized as separate species: C. parapsilosis sensu stricto and the two newly described species, Candida metapsilosis and Candida orthopsilosis. C. parapsilosis is usually susceptible to antifungal agents, but there are recent reports of clinical isolates with decreased susceptibility to echinocandins, and differences in antifungal susceptibility among the 3 species have been observed. The antifungal susceptibility profile of these new species is of clinical relevance as may influence the therapeutic choices for these infections.

Objectives: To compare the in vitro activities of anidulafungin (ANF), caspofungin (CAS) and micafungin (MIF) against clinical isolates of C. parapsilosis, C. orthopsilosis and C. metapsilosis.

Methods: In vitro activities of 3 echinocandins were assessed by Sensititre YeastOne 09 (Trek Diagnostics Systems, USA) against 117 C. parapsilosis (72 blood isolates and 45 isolates from other origins), 11 C. metapsilosis and 11 C. orthopsilosis from different anatomic origins. Isolates were categorized as susceptible (S) and non susceptible (NS) according to the CLSI M27-A3 and S3 supplement documents.

Results: ANF and MIF were more active against C. orthopsilosis and C. metapsilosis than against C. parapsilosis (p < 0.001). ANF MIC₉₀ values were 1, 0.5 and 2 μg/ml, and the mean values of MIC were 0.354, 0.182 and 1.245 μg/ml for C. orthopsilosis, C. metapsilosis and C. parapsilosis, respectively. MIF MIC₉₀ values were 0.5, 0.5 and 2 μg/ml, and the mean values of MIC were 0.193, 0.182 and 1.092 μg/ml respectively. CAS was the most active echinocandin against C. parapsilosis with MIC₉₀ and mean MIC values of 1 and 0.455 μg/ml. CAS MIC₉₀ was 0.5 μg/ml for C. orthopsilosis and C. metapsilosis, and the mean MIC values were of 0.249 and 0.170 μg/ml, respectively. However, 1 C. parapsilosis isolate from blood and other one from mouth were NS to MIF (MICs = 4 μg/ml). Moreover, 1 C. parapsilosis isolate from skin was inhibited only by ANF MICs ≥ 4 μg/ml.

Conclusion: Acquired echinocandin resistance is rare in Sweden but has now been demonstrated for the first time. Echinocandin Etest MICs against C. parapsilosis and C. guilliermondii were lowest for micafungin.

Use of proposed species-specific CLSI breakpoints to interpret anidulafungin MIC values. Results from three regional clinical studies of anidulafungin for the treatment of candidemia/invasive candidiasis

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Objectives: Pooled analyses of anidulafungin minimum inhibitory concentration (MIC) and clinical response data for 403 patients from three regional clinical trials were performed. These data provide a unique opportunity to assess the ability of the proposed anidulafungin Clinical Laboratory Standards Institute (CLSI) interpretive criteria to predict clinical outcome.

Methods: MICs were determined by CLSI broth microdilution as described in M27. Proposed CLSI interpretive criteria for anidulafungin were applied to interpret susceptibility data for baseline isolates from three open-label, non-comparative trials conducted in the US, Europe and Asia. Neutropenic and non-neutropenic patients with albicans and non-albicans Candida infections were treated with anidulafungin for at least 5 days followed by oral azole therapy with fluconazole or voriconazole. Clinical response was assessed by the investigator at the end of treatment visit in the modified intent-to-treat population.

Results: The majority of anidulafungin MICs for non-C. parapsilosis spp. were ≤ 0.25 mcg/mL. Clinical response data were available for 13 isolates with MICs above the susceptible species-specific breakpoint; 7/13 (54%) success, 4/13 (31%) failure and 2/13 (15%) unknown clinical response compared to 287/390 (74%) success, 32/390 (8%) failure and 71/390 (18%) unknown clinical response for susceptible isolates.

Conclusions: For this collection of clinical isolates, clinical failure occurred more frequently in patients infected with non-susceptible isolates compared to patients with susceptible isolates as defined by proposed species-specific CLSI breakpoints for anidulafungin.
In vitro activities of voriconazole, caspofungin and amphotericin B against clinical Candida krusei isolates by the time-kill method

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Objectives: C. krusei is inherent resistant to fluconazole and hence it is an important problem especially for immunocompromised patients. Amphotericin B (AMB) and voriconazole (VOR) act to the fungal membrane ergosterol via different mechanisms, and caspofungin (CAS) acts on the cell wall 1,3-β-D-glucan. We evaluated the in vitro antifungal activity of clinical C. krusei isolates to voriconazole, caspofungin and amphotericin B by time-kill method in the present study.

Methods: Thirty C. krusei isolates from various clinical samples were included in this study. Minimal inhibitory concentration (MIC) values of three antifungals were determined by reference CLSI M27-A2 broth microdilution methods. The time-kill tests were performed at 1/4x MIC, 1x MIC ve 4x MIC for all isolates.

Results: MIC values were 0.25–0.50 µg/ml for VOR, 1.0–2.0 µg/ml for AMB and 0.25–1.0 µg/ml for CAS in broth microdilution methods. VOR exhibited concentration-independent fungicidal activity against all isolates in time-kill tests. AMB showed entirely dose-dependent activity; it was fungistatic at 1/4−1 MICs for all isolates and fungicidal at 4x MIC for 26 isolates (86.6%). Also, efficiency of CAS was concentration-dependent; it was fungistatic at 1/4x MIC (73.3%) and fungicidal at 1−4x MIC (86.8–96.6%), respectively in general.

Conclusion: VOR, CAS and AMB were in vitro effective against C. krusei isolates. Although VOR was concentration-independent fungicidal effective, AMB and CAS exhibited entirely concentration-dependent activity; CAS had the most performance among these three antifungal agents.

Antifungal susceptibilities of Aspergillus fumigatus and sequence of the cyp51A gene of azole-resistant isolates in Nagasaki, Japan

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Objectives: Azole resistance of Aspergillus fumigatus is one of the concerns in treatment of aspergillosis. Epidemiology of azole resistance has been reported in some countries in recently. We examined MIC distributions for A. fumigatus and analyzed cyp51A gene mutations of azole-resistant isolates in Nagasaki, Japan.

Methods: Between January 1994 and April 2010, a total of 212 isolates of A. fumigatus were obtained from the Pneumology Department of Nagasaki University Hospital. The isolates were identified based upon macroscopic and microscopic morphological characteristics following standard mycological procedures. All isolates were screened for growth at 48ºC, thus confirming A. fumigatus. The large-subunit RNA gene (D1/D2 region) and internal transcribed spacers 1 and 2 (ITS1 and ITS2 regions) were sequenced for molecular identification of all azole-resistant A. fumigatus. The MICs of amphotericin B, micafungin, itraconazole and voriconazole were determined by using the Clinical and Laboratory Standards Institute M38-A2 reference method. The full sequence of the cyp51A gene was determined.

Results: Of 212 clinical isolates, nearly all isolates (98.1%) had a MIC ≤ 1 mg/L to amphotericin B, and 98.6% had a MEC ≤ 0.015 mg/L to micafungin. The distribution of azole MICs were as follows: itraconazole, <2 mg/L, 177 (83.5%); ≥ 2 mg/L, 13 (6.1%); ≥ 4 mg/L, 22 (10.3%); and voriconazole, <2 mg/L, 184 (86.8%); ≥ 2 mg/L, 15 (7.1%); ≥ 4 mg/L, 13 (6.1%). The frequency ofazole resistance did not change from 1994 to 2010. Of the 22 itraconazole-resistant isolates, 10 (45.5%) were cross-resistant to voriconazole. Of 23 azole-resistant isolates, 10 (43.5%) isolates had one or two point mutation and 13 (56.5%) isolates had no mutation in cyp51A gene. The mutations G54E, G54R, G54W, G54A, R55K, G54D and G54N were seen in the isolates. The frequency of cyp51A mutations was different from other countries. The frequency of cyp51A mutation and other unknown resistant mechanism may be different between regions.

Genotyping and cyp51A sequencing of consecutive Aspergillus fumigatus isolates suggest in vivo as well as ex vivo origin of azole resistance

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Objectives: We analysed the cyp51A gene and performed genotyping of consecutive Aspergillus fumigatus isolates from cystic fibrosis patients (CF) harbouring azole resistant isolates in order to explore the mechanism of resistance development.

Methods: A. fumigatus isolates were collected over a 2-year period from CF patients. Itraconazole screening agar (4 mg/L) was employed for the detection of azole resistant isolates. Isolates growing on the screening agar were susceptibility tested following the EUCAST methodology and
the cyp51A gene was sequenced. Both susceptible and resistant isolates from patients harbouring resistant isolates were genotyped by the short tandem repeat assay (de Valk, H.A., JCWI2005).

Results: A total of 6/133 patients (4.5%) harboured azole resistant A. fumigatus isolates (range, 1 to 9 resistant isolates per patient). Five different mutations or combinations thereof were found in the cyp51A gene (M220K, TR-L98H+S495I, M220V+F101F, and Y431C, respectively) (Table). Genotyping of 29 isolates from these patients revealed the following epidemiologically significant trends: 1) Microevolution in the short tandem repeat markers was observed for A. fumigatus isolates received consecutively over a two year period; 2) Susceptible and resistant isolates (not involving the TR+L98H combination) of identical or very closely related genotypes were found in two patients. 3) Two related susceptible isolates and a third resistant isolate with a unique genotype and the TR-L98H resistance combination were detected for one patient.

Conclusion: Genotyping of consecutive A. fumigatus isolates from Danish CF patients demonstrated long-term carriage of the same isolate as well as concomitant or sequential presence of several genotypes. Molecular microevolution occurred not only in the short tandem repeat markers but also in the cyp51A gene conferring azole-resistance suggesting in vivo evolution of resistance, whereas no susceptible isolates were found with a genotype with any TR-L98H isolates suggesting ex-vivo origin of this azole resistant A. fumigatus molecular type.

**Table 1. POSA plasma concentration after daily dose 600 and 800 mg.**

| Samples | 600 mg | 800 mg (2X400) | 800 mg (4X200) |
|---------|--------|----------------|----------------|
| Patients | 12 | 5 | 6 |
| POSA PC <0.5 | 48% | 45% | 71% |
| Mean POSA PC µg/ml | 1.16 | 1.03 | 0.415 |
| Median POSA PC µg/ml | 1.05 | 1.11 | 0.8 |
| Diarrhoea except of mucositis | 21% (n=3) | 10% (n=2) | 6.5% (n=3) |
| PC=plasma concentration; GIT=gastrointestinal tract |

**P2150** Posaconazole plasma concentrations in allogeneic haematopoietic stem cell transplant recipients

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Objectives: Posaconazole (POSA) is a broad spectrum azol antifungal used in treatment and prophylaxis of invasive fungal infection. POSA exhibits variability in plasma concentration, due to its different bioavailability and potential drug interactions.

Methods: POSA plasma concentrations were measured using a high performance liquid chromatography assay. Retrospective analysis of laboratory results and documentation from patients undergoing allogeneic hematopoietic stem cell transplantation treated with POSA from November 2006 to November 2010 in our institution was performed.

Results: 81 plasma samples from 13 patients were analyzed, 1–17 samples per patient. POSA plasma concentration was monitored 3–204 (4–48) days after start of POSA treatment. POSA was administered in 68% as prophylaxis, in 24.5% as preemptive antifungal treatment, in 2.5% as an empirical antifungal therapy and in 5% as a treatment of invasive fungal infection. POSA was administered as an oral suspension divided in 2–4 portions, the standard daily dose was 600 or 800 mg, in 2 patients was an individual dosing 480 mg and 960 mg daily. After 600 mg daily the plasma concentration was 0.21–1.7 µg/ml. The POSA plasma levels varied between 0.21–2.86 µg/ml and 0.2–1.14 µg/ml after 4x 200 mg and 4x 200 mg, respectively. In 27% (n=22) of all samples the POSA concentration did not achieved 0.5 µg/ml, which could be ineffective and the the antifungal treatment could be insufficient. In this analysis there is no correlation between the daily dose and achieved POSA plasma concentration. Low POSA plasma levels after 4x 200 mg could be due to more frequently diarrhea and other lesion of gastrointestinal tract. The results are in table 1.

**Conclusion**: Measurement of POSA plasma levels during its treatment enables to optimize the individual dosing and improve the response to antifungal treatment in high risk haematological patients after allogeneic hematopoietic stem cell transplantation.

**P2151** Chronic Aspergillus fumigatus colonisation of respiratory tract in cystic fibrosis: diagnosis, management and antifungal resistance in a French cohort of cystic fibrosis patients

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Introduction: Cystic fibrosis (CF) is the major genetic inherited disease in the European Caucasian population, with an average of 1 in 3000 living births in France. Prognostic depend essentially on the lung impairments. While considerable attention therefore has been paid over recent decades to prevent and treat bacterial respiratory infections, we observed emergence of fungi colonisation in CF respiratory tract. In particular, Aspergillus fumigatus represents the most common causative agent colonizing the airways of CF patients; it can be responsible for Allergic Bronchopulmonary Aspergillosis (ABPA). Since oral corticosteroids and itraconazole represent the mainstay of ABPA management, long-term therapy may increase the risk of acquired resistance to azoles that is mainly associated with amino acid substitutions in the CPS51A gene of A. fumigatus.

Objective: Because CF patients are chronically exposed to itraconazole, our study aimed to evaluate the prevalence ofazole resistance in isolates prospectively collected from CF patients followed-up in seven French hospitals involved in our national prospective study (“MucoFong” study – PHRC1902). To our knowledge, it is the first multicenter study focused on azole resistance of A. fumigatus in CF.

Methods: A total of 87 isolates of A. fumigatus was collected in 85 patients. The MICs of azole drugs were evaluated for each isolate using the E-test® strips. Isolates were characterized at the molecular level by targeting ITS, β-tubulin and MAT-A/α genes. The cyp51A gene as well as its promoter was sequenced.

Results and Discussion: A majority of isolates (88.1%) were found sensitive to itraconazole (MIC ≤2 µg/ml), and 2 new mutations were identified and localized within 3-dimensional Cyp51A protein model.
To obtain insight intoazole resistance of A. fumigatus, the results are analyzed taking into account clinical data, itraconazole exposition, and the potential correlation between the identified CYP51A mutations and azole resistance is discussed based on the Cyp51A protein homology model.

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Conflict of interest: None to declare

Role of caspofungin in enhancing chronic haemodialysed and kidney transplant patient PMN response against Candida glabrata

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Objectives: Taking into account that in immunocompromised patients successful resolution of fungal infections is often related to the ability of the patient’s immune system to cooperate with drugs, this study aimed to evaluate the potential immunomodulating activity exerted by caspofungin on polymorphonuclear cells (PMNs) from chronic hemodialyzed (HDS) and renal transplant patients (RTRs) against Candida glabrata, the most common life-threatening fungal pathogen in such immunocompromised hosts.

Methods: PMNs were separated from venous blood samples of HDS and RTRs. The effects of caspofungin on either phagocytosis of radiolabelled C. glabrata Bucalzone and voriconazole resistant strain or intracellular fungal killing by PMNs were investigated by incubating yeasts and phagocytes for 30, 60, 90 minutes with caspofungin at MIC level. Caspofungin-free controls were included.

Results: A diminished phagocytic efficiency was found in PMNs from HDS and RTRs, with both reduced phagocytosis and fungicidal activity towards intracellular C. glabrata, in comparison with that of healthy donor PMNs. As the majority of systemically acting antifungal drugs, caspofungin did not affect phagocytosis by granulocytes. Conversely, the fungicidal activity of PMNs from HDS and RTRs was significantly (p < 0.01) improved by caspofungin, resulting in increased numbers of killed yeasts for all three incubation times compared with those of the drug-free controls: during the 90 minute period the intracellular blastoconidial load was reduced by 56−68% for HDS and by 58−71% compared with those of RTRs.

Conclusion: In neutropenic high risk patients an empirical use of caspofungin has been described in literature. We previously demonstrated that caspofungin had beneficial properties in enhancing microbialic functions of PMNs from healthy subjects. The data of this study indicate that caspofungin possesses interesting immunomodulating properties that make it highly suitable for the treatment of C. glabrata multidrug-resistant infections in patients with impaired components of the immune system.

Development of amphotericin B deoxycholate-induced nephropathy is associated with induction of a net pro-inflammatory cytokine profile

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Objectives: It has been postulated that the toxic profiles of amphotericin B deoxycholate (AmBD), especially that of infusion related reaction, might be related to the drug's propensity to induce proinflammatory cytokines through Toll-like receptor (TLR) 2. Recently, induction of proinflammatory cytokine production through TLRs has been implicated as an underlying mechanism for drug-induced nephropathy. To date, this has not been studied in the clinical setting.

Methods: Circulating concentrations of interleukin (IL)-6, IL-8 and IL-10 were measured at baseline, weeks 1 and 2 of treatment, in a cohort of patients enrolled in a clinical trial comparing AmBD versus voriconazole for treatment of invasive aspergillosis. Serial cytokine trends were correlated with development of renal impairment. Renal impairment was defined as an increase in the serum creatinine level to double the base-line value, or more than 265 μmol/L if baseline value was higher than 133 μmol/L. Univariate and multivariate Cox regression analysis was performed to predict time to renal event.

Results: From the study cohort, 87 patients had received AmBD as the primary therapy, among which 35 patients (40.2%) developed renal impairment during treatment. The median time to development of renal event was 15 days. A progressive decline of proinflammatory serum IL-6 from baseline through week 2 of treatment was associated with decreased likelihood of developing renal impairment (hazard ratio 0.20, 95% CI: 0.06–0.70; p = 0.012). Conversely, persistent decreasing concentrations of the antiinflammatory cytokine IL-10 between baseline and week 2 was linked to an increased risk of nephrotoxicity (hazard ratio 5.72, 95% CI: 2.5–12.9, p = 0.001).

Diagnosis of viral infections

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Objectives: Rapid antigen tests (RAT) are used to screen patients with suspected influenza and provide results timely. RAT can also help to reduce unnecessary diagnostic testing, to facilitate antiviral treatment, and to decrease inappropriate use of antibiotics. However, the clinical sensitivity of RAT was poor for 2009 H1N1 influenza, showing an accuracy from 11.1% to 51%. Drexler et al. have suggested that the viral concentrations in clinical samples influence the outcome of RAT. Thus, the collection time of the samples may be an important factor for the accuracy of RAT.

Methods: Retrospectively, we tested 637 clinical samples from 637 different patients. Samples were collected during the pandemic 2009 H1N1 season by nasopharyngeal swab and were kept frozen at −80°C until use. The 120 controls were taken from H1N1 negative febrile subjects.

The 2009 H1N1 influenza was confirmed by real time reverse transcription-PCR. The RAT was done by SD Biosline Influenza A/B(A/H1N1) pandemic (Standard Diagnostics, Yongin, Korea). The RAT has 4 lines for the detection of 2009 H1N1, influenza A, influenza B, and controls, and distinguishes between seasonal influenza virus and 2009 H1N1. Samples were classified by the hours elapsed after the first symptoms appeared when they were collected. They were classified into ≤24 hours (D1), 24 to 48 hours (D2), 48 to 72 hours (D3), 72 to 96 hours (D4), and 96 to ≤168 hours (D5).

We calculated the sensitivity and specificity of RAT. Data analysis was performed using SPSS, version 16.0. ANOVA test and Tukey’s post hoc test were used to compare the mean log10 viral copy numbers. The study protocol was approved by Institutional Review Board of the Chonbuk National University Hospital.

Results: The mean age of the subject was 23.4±12.81 (male 51.0%). The control patients had a mean age of 34.6±20.8 (male 54.3%). The overall sensitivity and specificity of the RAT was 75.6% and 99.3%, respectively. The sensitivity of RAT at D1, D2, D3, D4, D5 was 75.0%, 76.8%, 79.9%, 77.4%, and 67.3%, respectively (Fig 1). The log quantity of virus copy numbers at D1, D2, D3, D4, D5 was 3.35, 3.60, 3.68, 3.46, and 3.17, respectively (p = 0.025). Only D3 and D5 showed significant difference by Tukey’s post hoc test (p = 0.026).
Clinical performance of a rapid antigen detection test for pandemic influenza A/H1N1

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Objectives: Recently, the clinical performances of rapid influenza antigen detection test (RIDT) have been evaluated during the influenza pandemic A/H1N1 (A/H1N1pdm). In particular, according to several studies, RIDT sensitivity is highly variable (range 10−70%), while specificity is generally high (range 86−100%).

The aim of the study was to evaluate the clinical performances of BinaxNOW Influenza A&B for influenza A/H1N1pdm with respect to the real-time RT-PCR method established by CDC during the influenza A/H1N1pdm 2009 pandemic.

Methods: Between April-November 2009, nasopharyngeal swabs from 662 consecutive suspect cases of influenza A/H1N1pdm were tested with BinaxNOW Influenza A&B rapid test. Nucleic acids were tested with the CDC molecular method, using pan-influenza A, swine influenza A-specific and A/H1N1pdm-specific primer/probe sets, targeting M, NP and H genes, respectively. Samples positive only to pan-influenza A were subtyped with H1N1 and H3N2 type-specific RT-PCR, as well as with A/H1N1pdm NP-specific RT-PCR, followed by nucleotide sequencing. Viral load (VL) in the pan-influenza A-RT-PCR-positive samples was calculated by plotting the M gene cycle threshold values on a regression curve, built on a standard influenza A/H1N1pdm preparation, containing a known genome-equivalent concentration.

Results: 260/662 (39.3%) cases, resulted positive to the pan-influenza A RT-PCR: 234 (96%) were typed as A/H1N1pdm, 6 (2.3%) as H3N2 and 20 (7.7%) remained untypable. Rapid test resulted positive in 47/260 pan-influenza A RT-PCR-positive cases and in one of 402 RT-PCR-negative samples (overall sensitivity 18.1%, specificity 99.7%, PPV 97.9%, NPV 65.3%). Sensitivity for A/H1N1pdm confirmed cases was 17.3% (44/254) and 50.0% (3/6) for H3N2. Median VL was 6.9 (IQR 6.3−7.3) and 5.1 (IQR 3.9−5.6) M gene Log cp/mL for RIDT-positive and negative cases, respectively (p=0.0001). among RIDT-positive samples, 92.1% showed VL values exceeding 6.0 M gene Log cp/mL.

Conclusions: The present study shows that RIDT has low sensitivity and high specificity for A/H1N1pdm, in agreement with previous reports. In particular, A/H1N1pdm viral load seems to be the major determinant for positive result in RIDT. Negative RIDT results should not be the basis for excluding influenza A/H1N1pdm diagnosis, and samples from suspect cases negative to RIDT should be analyzed with more accurate methods.
Based on reference tests, IgG ELISA sensitivity was 93.0% and specificity was 74.5%. Interestingly, 8.2% of post-pandemic sera, who resulted positive by the reference criteria, yielded an IgG ELISA borderline result.

Regarding IgA, 12.5% and 14.0% of samples resulted positive in ‘08 and ‘10 collections respectively.

**Conclusions:** These results suggest that the new IgG ELISA test is very sensitive in detecting specific immune response in patients with confirmed infection, and may be useful for the serological diagnosis of A/H1N1pdm infection, and to assess the individual response to A/H1N1pdm vaccination.

Considering the retrospective groups, sensitivity and specificity of IgG ELISA with respect to the reference tests are suboptimal, as expected. The significance of IgA in recent or remote infections, and in vaccine response, remains to be established.

### Study of norovirus infection from stool samples in transplant recipients or intensive care unit patients from a children’s hospital with an antigen detection enzyme immunoassay test

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**Objective:** The aim of this study was to compare the performance of the commercial antigen detection enzyme immunoassay RIDA® QUICK Norovirus, in comparison with the results obtained with conventional reverse-transcriptase polymerase chain reaction (RT-PCR), regarded as the gold standard, for detecting norovirus in stools samples from transplant recipients or intensive care unit (UCI) patients in a children’s hospital.

**Methods:** RIDA® QUICK Norovirus assay was performed on a total of 33 stool samples from 22 patients. The patients’ age ranged from 6 months to 3 years, 14 patients were males and 8 females. Samples were collected from January to August of 2010. Each sample was prepared for testing by RT-PCR (Kojima et al., J Virol Methods, 2002, 100:107–114) according to validated laboratory protocols.

**Results:** During the study period 16 samples from five patients with norovirus were detected by RT-PCR. Of these samples, 10 were also detected with the RIDA® QUICK Norovirus assay. Six cases of norovirus infection were not detected by the RIDA® QUICK Norovirus. With these results the calculated sensitivity of the RIDA® QUICK Norovirus, in this study, was 63% and the specificity was 100%. As a consequence the calculated negative predictive value was 74% and the positive predictive value was 100%.

**Conclusion:** The RIDA® QUICK Norovirus can be used as a rapid and simple first test in cases with suspected norovirus aetiology of a diarrhoea in children transplant recipients or hospitalized in the ICU. If the result is negative, the RT-PCR is recommended.

### Evaluation of new extremely rapid Epstein-Barr virus VCA-IgG, EBNA-IgG and VCA-IgM assays using immunofluorescence as reference

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**Objectives:** EBV specific serology is required to diagnose or exclude an infectious mononucleosis (IM). It is mostly tested with time-consuming methods often requiring specific instruments such as EIA, immunofluorescence (IFA) or blots.

The new Bio-Rad RDT EBV IgG and IgM Assays based on immunofiltration are evaluated using an in-house IFA as reference (German EBV Reference Center). The new assays provide results to VCA IgG, EBNA-1 IgG and EBV IgM within 2 min and require no specific instruments.

**Methods:** 340 clinical characterized samples were tested including 59 from patients with acute IM, 60 past EBV infection, 60 seronegative, 62 potential cross-reactive samples (CMV-, VZV-, HSV-IgM positive), and samples from patients with immunosuppression due to rheumatic disorders (51) or lung transplantation (48).

### Comparison of Epstein-Barr virus tests on Immunulite with Enzygnost/Novagnost tests

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**Objectives:** The aim of the study was to compare the performance characteristics of different Epstein-Barr Virus (EBV) tests (EBV-VCA IgG, EBV-VCA IgM, EBV-EBNA IgG) available on the random access analyzer IMMULITE (Siemens, Germany) with Enzygnost Anti-EBV/IgM II, Enzygnost Anti-EBV/IgG and Novagnost EBV-EBNA IgG ELISA tests performed on BEPIII (Siemens).

**Methods:** A total of 400 sera, collected at the University Hospitals Leuven, were evaluated on both analyzers. A first group contained 200 sera from hospitalized patients. The majority had a past EBV infection, sixteen were seronegative and none experienced a primary EBV infection. To compare the sensitivity for the detection of primary infection, 100 sera from patients with a primary EBV infection were included. Finally to investigate cross reactivity, we selected 100 sera: 20 sera positive for CMV IgM, 20 sera positive for Toxoplasma IgM, 20 sera positive for rheumatoid factor and 40 sera of pregnant women. In case of test discrepancies, additional Mikrogen recomLine EBV IgM/IgG immunoblot (Mikrogen, Germany) was performed for final classification of a specimen.

**Results:** In the first group (n=200) the specificity of the EBV-VCA IgM test on IMMULITE was 97.5%, compared to 100% with the Enzygnost Anti-EBV/IgM II kit. Sensitivity of the IMMULITE EBV VCA IgG test was very good (98.5%) and excellent (100%) for the Enzygnost IgG test.

The correlation between the IMMULITE EBV-EBNA IgG, EBV VCA-IgG and EBV VCA-IgM tests were respectively 98%, 98% and 94.5% with the corresponding Novagnost and Enzygnost kits. The correlation for positive sera was 94.2% for the EBNA-IgG and 95.1% for the VCA IgG determination. Detection of a primary EBV infection was moderate on IMMULITE with a sensitivity of 82.3%. There was no cross reactivity due to CMV IgM, Toxoplasma IgM, rheumatoid factor and pregnancy.

Five samples showed a secondary reactivation which was only detected on IMMULITE.

**Conclusion:** The different immunoassays on both analyzers have acceptable and comparable performance characteristics with exception of the IMMULITE IgM test which was less sensitive than the Enzygnost IgM test. As such, sensitivity of IMMULITE for the detection of a primary EBV infection was only moderate (82.3%).
cross-reactive samples. However, most of these samples similarly had a high positive EBNA-1 IgG and thus require further test methods. Similarly, majority of false positive EBNA-1-IgG was extremely weak and found within the primary infections in combination with a high positive VCA-IgM.

**Conclusion:** Our results indicate that the Bio-Rad RDT EBV IgG and IgM assays perform excellent comparable to gold standard IFA and many other ELAs. Additionally, Bio-Rad RDT EBV IgG and IgM Assays provide the practicability, ease-of-use and rapidity of Mononucleosis devices and only 2 minutes are needed to report EBV accurate profile with high level of performance. These assays can be recommended for EBV infection and IM detection.

**P1016** Analysis of the results of the SEIMC external quality control programme in the detection of gastrointestinal viruses

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**Objective:** To evaluate the results obtained by participants in the Programme of External Quality Control launched by the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC) in detection of gastrointestinal viruses.

**Materials and Methods:** From year 2004 to year 2010 four viral controls were sent to an average of 80 participating centers, comparing their results with those of a reference laboratory. In all cases, stool samples were sent with a clinical history, and participants were asked to operate the detection of gastrointestinal viruses. The samples submitted were positive for the following viruses: Astrovirus (2004), Rotavirus (2007), Adenovirus (2008) and Norovirus (2010).

**Results:** Participation rates were 73.2% (2004), 83.1% (2007), 85.1% (2008) and 74.7% (2010). When virus was Rotavirus or Adenovirus more than 97% of the centers performed the necessary tests to detect them, but if virus was Astrovirus or Norovirus only 23.7% or 59.3% of participants, respectively used appropriate techniques to detect them. The success rates were: 83.3% (Astrovirus), 100% (Rotavirus), 88.5% (Adenovirus) and 45.9% (Norovirus). The most common methods used were the immunochromatography (IC), latex agglutination (LA) and enzyme immunoassay (EIA). With EIA techniques the success rates were of 100.0% (Rotavirus), of 81.8% (Astrovirus), 92.2% (Adenovirus) and 20.0% (Norovirus). In case of IC techniques, the success rates were of 100.0% (Rotavirus) and 80% (Adenovirus). LA techniques, only were employed for Rotavirus (success rates of 100%) and for Adenovirus (success rates of 33%). Thus, for Norovirus, the most common techniques with better results were the molecular biology methods (PCR/real time PCR – success rate 100%, and sequencing – success rate 66.7%). Astrovirus just was detected by EIA and PCR.

**Conclusions:** (a) There’s an increase of centers that detected virus in feces, especially in Rotavirus and Adenovirus control due to rapid diagnostic techniques. Norovirus and Astrovirus are those with worse detection rates. (b) EIA techniques are particularly successful in the detection of all viruses with the exception of Norovirus and IC techniques obtained good results with Rotavirus and Adenovirus. AL techniques obtained acceptable results only in Rotavirus. PCR techniques obtained good results for all viruses. (c) There are few laboratories of all centers registered in virology control that are trained to detect Astrovirus and Norovirus.

**P1064** Occurrence of immunoglobulin M antibodies against several bacterial and viral pathogens in acute hantavirus infection

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**Objectives:** Elevated immunoglobulin M (IgM) antibodies against *Coxiella burnetti* and enterovirus without concurrent IgG antibodies were detected in a patient with acute hantavirus infection of serotype Puumala. Thus, we questioned whether detection of IgM antibodies to hantavirus non-related pathogens was a singular case in this patient or occurs more frequently in acute hantavirus infection.

**Methods:** Serum samples from 48 patients with acute hantavirus infection of serotype Puumala collected during the hantavirus epidemic year 2010 in Germany were available. All patients had clinical suspicion of hantavirus infection and were positive for hantavirus-specific IgM and IgG antibodies by immunoblot analysis. IgM and IgG antibodies against *Coxiella burnetti*, *Enterobacter cloacae*, *Epstein-Barr-Virus* (EBV), *Coxiella burnetti*, *Leptospira*, and *Borrelia burgdorferi* were determined by commercial immunoassay.

**Results:** IgM antibodies against one or more of the investigated pathogens were detected in 16 of 48 patients (33.3%). In detail, IgM antibodies were positive against *Coxiella burnetti* in 6 patients (12.5%), enterovirus in 5 patients (10.4%), *Leptospira* spp. in 1 patient (2.1%), and CMV in none of the patients. Isolated IgM antibodies against *Borrelia burgdorferi* that were not confirmed by immunoblot were found in 2 patients (4.2%). IgM antibodies against EBV were detected in 11 patients (22.9%), of whom all patients showed serological evidence of past EBV infection indicated by IgG antibodies against EBV nuclear antigen. Concurrent borderline or positive IgM antibodies against two or more of the investigated pathogens were observed in 10 of 48 patients (20.8%). During serological follow-up isolated IgM antibodies against *Coxiella burnetti* were detectable for more than 50 days in one patient.

**Conclusion:** IgM antibodies against several bacterial and viral pathogens, possibly caused by polyclonal B cell stimulation, can be detected quite frequently in acute hantavirus infection. This observation should be taken into account to avoid misinterpretation of serological test results.
**P2164** Evaluation of a rapid detection kit using immunochromatography for adenovirus infection

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**Objectives:** A newly described rapid chromatographic immunoassay was evaluated for adenovirus detection in nasopharyngeal aspirates of children with respiratory illness, compared to an indirect immunofluorescence method (IFA).

**Methods:** During a four year period (2006–2009), we screened 2337 nasopharyngeal aspirates collected from children, aged <2 years, hospitalized for acute lower respiratory infection, in a paediatric hospital in Greece. The screening was performed for respiratory viruses detection (respiratory syncytial virus, adenovirus, influenza A and B, parainfluenza 1, 2 and 3) using DFA (Respiratory Virus Panel – BioRad). Sixty-four specimens were found to be positives only for adenovirus. Positives specimens were divided, according to the number of fluorescing cells per optical field (fc/pof) in low (0–2 fc/pof), medium (2–4 fc/pof) and high positives (≥5 fc/pof). All adenovirus positives specimens, 16 specimens negatives for all the above respiratory viruses and 20 specimens negative for respiratory viruses other than adenovirus, were evaluated for adenovirus detection by the new chromatographic immunoassay (Adenovirus Resp. – CerTest).

**Results:** No false positives results were observed using the new assay. The specificity and positive predictive value for adenovirus detection by this new assay were 100%; in comparison with IFA. No cross reaction with the other respiratory viruses was detected. However the sensitivity ranged from 0%, 35.5% and 100% in low, medium and high positives specimens, respectively.

**Conclusion:** This new immunochromatographic assay is able to detect adenovirus infection simply and rapidly, but its usefulness is limited in early diagnosis when low or medium positives specimens are tested.

HIV/AIDS

**P2165** HIV-1 is able to infect resting TCD4+ cells efficiently in a short time of viral exposure

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HIV is the causative agent of the acquired immunodeficiency syndrome (AIDS). According to UNAIDS there are roughly 33 million people living with HIV. Several studies have addressed many aspects of HIV infections but elements pertaining to the relationship between the virus and target cells are still waiting for a better characterization. Most of the studies on early steps of viral infection showed that cellular activation is essential for HIV infection in TCD4+ lymphocytes and monocytes. The objective of the this work was to determinate if HIV-1 is able to infected resting TCD4+ lymphocytes purified from human peripheral blood. The virus was able to infect resting TCD4+ lymphocytes on MOIs ranging from 0.05 to 1.0. Our preliminary results showed that resting TCD4+ lymphocytes can be infected by HIV-1 within 30 minutes after the addition of virus to cell cultures. After infections, HIV-1 reverse transcription was allowed to proceed for 36h under IL-2 and PHA activation. Conventional PCR and RFLP of the protease gene showed that viral DNA can be detected for all MOIs and in all time periods from 30 minutes up to 180 minutes post-infection. Proviral load will be measured by qPCR for each MOI and period infection. Our results indicated that resting TCD4+ cells can be efficiently infected by HIV when different MOIs are used and even when cells were exposed to virus for only 30 minutes. Infection of resting cells does not represent a barrier for HIV since virus could be recovered 36 hours post infection. Our data may have some implications on how HIV can manage to establish infection on new hosts.

**P2166** Prevalence of primary resistance mutations and influence of integrase polymorphism on virological outcome of Raltegravir-containing anti-retroviral regimens

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**Objective:** To describe the prevalence of mutations conferring resistance to Raltegravir in a cohort of patients starting salvage therapy with this drug, and to evaluate the influence of polymorphisms in the integrase gene on virological outcome.

**Methods:** We have studied 173 patients form Eastern Andalusia, South of Spain, starting a Raltegravir containing regimen with at least 48 weeks of follow-up. Integrase from baseline and failure samples was amplified and then sequenced using the Trugene platform (Geno type2). The prevalence of resistance mutations in the integrase in the baseline samples (n=50) was 24% and only one patient carried a secondary resistance mutation (L74M, prevalence <1%). Most prevalent baseline integrase polymorphisms were K160R (11.1%), P145L (11.1%), E85G (5.5%), K103R (11.1%), Q216R (16.6%), as well as other changes near the catalytic site as A124V (5.5%), E138D/G (5.5), A179V (2.3), V201I (41.7%). No association with any baseline polymorphism and virological failure was observed. By week 48, 18 patients (10%) had virological failure to a Raltegravir, and only 6 patients (24%) had detectable integrase mutations. No relation with any baseline polymorphism and the detection of failure mutations was observed. Most prevalent selected polymorphisms (not present at baseline but detectable on failure) were K160R (11.1%), P145L (11.1%), E85G (5.5%), K103R (11.1%), Q216R (16.6%), as well as other changes near the catalytic site as A124V (5.5%), E138D/G (5.5), A179V (2.3), e i l62M (2.3). No-B subtypes did not show differences in virological failure compared to B subtypes.

**Conclusion:** The prevalence of resistance mutations in the integrase in patients treated with Raltegravir in Southern Spain is low, and there is no primary resistance to integrase inhibitors in our area. There is no association between baseline polymorphisms in integrase and virological failure to a Raltegravir containing regimen, or the selection of Raltegravir associated mutations.

**P2167** Regulation of APOBEC3G as therapeutic strategy to control HIV-1 infection

L. Ferreira*, A. Ramos, J. Gonçalves, I. Soeiro (Lisbon, PT)

APOBEC3G (A3G) was one of the first endogenous proteins reported to be able to impair HIV-1 replication. Recent studies show that A3G expression is raised in patients infected with HIV but presenting a slow disease progression; other studies showed that it is possible to enhance A3G expression through stimulation of specific cell surface receptors. The molecular mechanism responsible for this A3G expression variations are still not clear, thus our aim is to characterize the regulation of A3G expression upon specific T cell-receptor stimulation. FoxO3a belongs to the Forkhead family of transcription factors, which are involved in cell cycle arrest and apoptosis. Additionally, FoxO3a is regulated by the PI3K/AKT pathway that is usually found downstream of several T-cell receptors. In this context, the T-cell receptor stimulation was achieved using HIV-1 gp120 protein that binds to the CD4 receptor. Our results indicate that gp120 stimulation increases A3G expression and leads to inactivation of FoxO3a. To explore the A3G promoter region, bioinformatics tools were used leading to the identification of a sequence as the putative promoter containing four putatives FoxO3a binding sites. In order to validate this data, the putative A3G promoter was cloned into a Luciferase-reporter vector. Zinc-finger technology was used to validate this sequence and our preliminary results indicate that these zinc-fingers are able to trigger A3G expression.
To identify the role of FoxO3a in the A3G promoter and also to clarify the role of each putative FoxO3a binding site, all four sites were mutated and cloned into Luciferase-reporter vectors. Our results indicated that the A3G promoter has low activity, which might be indicative of a promoter that is usually under repression. Three of four mutants analyzed showed higher activity related to wt promoter reinforcing that FoxO3a might be binding to these sites with a repressive function.

To confirm this, Chromatin Immunoprecipitation analysis was performed, revealing the involvement of FoxO3a in this promoter. In conclusion, gp120 stimulation appears to activate the PI3K pathway, regulating FoxO3a activity. At the promoter level, preliminary results using zinc-fingers tools validate the sequence in study as a legitimate site to be investigated. In this context, A3G expression appears to be under repression by FoxO3a. The role of each putative FoxO3a binding site, all four sites were mutated and cloned into Luciferase-reporter vectors. Our results indicated that the A3G promoter has low activity, which might be indicative of a promoter that is usually under repression. Three of four mutants analyzed showed higher activity related to wt promoter reinforcing that FoxO3a might be binding to these sites with a repressive function.

Antiviral drug resistance is of rising concern in HIV-infected patients undergoing antiretroviral therapy (ART). Primary resistance in ART-naive individuals as well as transmission of resistant viruses are increasingly reported especially in communities with high incident HIV infections. Data regarding ART resistance in persons from low-endemicity regions are still sparse. This prospective analysis was conducted to describe the extent and level of genotypic resistance to antiretrovirals (HIV-GR) in a patient group presenting within three months of serologically documented seroconversion or clinically overt acute retrovirus syndrome (PHI) and in those naïve for ART scheduled for starting treatment (pre-ART) tested in the setting of a low-endemicity region in Germany.

Patients and Methods: Between 2008–2010 fifty individuals undergoing HIV-GR for (non-nucleoside) RT inhibitors (oNRTI) and protease inhibitors (PI) were capable for this prospective analysis. PHI or seroconversion was diagnosed in 13, pre-treatment testing was done in 20, and 17 individuals experiencing treatment failure served as comparison group. Crude ART resistance was observed as well as Stanford mutation score and compared between to either groups. HIV-GR was performed using commercially available assays, Stanford mutation score was calculated using published algorithms.

Results: Mutations (SNPs) in the reverse transcriptase (RT) and protease (PR) genes were found in nearly all subjects. Median number of mutations was 12 (range 4–29) for RT gene and 7 (range 3–14) for PR gene. Significant SNPs (both RT gene) were found in 2/13 PHI individuals conferring resistance against both, NRTI and oNRTI. No PR resistance could be detected. Stanford mutation score was 70 and 120 for these individuals compared to mean scores of 15 (RT gene) and 0.7 (PI gene) in all PHI patients. In the pre-ART group no evidence for RTI or PR resistance was detectable. Mean Stanford mutation scores were 0.7 (RT gene) and 0.6 (PI gene) in all 20 pre-ART patients. In those presenting with ART failure 47.1% had detectable resistance which included two (11.7%) with triple-class resistance. Mean Stanford mutation scores were 34.94 (RT gene) and 19.82 (PI gene) in ART-experienced individuals. These features prompted this study, which was conducted to characterize kinases and phosphatases identified as helper factors for HIV-1 replication in a previous shRNA screen. For this purpose, we attempted to assess: 1) the effect of the 13 of the 14 identified proteins in HIV-2 replication cycle; and 2) the mechanism by which two of the identified proteins, SGK and CIB2, affect the early phase of HIV-1 life cycle, more precisely in viral fusion and in post-reverse transcription pathways. To accomplish this, the constructed shRNA clones were infected with HIV-2 particles to evaluate the effect on HIV-2 replication. When we compared the amount of virus in all tested shRNA clones challenged with HIV-1 and HIV-2, all proteins exhibited a similar outcome from the one observed in HIV-1. This evidence could indicate that HIV-1 and HIV-2 share cellular pathways while hijacking these host factors to assure its survival. Since CIB2 potentially interacts with integrins, and both SGK and CIB2 are involved in regulation of several ion channels, we inquired if these proteins could affect virus-cell fusion and performed a virus-cell fusion assay. We assessed that both SGK and CIB2 are important in HIV-1 entry, since their knockdown reduces the number of fusion events. Furthermore, we conducted the quantification of 2-LTR circles by RealTime-PCR in SGK and CIB2 shRNA clones infected with HIV-1, in order to establish if SGK and CIB2 play a role in steps previous to integration. Preliminary data suggests that SGK and CIB2 could lead to 2-LTR circles formation, affecting HIV-1 replication. This study provides new insights for the complex host-HIV interactions and proposes several mechanisms by which some of these kinases and phosphatases affect HIV infection, instigating further studies and new possibilities for antiviral strategies.
Regulation of Vif by phosphorylation during HIV-1 infection

HIV integrase variability and genetic barrier in
Determination of drug resistance from HIV-1 RNA

Minorities in patients failing non nucleoside regimens is relatively frequent.

(a) The investigation of K103N by AS-PCR, in newly diagnosed patients, increases the rate of transmitted drug resistance to non-nucleosides in 1.7%, which, according to data published using population sequencing, remains to be cost-effective. (b) K103N to non-nucleosides in 1.7%, which, according to data published using population sequencing, remains to be cost-effective. (b) K103N could be additionally detected by AS-PCR in 15 of 89 patients in whom this mutation was not present by population sequencing, increasing the prevalence in 16.8%.

Conclusions:

(a) The investigation of K103N by AS-PCR, in newly diagnosed patients, increases the rate of transmitted drug resistance to non-nucleosides in 1.7%, which, according to data published using population sequencing, remains to be cost-effective. (b) K103N minorities in patients failing non-nucleoside regimens is relatively frequent.

Methods: The HIV-1 integrase variability was analyzed in 16 treatment naïve patients infected with subtype B strains and 25 with subtype non-B strains. In addition, the effect of treatment with inhibitors of integrase (INI), protease (PRI) and reverse-transcriptase (RTI) on the integrase variability was analyzed in 19 INI naïve patients with subtype B and 35 patients with subtype non-B. For comparison 13 patients infected with subtype B strains and experienced of all classes of antiretroviral drugs were analyzed. Moreover, the integrase variability was analyzed in 4 patients infected with HIV-2 naïve for treatment with INI. Finally, the genetic barrier for specific INI resistance mutations was calculated to compare the rate of drug resistance development.

Results: Primary mutations associated with resistance to INI were not detected in patients not previously treated with this class of drugs. However, some secondary mutations which have been shown to contribute to raltegravir resistance were found. In patients infected with subtype B strains, a lower genetic barrier for the acquisition of mutations G140S, S147G and V151I as compared with subtype non-B was observed. HIV-2 strains from INI naïve patients showed the presence of both primary and secondary resistance mutations.

Conclusions: The overall efficacy of INI do not appear to be reduced by spontaneous emergence of resistance polymorphisms. However, the lower genetic barrier in subtype B HIV-1 strains, and the natural resistance of HIV-2 strains should be taken into consideration when selecting INI-containing treatments.

Regulation of Vif by phosphorylation during HIV-1 infection

A. Ramos*, A. Couto, F. Luís, J. Gonçalves, I. Soeiro (Lisbon, PT)

The Human Immunodeficiency virus (HIV) is the causative agent of AIDS, a disease affecting around 33 million people worldwide. Current anti-retroviral therapy, although efficient in suppressing HIV-1 infection, cannot provide a cure. For this reason, several studies turn to the dynamic interplay of host-virus interaction as a tool for HIV eradication.

Viral Infectivity Factor (Vif) is an accessory protein codified in the HIV-1 genome and synthesized during its late life cycle. It is essential in infected cells for the formation of complexes (e.g. Vif-Cul5) that lead the cellular defence factor APOBEC3G (A3G) to degradation, preventing its inhibitory actions on HIV replication.

Previous studies reveal that several HIV-1 proteins are regulated by phosphorylation, raising the question whether Vif phosphorylation by host kinases could represent a valid option for novel antiviral therapy solutions.

Five previously unpublished residues were identified on Vif as targets for phosphorylation by cellular kinases, using 3 different bioinformatic tools. Those sites were mutated by site-directed mutagenesis and both bacterial and mammalian expression vectors constructed. The resulting mutants were purified so that their specific phosphorylation patterns can be confirmed by in vitro kinase assays. HEK293T cells were transfected with the mutant constructs and the expression levels of both Vif and A3G evaluated by Western Blotting. In order to assess the ability of the mutant proteins to bind to Cul5 and lead A3G to degradation, a co-immunoprecipitation assay was performed. Preliminary results show that the Vif mutants co-immunoprecipitate with Cul5, although with different abilities, indicating that the mutations do not completely influence the formation of the Vif-Cul5 complex required for A3G degradation. Because Vif also bears A3G-unrelated functions, we inquired if the infectivity of Vif-mutated viral particles was altered. Virions containing the mutated proteins were produced in HEK293T cells and used to infect HeLa-P4 cells. Viral production was evaluated by p24 ELISA assay and preliminary data suggests, in accordance to previous studies, that the replicative ability and infectivity of those virions was altered.

The identification of these phosphorylations and their specific biological role in regulating Vif activity may lead to a better understanding of the complexity of HIV-1 replication and eventually provide a new direction in antiviral research and development.

HIV integrase variability and genetic barrier in antiretroviral naïve and experienced patients

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Objectives: To analyze the HIV-1 integrase variability in treatment naïve and treatment experienced patients infected with different HIV-1 subtypes.

Methods: The HIV-1 integrase variability was analyzed in 16 treatment naïve patients infected with subtype B strains and 25 with subtype non-B strains. In addition, the effect of treatment with inhibitors of integrase (INI), protease (PRI) and reverse-transcriptase (RTI) on the integrase variability was analyzed in 19 INI naïve patients with subtype B and 35 patients with subtype non-B. For comparison 13 patients infected with subtype B strains and experienced of all classes of antiretroviral drugs were analyzed. Moreover, the integrase variability was analyzed in 4 patients infected with HIV-2 naïve for treatment with INI. Finally, the genetic barrier for specific INI resistance mutations was calculated to compare the rate of drug resistance development.

Results: Primary mutations associated with resistance to INI were not detected in patients not previously treated with this class of drugs. However, some secondary mutations which have been shown to contribute to raltegravir resistance were found. In patients infected with subtype B strains, a lower genetic barrier for the acquisition of mutations G140S, S147G and V151I as compared with subtype non-B was observed. HIV-2 strains from INI naïve patients showed the presence of both primary and secondary resistance mutations.

Conclusions: The overall efficacy of INI do not appear to be reduced by spontaneous emergence of resistance polymorphisms. However, the lower genetic barrier in subtype B HIV-1 strains, and the natural resistance of HIV-2 strains should be taken into consideration when selecting INI-containing treatments.

Determination of drug resistance from HIV-1 RNA stabilised at room temperature on solid matrix

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Objectives: Analysis of HIV-1 genomic sequences, the kinetic evaluation of high occurrence of nucleotide spontaneous mutations and the appearance of resistance induced by drug treatment appear to represent today the best index of clinical efficacy of antiretroviral therapy (HAART). The liability of the viral RNA requires the need to store or transport biological samples at controlled temperature (60–80°C) if not processed immediately after harvesting, giving a substantial limit to the current molecular methods for the determination of drug-resistance. The objective of our work is to make stable at room temperature (RT) the viral RNA on solid support for its safely transport to reference laboratories distant from places where they are collected to test drug resistance.

Methods: From 45 samples whole blood was collected from viremic patients either under drug treatment or not. Thirteen samples were processed as follows: 9 ml were used to perform, as routine procedure, the monitoring of hematologic and chemical parameters and the circulating lymphocyte subpopulation, viral-load and drug resistance; residual 3 ml were used to verify the stability and integrity of RNA that, after a brief treatment, was adsorbed on solid support and kept for 10 days at RT. Results from each fresh sample were compared with those from stabilized RNA on solid matrix. Genome sequences were analyzed by the HIV-1 genotyping TRUGENE and the DNA sequencing OpenGene systems which are able to analyze and identify all genotypic changes of the virus “naïve” or the drug-induced gene “pol” mutations in the region encoding the protease and reverse transcriptase.

Results: The same pol mutations associated to protease inhibitors (IP) and to Nucleoside/Nucleotide Reverse Transcriptase (NRTI) and not (NNRTI) inhibitors were highlighted both on fresh and stabilized samples. Furthermore, the same missense and silent point mutations were identified on both sample sets and were characterized as different from HIV reference sequences. These sequences may be substantial for HIV genotype phylogeny.

Conclusion: The purpose of this project is to develop a sampling method suitable to conduct HIV-1 drug resistance tests in reference laboratories even placed far from satellite centers, which are often located in developing countries. The technique may allow, after RNA stabilizing, to obtain the same characteristics of efficiency, sensitivity and specificity of procedures used in peripheral points of care.
**Prevalence of cognitive impairment in an Irish HIV-positive clinic population based on the use of surrogate markers for cognitive impairment**

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**Objectives:** A retrospective chart review was carried out at the HIV clinic in St. James's Hospital, Dublin, Ireland to examine the rate of cognitive impairment through the use of surrogate markers for cognitive impairment. The surrogate markers for cognitive impairment chosen for use in this chart review were subjective complaints from the patients documented in the charts, a new psychiatric diagnosis since the diagnosis of HIV seropositivity, neurological complications associated with HIV and radiological evidence of brain atrophy.

**Methods:** 500 consecutive hospital charts were reviewed of patients who were due to attend the outpatient clinics. All patients were included except for patients whose charts were missing or unavailable. The paper hospital charts were reviewed for the above surrogate markers and also the electronic patient record system was reviewed for the results of radiological investigations. Basic demographics including age, gender, nationality, mode of transmission of HIV, nadir CD4 count, current CD4 count, viral load, co-morbidities, medication history and antiretroviral history were all recorded.

**Results:** There were 306 men and 194 women included in the study. Median age was 37. 5.6% were diagnosed within the last year, 39% between 1 to 5 years ago and 33.4% 5 to 10 years ago. The most common mode of transmission was heterosexual followed by the use of intravenous drugs and then men who have sex with men. 45% had a nadir CD4 count of less than 200. 58% were virally suppressed. 77% were on antiretroviral therapy. Just under 30% were co-infected with hepatitis B (22/500), C (121/500) or both (4/500). 13.8% of patients had one or more positive surrogate markers for cognitive impairment. 9% had a new onset of a psychiatric disorder after the diagnosis of HIV. 4.2% had a neurological complication of HIV. Only 6 people had evidence of atrophy on either CT or MRI brain. 94% of these patients with positive surrogate markers for cognitive impairment were on antiretroviral therapy.

**Conclusions:** On univariate analysis significant relationships were found between the presence of positive surrogate markers for cognitive impairment and female gender, older age, nadir CD4 <200, longer duration of infection, Ireland as a country of birth, intravenous drug use as a mode of transmission, being on ART and co-infection with hepatitis B or C. Multivariate analysis using logistic regression showed significant relationships only with gender and year of diagnosis.

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**Durable outbreak of syphilis in HIV-infected patients: data from Northern Greece**

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**Objectives:** To describe the prevalence of syphilis and other sexually transmitted infections (STIs) and factors associated with HIV co-infection.

**Methods:** A review of infectious syphilis cases in HIV patients in Northern Greece during the period 2008–2010. Demographics of the patients, results of diagnostic tests, concurrent infections, possible route of acquisition for both infections and routine HIV screening tests were recorded.

**Results:** Of 800 HIV patients treated in our hospital, 331 were screened and 58 (17.5%) were syphilis positive, during a three year period. The vast majority of syphilis patients were MSM (55 patients, 94.82%) with a mean age of 38.4 years. The mean duration of HIV was 3.3 years and the way of transmission for all syphilis cases was sexual contact. At time of syphilis seropositivity, mean CD4-cells count was 440.26/mm3 and mean plasmatic HIV RNA virus load was 3.85 log/mL. Most patients with no syphilis infection were MSM with a mean age of 38.3 years. The mean duration of HIV was 3.01 years. At time of syphilis screening, mean CD4-cells count was 493.11/mm3 and mean plasmatic HIV RNA virus load was 3.7 log/mL. There was no associated between gender and syphilis (P=0.256). A significantly greater proportion of patients with syphilis were under HAART than no syphilis patients (P=0.026). A significantly greater proportion of patients with syphilis had co contaminant STIs than no syphilis patients (P<0.001). There was no association between syphilis and CD4 (P=0.216), syphilis and viral load (P=0.609), syphilis and age (P=0.943), syphilis and mode of transmission (P=0.484) or between syphilis and duration of HIV (P=0.693). Both ingestion of HAART and STIs existence were independent predictors of syphilis, showing that the odds of syphilis was 2.4 times higher in patients with HAART than in patients with no HAART (95CI: 1.26, 4.63, p=0.008) and it was 9.4 times higher in patients with STIs than in patients with no STIs (95%CI: 4.49, 19.64, p<0.001).

**Discussion:** Multiple sexual partners, unprotected sex, HAART intake and co-infection with other STIs are risk factors for this syphilis epidemic record. The overall high rate of unprotected sex demonstrates an increasing prevalence of unsafe sexual practices among MSM, probably attributed to faith in antiretroviral therapy. Therefore, continuous alert, prompt diagnosis, early treatment and contact tracing are essential in order to control this important outbreak among HIV population.
Late diagnosis of HIV infection in Ireland – a cause for concern
A. Jackson*, M. Brennan, F Mulcahy, W. Powderly, M. Codd, S. McConkey (Dublin, IE)

**Objectives:** HIV outcomes improve with earlier diagnosis: antiretrovirals can avert significant immunocompromise and reduce transmissibility. Unfortunately, many present late with low CD4 counts. Using data from 2000–2009 from the Dublin City HIV Cohort we aim to determine the proportion of people presenting with low CD4 counts and to see if this changes with time, gender, country of origin and route of transmission.

**Methodology:** The Dublin City HIV Cohort enrolled patients from 3 Dublin teaching hospitals from 2004 to 2009 collecting retrospective and prospective information. A retrospective cohort study was performed, reviewing cases diagnosed from 2000 to 2009 with a documented CD4 count within 12 months of diagnosis. CD4 distribution was approximately parametric data allowing use of parametric analyses with SPSS software.

**Results:** 1021 patients were analysed. 48% were Irish-born, 42% from Sub-Saharan Africa (SSA). 43% were female. Mean CD4 at diagnosis was lower in 2009 (280 cells/μL) than in 2000 (409 cells/μL) and 2001 (395 cells/μL), but this difference was not statistically significant. Mean CD4 was lower among those presenting from SSA (295 cells/μL), compared to Irish-born patients (390 cells/μL) (p < 0.001).

Considering CD4 <350 cells/μL at diagnosis as late presentation, 57% of our cohort presented late overall. 48% of Irish-born people, and 69% from SSA presented late. This did not change significantly over time for either subgroup. Men who have sex with men (MSM) were least likely (41%), and those infected through heterosexual contact were most likely (65%) to present late. 60% females were late presenters, and 55% men. Among Irish-born, commonest modes of transmission were MSM (37%), intravenous drug use (IVDU) (35%), and heterosexual contact (26%). In this group the relative frequency of IVDU among this group has declined: 13% in the past 2 years compared to 38% in the previous 8 years. Heterosexual transmission among Irish patients increased from 12% in 2000 to 38% in 2009.

**Discussion:** Late presentation of HIV is a problem both for individual patients and national prevention programs. Our data shows that people are not presenting earlier, despite current public health approaches. It supports increased HIV testing among heterosexual, non-IVDU and to Irish-born patients, reminder letters and text messages to be sent before clinic and digital electronic patient records specifically for HIV patients.

**Conclusion:** Risk management is a useful tool to identify and to prioritize areas where changes in clinical health care services should be made. Due to the effectiveness, complexity, cost and dangers associated with anti-retroviral therapy for people with HIV, areas related to therapy were prominent.

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Leprosy presenting as immune reconstitution inflammatory syndrome in patients on highly active anti-retroviral treatment. A case-series study from a tertiary care centre in Kerala, South India
T. John*, C. Jacob, M. Sadeep, K. Shobhanakumary, A. Jacob, A. George (Kottayam, Trivandrum, IN)

**Objective:** To find out the incidence and clinical profile of Leprosy presenting as IRIS “Immune Reconstitution Inflammatory Syndrome”, in HIV patients. IRIS is a unique syndrome which comprises of a collection of inflammatory disorders, associated with paradoxical worsening of preexisting infectious processes either previously diagnosed and treated or subclinical and later unmasked following the initiation of highly active antiretroviral therapy (HAART), in HIV-infected individuals. IRIS is usually associated with a low pretreatment CD4 count (often less than 100 cells/microl). Although the most frequently reported associated infections with IRIS are localized herpes zoster, M. tuberculosis, M. avium complex, cytomegalovirus, and Cryptococcus, rarely leprosy can also present in a similar fashion.

**Methods and Materials:** It was a prospective study where we evaluated patients with HIV started on HAART for evidence of Leprosy, from May 2007 to June 2010.

**Results:** 4 patients treated with HAART were diagnosed to have Leprosy (Incidence was 5.38/1000 HAART patients). Of these, 3 patients had Borderline Tuberculoid and one had Borderline Lepromatous with type 1 lepra reaction. The pretreatment CD4 counts were 25, 40.50 and 75 cells/microl (mean = 47.5 ± 21) and CD4 count at the time of disease detection was 198, 215, 245 and 230 respectively. All patients presented within 8 weeks of starting HAART. The clinical features were hypo pigmented lesions, erythematous tender plaques and foot drop. Skin biopsy of these patients showed granulomatous inflammation. These patients met the requirement to all its operational service areas. As a busy urban-based HIV clinic in a large teaching hospital, we have evaluated and introduced a risk management framework. The aim was to reduce the likelihood of adverse events and to improve clinical outcomes by changing practice and to educate staff regarding clinical and non-clinical risk and its management.

**Methods:** The project began in December 2009 and ran for one year, using a standardised tool developed by HSE to calculate risk, incorporating both the potential impact of a problem and its likelihood. Clinical staff, non clinical staff and patient representatives were involved. Four workshops were held: Risk identification: Members listed potential problems in specific areas of the clinic with which they were familiar; Risk analysis: The risks and current control measures were discussed in depth; Risk evaluation and rating: Risks were stratified into categories based on importance using a Risk Assessment Form; Identification of mitigating factors: For the highest risks a plan was developed to implement changes.

**Results:** 14 risks were identified in total: 1 scored low, 6 medium, and 7 high risks. High risk items were drug dispensing errors, medication prescription errors, serious drug interactions causing adverse events, unsafe sex and lack of disclosure to sexual partners of HIV patients, exposure to tuberculosis for staff and patients, patient non-attendance and medical records unavailability. The following changes are being implemented and pursued: additional pharmacy resources, simplify guidelines for prescribers, increase education to prescribers and increase staffing to pharmacy, increase resources and staffing for social work department and co-ordinate a ‘buddy system’ among willing patients, structural changes to clinic and early identification of possible TB patients, reminder letters and text messages to be sent before clinic and a digital electronic patient record specifically for HIV patients.

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Use of risk management as a tool for quality improvement in a public HIV service
M. Brennan*, A. Jackson, H. Tuite, S. McConkey (Dublin, IE)

Risk assessment and risk management is an important process in organisations ranging from corporations to public healthcare services. The Health Services Executive (HSE) in Ireland has made this a requirement to all its operational service areas. As a busy urban-based HIV clinic in a large teaching hospital, we have evaluated and introduced a risk management framework. The aim was to reduce the likelihood of adverse events and to improve clinical outcomes by changing practice and to educate staff regarding clinical and non-clinical risk and its management.

**Methods:** The project began in December 2009 and ran for one year, using a standardised tool developed by HSE to calculate risk, incorporating both the potential impact of a problem and its likelihood. Clinical staff, non clinical staff and patient representatives were involved. Four workshops were held: Risk identification: Members listed potential problems in specific areas of the clinic with which they were familiar; Risk analysis: The risks and current control measures were discussed in depth; Risk evaluation and rating: Risks were stratified into categories based on importance using a Risk Assessment Form; Identification of mitigating factors: For the highest risks a plan was developed to implement changes.

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**Conclusion:** Risk management is a useful tool to identify and to prioritize areas where changes in clinical health care services should be made. Due to the effectiveness, complexity, cost and dangers associated with anti-retroviral therapy for people with HIV, areas related to therapy were prominent.

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Leprosy presenting as immune reconstitution inflammatory syndrome in patients on highly active anti-retroviral treatment. A case-series study from a tertiary care centre in Kerala, South India
T. John*, C. Jacob, M. Sadeep, K. Shobhanakumary, A. Jacob, A. George (Kottayam, Trivandrum, IN)
Susac's syndrome in an HIV-infected patient

E. Vryonis*, E. Kakalou, A. Baxecanakis, C. Linardi, K. Papamikolou, V. Papastamopoulos, I. Baraboutis, A. Skoutelis (Athens, GR)

Objective: To present a case of Susac syndrome in an HIV infected patient.

Susac syndrome is a rare CNS vasculopathy characterized by the clinical triad of encephalopathy, branch retinal artery occlusions and cochlear hearing loss. Diagnosis is based on the clinical, laboratory and imaging findings.

There are no definitive treatment guidelines and treatment consists of corticosteroids, intravenous immunoglobulin, immunosuppressive treatment and anticoagulant therapy.

Methods-results: A 38 year old male HIV (+) with a high CD4 count and undetectable viral load without receiving antiretroviral therapy (ART) presented with gradual hearing loss and mild cognitive impairment. He was investigated for HIV related encephalopathy/dementia and was put on ART. Cerebrospinal fluid analysis was normal. Cerebrospinal fluid analysis was negative for CMV, EBV, JC and herpes viruses. Magnetic resonance imaging revealed multiple small white matter lesions in the corpus callosum and in the periventricular and supraventricular white matter. Audiometry confirmed severe hearing loss.

Four weeks later his cognitive function was further impaired. He presented ataxia and visual impairment. Fundoscopy revealed retinal vasculitis. Laboratory investigation failed to suggest any alternative diagnosis, thus the diagnosis of Susac syndrome was made. The patient was managed with iv immunoglobulin and pulse methyprednisolone. Within four weeks, neurological focal symptoms, cognitive deficits and psychiatric disturbances disappeared. There was minimal improvement of hearing loss and an improvement of visual loss. The result of re-evaluation at two months was indicative of no further disease activity, however hearing and visual mild improvement was considered residual damage. Treatment was subsequently stopped trying to safeguard immune status and avoid unnecessary toxicity bearing in mind the HIV status of the patient.

Conclusions: To the best of our knowledge this is the first report of Susac syndrome in an HIV patient. Our patient was of good immune status before he presented with Susac syndrome. Prompt diagnosis and treatment are essential in reducing neurological and sensory sequelae. Susac syndrome should be included in the differential diagnosis of unexplained encephalopathies, even in the case of HIV infected patients.

HIV/AIDS

Susac's syndrome in a HIV-infected patient

Newly diagnosed HIV-infected patients in Romania – late presentation

V. Arama, R. Mihalescu*, A. Streinu-Cercel, A. Hristea, A.M. Tudor, S. Parascusi, D. Oteleu, M. Radulescu, D. Munteanu, L. Benea, C. Tiliscan, S.S. Arama (Bucharest, RO)

Objectives: First presentation suggests the burden that is to be carried by the patients and health systems. We evaluated the clinical, immunological and virological features of Romanian newly-diagnosed HIV-infected patients and short-term course of the disease.

Methods: Prospective Romanian grant (CNCSIS no. 848/2006) on newly-diagnosed HIV-infected patients in a tertiary care hospital, over a period of 3 years. Clinical, immunological and virological screening was performed every 3 months. Chi-square tests and multiple logistic regression were used when analyzing the data by means of SPSS software.

Results: We included 105 newly-diagnosed HIV-infected subjects, 53.3% men, with overall average age of 30 years, who were followed-up a mean period of 18 months. Clinical CDC stage was A, B, C in 15%, 49%, 45% of cases, respectively. At baseline, we found CMV replicative disease in 19% of subjects, tuberculosis-14.2%, hepatitis B-12.3% (including B-D 2%), hepatitis C-2%, Pneumocystis carinii pneumonia PCP-7%, cerebral Toxoplasmosis-1%, syphilis-1%; also 3 lymphomas, 1 Kaposi sarcoma, 2 progressive multifocal leukoencephalopathies. Among the 6 deaths, 4 occurred in the first 6 months from diagnosis in patients aged 28−47 years (leukoencephalopathy, lymphoma and 2 Kaposi sarcoma). The 6 deaths were subjected to a shan procedure. Endothelial function and inflammatory markers were assessed prior to, 8 and 48 hours post vaccination.

Results: Endothelial function, as assessed by FMD values, deteriorated following vaccination (baseline: 6.2 ± 1.1%, 8 hours: 2.0 ± 1.3%, 48 hours: 1.5 ± 1.4%, P < 0.05). White blood cell count increased at 8 hours and remained elevated at 48 hours (baseline: 7060 (6385, 7385) cells/μL, 8 hours: 7650 (7275, 8925) cells/μL, 48 hours: 7615 (6653, 8216) cells/μL, P = 0.01), sICAM-1 levels decreased (baseline: 520 (143 mg/mL, 8 hours: 388 (35 mg/mL, 48 hours: 333 (28 mg/mL, P < 0.001) and there was a trend for increase in CRP levels (baseline: 1.32 (0.55, 3.65) mg/dL, 8 hours: 1.79 (0.54, 4.22) mg/dL, 48 hours: 2.53 (0.85, 5.13) mg/dL, P = 0.057). IL-6 and ADMA levels did not change. Conversely, the sham procedure did not induce significant changes in endothelial function or inflammatory markers, apart from a fall in white blood cell count at 48 hours.

Conclusions: Vaccination against the influenza A/H1N1 virus induces endothelial dysfunction in HIV infected patients and this effect is sustained for at least 48 hours. While our study provides a new model for inflammation-induced endothelial dysfunction, our findings may have additional important implications in view of the high cardiovascular risk that HIV infection implies and should be weighed against the immunological protection conferred by the novel vaccine.

Adverse effect of influenza A/H1N1 vaccination on endothelial function of HIV infected patients

H. Sambatakou* (Athens, GR)

Background: Vaccination against the influenza A/H1N1 subtype had been proposed as a mandatory precaution measure for HIV infected patients during the 2009 pandemic. The immediate cardiovascular effects of the novel vaccine have been largely unexplored. We investigated the impact of vaccination on indices of endothelial function in a cohort of HIV infected patients.

Methods: Twenty-four HIV infected patients were studied (mean age 37 years, all male) in a randomized, sham procedure-controlled design. A monovalent, adjuvanted vaccine against influenza A/H1N1 was used for patients in the vaccine arm (n=16); the control group (n=8) was subjected to a sham procedure. Endothelial function and inflammatory markers were assessed prior to, 8 and 48 hours post vaccination.

Results: Endothelial function, as assessed by FMD values, deteriorated following vaccination (baseline: 6.2 ± 1.1%, 8 hours: 2.0 ± 1.3%, 48 hours: 1.5 ± 1.4%, P < 0.05). White blood cell count increased at 8 hours and remained elevated at 48 hours (baseline: 7060 (6385, 7385) cells/μL, 8 hours: 7650 (7275, 8925) cells/μL, 48 hours: 7615 (6653, 8216) cells/μL, P = 0.01), sICAM-1 levels decreased (baseline: 520 (143 mg/mL, 8 hours: 388 (35 mg/mL, 48 hours: 333 (28 mg/mL, P < 0.001) and there was a trend for increase in CRP levels (baseline: 1.32 (0.55, 3.65) mg/dL, 8 hours: 1.79 (0.54, 4.22) mg/dL, 48 hours: 2.53 (0.85, 5.13) mg/dL, P = 0.057). IL-6 and ADMA levels did not change. Conversely, the sham procedure did not induce significant changes in endothelial function or inflammatory markers, apart from a fall in white blood cell count at 48 hours.

Conclusions: Vaccination against the influenza A/H1N1 virus induces endothelial dysfunction in HIV infected patients and this effect is sustained for at least 48 hours. While our study provides a new model for inflammation-induced endothelial dysfunction, our findings may have additional important implications in view of the high cardiovascular risk that HIV infection implies and should be weighed against the immunological protection conferred by the novel vaccine.

Diagnostic criteria of IRIS

The presence of AIDS with a low pretreatment CD4 count (often less than 100 cells/mm3).

A positive virological and immunological response to HAART therapy (defined as a greater than 1 log reduction in viral load or a two to fourfold or greater rise in CD4 count within eight weeks).

A temporal association between HAART initiation and the clinical features of illness.

The absence of evidence of drug-resistant infection, bacterial super infection, drug allergy or other adverse drug reactions, patient noncompliance, or reduced drug levels due to drug-drug interactions or malabsorption after appropriate evaluation for the clinical presentation.
reached normal range after 21 months of follow-up. HIV viral load was >100,000 copies/ml in 35.2% of the cases at enrollment and remained so in less than 3% after 3 years. The reasons of HIV tests were: non-specific symptoms or opportunistic infections in 73% of cases, HIV-infected partner-14.5% and screening tests-12.5%.

Conclusions: In our study, more than two thirds of HIV-infected patients were late presenters, despite their young age, letting their disease progress and disseminating HIV for years. National programme for serological screening should be implemented in order to discover HIV seropositives before the disease has advanced.

**High prevalence and incidence of oncogenic human papillomavirus infection in a cohort of African HIV-positive women in Belgium**

D. Konopnicki*, Y. Manigart, C. Gilles, J. de Marchin, M. Delforge, F. Foss, P. Barlow, S. De Wit, N. Clummeck (Brussels, BE)

Objectives: HPV may induce different diseases according to its genotype: high risk (HR) genotypes are associated with cervical cancer (CC) and low-risk (LR) with benign condyloma. Worldwide, 12% of women with normal cervical cytology are infected with HR HPV but HIV positive women have higher rates: in a meta-analysis, prevalence of infection by both LR and HR HPV was 31% in USA, 32% in Europe and 57% in Africa. This leads more frequently to cervical precancerous lesions (CPL) and CC than in HIV negative women. Most studies performed in women with HIV have reported HPV rates mixing both LR and HR HPV genotypes and very few have published incidence rates. We aimed to determine prevalence and incidence of HR HPV infection in a cohort of HIV positive women in Belgium.

Methods: A prospective program of screening and follow-up of HR HPV infection is systematically offered to all women followed for HIV-1 infection in our AIDS reference clinic since 2002. Women are seen by a dedicated gynaecologist within the AIDS reference centre. At each visit, Pap smear and HPV screen are performed by HC2 High-Risk HPV DNA Test (Digene®, USA). A prospective cohort database collects all results including surrogate markers of HIV infection.

Results: We enrolled 808 women in the HPV screening program: 142 were excluded because of previous CPL or CC and 74 because of non oncologic hysterectomy leaving 592 for analysis. 85% of women were from sub-Saharan Africa and 93% acquired HIV heterosexually. The median number of HPV tests was 2 per woman (IQ10–90:1–4) with a median interval of 15.5 months. At time of first screen, prevalence of HR HPV infection was 41% (median age 38 years, median CD4 429/μL, 78% of women on highly active antiretroviral therapy (HAART) for a median time of 25 months). The prevalence according to age is 67% for women <25 years (y), 58% (25–34 y), 31% (35–44 y), 27% (45–54 y) and 29% after 55 y. Among the 151 patients with a first negative screening followed by at least another test, 51 women presented a new HPV detection during 355 patients-years follow up leading to an incidence of 14.7 per 100 patient-years.

Conclusion: We found a prevalence of high-risk oncogenic HPV of 41% and a yearly incidence of 15% in an HIV Belgian cohort mainly composed of African women on HAART. These high rates should be taken into account when designing screening and vaccination programs for cervical cancer.

**Characteristics of women with a new HIV diagnosis in Italy, 1985–2009**

L. Camoni*, V. Regine, M.C. Salia, M. Raimondo, S. Boros, B. Saligoi and HIV Surveillance referees

Objective: To assess the incidence of new HIV diagnoses among women over time and the characteristics of women with a newly diagnosed HIV infection.

Method: Descriptive analysis of new diagnoses of HIV infection among Italian women reported between 1985 and 2009 using regional surveillance data from 15 Italian regions and provinces (which account for approximately for 72% of the Italian population).

Results: Between 1985 and 2009, 45,201 new HIV diagnoses were reported. Among these, 13,062 (28.9%) were women. Among women, the median age at diagnosis progressively increased from 24 years in 1985 to 35 years in 2009. Overall almost three-fourth of women accounted for 44.3% of the reported female cases. The proportion of new HIV diagnoses among non-national women increased from 28.5% in 1992 (first year of available data for non nationals) to 51.0% in 2009. Overall almost three-fourth of women acquired HIV infection through unprotected sexual contacts. The proportion of women that acquired HIV infection through unprotected sexual contacts increased from 0.7% in 1985 to 87.7% in 2008. The incidence of new HIV diagnoses among women (from 4.7 per 100,000 resident women in 1985 to 3.4 per 100,000 resident women in 2009) as well as among men. The male to female incidence ratio (inc. M/inc. F) showed a decrease over time: from 3.4 in 1985 to 2.1 in 1999, increasing subsequently and reaching 3.2 in 2009 (Figure).

During 2009, 5 cases of mother to child transmission were reported: 1/3 of HIV-infected mothers were non-national. These data underline the need to encourage the adoption of safe sexual behavior among women as well as the implementation of HIV testing, especially among pregnant women.
Prevalence and persistence of anal infection by oncogenic human papillomavirus virus and co-infection with Chlamydia trachomatis among HIV-infected homosexual males

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Objectives: The incidence of anal cancer among HIV-infected men who have sex with men (MSM) is very high. The main risk factor associated are the promiscuity, the tobacco, the immunoospression and HPV infection. On the other hand, it has been reported the relation between the HPV-C. trachomatis co-infection and progression to cervical cancer. Our objective was to establish the prevalence and persistence of anal infection by oncogenic HPV and the co-infection with C. trachomatis among HIV-infected MSM.

Methods: We studied the presence of HPV and C. trachomatis infections in samples of anal canal among a cohort of 62 HIV-infected MSM. For HPV infections the samples were collected in ThinPrep Preservcyt medium (Roche Diagnostics). We used the AMPLICOR HPV Test (Roche Diagnostics) for genotype. For C. trachomatis infection we used the CT/NG Amplification test and the Linear Array HPV Genotyping test (Roche Diagnostics) for genotype. The patients were reclaimed for a second determinations at six month.

Results: The median age was 40 years (range 21–78). The median of CD4+ was 560/mcL (range 310–1230) and the percentage of patients >20 patients), 51 (17 patients), 45 (14 patients) and 18 (13 patients). In 29 patients a second determination was available: there were 2 patients (7%) that were HPV negative at first and second determination; 25 (86%) persisted HPV infected; and 2 patients (7%) HPV-positive initially were HPV negative six month later. Six patients (10.3%) harboured HPV-C. trachomatis co-infection. Of them, 2 persisted positive at six month, 2 reverted to negative and two patients were missed on study.

Conclusions: Prevalence of anal infection by oncogenic HPV genotypes among HIV-positive MSM was very high and an elevated frequency of HPV mixed infections (more than one genotype) was found. Moreover, in this preliminary analysis a significative number of anal co-infection of HPV and C. trachomatis was detected, but more extensive studies should be needed to elucidate their prognostic relevance.
**P2189** Inflammatory markers and metabolic syndrome in HIV-positive adults undergoing highly active anti-retroviral therapy

D. Munteanu*, Y. Arama, R. Mihaiescu, A. Stremin Cercei, S.S. Arama, I. Olaru, A. Hristea, C. Popescu, R. Moroiti, D. Ion, C. Tiliscan, L. Benea, M. Lazar (Bucharest, RO)

Background: HIV infection induce a chronic inflammatory status that is frequently associated with metabolic syndrome. Although highly active antiretroviral therapy reduces viral immune activation, inflammatory markers can remain elevated in HIV positive patients, increasing the cardiovascular disease risk. Our aim is to evaluate the inflammatory markers in HIV positive patients undergoing HAART and to assess the correlation with metabolic syndrome and cardiovascular risk.

Methods: Prevalence of sFas and sFasL with the metabolic syndrome, but not with a high cardiovascular risk was not correlated with the presence of inflammation. Values of MPC1 (p=0.026) in uni- and multivariate analysis. High cardiovascular risk more than 10% according to Framingham score. The presence of inflammatory markers showed elevated values of TNF (42%), high sensitive PCR (36%), IL6 (12%) and MPC1 (6%). The prevalence of metabolic syndrome in HIV patients with herpes simplex type 2 (HSV-2) and human herpesvirus type 8 (HHV-8) infection.

Results: Up to date we included 106 patients with median age of 31 years; 59% men; 75% multixperienced, 80% receiving a protease inhibitor (PI) for a minimum of 6 months; median CD4 cell count 454/mm3; and HIV viremia undetectable in 70% of cases. The inflammatory markers showed elevated values of TNF (42%), high sensitive PCR (36%), IL6 (12%) and MPC1 (6%). The prevalence of metabolic syndrome was 14.3% and 20% of patients had a cardiovascular risk more than 10% according to Framingham score. The presence of metabolic syndrome was significantly correlated with increased values of MPC1 (p = 0.026) in univariate analysis. High cardiovascular risk was not correlated with the presence of inflammation.

Conclusion: Despite antiretroviral therapy, we observed the presence of chronic inflammation in up to 42% of patients and this was correlated with the metabolic syndrome, but not with a high cardiovascular disease risk.

**P2190** Impaired antibody response to pandemic H1N1 influenza vaccination in HIV-positive patients

C. Noah*, G. Mohrmann, G. Beckmann, H. Suhly, H.J. Stellbrink (Hamburg, DE)

Objectives: A new strain of the H1N1 Influenza A virus emerged in North America in April 2009 and spread around the world. Components of the seasonal H1N1 influenza vaccine of 2009/2010 did not protect against the pandemic variant, thus vaccination specific for the pandemic (H1N1) influenza was recommended. Monitoring of as yet vaccinated individuals showed no excess of serious adverse events. Vaccine induced H1N1-specific antibodies in healthy persons are expected to protect against infection. In contrast, little is known about the vaccine's immunogenicity in HIV-positive patients.

Individuals and Methods: Forty seven HIV-positive patients and 71 healthy individuals who received a single vaccination against pandemic influenza (adjuvanted Pandemrix® vaccine) between Oct. and Dec. 2009 were included. Serum was available from HIV patients before vaccination and pandemic outbreak and from patients and healthy controls at least 4 weeks following vaccination. The Pandemic New Influenza A IgG ELISA (Genzyme Virotech) was used to detect specific antibodies and the magnitude of antibody response. The Virotech Unit (VU=OD sample/OD cut-off X 10) is regarded as a measure of antibody content of the sample, with a VU > 11 indicating a positive response.

Results: Twelve of the 47 HIV patients but only 2 of the healthy individuals (25.5% vs. 2.8% respectively) did not show sufficient antibody response after vaccination (p = 0.0003; OD 11.829; 95%CI 2.5–55.8). Responses 4 weeks following vaccination were significantly higher among healthy individuals (16.8±4.2 VU) as compared to HIV patients (13.8±5.3 VU) (p = 0.0004; 95%CI 1.4–4.5). Antibodies were detected in 8 HIV patients prior to the pandemic outbreak, with a significantly lower mean VU (13.45±2.3 VU) than that of vaccinated healthy individuals (p < 0.0001). There was no correlation of antibody responses with current or nadir CD4 cell count, total IgG or plasma HIV RNA (p < 0.089; p > 0.5).

Conclusion: Regardless of antiretroviral therapy, HIV-infected patients showed a weaker antibody response to a single adjuvanted pandemic H1N1 vaccination, indicating limited immunogenicity and protection of the vaccine. The mechanisms of the impaired antibody responses, as well as alternative vaccines or vaccination strategies require further investigation. Some HIV-infected subjects apparently had cross-reactive antibodies prior to the epidemic, indicating previous exposure to related viral antigens.

**P2191** Differences in serum levels of soluble Fas and FasL in patients with human immunodeficiency virus, human herpesvirus type 8 and herpes simplex virus type 2 infections

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Objectives: Fas mediated apoptosis is considered to be an important T cells depletion mechanism in HIV-infection. Soluble Fas (sFas) prevents apoptosis by inhibiting Fas binding with Fas ligand or soluble FasL (sFasL). We investigated possible differences in sFas and sFasL serum levels in HIV patients with herpes simplex type 2 (HSV-2) and human herpesvirus type 8 (HHV-8) confection.

Methods: Levels of sFas and sFasL were measured by EIA in 136 HIV patients sera divided according to HHV-8 and HSV-2 antibody status. Four HIV patients groups were studied: 1) without HHV-8 and HSV-2 (n = 48), 2) with HHV-8 and HSV-2 (n = 21), 3) with only HSV-2 (n = 61), 4) with only HHV-8 (n = 6). HHV-8 negative persons (n = 20) served as controls. In statistical analyses we used the Kruskal-Wallis and Mann-Whitney-U tests. The Bonferroni correction was applied for pairwise comparisons.

Results: The median concentrations of sFas and sFasL were: 589.3 pg/ml and 47.8 pg/ml in HIV patients without HHV-8 and HSV-2; 982.3 pg/ml and 72.2 pg/ml in HIV patients with HHV-8 and HSV-2; 717.6 pg/ml and 40.9 pg/ml in HIV patients with only HSV-2; 1010.6 pg/ml and 70.9 pg/ml in HIV patients with only HHV-8; 678.9 pg/ml and 61.4 pg/ml in HHV-8 negative controls; 695.2 pg/ml and 59.9 pg/ml in HHV-8 negative controls with HSV-2. Higher sFas and sFasL levels were found in HIV patients with HHV-8 and HSV-2 compared to HIV patients without HHV-8 and HSV-2 (Ab (P < 0.001; P = 0.001, respectively) and also compared to HIV patients with only HSV-2 Ab (P = 0.034; P < 0.001, respectively). Higher sFas was also found in HIV patients with HHV-8 and HSV-2 Ab compared to HIV-negative controls without HHV-8 and HSV-2 Ab (P = 0.046). HIV patients with only HHV-8 Ab had higher sFas than HIV patients without HHV-8 and HSV-2 Ab (P = 0.006). Lower sFasL levels were found in HIV patients with only HSV-2 Ab than in HIV-negative controls with and without HSV-2 Ab (P < 0.001; P < 0.001, respectively).

Conclusion: Our results suggest that HHV-8 and HSV-2 infections may mediate apoptosis signalling molecules sFas and sFasL. The increased sFas concentrations found in HIV patients with positive HHV-8 and HSV-2 Ab suggest suppression of Fas mediated apoptosis. Our findings need to be confirmed by additional in vitro data and further studies are necessary to define the clinical significance of these findings.
Disclosure of HIV serostatus and quality of life among people living with HIV/AIDS in Thailand

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Background: HIV disclosure increases social support and avoid the enhanced risk of transmission among sexual partners due to concealment. However, it can impact the quality of life (QOL) of HIV-infected patients.

Objectives: To determine the rate of HIV disclosure and examine the association between the HIV disclosure and QOL among HIV infected patients in Thailand.

Methods: A cross-sectional study was conducted at the outpatient departments of 16 community hospitals in Thailand during April 1–30, 2007. Sociodemographic characteristics, illness history, current health status, HIV disclosure, and duration from knowing HIV infection to disclosure were obtained by questionnaire interview. QOL was assessed by participants' rating score using visual analogue scale.

Results: There were totally 1,279 HIV-infected participants with the mean age of 36.4 years and 53.9% female. We found that 82.5% and 70.7% of participants disclosed their HIV serostatus to family members and neighbors. For 796 married participants, 84.2% disclosed the serostatus to their spouse. Among 580 clients who had coworkers, 46.9% disclosed the serostatus to their colleagues. The median duration from knowing HIV infection to disclosure was 1 month. The mean QOL scores among participants who did not disclose the serostatus to family member, spouse, coworker, and neighbor were 82.0, 83.4, 85.5, and 83.0 respectively compared to 78.9, 79.9, 79.2, and 78.9 among participants who disclosed. Multivariate analysis showed that participants who disclosed their serostatus to all family members or coworkers had significantly lower mean QOL scores than participants who did not.

Conclusion: HIV disclosure can have negative impact on QOL and should be integrated into the overall counselling and psychosocial support of people living with HIV. Comprehensive community education for social support of the infected patients should also be implemented.

Evaluation of experienced patients treated with raltegravir

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Purpose of the study: In the past few years several new antiretroviral drugs have been used for the treatment of HIV infected patients, particularly in those with severe adverse events or on salvage regimens. Evaluating drug performance in clinical settings is of the utmost importance for the improvement of patient care. The aim of this study was to identify the main reasons for raltegravir (RAL) prescription in a cohort of heavily pre-treated HIV-infected patients followed in our centre and to evaluate the safety, efficacy and tolerability of RAL-containing regimens.

Methods: We performed a retrospective analysis of all patient files, between 1st January 2008 and 31st June 2010, which included raltegravir in their therapeutic regimen. Demographic, epidemiologic and clinical parameters were recorded and analyzed.

Summary of the results: 56 patients were on RAL-containing regimens; 5 patients were excluded due to insufficient data. The average time since prescription of Raltegravir was 14 months (2–26 months). Of the 51 patients analysed, 41 were male (80.4%), 46 were HIV-1 and 5 were HIV-2 (9.8%) infected patients; 39 (76.5%) were born in Portugal, and 12 (23.5%) were from other countries. The mean patient age was 51 years (35–77 years). On average, HIV-infection had been diagnosed 15 years earlier, and most of the patients were heavily pre-treated, with a median of 5 previous treatment regimens (1 to 14). At the time RAL was prescribed, patients had a mean 332 (4–1147) and a median of 289 TCD4+/ cells/μL. Main reasons behind RAL prescription were a failing regimen in 41 patients (80.4%), a switch from enfuvirtide in 5 patients (15.7%), or due to adverse events to the previous regimen in 2 patients (3.9%). The new regimen was generally well tolerated. Most (94.1%) of the patients are still on the same regimen, 2 (3.9%) discontinued due to regimen failure, and 1 due to regimen-related toxicity. On those kept on a RAL-containing regimen, there was a mean increase of 62.7 TCD4 cells/μL at 24 weeks (in those who were on the RAL-containing regimen for less than 24 weeks, the last absolute T CD4 cell count was considered).

Conclusions: The main reason behind RAL prescription in this population was failure to ongoing treatment. There were no significant adverse events associated with RAL-containing regimens, which were generally well tolerated. A trend towards an increase in T CD4 cell count was observed, both in HIV-1 and HIV-2 infected patients.

Underreporting and non-adherence to post-exposure prophylaxis among healthcare workers in Iranian university hospitals

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Objectives: To determine the rate of non-adherence to post-exposure guidelines in occupationally exposed healthcare workers (HCWs) in Iran.

Methods: Questionnaire study performed on a randomly selected sample of HCWs (residents, interns, nurses, and nurse aides) in the teaching hospitals of Shahid Beheshti University of Medical Sciences (Tehran, Iran) during the years 2006–2007.

Results: Among 650 participant, 347 (53.4%) reported at least one NSI, with 181 (27.8) reporting splash exposures. Of the 347 HCW with NSI or splash exposure, 143 took no further action, 135 cared for themselves, and 69 reported their exposures. 80.1% of persons (278 of 347) did not report exposures. Of the known total 75 HIV, HBV, and HCV exposures, 40% adequately followed the guidelines. Those who presented to physicians were no more likely to follow guidelines than those who self-treated (p=1.0). There were no cases of seroconversion among these 75 known exposures. The total rate of non-adherence (number of underreported and noncompliant with recommendations) in this study was 88.2%.

Conclusion: This study highlights the problem of occupational exposure to bloodborne pathogens in HCWs in the developing world. Although none of the people in our study knowingly contracted an occupational illness, the sheer number of under-reported exposures and inadequate post-exposure care demonstrate the continuing risk of working in a health care setting in developing countries. Continuing educational efforts directed at HCWs and those who care for them are needed to address this problem.

Factors influencing the duration of illness in pandemic H1N1/2009

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In order to devise adequate public health plan for mitigating impacts of the pandemic influenza, there is need to predict the potential longevity of the disease. The auxiliary police (AP) are conscripted policemen of Korea, healthy young men in their early 20s and living in barracks but have frequent contact with general population unlike military personnel. Therefore, they are vulnerable in the epidemic of infectious diseases. During the pandemic of 2009 H1N1 all of them with influenza like illness (ILI) around Seoul were admitted in our hospital for isolation and treatment.

We performed real time RT-PCR (rRT-PCR), rapid influenza antigen test (RIAT, BinaxNOW®), complete blood cell count, C-reactive protein (CRP), chest X-ray (CXR) and got the history of symptoms and vital signs of all admitted patients. From 10th August 2009 to 14th January 2010 (prior to vaccination), 744 patients were presented with ILI among total 23,000 AP in the target area. 317 were confirmed 2009 H1N1 by rRT-PCR. The estimated incidence rate of confirmed 2009 H1N1 in AP was 596.6 cases per 100,000 person-years. The median age of patients was 21
Secondary bacterial infections in patients with seasonal influenza A and pandemic H1N1

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Objectives: To analyse and compare the secondary bacterial infections related to seasonal influenza A and pandemic H1N1.

Methods: The detection of influenza virus was made from nasopharyngeal aspirate or nasopharyngeal swab samples by immunofluorescence (IF) and in some cases also in cell culture for seasonal influenza A and by rRT-PCR for pandemic H1N1. Detection of bacteria from sputum, bronchoalveolar lavage and blood samples were made by conventional methods.

Results: A total of 352 patients with seasonal influenza A (between 2005–2008) and 742 patients with pandemic H1N1 (2009) were included in the study. All patients were cross examined in the clinical microbiology laboratory database of Karolinska University Hospital in Huddinge for the presence of positive bacteria cultures from lower airways or blood. Positive bacterial cultures had underlying diseases. The bacterial profiles of the different groups are shown in the table below.

| Bacterial Species       | Seasonal Influenza A | Pandemic Influenza H1N1 |
|------------------------|----------------------|------------------------|
| *Streptococcus pneumonia* | 10/352 (2.8%)        | 1/742 (0.1%)           |
| *Staphylococcus aureus* | 5/352 (1.4%)         | 1/742 (0.1%)           |
| *Other bacteria*        | 29/352 (8.3%)        | 73/742 (9.8%)          |

Conclusion: The results indicate that secondary bacterial infections are more common in patients with seasonal influenza A than in patients with pandemic H1N1. In both groups most of the patients with positive bacterial cultures had underlying diseases. The bacterial profiles of the positive cultures were similar in both groups.
because of seasonal influenza had more underlying illness (85% vs 69%, not significant). In both groups, admission at the Intensive Care Unit (ICU) was necessary in approximately 30% of patients. Patients admitted to the ICU with seasonal influenza were younger than those admitted because of pandemic influenza (median age 4.5 vs 14 yrs). Both groups were admitted to the ICU within 2 days after the first day of illness; both groups had equal amount of underlying illness. Duration of hospitalization was longer in patients with pandemic influenza (median 23.5 days vs 9 days). The most striking difference was antiviral treatment with oseltamivir: 10% of the patients with seasonal influenza were treated with oseltamivir, compared to 81% in patients with pandemic influenza. Similarly, patients admitted to the ICU with seasonal influenza were less treated with oseltamivir (12.5% vs 84% in patients with pandemic influenza). Seven patients (8%) died because of infection with Influenza A H1N1v, compared to three (5%) with seasonal influenza. Resistance to oseltamivir was detected in four patients with pandemic influenza, compared to 5 with seasonal influenza. Conclusion: Comparison of seasonal with pandemic influenza in patients hospitalized in our center shows similarities in severity and course of illness. However, antiviral treatment was more frequently started in pandemic influenza as compared to seasonal influenza.

**P2199** Human parechovirus infections in a hospitalised patient population in Northern Italy, 2008–2010
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Objectives: Human parechoviruses (HPeVs) infection are associated with a wide range of clinical syndromes such as respiratory, gastrointestinal and neurologic disease. In particular, HPeV3 have been associated with neonatal sepsis-like illness and neurologic illness. The aim of this study was to investigate the epidemiology and the clinical manifestations of HPeVs infection in a hospitalized patient population.

Methods: Respiratory samples (n=5288) from 3525 patients and cerebrospinal fluid samples (CSF, n=356) from 345 patients collected during a two years period (October 2008–October 2010) were tested retrospectively by HPeV-specific real-time RT-PCR. Phylogenetic analysis of VP3/VP1 region was applied to genotyping the positive samples obtained. Clinical data were retrospectively analyzed to better define the clinical impact of HPeV infection.

Results: Overall, 16/3525 (0.4%) patients had respiratory samples positive for HPeV. Of these, 12 had a single positive sample, while 2 had multiple positive samples. 3/345 (0.9%) patients had positive CSF samples. In 4/19 HPeV-positive patients multiple samples (e.g. stool, plasma, CSF or respiratory samples) were available, and HPeV positivity was showed in all specimens. Eleven patients (57.9%) were infected with HPeV1 strain, 7 (36.8%) with HPeV3 and 1 (5.3%) with HPeV6 strains. Ten of the 16 HPeV patients with positive respiratory samples were found to be co-infected with other respiratory viruses (8 with rhinovirus and 2 with coronavirus OC43). Five of HPeV3 infected patients were less than 1 month of age and HPeV infections was associated with sepsis-like illness.

Conclusion: HPeV does not circulate with high prevalence in hospitalized patient population in northern Italy. HPeV is associated with severe clinical syndromes in newborns. HPeVs should be enclosed in diagnostic panels of virus detection in CSF and respiratory samples.

**P2200** Type-specific human papillomavirus infection in female anal and cervical brushings
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Objectives: Despite the recognition of HPV anal infection as strongly associated to progression to anal carcinoma, epidemiologic data have shown no reduction in the incidence of this cancer so far, probably due to an increase in high-risk sexual behaviours. Recent data showed that in immuno-competent individuals HPV infection occurs in the anal canal even in the absence of anal intercourse and, with a higher frequency, in cervical HPV-positive women. However to date, the prevalence of specific HPV genotype infection in anal infection in relation to genital HPV have been only marginally addressed.

Methods: We here report preliminary results of a study on women attending a colo-proctologic clinic over the period 2006–2010, for screening programs or referred by general physicians. The presence of specific HPV genotypes in anal and cervical brushings was determined by two PCR assays followed by sequencing; this method allows the identification of a wide range of mucosal HPV types.

Results: From a total of 145 anal brushings, HPV genotypes detected in anal samples were: HPV 6 (13 cases), HPV 16 (8 cases), HPV 53, HPV 66 and HPV 84 (3 cases each), HPV 31 (2 cases), and HPVs 11, 58, 61, 62, 74 and 87 (1 case each).

Considering 117 women for whom anal and cervical brushings were concomitantly obtained, HPV was equally common in cervical and in anal samples (25% vs. 24%). Despite the fact that HPV 6 was the most frequent genotype in anal infections, percentage of high-risk HPVs in anal samples were similar to the corresponding cervical samples (43% vs. 40%).

Half patients presenting HPV positive anal samples (13/26) were HPV negative at the cervical sample, and different HPV genotypes occurred in 4/13 (31%) women positive at both anal and cervical samples. These results suggest a different way of acquisition of the infection or, alternatively, anal HPV may be acquired from vaginal/cervical HPV infection but may be more persistent in infection.

Conclusion: Our results support the need of screening programs for HPV anal infection directed not only to cervical HPV-positive women, but also to general population with the aim to further evaluate anal HPV genotypes distribution and to monitor their potential persistence and progression to anal dysplastic lesions.

**P2201** Antiviral effect of siRNA against Langat virus in cell culture and organotypic hippocampal rat brain slices
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Objectives: Tick borne encephalitis virus (TBEV) is the causative agent of tick borne encephalitis (TBE), a potentially fatal neurological infection affecting humans in Europe and Asia. TBEV, which belongs to the mammalian group of tick-borne flaviviruses, is transmitted by the bite of infected ticks and despite the availability of a vaccine, approximately 2000 infections occur every year in Europe. To date there is no effective antiviral therapy for TBE.

Methods: The antiviral effect of RNA mediated interference (RNAi) by small interfering RNA (siRNA) was evaluated in cell culture and organotypic rat hippocampal cultures (OHC). Langat virus (LGT), a naturally attenuated flavivirus strain closely related to TBEV exhibits a low-pathogenicity for humans but retains neurovirulence for rodents. LGT was used herein for the establishment of an in vitro model of TBE. LGT productively infected both, dissociated HeLa cells and 400μm-thick organotypic tissue slices of rat hippocampus cultured on a porous membrane. Once infection was established, we analyzed the efficacy of 19 different siRNA sequences targeting defined regions of the Langat genome to inhibit virus replication in the 2 in vitro systems.

Results: The most efficient suppression of virus replication in both in vitro systems was achieved by siRNA sequences targeting genes within the envelope and the 3′ untranslated region (UTR). When siRNA was administered to HeLa cells before infection with LGT, a 1000-fold reduction of infectious virus particles was observed while delayed treatment after infection decreased the viral replication by 90%. In OHC the replication of LGT was reduced by up to 97% in the pretreatment paradigm compared to OHC transfected with non-targeting siRNA used as controls.

Conclusion: Organotypic rat hippocampal cultures represent a suitable in vitro model to investigate neuronal infection mechanisms and treatment strategies in preserved three-dimensional tissue- and cyto-architecture and cellular composition of the brain. Our results demonstrate that siRNA is an efficient approach to limit LGT virus replication in vitro.
Shellfish were identified as a vector for human enteric pathogen transmission more than 150 years ago, as human pathogens may be accumulated by shellfish, during their filter-feeding activity. Contamination of shellfish-growing waters with human sewage was recognized as a contributing cause of such contaminations. Owing to the high densities of cows bred in some coastal areas the hypothesis of seawater contamination may be raised and thus the potential oyster contamination by bovine pathogens. This is of special interest regarding norovirus (NoV) main cause of acute gastroenteritis in human (genogroups (G)I and II) but also present in cattle (GIII). A study was conducted to evaluate farm and urban activities impact on shellfish contamination in a coastal area devoted both to intensive agriculture and shellfish aquaculture (Northern Brittany, France). Bovine stool collected in this area were found contaminated by GIII NoV (18%). Environmental investigation was conducted over a one year period. River and estuarine waters were found contaminated by both human NoV (7% for NoV GI and 24% for NoV GII) and bovine NoV (14%), whereas NoV GI were detected in 4%, GII in 21% and GIII in 2% of oyster samples. However, more interestingly, when comparing concentration detected in water and oyster samples important differences were noticed with NoV GI being much more efficiently concentrated than GII or GIII.

To explain such variations, we evaluated the binding specificity of these NoV strains to oyster tissues. Clear difference were detected with NoV GI binding via an A-like Ag to digestive tissues, whereas GII 4 binds to digestive tissues, gills and mantle via two ligands. No ligand could be identified for NoV GIII in oyster tissues nor to human, explaining the low frequency of these strains in oyster samples. This suggest also that the NoV GIII are unlikely to infect human.

Results: 367 cases of TBE were reviewed. The patients ranged in age from 14 to 80. The male/female ratio was 196/171. The specific IgM Ab were detected in the blood − 94%, in the CSF − 56% of all cases and in 51% cases Ab were detected in both. Four different clinical forms of illness were observed. The most frequent form was meningoitis and it was determined in 68% of cases, meningoencephalitis in 14% and meningoencephalomyelitis in 8%. The median illness day at admission was 14. Different levels of altered consciousness (scores of GCS ≤12) have been developed in 19% of patients with encephalitis features. Acute CSF samples were obtained at admission and convalescence samples 2 weeks later or at discharge. The mean pleocytosis of CSF at admission was 265 and protein level was 0,66 g/l. There were no differences regarded to clinical forms or altered consciousness. Analyzed neut/ly ratio, we had found that it was higher in cases with focal brain damage features (p = 0,014) and with altered consciousness (p = 0,018). Convalescence CSF findings had not significant differences regarding to clinical forms. 76,6% of all patients had received glucocorticoids. The convalescence CSF findings had not significant differences with patients who had not received them.

Conclusion: CSF findings have not been correlated with severity of tick born encephalitis. Using corticosteroids did not impact CSF sanitation in TBE.

**P2203**

Cerebrospinal fluid findings in patients with different clinical features of tick-borne encephalitis treated with glucocorticoids

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Objectives: Analysed cerebrospinal fluid (CSF) findings in patients with different clinical features of tick born encephalitis (TBE) and compared them in regard to using glucocorticoids.

Methods: 367 medical records of patients, treated at the Infectology Center of Latvia during 2000 to 2004, were analysed. Clinical features of illness, CSF pleocytosis, neutrophils/lymphocytes (neu/ly) ratio and protein level were compared. The case definition was confirmed by detecting specific antibodies (IgM Ab ELISA) in the blood and in the CSF.

Results: All routine virological studies from the two cases were negative. The presence of LCMV-specific IgM and IgG antibodies (indirect fluorescence assay) was observed in the serum samples of both patients. Generic RT-PCR for detection of arenaviruses was negative. The presence of LCMV was confirmed by specific RT-PCR. Viral isolate was only recovered from the first case. A slight cytopathic effect was suspected, although the slow progression of the virus in cell culture made this effect indistinguishable from the effect due to ageing of cell monolayers. The growth of LCMV was confirmed by positive specific RT-PCR in serial 10-fold dilutions from the Vero culture supernatant. Case 1 (female, 24 years old) was detected in 2008, and case 2 (male, 39 years old) in 2010. Both individuals lived in the same neighbourhood of Granada (southern Spain), characterized by its low socioeconomic level.

Conclusion: 1. Serologic tests are especially useful for detecting less frequent viruses, thus they should always be considered good tools in suspected cases of viral meningitis without an etiologic diagnosis. 2. Higher sensitivity is obtained with molecular methods for the recovery of viral isolates. 3. An epidemiological study is being conducted to investigate the presence of reservoirs of the virus and the mechanisms of transmission involved in human LCMV cases.
Sporadic detection in children during a 25-year surveillance in Palermo, Italy, clarifies the zoonotic potential of G6 rotavirus strains

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Objectives: G6 rotavirus is frequently isolated from cattle but has been sporadically detected in humans. Emerging rotaviruses such as G8, G10 and G12 strains, are believed to have originated from animal rotaviruses that were introduced into the human population through interspecies transmission. In order to define the zoonotic capability of G6 rotaviruses we conducted a retrospective study of the G6 strains circulation in children hospitalised with diarrhoea over 25 years.

Methods: 4,934 faecal samples were obtained from children, less than five years, hospitalised for gastroenteritis in Palermo, Italy, at the “G. Di Cristina” paediatric Hospital from 1989 to 2009. The samples were screened for the presence of rotaviruses by EIA and/or RT-PCR and positive specimens were G/P genotyped through multiplex nested RT-PCR. G6 isolates were further characterised by sequence and phylogenetic analysis of the VP6, NSP4 and NSP5 genes.

Results: During the 25-year surveillance only seven strains were characterised as G6. The sequence analysis of the VP7, VP4, VP6, NSP4 and NSP5 genes included the Italian G6 strains in either of two genomic constellations: G6-P[9]-I2-E2-H3 and G6-P[14]-I2-E2-H3. The phylogenetic analysis of the five genome segments revealed genetic diversity at lineage or sub-lineage level for most of the isolates, but some lineage/sublineage combinations were retained over time. Two G6P[9] strains isolated in 2003 shared >99.5% nt identity in all the five segments analysed, which indicates a possible clonal origin. Genome segments of human G6P[14] strains revealed closer relatedness to ovine and antelope rotavirus strains, while the G6P[9] Italian rotaviruses were close to feline or human/feline strains.

Conclusion: The data obtained in this study confirm that G6 rotaviruses can still be considered accidental human pathogens occasionally transmitted from animal sources and some defined genotype combinations may be required for transmission to humans. The finding of two G6 isolates clonally related demonstrates that these strains have the capability for human-to-human transmission. Circulation in children even at low rates may provide a good opportunity for animal and human rotaviruses to reassort and generate novel strains. Continuous surveillance will be paramount to detect the eventual introduction in the human population of unusual strains with high transmissibility among humans that may escape immunity induced by the vaccines.

Human papillomavirus infection prevalence in Spain.

Preliminary results of a screening programme in Castilla y León, Spain

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Objectives: Human papillomavirus (HPV) is the main cause of genital cancer in women. The Castilla y León Autonomic Community (SPAIN) is developing a program of genital cancer prevention in women, which includes screening for HPV and genotype (GT) identification. Here we report HPV infection prevalence and features among >10,000 women involved in this program during 2010.

Methods: The Genital Cancer Prevention Program includes 35−55 years old women attending to our centers. We determined the presence and GTs of HPV in cervical brushing samples obtained specifically for HPV screening, from women attending to centers involved in this program, between January 2010 and November 2010. HPV presence and GTs were determined by PCR and microarray hybridization (Genômica, SPAIN).

Results: We studied 10,442 samples during 11 months. 806 (7.7%) were positive for HPV. Among samples positive for HPV, 593 (73.6%) were positive for one GT, and 213 samples (26.4%) were positive for between 2 and 7 GTs. 160 samples (19.9% of positive samples) were positive for 2 GTs, 29 (3.6%) for 3 GTs, 17 (2.1%) for 4 GTs, and 7 (0.9%) for 5−7 GTs. Among samples positive for 1 GT, 164 (27.7%) were positive for low risk GTs (LRGT), and 429 (72.3%) for high risk GTs (HRGT). Among samples positive for several GT, 110 (51.6%) were positive both for LRGTs and HRGTs, 16 (7.6%) for LRGTs, and 87 (40.8%) only for HRGTs. As whole, the most frequent LRGTs were GTs 61 (7.3%) and 6 (2.6%). GT 11 prevalence was low (0.6%). The most frequent HRGT were GT 31 (11.4%), 16 (11.3%), 53 (9.1%), 51 (8.2%), and 66 (6.7%). GT 18 prevalence was much lower (2.6%).

Conclusions: HPV infection prevalence is similar to prevalences reported previously in other countries. There is a high GTs diversity. GT 31 prevalence is similar to GT 16, and GT 18 frequency is low. Infection by multiple genotypes is frequent, and >90% cases include HRGTs.

Rubella susceptibility among women of childbearing age

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Background: Although rubella is a mild exanthematous disease, an infection during the first trimester of pregnancy can cause the congenital rubella syndrome.

Objective: To estimate the susceptibility to rubella virus infection of women of childbearing age.

Materials and Methods: During a two-year period (from January 2009 to November 2010) 1840 women of childbearing age were tested for rubella virus-specific IgG and IgM antibodies. Of the 1840 women, who attended the department of Obstetrics and Gynecology of “Alexandra” General Hospital of Athens, the 991 were Greek and 849 were immigrants from Africa, the Middle East, and Eastern Europe. Detection of rubella virus IgG and IgM antibodies was carried out by EIA (ABBOT, AxSYM Rubella IgG and IgM Microparticle Enzyme Immunoassay) according to the manufacturer’s instructions.

Results: Rubella virus-specific IgG antibodies were detected in 864 (87.19%) out of 991 Greek women examined, whereas they were not detected in 127 (12.81%) women. Rubella virus IgM were detected in 735 (86.57%) out of 849 immigrant women, while 114 (13.43%) were found negative for rubella virus IgG antibodies. Both groups were negative for rubella virus-specific IgM antibodies.

Conclusions: (a) In spite of the widespread application of MMR (measles, mumps and rubella) vaccination, a significant proportion of Greek women of childbearing age remain susceptible to rubella virus infection. (b) The immigrants present almost the same proportion of susceptibility to rubella virus infection, even though they come from developing countries, where the MMR vaccination is restricted or unavailable. (c) The non-interrupted transmission of rubella virus and the natural rubella immunity may be responsible for high prevalence of rubella virus-specific antibodies in immigrant women of childbearing age.
P2208 Selection of molecules disrupting protein-protein interactions within the vaccinia virus replication complex

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Background: Variola virus (smallpox agent, Poxviridae family, Orthopoxvirus genus) can potentially be used as a biological weapon (Category A pathogenic agent). We are actually searching for new active molecules against this virus.

Vaccinia virus (VV), the prototypic member of the Orthopoxvirus genus, shares around 90% similarity with variola virus; it is admitted that a compound inhibiting VV would also inhibit Variola virus. Proteins involved in poxvirus DNA replication are relevant targets to obtain specific inhibitors of viral multiplication. The VV A20 protein interacts with the essential protein involved in DNA replication: the E9 protein (replicative DNA polymerase), the D4 protein (uracil DNA glycosylase) and the D5 protein (helicase/primase).

Methods: In order to screen for compounds able to specifically interrupt interactions between A20/D4 and/or A20/D5, a yeast two-hybrid high-throughput screen, based on the dual luciferase reporter system, was performed. The interaction between A20 with either D4 or D5 is detected independently by the sequential activity measurement of the fireplace and Renilla luciferase proteins, used as respective reporters for each interaction. Thus, this test allows the selection of molecules able to disrupt one of the two interactions in the same well. About 27000 compounds were screened from commercial chemical libraries.

Results: 33 molecules with good solubility and stability have been identified as potential inhibitors. Among these compounds, we identified two molecules that exhibit antiviral activity in VV or cowpox virus infected Vero cell but not against herpes simplex virus type 1.

Conclusion: Thus, the screening of 27000 compounds from collections of diverse commercial libraries led to the selection of 2 potent inhibitors that display specifically antorthopoxvirus effect in infected cell culture. We are pursuing the characterization of these two compounds by testing their activity against viral genome replication.

P2209 Systematic control of rubella virus infection in pregnancy in the childbearing population in Emilia-Romagna region, Italy

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Objective: Prevention of congenital rubella syndrome is one of the priorities set by the World Health Organization (WHO) Regional Office for Europe. In 1998, the target of one case of Congenital Rubella Syndrome (CRS) per 100,000 live births by 2010 was approved as a goal of immunisation programmes in the European Region. The objective is to evaluate the rubella immunity coverage in Emilia Romagna region and assess valuable criteria to prevent the risk of infection and offer receptive women vaccination after delivery.

Methods: Between January and June 2010 more than 11,000 samples have been received for rubella testing by two large regional laboratories located in Pievesestina di Cesena (FC) and in Bologna Maggiore Hospital. These laboratories manage the workload relative to about half of the population of the Emilia Romagna region. All sera were screened for anti-rubella IgG and IgM with fully automated LIAISON® chemiluminescent immunoassay analyzer (DiaSorin, Saluggia, Italy) in use in both laboratories. All results were reported in International Units for IgG and Arbitrary Units for IgM.

Results: The results have been assessed according to age, sex and country of origin of the subject (the area hosts a huge community of immigrants coming both from Eastern European Union and from Africa and Asia).

A total of 88.9% immune population was found in Italian people while among immigrants the immunity was 83.5%. People of childbearing age were much younger, if immigrants, than the Italians. Quite a relevant percentage of non immune persons were found also in male Italian population (16%) probably due to the fact that only female persons, in the near past, were vaccinated.

Conclusion: The data workflow coming from the Laboratory observatory can be a very useful tool in handling a correct vaccination policy in the population. Women should be aware of the risk connected with the receptive status in pregnancy.

Vaccination should be offered to young persons and a sensitization campaign done in order to avoid the risk of rubella infection in pregnancy.

P2210 Inter-α inhibitor proteins exhibit antiviral activity against influenza virus strains in vitro

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Objectives: Inter-α inhibitor proteins (IaIp) are a complex of endogenous molecules consisting of inter α trypsin inhibitor, pre-α inhibitor and bikunin (urinary trypsin inhibitor). Although it is found in high concentrations in plasma, its role is still not fully understood. Research work in anthrax and sepsis has showed that it may have anti-inflammatory properties. Our hypothesis was that IaIp, as a serine protease, may inhibit viral infection by inactivating furin which is necessary for the cleavage and activation of certain envelope proteins critical in the cellular attachment of dengue and influenza viruses to host cells.

Methods: We infected Madin Darby Canine Kidney (MDCK) cell cultures infected with influenza A/H3N2 and H1N1 virus. IaIp was then added at a dose of 250mcg/ml or 500mcg/ml for 2 days post-inoculation. The protective effect of IaIp was present with both influenza virus strains (H1N1 and H3N2) and its antiviral activity was confirmed when both doses of IaIp (500mcg/ml and 250mcg/ml) were used.

Conclusion: The addition of IaIp had an antiviral effect on MDCK cell cultures infected with influenza virus H1N1 and H3N2. Hence, these antiviral properties of IaIp reveal its promising therapeutic potential in the fight against influenza.

P2211 In vivo protection against Old World arenaviruses using phosphorodiamidate morpholino oligomers with positionally specific positive molecular charges added to monomers to backbone

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Objectives: The Arenaviridae family represent severe threats to human health not only from the possible use as weapons but also from the risks to populations that live in endemic regions. At present, no vaccines or therapeutic agents have been licensed for use against these viruses. We explored the in vivo antiviral efficacy of AVI-7012, a positively charged phosphorodiamidate morpholino oligomer (PMOplus™) designed to target conserved genetic sequences common to both L and S strands of New World and Old World arenaviruses.

Methods: Mice were treated i.p. daily starting either 2 days before inoculation of lymphocytic choriomeningitis virus (LCMV) inoculation with 60ng/mouse of scramble or AVI-7012 PMOplus™ or 2 days post LCMV inoculation and treatment was continued until day 7 post-inoculation. Mice were bled at day 8 post-inoculation and viral load in kidney was quantified by qRT-PCR amplification of viral GPC. Strain 13 guinea pigs were infected with Lassa virus (LASV) and AVI-7012 inoculation and treatment was continued until day 7 post-inoculation.

Conclusion: The data workflow coming from the Laboratory observatory can be a very useful tool in handling a correct vaccination policy in the population. Women should be aware of the risk connected with the receptive status in pregnancy. Vaccination should be offered to young persons and a sensitization campaign done in order to avoid the risk of rubella infection in pregnancy.
was administered by daily i.p. injections at either 15, 30, or 60 mg/kg from the time of inoculation to day 21. Animal survival, weight, and clinical index were recorded daily for 28 days and two animals from each treatment group were sacrificed at day 15 post-inoculation for assessment of viral burden by standard plaque assay.

**Results:** In mice, AVI-7012 reduced viral burden in kidney, brain, spleen and liver by approximately 1 log when administered either prior to or after inoculation with LCMV clone 13. Administration of AVI-7012 at 30 and 60 mg/kg dose levels conferred a high degree of protection in guinea pigs inoculated with LASV, with 92% (11 out of 12 animals, cumulative) surviving infection, compared with 50% survival in saline-treated animals. Moreover, administration of AVI-7012 at all dose levels reduced blood and spleen viral burden and protected against LASV-induced weight loss and clinical signs of disease relative to that observed in saline-treated animals. In a separate evaluation, AVI-7012 conferred no protective advantage against Junin virus (JUNV), a human pathogenic New World arenavirus, in a lethal guinea pig model of infection.

**Conclusions:** Taken together, these results suggest that targeting conserved genetic sequences using PMOplus™ therapeutics may be a promising approach to counter Old World arenaviruses. Further investigations will be required to determine whether this approach can protect against New World arenaviruses besides JUNV.

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**Infection of human intestinal cells and mice with pepper mild mottle virus, a plant virus**

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**Objectives:** Pepper mild mottle virus (PMoV) is a plant RNA virus. Recently, it was detected in fresh and processed peppers for human consumption. Besides, it was identified as the most prevalent RNA virus in human stools by metagenomics and its presence in patients’ stools was significantly associated with a specific immune response and clinical symptoms (fever, abdominal pains and puriritus). Moreover, the virus was viable in food products and stools. These findings question if PMoV may be pathogenic in humans. We studied PMoV infection of human intestinal cells and in an experimental mice model.

**Methods:** In the culture assay, 4.10^5 Caco2 cells (human epithelial colorectal adenocarcinoma cells) were incubated during 12 days with 1010 infectious PMoV recovered by culture on host plants. In the experimental model, mice received orally 10^{11} infectious viruses with/ without pretreatment with capsaicin (the irritant pepper component; 0.2 mg/mouse for 5 days before PMoV ingestion). Four groups of mice included controls (n=9), capsaicin-treated (6), PMoV-treated (10) and capsaicin/PMoV-treated (5). PMoV detection was performed daily in mice stools by in house real-time PCR. At day 7 and 14 after virus ingestion, mice were sacrificed to detect PMoV in organs (liver, spleen, colon, small intestine). Weight of fresh/dried feces collected in colon was measured to assess diarrhea. Viral infectivity was tested by culture on plants. Anti-PMoV antibody testing was performed using an in house immuno-enzymatic assay.

**Results:** In Caco2 cells, PMoV was detected until day 12 after infection in culture medium and in washed cells. In the medium, a 3.5 Log (copies/ml) decrease of the viral titer was observed between Day 0 and 6 (from 11.1 to 7.6 Log), then a 1.4 Log decrease was observed between Day 6 and 12. In washed cells, a 2.2 Log decrease was observed between Day 0 and 6 (from 8.4 to 6.2 Log), then viral titer did not decrease between Day 6 and 12. In the experimental mice model, PMoV was detected in stools between day 1 and 7 after virus ingestion. The viral titer decreased of 5 Log (from 10^{10} to 10^{5} copies/ml). Stools suspension remained infectious up to 2 days post-infection. PMoV was not detected in mice organs. No specific anti-PMoV IgG antibodies could be detected in mice sera with our assay. No diarrhea was observed.

**Conclusion:** These findings confirm that PMoV remains infectious after their transit in the gut. PMoV may infect vertebrate cells.

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**Kaposi sarcoma-associated herpesvirus saliva shedding in children and mothers in Uganda**

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**Objectives:** Kaposi sarcoma-associated herpesvirus (KSHV) seroprevalence rate in Ugandan children is elevated, ranging from 8% to 57%. This suggests that KSHV infection can occur via horizontal nonsexual route through saliva in which viral load is higher than in other body fluids. According to our hypothesis KSHV is transmitted from mother-to-child through infected saliva, applied on child’s skin to relieve itching and scratching following bloodsucking arthropod bite which induces a skin inflammatory/immune response that could facilitate viral replication. We investigated prevalence of viral DNA in mothers’ and children’s saliva and how is spread the use of saliva.

**Methods:** Overall 270 saliva samples on Whatman® grade 1 filter papers were collected in North-East Uganda (Karamoja: rural community, 76 women and 76 children) and in Central Uganda (Kampala and Entebbe: urban community, 55 women and 63 children) to detect the presence of KSHV DNA by Real Time PCR. Moreover, questionnaires directed at 533 children (211 in rural and 322 in urban communities) were administrated at schools and health centres. Yates-corrected chi square test, odds ratios (OR) and confidence intervals (CI) had been used to test differences in KSHV DNA presence between rural and urban groups.

**Results:** KSHV DNA had been detected in 17.9% among rural community (9.2% children, 26.7% mothers) and in 18.6% among urban community (15.1% children, 22.4% mothers) (p<0.05). The frequency of saliva use was 20.4% and 57.1% in rural and urban communities respectively (p<0.001).

**Conclusions:** Molecular data show that the higher viral load was among rural mothers (26.7%), even though in the urban community the use of saliva is more widespread than in rural one (57.1% and 20.4% respectively). So we observe that in urban community could exist more favourable conditions for a mother-to-child KSHV transmission. This trend is confirmed by preliminary molecular data of saliva KSHV DNA detection which reveal an higher positivity rate among children from urban community (15.1%).

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**Prevalence of anti-hepatitis E virus antibodies in HCV chronically infected patients in southeastern France**

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**Objectives:** Hepatitis E virus (HEV) infection was associated with a high mortality rate in patients with liver diseases in developing and developing industrialized countries. Patients chronically-infected with HCV could be therefore at particular risk for severe outcome in case of HEV superinfection. The aim of this study was to determine the prevalence of anti-HEV antibodies and HEV RNA in HCV RNA positive patients.

**Methods:** Serum samples from 96 HCV RNA positive patients collected in our institution in 2005 were tested for anti-HEV IgG and IgM antibodies (Adaltis EIAGen kits) and HEV RNA (in-house real-time PCR assay targeting the open-reading frame-2 region of HEV genome). Detection of total anti-hepatitis A virus (HAV) antibodies (Axsym Abbott) was concurrently performed.

**Results:** Mean age of patients was 48 (range, 21–73), 61 were male. A total of 42 patients (44%) had cirrhosis of the liver. Ten patients (10.4%) were positive for anti-HEV IgG antibodies. Mean test/cut off optical density ratio was 2.4±2.2 (range, 1.14–8.12; ratios >1.1 were classified as seropositive). The mean age of these patients was 41 (24–57). Four of them had cirrhosis. Neither anti-HEV IgM nor HEV RNA were detected in the serum of the 10 patients. No correlation between age or cirrhosis and anti-HEV IgG prevalence was found. 67 of the 96 patients (70%) were positive for anti-HAV total antibodies. Six (9%) of them were positive for anti-HAV IgG. Conversely, six of the ten anti-HEV positive patients were positive for anti-HAV total antibodies. The seroprevalence
for anti-HAV antibodies increased statistically significantly with age (86% vs 59% in patients older or younger than 50, respectively; p < 0.01).

Conclusion: Our data suggest that the majority of HCV chronically-infected patients in southeastern France are HEV seronegative, and thus at risk for severe and fulminant hepatitis E. In comparison, a majority of these patients are HAV seropositive, although vaccination against HAV should be proposed to increase HAV seroprevalence. No relationship between anti-HEV seropositivity and the severity of fibrosis could be noted. No case of concurrent HCV and HEV RNA detection was observed in our population.

Conclusion: Premature stop codon in S gene was observed in all cases with appropriate sequences for analysis. The frequency of mutated sequences to total evaluated sequences were 17/884, 1/2, 4/110 and 1/30 for Iran, Iraq, Turkey and Yemen respectively. In other countries (Pakistan, Afghanistan and UAE), there were no submitted sequences or the submitted sequences were inappropriate for analysis. All submitted sequences were from patients with positive HBsAg by Elisa technique. The population of patients with premature stop codon in S gene included blood donors.

Methods: Submitted HBV sequences to NCBI genome database from Middle East countries (Iran, Iraq, Turkey, Pakistan, Afghanistan, UAE and Yemen) were retrieved. The S gene of HBV submitted sequences were analyzed by Bioedit ver. 7 software to evaluate the genotype and premature stop codon in S gene.

Results: Premature stop codon in S gene was observed in all countries with appropriate sequences for analysis. The frequency of mutated sequences to total evaluated sequences were 17/884, 1/2, 4/110 and 1/30 for Iran, Iraq, Turkey and Yemen respectively. In other countries (Pakistan, Afghanistan and UAE), there were no submitted sequences or the submitted sequences were inappropriate for analysis.

Viral hepatitis

**P2215** Increasing CD4+CD25hiFoxP3+ regulatory T-cells in subjects after repeated booster doses of rabies vaccination

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We have experience with subjects, who have had frequent rabies vaccine boosters, have a decrease in their rabies neutralizing antibody (Nab) response when given booster injections. Accumulating evidence indicates a crucial role of CD4+CD25hiFoxP3+ regulatory T cells (Tregs) in inducing antigen-specific immunological tolerance. We hypothesized that an increased frequency of Tregs could be involved in decreased antibody response in subjects who received frequent booster vaccination. We analyzed peripheral blood lymphocytes from subjects receiving repeated doses of rabies vaccine for Tregs and correlated their levels with rabies Nab. Forty-one subjects aged between 20–55 yrs, who regularly donate serum for human RIG production, have received 2-site intradermal booster doses of rabies vaccine every 3 months with 0.1 mL of PVRV more than 2 years were enrolled. Blood samples were obtained before booster vaccination (day 0) and on day 14 after vaccination for rabies Nab titers and Tregs. To determine Nab titers, we performed rapid fluorescent focus inhibition test for all serum samples individually. We collected peripheral blood mononuclear cells (PBMCs) from ACD whole blood by Ficoll separation then detected CD4+, CD25hi and intracellular FoxP3 level by flow cytometry.

**Results:** Thirty-seven subjects (low-responder group: age range, 20–55 yrs) had a poor or no Nab response after booster vaccination [GMT of Nab titers = 8.36 (1.69–50.0) IU/mL on day 0 and GMT of Nab titers = 9.7 (1.93–42.04) IU/mL on day 14] while only 4 subjects (responder group: age range, 22–55 yrs) have Nab titers increased more than 2 fold [GMT of Nab titers = 3.87 (0.60–14.86) IU/mL on day 0 and GMT of Nab titers = 13.63 (6.25–31.05) IU/mL on day 14]. The frequency of Tregs was increased significantly in low-responder group on 14 days (2.53±1.29% vs. 3.27±1.59%, p < 0.01). Tregs level in responder group did not differ on day 0 and 14 days after booster vaccination (3.21±0.75% vs. 3.61±0.9%, p > 0.05). There were no significantly statistical differences between two groups in terms of age, numbers of previous booster doses, Nab titers and Tregs level on day 0.

**Conclusion:** We demonstrate that the frequency of Tregs increased significantly in subjects who had a poor Nab response after booster vaccination but not in responder group. However, whether the up-regulation of Tregs is involved in induction of immunologic tolerance in subjects who received frequent booster vaccination need further investigation.

**P2216** Premature stop codon in HBV S gene among several patients groups from the Middle East

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**Objectives:** We previously reported premature stop codon in S gene among Iranian patients. This mutation can render HBV undetectable by conventional Elisa methods. In this study we aimed to determine the presence of premature stop codon in S gene from Middle Eastern countries with predominant HBV genotype D.

**Methods:** Submitted HBV sequences to NCBI genome database from Middle East countries (Iran, Iraq, Turkey, Pakistan, Afghanistan, UAE and Yemen) were retrieved. The S gene of HBV submitted sequences were analyzed by Bioedit ver. 7 software to evaluate the genotype and premature stop codon in S gene.

**Results:** Premature stop codon in S gene was observed in all countries with appropriate sequences for analysis. The frequency of mutated sequences to total evaluated sequences were 17/884, 1/2, 4/110 and 1/30 for Iran, Iraq, Turkey and Yemen respectively. In other countries (Pakistan, Afghanistan and UAE), there were no submitted sequences or the submitted sequences were inappropriate for analysis.

**P2217** Molecular epidemiology of HCV in Morocco

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**Objectives:** Hepatitis C is a major public health problem worldwide and is a leading cause of liver disease that may silently progress toward cirrhosis and hepatocellular carcinoma. The estimated worldwide prevalence of HCV infection is 170 million, approximately 3% of the world's population. Genetic heterogeneity of HCV strains have led to a consensus classification of six major genotypes (1–6) and several subtypes whose geographical distributions differ significantly according to transmission modes. HCV genotypes are clinically relevant in terms of diagnostics, impact on natural history of liver disease, treatment regimen required, and response to pegylated interferon treatment.

**Methods:** A large scale serology screening (24 646 individuals) was made by ELISA HCV V4.0. Qualitative HCV RNA was detected in the fully automated Cobas Amplicor/Cobas Ampliprep (Roche Diagnostics), HCV viral load was measured by the Cobas Amplicor/Cobas TaqMan real-time PCR (Roche Diagnostics). HCV genotyping (1473 patients) was tested with Versant LIPA HCV II (Siemens).

**Results:** We have found that HCV prevalence was about 1.1% and increased with age. Genotype 2a/2c identified in total 41.5% was the most prevalent. The genotype 1b identified in 40.33% was the second one distributed. HCV genotypes 3a, 3b, 4b and 5 were identified in 3.45%, 0.95%, 0.61%, 0.07% and 0.07% of cases respectively. Sequencing of HCV NS5B region of 2a/2c and unclassified 2 genotype have permitted us to analyze 16 samples. Surprisingly, 15 samples were assigned to subtype 2a, combined in one sample with 2a/2b subtype. These findings suggest that HCV subtype 2a infects also Moroccan patients as French ones.

**Conclusion:** Preliminary results suggest that intermediate anti-HCV prevalence was found in Moroccan population studied. Molecular data have shown a predominance of genotypes 1 and 2 in Moroccan patients while HCV genotypes 3, 4 and 5 are rarely found. Parenteral transmission of HCV was observed in the population studied. Nosocomial transmission of HCV was important, especially in high risk groups such as hemodialysis patients where genotype 1b seems to be homogenous (75%) and also genotype 4 that we have found in 4 patients at the same dialysis center. HCV genotype 3 seems to be imported from Europe by young Moroccan migrants while HCV genotype 4 was found in patients who have been hospitalized in Saudi Arabia hospitals during their pilgrimage.
Evaluation of a novel quantitative HCV Core Ag immunosassay for the study of chronic hepatitis C

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Objectives: To evaluate the performance of a novel automated chemiluminescence immunosassay (CLIA) for HCV core Ag in comparison to quantitative determination of HCVRNA in patients with chronic hepatitis C (CHC).

Methods: HCVAg was measured in sera of 121 patients with CHC and six healthy individuals by using the new Architect HCVAg CLIA (Abbott), an automated assay with a cut off value of 3.0 fmol/L and a gray zone (GZ) of 3–10 fmol/L. Anti-HCV detection was performed with CLIA (Architect, Abbott) and HCVRNA was quantified by bDNA (Siemens) with detection limits of 615 IU/mL. All HCV genotypes (1–4) and subtypes were detected. Values of HCValphag (+) ranged between 15 and >20,000 fmol/L. Seven patients (6%) had anti-HCV (+), HCVRNA 700–8000 IU/ml and HCValphag in GZ, between 3.65 and 9 fmol/L. One patient (1%) with anti-HCV (+), HCVRNA = 3000 IU/mL, and genotype 3a had HCValphag positive, (0.65 fmol/L). The six healthy individuals had undetectable HCVAg (0.06–2.52 fmol/L), anti-HCV (+) and HCVRNA (-).

Conclusions: The new CLIA HCVAg represents an easy to perform, comparable to HCVRNA determination and useful method for the study of CHC. It shows a high sensitivity (99%), and excellent correlation (rho = 0.943) with viral load quantification by bDNA method. HCVAg CLIA represents also an alternative approach to routine diagnosis of acute hepatitis C, as well as, in cases of CHC of immunocompromised patients (haemodialysis or HIV +) that sometimes show undetectable anti-HCV antibodies.

The prevalence of HCV infection among surgical nurses, midwives, their patients and blood donors: a cross-sectional sero-survey

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Surgical nurses and midwives might be considered at most risk of accidental HCV infection due to their higher exposure to blood. One of the most important risk factors of viral infection among the healthcare workers is the prevalence of infection in the general population, or more specifically, in patients.

Objectives: To compare the prevalence of HCV infection in surgical nurses/midwives and in a control groups: consecutive surgical/gynecological female patients from the same hospitals and female blood donors in a cross-sectional sero-survey and to evaluate the alleged risk factors for acquiring an occupational infection.

Methods: Serum samples from nurses/midwives (study group) and their female patients (first control group) were collected in 16 randomly selected hospitals in West Pomeranian region of Poland, between February 2008 and June 2009, assayed for anti-HCV antibodies using in house real-time PCR and amplification/sequencing assays. Serum samples obtained from blood donors from the Regional Center for Blood Donation served as the second control group.

Results: Of the 590 personnel eligible, 414 (70.2%) consented to participate, all of them were female. Anti-HCV were found in 6 staff members (1.4%; 95% CI 0.7–3.1%). A stepwise multivariate model indicated that for anti-HCV sero-positivity, only the length of employment was associated with an increased odds of being infected (p = 0.006). The prevalence of anti-HCV in 1118 female patients was 0.4% (4/1118; 95% CI 0.1–0.9%), while in 801 female blood donors 0% (0/801; 95% CI 0–1.1%). Comparison of anti-HCV prevalence with blood donors from the region of Western Pomerania and the female patients’ population indicated an increasing trend in this order: blood donors, patients, nurses/midwives.

Conclusions: The prevalence of HCV-antibodies in surgical nurses and midwives was more than three times higher than in the population of their patients which may indicate an important occupational risk. Longer work at a surgical/gynecologic ward may predispose to acquire an occupational infection.

Viral hepatitis B and C among homeless people living in south-eastern France

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Objectives: Homeless people (HP), as a result of their lifestyle and other social factors, are at risk for infectious diseases. Few data about infections with hepatitis B and C viruses (HBV and HCV) in these individuals are available. We studied HBV and HCV prevalence in HP living in a large city of south-eastern France.

Methods: Sera were collected after individual consent from 190 HP living in Marseille during a two-day survey in 2005. HBV and HCV serologies were tested using AxSYM Abbott assays. HBV-DNA and HCV-RNA were tested in HBsAg-positive and HCV seropositive HP, respectively, using in-house PCR amplification/sequencing protocols. The study has been reviewed and accepted by an ethical committee.

Results: Mean age of HP was 41 ± 14 years, 182 were male, and 74 were born in France. Mean homeless life duration was 43 ± 85 months. 17 and 39 HP reported piercing and tattooing, respectively. Excessive alcohol intake was noted in 66% of HP. Only 6 (3.2%) HP reported past or current intravenous drug use, and only 2 (1%) HP were HIV-infected. Mean alanine aminotransferase level (ALT) was 35 ± 44 IU/L (5–470). ALT were >2x the upper normal value (UNV) in 9.8% of HP. HBsAg was positive in 4.2% (n = 8) of HP. HBV genotype could be determined from 4 sera, and was A, D (n = 2), or E. HBV serologies indicating past-HBV infection were observed in 25% of HP. HBV serology indicating vaccine immunization was observed in only 8.9% of HP. A total of 4.7% (n = 9) of HP were HCV-seropositive. HCV viremia was detected in eight cases. One of them was co-infected with HIV. HCV genotype was 1 (n = 4 HP), 3 (n = 3), or 4 (n = 1). HCV infection was significantly more frequent in HP with ALT >2x the UNV (22 vs 2.4%; p < 0.0001). Concurrent HBsAg and anti-HCV detection was not observed in any HP.

Conclusions: The present data underscore that HP are highly exposed to infections with hepatitis B and C viruses. They prompt to extend the use of HBV vaccine and the diagnosis of infections with hepatitis viruses in this special population.
in 61 (7.9%) and 23 (3.0%) pts, respectively. The proportion of HIV-negative pts was statistically significantly greater for HDV seropositive than seronegative pts (48 vs 19%; p < 10^-6). Besides, the proportion of men was statistically greater in HDV seropositive than seronegative pts (82 vs 63%; p = 0.003), and HDV seropositive pts were statistically more frequently in the 30–60 years group of age than HDV seronegative pts (64 vs 41%; p < 10^-3). One primary HDV infection could be documented in 2008. HBV DNA was measured in 679 pts concurrently tested on HDV virologic markers. No statistically significantly difference was observed regarding HBV load in total or IgM anti-HDV/positive and HDV seronegative pts. Overall, HDV RNA was detected by real-time PCR and/or sequencing in serum samples from 24 pts. HDV RNA sequences were available for 19 pts; HDV genotype was I in 18 cases and V in one pt.

Conclusions: HDV infects 3% of HBV chronically-infected pts monitored in public hospitals of Marseille, more predominantly those co-infected with HIV. These data more accurately reflect the clinical impact on the progression of liver disease of hepatitis Delta.

**P2224** Frequency and clinico-laboratory features of mix-infections, caused by CMV and HSV (1/2) in pregnant women with chronic HBV- and HCV-infection in Ukraine

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Objectives: To define frequency of mixt-infections, which caused by Cytomegalovirus (CMV) and Herpes simplex virus (HSV) (1/2) in pregnant woman with chronic HBV- and HCV-infection, to study its clinico-laboratory features.

Methods: Clinical, biochemical, immunochromatographic test, reaction of direct immunofluorescent, ELISA, quantitative polymerase chain reaction (PCR), ultrasonic research.

Results: Between January 2008 and December 2010 a total of 171 pregnant women were evaluated (the 1st group − 84 pregnant woman with chronic HBV-infection, the 2nd group − 37 patients with chronic HCV-infection, control group − 50 women with physiological course of pregnancy without chronic HBV- and HCV-infection). All women in the study were screened for HBsAg, HBeAg, antiHBe, HBCgM, HBCeG, DNA of HBV and RNA of HCV, HSV IgG (1/2), HSV IgG (1/2), CMV IgM, CMV IgG, CMV or HSV (1/2) DNA in blood, mucus of cervical channel, urine and saliva. In 56 (66.7%) pregnant woman of the 1st group were confirmed reactivation of chronic HSV-infection (1/2) (n = 35) and CMV-infection (n = 21), in the 2d group in 23 (62.2%) woman were revealed reactivation of HSV-infection (1/2) (n = 14) and CMV (n = 9) in replication stage. 3 (6%) women in control group had relapse of HSV-infection (1/2), but CMV-infection had not revealed.

Conclusion: Frequency of detection of HSV- (1/2) and CMV-infection in pregnant chronic HBV- and HCV-infection is high (>60%).

Patients with mixt-infection have considerably more frequent specific signs of chronic viral hepatitis (jaundice, hepatosplenomegaly, lesion of hepatic structure on ultrasonic research, increase of markers of cytolytic syndrome), then nonspecific syndromes (intoxication, asthenovegetative, dyspeptic and arthralgic). HSV (1/2) and CMV in replication stage promote more prolonged lesions of liver functions (increase of ALT, AST level and thymol test). Received results of study indicate hepatotropic influence of CMV and HSV.

**P2225** Acute and fulminant hepatitis B in public hospitals of Marseille, southeastern France

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Objectives: Fulminant hepatitis has been shown to occur in about 1% of acute hepatitis B virus (HBV) infections, and its mortality rate is nearly 70%. Specific HBV genotype features have been pointed out in fulminant acute hepatitis B worldwide, but these associations remain controversial. In France, hepatitis B remains a public health concern with an estimated prevalence of HB surface antigen (HBsAg) carriage of 0.68% and a low vaccine coverage rate (around 33–41%). We describe primary HBV infections diagnosed in 2008 in public hospitals of Marseille, the main city of southern France.

Methods: HBV serologic status was assessed using Assym Abbott assays on all sera sent in 2008 to the virology lab of public hospitals of Marseille. Primary HBV infections were diagnosed on the basis of strongly elevated alanine aminotransferase level (ALT) (>20-fold the upper normal value), HBV DNA positivity in serum, and strongly elevated anti-HB core (HBC) IgM antibodies (>200 IU/mL) (Vidas BioMérieux assay). Serum HBV DNA was quantified using the Cobas TaqMan Roche assay. HBsAg encoding gene and the pre-core/core region were amplified/sequenced using in house protocols.

Results: 18223 HBV serologies were performed in 2008 in our lab on sera from 15774 patients. Serology indicating past HBV infection and vaccine immunization were observed in 13.8% and 40.1% of sera tested, respectively. HBsAg was positive in 427 cases (2.3%) and HBsAg-positivity was newly-diagnosed in our lab in 146 cases (0.9%). 4 (2.7%) of the 146 newly-diagnosed HBsAg-positive cases were acute hepatitis B. Three were men; mean age was 38 years. Serum HBV DNA titres ranged between 116-1,272,696 IU/mL. Fatal fulminant hepatitis B occurred in 2 cases, a 60-year old woman and a 38-year-old man. Prothrombin index was <20% at admission. Precoces G1896A HBV precore mutants were detected in both fatal fulminant cases. Mutation G1899A was detected from one fulminant and one non-fulminant case. Nucleotides at positions 1762 and 1764 in the basal core promoter region were wild-type in the four cases. HBV genotypes were D or E. HB e antigen was negative in the two fatal cases, and hepatitis B surface antigen was negative in one fatal observation, despite HBV DNA detection.

Conclusions: Acute hepatitis B remains a cause of death in France. This prompts to extend the use of the HBV vaccine. The substantial prevalence in France of G1896A precore HBV mutants suggests that they may be frequently implicated in acute hepatitis B.

**P2224** Liver damage in pandemic influenza A/H1N1

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Objective: Although liver may not be primary target organ affected with viral infection, it can be collaterally damaged in viral upper respiratory tract infections. In these patients’ hepatic changes is a consequence of immune response to viral antigens with a close topographic association between the presence of viral antigens and the associated inflammatory infiltrates in liver. In novel pandemic influenza A H1N1, it is still unclear how large is liver damage in this new type of respiratory disease. The aim of our study was to investigate collateral damage of the liver during pandemic influenza A H1N1 infection.

Methods: This was a retrospective study conducted at the University hospital for Infectious Diseases Zagreb. We included confirmed cases of pandemic influenza hospitalized during outbreak of influenza A H1N1. Other group of patients were patients with seasonal influenza hospitalized from 2007–2009. Patients younger than 15 years and with previous liver diseases were excluded from the study. Statistical analysis was performed by the Fisher’s exact test and t-test as appropriate.

Results: We examined 97 patients with confirmed pandemic H1N1 influenza and 86 patients with seasonal influenza. The pandemic group consisted of 54 men and 43 women, mean age 46.79 years (range 17–75), and the seasonal group included 52 men and 34 women, mean age 47.06 years (range 15–87). There was no significant difference in age and sex between the two groups. Serum levels of AST were in pandemic group 48.66±61.08, in seasonal 31.91±22.10 (p-value 0.0172); ALT were in pandemic group 20.89±54.16, in seasonal 27.33±22.32 (p-value 0.0035) and GGT were in pandemic group 71.79±130, in seasonal 34.52±32.76 (p-value 0.0115).

Conclusion: Liver damage is not tipically present in seasonal influenza. According to our study serum levels of aspartate aminotransferase, alanine aminotransferase and γ-glutamyl transpeptidase were significantly higher in the pandemic influenza. Most of patients had mild hepatic lesion, representing pattern of hepatocellular injury. Although platelets
and D-dimer values were higher in pandemic influenza, suggesting endothelial damage, the differences weren’t statistically significant. These findings support the concept of the pandemic H1N1 influenza as a new illness with significant immune response to infection leading to hepatocellular injury, different clinical and laboratory features than in previous seasonal epidemics.

Results: In the HAV non-contact group, none of the subjects had anti-HAV antibodies. In the HAV contact group, subjects had both IgM and IgG anti-HAV antibodies, and two had only IgG anti-HAV antibodies. Two of three (or five) HAV-infected subjects were treated for hepatitis A.

Conclusion: The secondary attack rate (infectivity) of HAV was 4.3–7.1% (3–5/70) and the pathogenicity rate was 40.0–66.7% in the group of young adults.

### Table: Presumed Viral Transmission Time

| Presumed Viral Transmission Time | Male | Female |
|---------------------------------|------|--------|
| 1 day                           | 54   | 52     |
| 2 days                          | 34   | 34     |
| Age                             | 46.70±15.69 [37–75] | 47.06±22.55 [15–87] |
| C-reactive protein              | 76.18±79.17 | 57±60.95 |
| WBC (white blood cells)         | 8.14±6.7 | 8.24±4.05 |
| Platelets                       | 254.09±85.54 | 470±67.52 |
| Fibrinogen                      | 6.006±6.13 | 5.31±2.51 |
| D-dimer                         | 0.58±0.59 | 0.58±0.40 |
| Total bilirubin                 | 10.04±8.83 | 12.54±14.72 |
| AST (aspartate aminotransferase) | 48.08±90.01 | 13.1±22.10 |
| ALT (alanine aminotransferase)  | 45.90±24.19 | 27.33±22.32 |
| GGT (γ-glutamyl transpeptidase) | 73.70±13.90 | 34.5±32.76 |
| ALP (alkaline phosphatase)       | 44.22±67.68 | 77.08±53.88 |
| LDH (lactate dehydrogenase)      | 550.82±550.32 | 750.32±339.09 |
| Peak ALT level                  | 7.65±0.14 | 7.75±0.16 |
| Prothrombin time                | 0.76±0.14 | 0.75±0.16 |

### Objectives:

Acute viral hepatitis A infection in adults is an emerging public health problem in Korea. The infectivity and pathogenicity of hepatitis A virus (HAV) in close contact have not been studied previously. This study investigated the secondary attack rate and pathogenicity rate of HAV during an outbreak among auxiliary police in a communal living setting.

Methods: A total of 70 people in close contact with a hepatitis A patient (index case) were enrolled in the study, which included a thorough oral history, physical examination, and laboratory testing. The subjects were part of an auxiliary police unit living in a communal setting (HAV contact group). Specific antibodies in the sera were determined at the time when the last hepatitis patient was discharged from hospital and at the end of the longest incubation periods. Subjects in another auxiliary police unit without exposure to hepatitis A were examined as a control group (HAV non-contact group). The secondary attack rate and pathogenicity rate were calculated from the data.

Results: In this retrospective study, 11 patients with acute viral hepatitis type E were included. To describe the clinical and demographic characteristic, we used information from cases recorded at the Clinic of Infectious, Parasitic and Tropical Diseases, Military Medical Academy – Sofia, between January 2004 to December 2010. The diagnosis of HEV infection was confirmed by the finding of anti-HEV antibodies of class IgG and IgM (ELISA) in acute phase, and by disappearing of IgM anti-HEV in convalescent period. Statistical analysis was performed using the chi-square test.

Objective: The seroprevalence of hepatitis A (HVA) has decreased in recent years in developed countries. The subpopulation of adults less than 40 years has converted to a group susceptible to contagion. Recognising the epidemiologic profile of the infected population in our country is of great interest to determine the population in risk, that will benefit of a program to vaccinate for hepatitis A.

Methods: We analyzed retrospectively the cases diagnosed with acute hepatitis A in the Microbiological department of ‘Hospital Universitario Insular de Gran Canaria’ in the period from July 2007 to November 2010. The microbiological diagnosis of HVA was realised using a qualitative detection of IgM anti-VH virus antibodies using enzyme immunoassay (AXSYM) or using chemiluminescent microparticle immunoassay (ARCHITECT, Abbott Laboratories) in human serum. In the patients with suspected clinic we completed serologic research with markers of hepatitis B virus, hepatitis C virus (HCV) and Human Immunodeficiency Virus (HIV) with the techniques previously described. Using a screening for syphilis RPR, completing the positive results of the FTA-ABS test (bioMérieux) and line immunoassay LIA (Innogenetics). The positive results for HCV and HIV were confirmed using line immunoassay LIA (Innogenetics).

Results: We diagnosed 53 cases of acute hepatitis A, whose distribution for gender was approximately 5:1, with a predominance for male (45 cases). The age range was from 18 to 80 years, with the most cases between 20–40 years (75.47%). The coinfection with other hepatotropic viruses, HIV or syphilis was not significant. The detected cases in the laboratory were communicated to Epidemiology and Prevention Section.
Hepatitis E virus in Italy: molecular analysis of travel-related cases

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Human hepatitis E virus (HEV) causes acute liver disease, and is a major public health concern in non-industrialized countries where it causes large outbreaks, significant morbidity and deaths. In industrialized countries, the disease is non-endemic, usually confined to travelers returning from endemic areas. Nevertheless, a growing number of sporadic cases have been identified, involving also patients with no travel history. The aim of the present study was to contribute to the body of knowledge available on the molecular epidemiology of acute hepatitis E in Italy. Three sets of HEV-specific primers targeting the ORF1 and ORF2 regions were used to examine serum samples collected from 43 acute hepatitis patients positive for anti-HEV IgG and/or IgM, between 2007 and 2010. The following information was collected from the patients’ hospital charts, where available: age, sex, symptoms and diagnosis on admission, exposure history (food, water, travel, activities, and recent contact with infected individuals). Seventeen patients (39.5%) resulted HEV RNA-positive: 12 infections, due to genotype 1, were associated to travel to endemic areas (Bangladesh, India and Pakistan), while 5 infections, due to genotype 3, were presumably autochthonous, whose risk factors included exposure to raw seafood, pork liver sausages, and wild boar. Patients with genotype 3 tended to suffer less severe symptoms and none was hospitalized, while patients with genotype 1 were all hospitalized due to severe symptoms (jaundice, vomiting and/or fever), and had ALT levels significantly above the normal. Results from this study confirm that human HEV infection in Italy is due to different genotypes, depending on travel-related or autochthonous origin. Further studies are necessary to gather information on the occurrence and on the genetic diversity of the strains circulating in humans, animals and the environment, in order to gain a better understanding of the epidemiology of HEV, and to plan adequate preventive measures.

Occult hepatitis B infection in couples, candidates for assisted reproductive technology

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Objective: In Tunisia, identification of hepatitis B virus (HBV) infection in infertile couples candidates to Assisted reproductive technology (ART) is based on HBs antigen (HBsAg) detection. However this marker can be absent in case of occult hepatitis. The objective of this study is to document the prevalence rate of occult hepatitis B in 808 infertile couples candidates to ART.

Methods: All sera of the candidates to ART were prospectively screened for HBsAg and anti-HBe antibody (anti-HBc). Negative HBsAg sera were systematically tested for anti-HBs antibody (anti-HBs). Detection of HBV-DNA was performed for “anti-HBs isolated” sera. The presence of HBV genome was tested by a single-step PCR in Pre-S gene and two nested PCR in X and C regions. The sensitivity of the PCR assays was evaluated using samples with known viral load.

Results: Seroprevalence of positive HBsAg was 3.2%, Vaccinal immunity was detected in only 3.3% of HBsAg negative sera. Anti-HBc was detected in 22.3% of candidates and was significantly higher in men (<10–4). Anti-HBc was isolated in 5% of candidates sera. Detection of HBV-DNA was performed in 77 cases (with anti-HBs lower than 5mUI/ml) and was positive in two cases (2.5%) by the three PCR assays. Sensitivity of HBV-DNA detection was 10^3 copies/ml.

Conclusion: Occult hepatitis B can be misdiagnosed by selective HBsAg screening in ART candidates. This can lead to a lack of appropriate prophylaxis in couple and in newborns. Anti-HBc antibody should be tested routinely before ART procedures especially in a country of intermediate endemicity for HBV infection.

Thyroid dysfunction related to pegylated interferon-α and ribavirin treatment for HCV hepatitis

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Pegylated interferon-α (IFN) is well known to cause both hyper- and hypothyroidism.

Objective: To evaluate the prevalence and predictive factors for the development of thyroid dysfunction in patients treated with IFN and ribavirin for HCV hepatitis.

Methods: Prospective cohort study in patients treated with IFN for HCV hepatitis. All patients were monitored every three months for: thyrotropin (TSH), free triiodothyronine (FT3), anti thyroperoxidase antibodies (ATPO) and anti thyroglobulin antibodies until the completion of treatment and then at 3 and 6 months during the follow-up period. The patients with previous positive ATPO antibodies were monitored for TSH, monthly.

Results: 83 patients have met the inclusion criteria. 19 patients developed thyroid dysfunction (22.89%), 8 patients developed hyperthyroidism and 11 developed hypothyroidism. The mean age was similar in both groups of patients (with vs. without thyroid disorders: 43.35 years vs 48.12 years). Sex ratio for patients with thyroid disorders was F:M ~ 5:1. 1 (0.66; 17.12). ATPO antibodies were present before the initiation of antiviral treatment in 8 patients (9.63%) and 7 of them developed hypothyroidism (p = 0.00003, RR = 5.47 (3.06; 9.78)). All the patients with hyperthyroidism had previous negative ATPO antibodies (p = 0.04, RR = 2.40 (1.23; 4.69)); the hypothyroidism seems to be caused by a non-autoimmune destructive thyroiditis. 5 patients with hypothyroidism and 3 patients with hyperthyroidism had severe clinical manifestations. For 2 patients with hypothyroidism the IFN was temporary stopped. 6 patients developed subclinical thyroiditis.

Conclusions: Female gender and positive ATPO antibodies are risk factors for the development of thyroid dysfunction during interferon therapy. TSH level should be monitored monthly especially in patients at risk for thyroiditis Early detection and therapy of thyroiditis are important in order to avoid discontinuation of IFN treatment.

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**Hepatitis A and HIV co-infection**

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**Objectives:** To describe the clinical and epidemiological characteristics of patients diagnosed with acute Hepatitis A and HIV coinfection in the HCU Virgen de la Victoria in Malaga over a period of 5 years.

**Methods:** A retrospective study of cases of Hepatitis A from January 2006 to December 2010. Sex, age, health center of origin, nationality, risk factor for transmission, coinfection with HIV and admission to the hospital were analyzed. The diagnosis was made by detection of IgM anti-HAV and anti-HIV by ELISA (Dia Sorin®) and the statistical analysis was performed using SPSS 15.0.

**Results:** We obtained a total of 419 positive (2.4%) of the 17163 samples studied, with a reference population of 450,000 people; we estimated that it has remained stable throughout the study period. 69% were men with a mean age of 28.9±13.9 years (range 2–84). The patients came from 4 health centers (11.9% of Alhaurin el Grande, 10% Típo Pichón, 10% Cruz Humilladero and 9.7% Torremolinos) varying the distribution depending on the year (Table 1). 92.6% of patients were of Spanish nationality. The main transmission factor risk were: contact with HAV-infected (fecal-oral route) (17.2%), followed by travelling to endemic areas (3.3%), sexual risk (3, 1%) and others (seafood consumption, IVDU, piercing, ...) (2.8%). 24.3% needed to be hospitalized.

It was developed an intervention program on immunization of groups with risk practices given the progressive increase in reported cases until 2009.

**Conclusions:**
- The infection predominates in men with a middle age of 29 years was hospitalized a quarter of them.
- The increase in the number of cases of Hepatitis A until 2009 was followed by a decrease in the last year, since the intervention program was developed.
- In 2006–2009 a progressive increase in the number of cases coinfected with HIV in Torremolinos was observed.

**Table 1**

| Location            | 2006 | 2007 | 2008 | 2009 | 2010 |
|---------------------|------|------|------|------|------|
| Patients            | 89   | 81   | 106  | 146  | 27   |
| Hospital centers    |      |      |      |      |      |
| Alhaurin el Grande  | 45%  | 4%   | 4%   | 4%   | 3%   |
| Típo Pichón         | 10%  | 18%  | 11%  | 8%   | 3%   |
| Cruz Humilladero    | 12%  | 16%  | 8%   | 9%   | 7%   |
| Torremolinos        | 5%   | 6%   | 5%   | 5%   | 7%   |

**Conclusion:** The overall prevalence of sero-positive HBsAg among pregnant women in Tirana, Albania and to identify the target group for perinatal post exposure prophylaxis.

**Methods:** A total of 6000 pregnant women who attended the antenatal clinic at Obstetrics Gynecologic University Hospital, Tirana, Albania from January till September 2010 were included in the study. The age range was 15 till 43 years. Blood samples in gel tubes were drawn, centrifuged and worked with serum. HBsAg test was done using ELISA method with SIRIUS.

**Results:** The overall prevalence of sero-positive HBsAg among pregnant women was 6.8% (410 positive cases out of 6000 tested). According to age group, the number and percentage of HBsAg positive cases were: <20 years, 12 out of 250 tested (4.8%); 21–30 years, 283 out of 3940 tested (7.2%); 31–40 years, 106 out of 1710 tested (6.2%); and >40 years, 9 positive out of 100 cases tested (9%).

**Conclusions:** Even that we cannot generalize these findings to the general population, these are consistent with data shown in other studies, suggesting that Albania falls in the intermediate endemicity WHO category for hepatitis B. Besides routine infant immunization which was introduced in mid 90’ in the country, other preventive measures such as targeted adult vaccination and screening of pregnant women should be considered.
Multicentre trial of the new Siemens HBsAgII assays for qualitative detection of HBs antigen on the ADVIA Centaur systems

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Objectives: The ADVIA Centaur HBsAgII assay is an in vitro sandwich chemiluminometric immunoassay for qualitative detection of hepatitis B surface antigen (HBsAg) in human serum and plasma using the ADVIA Centaur System. The assay may be used in conjunction with other serological and clinical information to diagnose individuals with acute or chronic hepatitis B infection, and as a screen for hepatitis B infection in pregnant women to identify neonates at risk of acquiring hepatitis B during the perinatal period. This study compared assay performance and concordance to established commercial assays.

Methods: Assay sensitivity and specificity were evaluated at two independent sites. Two kit lots were used at each site to test a total of 5689 fresh and frozen samples from HBV-infected, blood donor, and hospitalized patient populations. HBV+ samples comprised genotypes A–E, multiple subtypes, and mutations. Twenty seroconversion panels (202 individual samples) were evaluated between both sites and lots, and compared to the Abbott Architect HBsAg assay. Ten additional seroconversion panels were tested by Siemens Healthcare Diagnostics and compared to supplier data for Abbott AxSYM and PRISM systems. For all studies, ADVIA Centaur results ⩾1.00 index are positive; <1.00 index are negative. Samples with discordant results between methods were retested in duplicate and subjected to confirmatory assays on both systems.

Results: For donor and hospitalized patient samples, final ADVIA Centaur results were compared against disease classification according to the Abbott Architect, with retesting and confirmatory testing where needed (only two results were discordant). The assay detected all genotypes, subtypes, and mutations tested. Overall sensitivity was 100%. Specificity was 99.51% upon initial testing, 99.91% after retesting, and 100% after confirmatory testing. Eleven of the 20 seroconversion panels were reactive at the same bleed for the ADVIA Centaur and Architect assays and 9 were reactive one bleed date earlier using the ADVIA Centaur assay. For the remaining 10 panels, there was no difference in reactivity between ADVIA Centaur and PRISM assays for 8/8 panels, while for 5/8 panels, ADVIA Centaur was reactive 1 to 2 bleed dates earlier than the AxSYM.

Conclusions: The ADVIA Centaur HBsAgII assay meets the common technical specifications (CTS) requirements for sensitivity and specificity and is comparable to several commercially available predicate assays.

Assessment of a new Liaison® XL automated immunoassay for the quantitative and highly specific detection of HBsAg in human serum/plasma

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Objective: The aim of the work was to develop and assess a highly specific quantitative immunoassay for HBsAg detection in human serum/plasma, capable of recognition of all mutants, to be used on the new LIAISON® XL analyzer. Quantitative HBsAg determination has been recently suggested as predictor of response to anti-HBV treatment.

Methods: The quantitative detection of Hepatitis B virus surface antigen is a two-step sandwich chemiluminescence immunoassay (CLIA). Comparable sensitivity for detection of different mutants and genotypes is assured by mouse monoclonal antibodies directed to highly conserved epitopes of HBsAg inner region that can detect HBsAg when used in combination with a complex detergent mixture. A mixture of mouse monoclonal antibodies are used for coating magnetic particles. The assay automatically calculates the HBsAg concentration (as IU/mL) and grades the results.

Results: 5,201 serum and plasma specimens collected in two blood banks were run. The assay showed 99.88% diagnostic specificity at screening and 99.98% after retest of initially reactive samples. 911 specimens from hospitalized patients, dialysis patients, pregnant women, high-risk subjects, and another population of 2,000 specimens from a laboratory routine, were also tested obtaining a diagnostic specificity of 99.78% and 100% respectively. The assay showed 100% diagnostic sensitivity by testing a panel of 10 recombinant mutants and 424 specimens from preselected HBsAg positive patients (86 of whom with defined HBsAg subtypes). 32 seroconversion panels were also evaluated in comparison CE-marked HBsAg assays. The results show that the LIAISON® XL Murex assay detected HBsAg one bleed earlier in five out of 32 panels and one bleed later in two out of 32 panels. Both assays exhibited equivalent HBsAg detection in 25 out of 32 panels.

Conclusions: The LIAISON® XL Murex HBsAg Quant assay shows high analytical performance in the quantitative determination of HBsAg in serum/plasma specimens. The test has to be performed on the LIAISON® XL analyzers only. Additional investigations with clinical samples should support algorithm definition with this quantitative application.
**The serological profile of the samples with HBsAg concentration below detection limit of best currently available EIA kits**

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**Objectives:** The aim of the study was to characterize the serological profile of the samples tested as HBsAg negative by the best available tests with sensitivity 0.05–0.1 IU/ml (Second International Standard for HBsAg subtype adw2, genotype A, NIBSC code number: 00/588). All these samples were HBsAg-positive by the EIA with sensitivity 0.01 IU/ml DS-EIA-HBsAg-0.01.

**Methods:** Different samples cohorts (n = 307) tested as HBsAg negative by the currently available assays with sensitivity 0.05–0.01 IU/ml were analysed. The presence of HBsAg in these samples was estimated by DS-EIA-HBsAg-0.01 and confirmed by neutralization. All specimens containing HBsAg were characterized for HBV-specific serological markers.

**Results:** Among anti-HIV positive samples (n = 172) 14 were detected as HBsAg positive by the EIA kit with sensitivity 0.01 IU/ml. Out of 14 specimens 12 contained HBV DNA. 1 HBV DNA-negative sample showed presence of anti-HBc, anti-HBC-IgM and anti-HBe, another contained anti-HBC. Out of 72 isolated anti-HBC-positive specimens 12 were HBsAg-reactive in EIA kit with sensitivity 0.01 IU/ml. Out of 12 samples 4 were anti-HBC-IgM positive. Other 63 samples from different cohorts (the primary asymptomatic blood donors (accidental selection), clinical patients, pregnant women, patients infected with various bacteria and viruses) previously detected as HBsAg-negative were positive for HBsAg by DS-EIA-HBsAg-0.01. 21 samples of them were positive for anti-HBc. The serological profile of 7 samples (anti-HBc and anti-HBe positive, anti-HBC-IgM and anti-HBs negative) is compatible with that of an HBV carrier. 2 samples showed the serological profile of acute HBV infection (anti-HBc and anti-HBC-IgM negative, anti-HBe, HBcAg and anti-HBs negative). The serological markers of 1 sample was compatible with that of acute HBV infection with active replication (anti-HBc, anti-HBC-IgM and HBcAg positive, anti-HBe and anti-HBs negative). 2 samples showed the serological profile of an aggravation of a chronic HBV infection (anti-HBc, anti-HBC-IgM and anti-HBe positive, HBcAg and anti-HBs negative). 9 samples from 31 tested for HBV DNA with HBsAg concentration below 0.05 IU/ml were HBV DNA positive.

**Conclusion:** The enhancement of sensitivity of the currently available kits for HBsAg detection will allow to reveal HBsAg more effectively in samples which tested negative now, will improve quality of blood donor screening and reduce the risk of posttransfusion hepatitis B infection.

**Infection in cancer patients**

P22320 Human rhinovirus-C: a frequently detected species in adult haematopoietic stem cell transplant recipients with lower respiratory tract infection

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A previously unidentified species of rhinovirus, HRV-C, was detected in 2006, in addition to the two known species A and B. While HRV-C is associated with lower respiratory tract infection (LRTI) and wheezing in childhood, there are no reports of HRV-C infection have been reported in adults, and none associated with LRTI.

**Objectives:** To describe the clinical features of HRV-A, B and C in adult haematopoietic stem cell transplant (HSCT) recipients.

**Methods:** A prospective cohort study of 193 adult HSCT recipients (141 (73.5%) allogeneic, 52 (27%) autologous) admitted to Westmead Hospital, Australia, over 27 months (July 1 2005 to September 30 2007) was undertaken. Respiratory samples (596 nose/throat swabs, 40 bronchoalveolar lavage samples, two sputa) were tested for HRV and 15 other respiratory viruses (RV) by polymerase chain reaction followed by HRV genotyping. Viral culture and immunofluorescence for influenza A&B, parainfluenza 1–3, adenovirus, respiratory syncytial virus and human metapneumovirus were also performed.

**Results:** HRV was the virus most frequently detected in 32/435 (7.4%) and 4/205 (2.0%) samples from symptomatic and asymptomatic patients respectively, with 36 positive results from 23 clinical episodes. Sixteen of 22 HRV viruses (73%) that were positive by PCR were speciated. HRV-C was most frequent (nine, 56%), followed by A (five episodes) and B (two episodes). All but the two episodes of HRV-B infection were in allogeneic recipients. Pneumonia was diagnosed in 8/16 episodes (C: 5/9, A: 1/5, B: 2/2). Co-infection with another RV was most common with HRV-C (C: 4/9, A: nil, B: 1/2). Polyomavirus K1 and HRV-C were present in 3 episodes; adenovirus and HRV-A were present in one. Wheezing was noted in three episodes of LRTI (2/5 with HRV-C and 1/2 with HRV-B). Two of the patients with wheezing had a history of asthma. Concomitant bacteremia and/or candidaemia occurred in three episodes of HRV-C infection; two of these episodes were fatal. HRV-Cs were detected in 5/9 cases with LRTI, and were the sole pathogen in two.

**Conclusion:** HRV-Cs were detected in adult HSCT recipients with viral respiratory infection including LRTI, with the worst outcomes occurring in the presence of co-pathogens. Further evidence is required to clarify the clinical importance of specifying rhinoviruses in this patient population.

**Influence of A virus H1N1v in allogeneic haematopoietic stem cell transplant recipients: comparison of the antibody response to monovalent influenza A/H1N1 virus vaccine versus the response in natural infection**

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**Background:** Vaccination against influenza is less effective in hematopoietic stem cell transplant (HSCT) recipients than in the immunocompetent population. In addition, little is known about the antibody response in natural infection. The presumed lack of background antibody against influenza A (H1N1v)-virus was an opportunity to compare the antibody response to illness with that observed after vaccination.

**Methods:** This prospective study included 90 allogeneic HSCT recipients aged from 18 to 65 years, either vaccinated with 2, 3-week interval doses of monovalent influenza A(H1N1v) vaccine (Grl), or with PCR positive influenza A(H1N1v) illness (GrII). H1N1v-specific antibody titers were measured by a hemagglutination-inhibition assay, at days 0, 21 and 42 after the first vaccine dose of in Grl patients (pts), and in both frozen pre-pandemic and post-infection serum in GrII.

**Results:** 70 pts were included in Grl and 20 in GrII. In Grl, 59 pts received an adjuvanted vaccine (GrAdj+) and 11 a non-adjuvanted vaccine (GrAdj–). There were no significant differences in characteristics (pts) according to the group (sex, type of conditioning regimen, source of stem cells, type of donor, incidence of acute- or chronic graft-versus-host disease (GVHD)). GrII pts were younger. The time interval between HSCT and vaccination was longer than between HSCT and vaccination. The percentage of seroprotection (1/40) at day 42 after vaccine, or post infection, was similar in the 3 groups: 66%, 50% and 60% in GrAdj+, GrAdj– and GrII respectively (p = 0.59). The ratio of geometric mean titers (RGMT) at day 42/day 0 of vaccination or post infection were 13.6, 3.9 and 9.1 in GrAdj+, GrAdj– and GrII respectively, with a significant difference between GrAdj+ and GrAdj– (0.04). In the multivariate analysis, 3 factors were associated with higher rate of post-infection/second vaccine seroprotection: a longer time interval between HSCT and vaccination/infection, the absence of chronic GVHD and a myeloablative conditioning regimen (p < 0.05). RGMT were correlated with the same factors (p < 0.05).

**Conclusions:** (1) More than half of the HSCT recipients reach a sero-protective hemaglutination titer after natural infection. (2) An equivalent
response is obtained after 2 doses of vaccine. (3) Moreover, adding an adjuvant improves the efficacy of the vaccine.

Development of quinolone-resistant Escherichia coli bacteremia in neutropenic cancer patients receiving quinolone prophylaxis: data from a high-resistance prevalence setting

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Objectives: Quinolone prophylaxis (QP) is commonly used in neutropenic cancer patients (NCPs) to prevent bacteremia. However, this practice has been criticized to increase colonization with quinolone-resistant Escherichia coli (QREC). We recently showed in our institution that the rate of quinolone resistance (QR) in E. coli bacteremia is 58% in NCPs. We set out to evaluate the role of QP on both colonization and infection with QREC in such a high-resistance setting.

Methods: Between November 2009-December 2010 perirectal swab specimens from 53 NCPs with 105 episodes of neutropenia were taken at first admission before QP and then repeated after QP, once weekly, for 3 weeks. All patients received 500mg levofloxacin, irrespective of their colonization status. Quinolone prophylaxis was continued until fever ensued or recovery of neutropenia. MacConkey agar (Oxoid, UK) containing 1 microg/ml ciprofloxacin was used for screening and API 20E (BioMerieux, France) for identification. Quinolone resistance was determined by disk diffusion method according to CLSI criteria. Blood cultures were taken to identify a presumable bacteremia in febrile patients.

Results: Of the 53 patients, 24 (45%) had QREC colonization at first admission before QP. Among patients that were not colonized initially, 16 (55%) developed QREC colonization after prophylaxis. Bacteremia admission before QP. Among 29 patientsthat were not colonized initially, patients.

Bloodculturesweretakentoidentifyapresumablebacteremiainfebrile episodes may indicate suppression of Gram-negatives by QP. However, the fact that all these infections were by QREC and significant increase in colonization by this bacteria after prophylaxis seem to counterbalance this protective effect.

Enterobacter bacteremia in adult patients with solid tumours at a tertiary care cancer centre in Japan

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Objectives: Very few studies have been carried out on the characteristics of Enterobacter bacteremia in adult patients with solid tumours. Based on our experience, we reviewed the characteristics of Enterobacter bacteremia in adult patients with solid tumours as compared with those in other populations reported previously.

Methods: We conducted the hospital records of adult patients with solid tumours whose blood cultures yielded Enterobacter species at the Shizuoka Cancer Centre (569-beded tertiary care cancer centre in Japan) from September 1, 2002, through December 31, 2009.

Results: Seventy-six positive blood cultures were documented in 71 patients (Enterobacter aerogenes 25, E. cloacae 47, E. sakazakii 1, Enterobacter sp. 3). Fifty episodes were associated with hepatobiliary problems (related to biliary drainage in 22, hepatic metastasis in 14, pancreatic tumour in 13, and cholangiocarcinoma in 12 episodes). Among the 58 episodes for which the sites of infection could be documented, the most common site was intra-abdominal (29 episodes [cholangitis 19, hepatic abscess 6, abdominal abscess 4]). E. aerogenes was associated more frequently with intra-abdominal infection than E. cloacae (E. aerogenes 15 out of 25 [60.0%] vs. E. cloacae 13 out of 47 [27.7%], odds ratio, 3.92; 95% confidence interval, 1.4–10.9; P < 0.01).

Isolates of Enterobacter species from 18 of the episodes were resistant to β-lactams and/or quinolones. The 18 episodes included 16 of the 51 (31.4%) for which antimicrobials had been administered within the previous 4 weeks and 2 of the 25 (8.0%) for which no antimicrobials had been administered within the previous 4 weeks (odds ratio, 5.26; 95% confidence interval, 1.1–25.0; P = 0.02). Twenty-one out of 71 patients (29.6%) died within 30 days (21 out of 76 episodes [27.6%]).

Conclusion: Enterobacter bacteremia in adult patients with solid tumours may be associated with problems in the hepatobiliary system. Careful consideration is needed when selecting antimicrobials for empiric therapy of Enterobacter bacteremia, because of the high frequency of drug resistance, especially in cases with recent exposure to antimicrobials.

Healthcare-associated infection in haematopoietic stem cell transplantation patients during a ten-year period (2001–2010)

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Objectives: Infection remains a major cause of morbidity and mortality in hematopoietic stem cell transplantation (HSCT) patients.

We conducted a 10 years (2001–2009) retrospective cohort study of neutropenic patients submitted to HSCT at a reference Center in São Paulo/Brazil. A database was built using Epiinfo program, variables with P < 0.01 were selected to multivariate analysis, Kaplan-Meier Survival was performed to evaluate impact of infection on outcome.

Results: Over the 120-month period there were 476 neutropenic episodes among 429 patients, with a total of 6,816 neutropenic-days. Of these patients 358 (75.2%) had undergone allogenic HSCT. BSI (blood stream infection) was the most frequent infection presented in 86 (18%) followed by pneumonia in 55 (12%) of the cases. Invasive aspergilosis was possible in 30 (6%), probable in 12 (3%) and definite in 6 (1%) of cases. Most bacteremia were due to Gram-negative bacteria: 45 (52.3%), while 36 (41.9%) were caused by Gram-positive bacteria, and 5 (5.81%) by fungal species. Among Gram-negative bacteria, Pseudomonas aeruginosa (22.1%) accounted for the majority of cases. Risk factors associated with healthcare-associated infection among HSCT patients by multivariate analysis were prolonged neutropenia, duration of fever (both with p ≤ 0.00001) and allogenic transplant (p = 0.0001). Being submitted to an allogenic transplant, having a microbiological documented infection, invasive aspergilosis disease and acute leukemias were related with death in multivariate analysis. The intravascular catheter-related BSI was associated with decreased survival of patients during hospitalization by Kaplan-Meier Survival (p = 0.01).

Conclusions: The type of the transplant, and the primary diagnoses are able to select a higher risk population, and the microbiological identification is also an alarm signal. These findings may have important clinical implications in decisions relating to treatment. Our results showed that BSIs play an important hole on the post-transplant outcome, and also that healthcare-related infections are closely related with prolonged neutropenia and hospitalization.

18F-fluorodeoxyglucose positron emission tomography/computerised tomography (FDG-PET/CT) scanning in persistent febrile neutropenia: a pilot study

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Objectives: Febrile neutropenia frequently complicates cancer care, with evaluation often failing to identify the cause. It is not known whether FDG-PET CT would be useful in addition to the routine investigations performed for persistent febrile neutropenia. The aim of this study was to evaluate the clinical utility of FDG-PET CT in patients with 5 or more days of persistent fever and neutropenia despite broad spectrum antibiotic therapy.
**Methods:** Adult patients with a diagnosis of an underlying malignancy and with persistently febrile neutropenia (temperature ≥38°C and neutrophil count <500 cells/ml for 5 days) underwent FDG-PET CT as an adjunct to conventional evaluation (including clinical, laboratory, microbiology, molecular and standard imaging tests). FDG-PET CT results were made available to treating physicians and the impact of the scan result on patient care was evaluated adapting previously published criteria.

**Results:** Twenty highly immunocompromised patients (16 with acute leukaemia, 4 with other malignancies) fulfilled eligibility criteria and underwent FDG-PET CT scanning in addition to conventional evaluation. The median neutrophil count on day 5 of the febrile neutropenia episode was 30 cells/ml (range 0–370 cells/ml) and the median neutrophil count on the day of the FDG-PET CT scan was 30 cells/ml (range 0–730 cells/ml). The median duration of the febrile neutropenia episode at the time of FDG-PET CT scan was 7 days (range 5–14 days). Fourteen distinct sites of infection were identified by conventional evaluation and 22 infection sites by FDG-PET CT. Thirteen of 14 (93%) infections diagnosed by conventional evaluation were identified by FDG-PET CT, including all deep tissue infections. Nine additional sites of infection were identified by FDG-PET CT, 8 of which were subsequently confirmed “true positives” by further investigations. The FDG-PET CT scan impacted on patient management for 15 of 20 (75%) patients, prompting surgical review of 3 patients and altering antimicrobial management in 13 patients, including withholding of empiric antifungal agents in 5 patients. No adverse events to FDG-PET CT scanning occurred.

**Conclusion:** This study supports the usefulness of FDG-PET CT scanning in severely immunocompromised patients with 5 or more days of neutropenia and fever. Larger studies evaluating the contribution of this technique for management of febrile neutropenia are indicated.

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**Bacteraemia due to Enterococcus faecium in cancer patients: clinical features, antimicrobial susceptibility, and outcomes**

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**Objectives:** We aimed to ascertain the characteristics, antimicrobial susceptibility, and outcomes of bacteraemia due to *E. faecium* in cancer patients.

**Methods:** All episodes of *E. faecium* bacteraemia prospectively documented in a university cancer center from Jan 2006 to November 2010 were included in the study. Antibiotic susceptibility was studied by a microdilution method (MicroScan®). Results: We documented a total of 907 episodes of bloodstream infection. Among 370 episodes of Gram-positive bacteraemia (41%), 60 episodes (16%) were caused by *E. faecium*. Eighteen patients were male (62%), mean age 57.8 yrs. (23–83 yrs). Underlying diseases were hematological malignancies in 23 patients (79%) and solid tumors in 6. Nineteen episodes (65%) occurred in neutropenic patients. The most common sources of bacteraemia were endogenous/unknown origin in 48% of cases, followed by intravascular catheter (21%) and cholangitis (17%). All patients had previously received two or more antimicrobial agents, specially cephalosporines and carbapenems. All isolates were vancomycin and teicoplanin susceptible. One strain was ampilillin susceptible. All the others strains had high level resistance to ampicillin, with MIC up to 256 mcg/mL. Rates of high level resistance to gentamycin and streptomycin were 28.6% and 96.4%, respectively. Twelve patients (41%) received inadequate empirical antibiotic therapy, with cephalosporines in 17 cases, and carbapenems in 17 cases. Definitive antibiotic therapy consisted in vancomycin in 17 cases (59%), teicoplanin in 7 cases (24%) and daptomycin in 3 cases (10%). Among 4 patients (14%) with persistent bacteraemia, 3 had catheter-related infection and 2 had received inadequate empirical antibiotic therapy. ICU admission was needed in 4 patients. Early mortality (<48 hours) was 14% and overall mortality (<30 days) was 38%.

**Conclusions:** *E. faecium* is a frequent cause of enterococcal bacteraemia in cancer patients, mainly among those with hematological malignancies and neutropenia. In our institution, most *E. faecium* strains are highly resistant to ampicillin but susceptible to glycopeptides. Cancer patients with bacteraemia receive frequently an inadequate empirical therapy and the infection is associated with high mortality rates. Further studies are needed to identify risk factors for *E. faecium* bloodstream infections.

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**The impact of microbiological factors on course and outcome of febrile neutropenia in paediatric cancer patients**

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**Objectives:** Over the past decade, it has become evident that neutropenic cancer patients are not a homogeneous group and practice guidelines may vary on their risk status. Thus, we aimed in this study to evaluate the significance of a positive blood culture in febrile pediatric cancer patients with chemotherapy induced febrile neutropenia, and to study the impact of microbiological factors on clinical course and outcome of these febrile episodes.

**Methods:** This prospective cohort study was carried out on febrile episodes occurring in a large group of pediatric patients with chemotherapy related neutropenia at National Cancer Institute in...
Egypt over a period of one year. The association between underlying malignancies, age, absolute neutrophil count (ANC), clinical foci of infection, microbiological documented infection, blood stream infection (BSI) were analyzed in relation to duration and outcome of episodes.

Results: Among 1229 episodes of fever and neutropenia, bacteraemia was detected in 336 episodes. A lengthy episode was observed in 56 (37%), 90 (62%), 35 (94%) and 160 (18%) of Gram positive BSI, Gram negative BSI, polymicrobial BSI and non-BSI episodes, with mean values of 7.6±4.6, 9.4±5.0, 12.7±5.0, and 6.9±4.1 days, respectively, p-value 0.001. Coagulase negative staphylococci were associated with the least complicated BSIs and Klebsiella spp was associated with the most severe BSIs. A microbiological documented infection (MDI), other than BSI, was significantly associated with a long episode, p-value 0.001. The overall mortality rate was 7.5% (n=55) and was higher among bacteremic (n=37/336) patients than those who were not bacteremic (18/893) (11% versus 2%, p 0.001). In addition, the mortality was significantly higher in Gram-negative and polymicrobial BSI than in patients with Gram positive BSI, p 0.001. Among clinical foci of infection, lower respiratory tract infection was significantly associated with a long episode, p-value 0.001. The most severe BSIs. A microbiological documented infection (MDI), other than BSI, was significantly associated with a long episode, p-value 0.001. The overall mortality rate was 7.5% (n=55) and was higher among bacteremic (n=37/336) patients than those who were not bacteremic (18/893) (11% versus 2%, p 0.001). In addition, the mortality was significantly higher in Gram-negative and polymicrobial BSI than in patients with Gram positive BSI, p 0.001. Among clinical foci of infection, lower respiratory tract infection was significantly associated with a long episode, p-value 0.001. The overall mortality rate was 7.5% (n=55) and was higher among bacteremic (n=37/336) patients than those who were not bacteremic (18/893) (11% versus 2%, p 0.001). In addition, the mortality was significantly higher in Gram-negative and polymicrobial BSI than in patients with Gram positive BSI, p 0.001. Among clinical foci of infection, lower respiratory tract infection was significantly associated with a long episode, p-value 0.001. The overall mortality rate was 7.5% (n=55) and was higher among bacteremic (n=37/336) patients than those who were not bacteremic (18/893) (11% versus 2%, p 0.001). In addition, the mortality was significantly higher in Gram-negative and polymicrobial BSI than in patients with Gram positive BSI, p 0.001. Among clinical foci of infection, lower respiratory tract infection was significantly associated with a long episode, p-value 0.001. The overall mortality rate was 7.5% (n=55) and was higher among bacteremic (n=37/336) patients than those who were not bacteremic (18/893) (11% versus 2%, p 0.001). In addition, the mortality was significantly higher in Gram-negative and polymicrobial BSI than in patients with Gram positive BSI, p 0.001. Among clinical foci of infection, lower respiratory tract infection was significantly associated with a long episode, p-value 0.001. The overall mortality rate was 7.5% (n=55) and was higher among bacteremic (n=37/336) patients than those who were not bacteremic (18/893) (11% versus 2%, p 0.001). In addition, the mortality was significantly higher in Gram-negative and polymicrobial BSI than in patients with Gram positive BSI, p 0.001. Among clinical foci of infection, lower respiratory tract infection was significantly associated with a long episode, p-value 0.001. The overall mortality rate was 7.5% (n=55) and was higher among bacteremic (n=37/336) patients than those who were not bacteremic (18/893) (11% versus 2%, p 0.001). In addition, the mortality was significantly higher in Gram-negative and polymicrobial BSI than in patients with Gram positive BSI, p 0.001. Among clinical foci of infection, lower respiratory tract infection was significantly associated with a long episode, p-value 0.001. The overall mortality rate was 7.5% (n=55) and was higher among bacteremic (n=37/336) patients than those who were not bacteremic (18/893) (11% versus 2%, p 0.001).

Conclusion: Patients’ overall condition (co morbidities and autonomy) and elevated CRP level were associated with an unfavourable clinical outcome after an intravenous catheter-related infection. Co-NS, in this setting, may be responsible for severe sepsis or septic shock.

[21st ECCMID/27th ICC, Posters]

**Outcomes**

**P2248**

Antibacterial prophylaxis for patients receiving high-dose chemotherapy with autologous haematopoietic stem cell transplantation: a randomised-placebo controlled trial on the efficacy and safety of moxifloxacin

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Patients receiving high-dose chemotherapy with autologous peripheral blood stem cell transplantation (PB SCT) are at a high risk of infections, especially bacteraemia. We performed a prospective, double-blind, randomized, placebo-controlled, single-centre pilot study on oral moxifloxacin 400 mg versus placebo for preventing bacteraemia in PB SCT recipients. Patients received moxifloxacin or placebo for the duration of neutropenia or until emergence of fever or other infections necessitating intravenous antibiotic treatment. Of 68 patients included into the trial, two were removed from the trial before taking their first dose. The remaining 66 patients were eligible for evaluation in the intention-to-treat analysis set. Neutropenia with neutrophil counts <500/µl developed in 30 (88.2%) and 21 (65.6%), respectively (P<0.03). Ten (29.4%) and 8 (25%) patients, respectively, were prematurely discontinued from study treatment (NS). Breakthrough bacteraemia occurred in 3 (8.8%) and 9 (28.1%) of the study patients, respectively (P=0.042). The amount of days without fever was 9.5 (95% CI: 8.06–10.94) and 7.69 (95% CI: 6.51–8.85), respectively (P=0.0499). There was no significant increase of adverse events or toxicities in the moxifloxacin group. Moxifloxacin is a well-tolerated and effective drug for preventing bacteraemia and shortening febrile episodes in patients receiving autologous PB SCT.

**Conclusion:**

 Patients’ overall condition (co morbidities and autonomy) and elevated CRP level were associated with an unfavourable clinical outcome after an intravenous catheter-related infection. Co-NS, in this setting, may be responsible for severe sepsis or septic shock.

**P2247**

Outcome after a long-term intravenous catheter-related bacterial infection in oncology: results from a prospective monocentric study

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**Objectives:**

Long term intravenous catheter (LTIVC)-related infections in oncology are responsible for their own morbidity and mortality but no prospective data are available to quantify them.

**Methods:**

We conducted a prospective monocentric observational study and included all patients with solid cancer who experienced a LTIVC-related infection (defined according to the Infectious Diseases Society of America guidelines) between February 1st 2009 and February 1st 2010. Variables of interest were prospectively collected and patients were prospectively followed during 12 weeks. Main endpoints were: prevalence and factors associated with severe sepsis and septic shock, mortality at week 12 and delay or cancellation of antineoplastic chemotherapy.

**Results:**

Forty-two episodes of LTIVC-related infections were included (mean age, 59.7 years (± 13.6)). Mean Charlson comorbidity index (CCI) and Karnofsky index were respectively 62.6 (± 17.6) and 6.8 (± 2.7). All but one patients were carrying a totally implantable access port catheter. Among 48 microorganism, 44% were coagulase negative staphylococci (CoNS) (21/48), most of them being methicillin resistant (62%). Eleven patients (11/42, 26%) exhibited local signs with 5 complications leading to LTIVC removal (tunnel or port pocket infection).

At diagnosis of infection, prevalence of complications were 19% for severe sepsis or septic shock (8/42 patients), 38% for death at week 12 (16/42 patients) and 30% for definitive cancellation of antineoplastic chemotherapy (8/27 patients). Mean delay of antineoplastic chemotherapy was 14 days (± 7.7). Main factor associated with severe sepsis or septic shock was an elevated C-reactive protein (CRP) level; three episodes (3/8, 37.5%) were due to CoNS. Factors associated with death at week 12 were a limited autonomy, an elevated CCI, the metastatic evolution of cancer and an elevated CRP level. Among cases of catheter-related bloodstream infections during wich catheters were removed, sensibility of catheter tip and port cultures were comparable and similarly influenced by antibiotic pretreatment.