The LAMP technology has been used to detect virus, bacteria, fungi and parasites directly from clinical samples and offers an alternative to bacterial cultivation and PCR. In addition to sensitivity, it is cheap in comparison with PCR techniques and allows the detection of microorganisms directly from blood cultures, BAL, blood and other samples. There have been several studies presented at this meeting related to the application of LAMP. In one study, the LAMP technology was used to detect Staphylococcus aureus and methicillin-resistant S. aureus (MRSA) directly from blood cultures, demonstrating that the test is fast, accurate and cost-effective (Yarayatne P, et al; EV0215). A total of 200 blinded blood culture samples that were positive for Gram-positive cocci (80 methicillin-susceptible SA, 40 MRSA, 55 coagulase-negative staphylococci (CoNS), and 25 other organisms (Enterococcus spp. and Streptococcus spp.) identified by routine culture-based methods were tested by LAMP assay. All S. aureus and MRSA positive DNA specimens were detected within 20 minutes of amplification and the total turn-around-time was less than 60 minutes. The CoNS required longer time of amplification for detection. The streptococci were identified as non-staphylococci. The sensitivity and specificity for detection of S. aureus by LAMP were both 100% as compared to culture. Sensitivity and specificity for detection of CoNS were 89.0% and 96.0%, respectively. The positive predictive value (PPV) and the negative predictive value (NPV) for CoNS were 98.0% and 80.0%, respectively. The test performance values for MRSA were 94.7% sensitivity, 97.5% specificity, 94.7% PPV, and 97.5% NPV. The cost per test was around 5 euros and might be considered as a diagnostic option.

Another study analyzed the performance of LAMP for the detection of Helicobacter pylori directly from stools (Yarayatne P, et al; EV0218). One-hundred and fifty nine paediatric (<5-years-old) and 60 adult diarrhoeal stools submitted for routine microbiological testing were tested by LAMP and by real time PCR. Out of 219 specimens tested, 74 were positive and 136 were negative for H. pylori by both methods. There were 9 discrepant specimens that were all negative by LAMP.
but positive by PCR. The test performance characteristics of the LAMP method as compared to the PCR were as follows: sensitivity, 89.1%; specificity, 100%; NPV, 93.7%; and PPV, 100%. The limit of detection for LAMP was 10^5 colony forming units per millilitre (CFU/ml), the turn-around-time was 90 minutes, and the estimated cost per test was less than 5 euros, being a cost-effective, sensitive, specific and fast method for detection of *H. pylori* from stools. In similar studies, the LAMP was used for the detection of *Mycobacterium tuberculosis* in sputum (Law 1, et al; P0076) detecting a bacterial concentration as low as of 10^2 CFU/ml sputum sample in 60 minutes. The test was also used for the detection of *Campylobacter* spp. directly from fecal samples (Florea D, et al; P1009). In a multicenter study, in which a commercialized LAMP test (Orion GenRead Campylobacter) was analyzed, the sensitivity and specificity were 98.02% and 94.4%, respectively and the results were obtained in 50 minutes, demonstrating that this is a rapid and reliable method for the identification of *Campylobacter* species directly from faecal samples and could be an alternative for the conventional phenotypic methods. In other studies the LAMP methodology was used for the detection of virus, fungi, and parasites. In one study (Vergara A; P1901) it was evaluated as a screening tool to detect CMV in 52 critically ill patients in comparison with real-time PCR. The test was performed directly in bronchoalveolar lavage (BAL) samples, boiled BAL samples and after the extraction of DNA. In all cases, the specificity was 100%, and the sensitivities were 58.3%, 66.7%, and 95.8%, respectively.

In another study the LAMP methodology was evaluated for the detection of *Pneumocystis jirovecii* (Alejo I, et al. P0983). A total of 10 BAL samples were analyzed also by real-time PCR and a staining with methenamine silver stain (microscopy) by using different extraction methods. Ten BAL samples positive for *P. jirovecii* by microscopy were processed for DNA extraction by four different methods. The specificity was 100%; however, the test showed a lower sensitivity than the PCR (64% in boiled samples vs 95% by using an automated extraction method). The test was performed in 60 minutes. Finally, a commercialized LAMP test was used for the detection of malaria directly from blood (Deleplanque AS; P0786). Although the test does not differentiate the different *Plasmodium* species, it showed a higher sensitivity than culture and if negative it could be used to exclude malaria.

At present, molecular assays based on the detection of microorganisms directly from clinical samples by real-time PCR have reduced the time to organism identification, contributing to optimize antimicrobial therapy and to decrease mortality rates [3, 4]. Among these, the syndromic microarray-based nucleic acid assays have shown high positive-predictive values for detection of organisms in blood, CSF, stool, and other samples. Several studies presented at the ECCMID have assessed the performance of PCR panels (FilmArray, Biofire, bioMérieux) for the diagnosis of meningitis and gastroenteritis. In one multicenter study (Ottiger C, et al; OS0112) a total of 195 CSF samples (136 negative and 59 positive) were analyzed by using a panel that covers six bacteria, seven viruses, and one yeast. Results were obtained in 3 hours and the sensitivity, specificity, positive and negative predictive values were 100%, 95.8%, 86.3%, and 100%, respectively. In the case of gastroenteritis the system, which covers 22 pathogens, was performed in 190 rectal swab specimens and compared with standard procedures (Chapin K, et al. P0999). Percent agreement for bacterial, viral, *C. difficile* and parasitic targets was 93.8%, 70.3%, 57.8% and 40%, respectively.

Antigen-based detection using rapid immunomagnetic tests (ICT) are attractive alternatives for the detection of microorganisms, carbapenemases and other proteins and toxins, because they are easy to perform, rapid, and cheap [5]. Several commercial ICT strip tests have been developed for these purposes. In one study an ICT was evaluated for the simultaneous detection of both *Giardia intestinalis* and *Cryptosporidium* spp. (Goudal A, et al. P0761) and showed 89.2% and 86.7% sensitivity and 99.3% and 100% specificity for the detection of *G. intestinalis* and *Cryptosporidium* spp., respectively. Another commercialized ICT (Synovasure®) was evaluated in a multicenter study for the detection of alpha-defensin in the diagnosis of prosthetic joint infection. The ICT was an easy to conduct and fast diagnostic tool with a high specificity (from 94% to 100%) and a sensitivity from 48% to 75% (Renz N, et al. P1808). Finally an ICT was analyzed for the simultaneous detection of carbapenemases (OXA-48, KPC, and NDM) from cultured bacteria (Glupczynski Y, et al. P0299). In comparison with PCR, the ICT showed 100% sensitivity, positive and negative predictive values, the results were obtained in 10 minutes, it was very easy to perform and could be a cost-effective alternative to PCR methods.

New technologies based on multiplexed automated digital microscopy (Accelerate Pheno™ system) have been developed for the rapid identification of pathogens (1.5 hour) and antibiotic susceptibility (7 hours) directly from positive blood culture broths [6]. In this meeting, a study (De Angelis G, et al; P0997) evaluated this system in comparison with culture-based methods and found that the overall species identification agreement was 88.4% (23/26). A total of 132 microorganism-antimicrobial combinations were included. Category agreement was 93.2% (123/132) and false susceptibility emerged in 3 cases with the combination *Enterobacteriaceae*piperacillin-tazobactam. The system could be useful in selected patients but at present it is an expensive technology.

In our days, MALDI-TOF (matrix-assisted laser desorption ionization time-of-flight) mass spectrometry is the first-line diagnostic tool in the identification of microorganisms [7]. Future applications of this technique include rapid detection of antimicrobial resistance and bacterial typing. The purpose of a study presented at this meeting was to search, by using MALDI-TOF, for specific biomarker proteins of the ST131 clonal group of multiple drug resistant *E. coli*. They found that the YahO protein with the E34A substitution was a common biomarker protein with prominent ability to discriminate this high-risk bacterial clone responsible for worldwide pandemics (Nakamura A et al; OS 0139).
The high speed and throughput of data generation obtained with the nucleic acid sequencing technologies, “next-generation” sequencing (NGS), constitute a revolution with new diagnostic applications [8]. One study evaluated the diagnostic utility of NGS of circulating cell-free DNA in septic patients (Stevens P, et al; OS0746). Among 254 plasma samples, only 28 (11%) showed a positive blood culture. In contrast, by NGS, 140 samples (55%) showed at least one species (bacteria, viruses and fungi) and time from sample to diagnosis was 24 hours. In the near future, NGS based diagnostics will be a sensitive and specific approach for the diagnosis in septic patients.

**ANTIMICROBIAL RESISTANCE**

Nowadays, antimicrobial resistance is a major concern for human health. The array of resistant organisms is increasing every day and contributes to high rates of mortality and high economic costs [9]. In recent years, the emergence of chromosomal and plasmid-mediated resistance to polymyxins, which are antibiotics of last resort for the treatment of carbapenemase-producing organisms results in a pan-resistant organism that is potentially untreatable. Plasmid-mediated colistin-resistance is due to the mcr-1 gene encoding for a phosphoethanolamine transferase [10]. A study presented at ECCMID (Hadjadj L, et al; OS0871) analyzed 32 mcr-1 strains (25 E. coli and 7 K. pneumoniae) isolated from different geographical origins from animals and humans. Among E. coli, several clones were found, being the most frequent ST4015, ST3997, ST10, ST93, ST48, and ST648. Plasmid carrying mcr-1 gene was detected in 22 E. coli strains (88%) and 2 K. pneumoniae strains (28.6%), and one human isolate carried the mcr-1 gene in the chromosome. Since a wide diversity of insertion sequences allowed this gene to be translocated into the chromosome, the emergence of endemic colistin resistant strains with chromosomic location of mcr-1 gene must be monitored. Another cause of concern is the dissemination of carbapenemases and the description of multiple outbreaks around the world with the persistence of isolates difficult to eradicate.

One study analyzed the dissemination of the carbapenemase NDM-5 by vertical (E. coli ST167) and horizontal (an IncX3 plasmid) transfer during an outbreak. The same plasmid was identified among all isolates (E. coli, Klebsiella spp. and Enterobacter spp.) and simultaneously there was an outbreak of a common E. coli clone. Both vertical and horizontal dissemination were responsible for the persistence of blaNDM-5 in one institution (Feng Y, et al; OS0292). Carbapenemases are also recovered from microorganisms in the environment and can act as reservoirs. One study explored the presence of carbapenemases in a river in Barcelona (Spain). After analyzing 11 sediments and 12 water samples they found different carbapenemases in different Enterobacteriaceae carried in different plasmids. KPC-2 was the most prevalent enzyme, they reported a K. oxytoca isolate coproducing VIM-1 and KPC-2, and the first VIM-1-producing R. ornithinilytica of environmental origin. The STs identified had not been previously identified in isolates from clinical origin (Piedra N, et al; P1091). In another study, during an active surveillance-screening program for detecting extended-spectrum β-lactamase (ESBL)-carriers, an OXA-48+CTX-M-9-like producing Kluvyera ascorbata was detected in 6 unrelated patients. The carbapenemase was carried in a plasmid also found in other species. The authors highlight the threat of further nosocomial OXA-48 dissemination through K. ascorbata either by clonal transmission or by lateral transfer of a plasmid (Hernández Garcia M, et al; OS0289).

Ceftazidime-avibactam (CAZ-AVI) is one of the few new antibiotics with activity against carbapenemase-producing organisms, however, the emergence of resistance during the treatment with this antimicrobial has been described [11]. One study demonstrated that mutations in blaKPC-3 that emerged during CAZ-AVI treatment of carbapenem-resistant K. pneumoniae infections encode novel KPC-3 variants that confer CAZ-AVI resistance, restore carbapenem susceptibility, and function and may be misidentified as ESBL (Nguyen MH, et al; OS0490). Other mechanisms of resistance can also decrease the efficacy of this antimicrobial. In one study, the authors found that AVI is a substrate for the efflux pump mexAB-OprM in P. aeruginosa. The overexpression of this efflux pump increased CAZ-AVI MICs of 5 two-fold dilutions without increasing that of CAZ but reducing the activity of AVI against P. aeruginosa and demonstrating a role of this transporter in AVI efflux (Chalhoub H, et al; EV0469). The emergence of 16S rRNA methyltransferases (16S RMases) is a resistance mechanism that confers high-level resistance (MICs ≥256 mg/L) to all clinically-relevant aminoglycosides in Gram-negative bacteria. One study performed in the United Kingdom (Taylor E, et al; OS0298) identified the prevalence of 16S RMase genes (armA, rmtA-H and npmA) in isolates received at a reference center: 527 (95.8%) A. baumannii and 755 (92.4%) Enterobacteriaceae isolates were positive for 16S RMase genes; armA, rmtB, rmtC, rmtE, rmtF and various two gene combinations were identified. The vast majority (94.5%, 1211/1282) of 16S RMase-positive isolates also produced a carbapenemase.

Another multicenter study performed in Greece, showed that among 300 carbapenemase-producing Enterobacteriaceae, 23 isolates (7.7%) carried the rmtB (n=22) and armA (n=1) genes, and these isolates were resistant to the next generation aminoglycoside plazomicin (Galani I, et al; P0406). This combination of carbapenemase and 16S RMase genes poses a serious threat to the treatment of multidrug-resistant Gram-negative isolates.

Among Gram-positive bacteria, new resistance mechanisms have also been emerged in the last decade. Among 539 linezolid-resistant Enterococcus spp. received in a reference center in Germany, 18 isolates (32% of linezolid-resistant E. faecalis and 1.4% of linezolid-resistant E. faecium) carried the plasmidic and transferable transporter optrA gene, enhancing the risk of dissemination of linezolid resistance (Bender JK, et al; P1382).
NEW ANTIMICROBIAL AGENTS

The development and introduction of new antimicrobials has slowed considerably in the last decade. Since 16 antibiotics were approved by the FDA between 1983 and 1987, only 2 were approved between 2008 and 2012, and a total of 7 new antimicrobials have been approved since the end of 2012 [12,13]. Some of the new antimicrobials developed and/or approved in recent years are summarized in table 1. Among the new antimicrobials, zidebactam is a penicillin-binding protein (PBP) inhibitor showing potent beta-lactam enhancer activity against the PBP2 of *Klebsiella pneumoniae*, but it is not active against metallo-beta-lactamases (Moya B, et al; P1300).

Cefiderocole is a novel siderophore cephalosporin active against multidrug-resistant Gram-negative microorganisms including *P. aeruginosa*, *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* with MIC90 values ranging from 2-4 mg/L (Dobias J, et al; OS0561). Cefiderocole is also active against colistin-resistant, ceftolozane-tazobactam-resistant and ceftazidime-avibactam-resistant isolates (Yamano Y, et al; P1316). Eravacycline is a fluorocycline antibiotic of the tetracycline class with activity against multi-drug-resistant Gram-negative and multi-drug resistant Gram-positive organisms (Morrissey Y, et al; P1260). Omadacycline is a broad spectrum aminomethylcycline of the tetracycline class with similar spectrum to that of eravacycline, with intravenous and oral formulations, developed for the treatment of community-acquired bacterial pneumonia and acute bacterial skin and skin structure infections (Huband M, et al. P1253). Octapeptins are new lipopeptides active against *Helicobacter pylori* infection is difficult to treat due to increased resistance to antibiotics. In order to define the prevalence of antibiotic resistance in *H. pylori* in the world, the authors conducted a systematic search of the literature published in the last ten years (Savoldi A, et al; P0719). A total of 59,478 *H. pylori* isolates were included. The prevalence of resistance (2013-2014) was: metronidazole 54.4%, levofloxacin 25.3%, and clarithromycin 25%, observing an increasing time trend of drug-resistant *H. pylori*. Co-resistance to clarithromycin and metronidazole ranged from 1% in Europe to 30% in Southern Asia, confirming the worrisome tendency towards high level of drug resistance to the first and second line antibiotics used for the treatment of *H. pylori*.

Another concern is the emergence of resistance in microorganisms causing sexually transmitted infections. One study analyzed the prevalence of resistance of *Mycoplasma genitalium* to macrolides and fluoroquinolones in Barcelona. Among a total of 85 *M. genitalium* isolates, macrolide resistance mediating mutations were detected in 35% of the *M. genitalium*-positive episodes whereas 8% carried fluoroquinolone resistance mutations, and 3 cases harbored multi-drug resistance to both classes of antibiotics. Men who had sex with men and previous azithromycin treatment were strongly associated with azithromycin resistance. This alarming rate of resistance makes necessary the implementation of combined diagnostic-resistance detection assays for *M. genitalium* (Fernández Huerta M, et al; P1884).

### Table 1

| Enterobacteriaceae | Other Gram-negative bacilli |
|--------------------|----------------------------|
| **ESBL** | **AmpC** | **KPC** | **OXA-48** | **MBL** | **Pseudomonas aeruginosa** | **Acinetobacter baumannii** |
| Ceftolozane/ tazobactam | + | - | - | - | + | - |
| Cefazidime/ avibactam | ++ | ++ | ++ | + | - | + |
| Aztreonam/ avibactam | ++ | ++ | ++ | + | ++ | + |
| Imipenem/ relebactam | ++ | ++ | ++ | - | - | + |
| Meropenem/ vaborbactam | ++ | ++ | ++ | - | - | + |
| Cefepime/ zidebactam | ++ | ++ | ++ | + | + | - |
| Cefiderocol | ++ | ++ | ++ | ++ | ++ | + |
| Eravacycline | ++ | ++ | ++ | ++ | ++ | + |
| Plazomicin | ++ | ++ | ++ | ++ | ++ | + |
| Omadacycline | + | + | + | + | + | + |
| Octapeptins | + | + | + | + | + | + |
| Novanfyn | - | - | - | - | - | + |
| Murepavadin | - | - | - | - | ++ | - |

*Carbapenemases: KPC, OXA-48, metallo-beta-lactamases (MBL)*

**H. pylori** infection is difficult to treat due to increased resistance to antibiotics. In order to define the prevalence of antibiotic resistance in *H. pylori* in the world, the authors conducted a systematic search of the literature published in the last ten years (Savoldi A, et al; P0719). A total of 59,478 *H. pylori* isolates were included. The prevalence of resistance (2013-2014) was: metronidazole 54.4%, levofloxacin 25.3%, and clarithromycin 25%, observing an increasing time trend of drug-resistant *H. pylori*. Co-resistance to clarithromycin and metronidazole ranged from 1% in Europe to 30% in Southern Asia, confirming the worrisome tendency towards high level of drug resistance to the first and second line antibiotics used for the treatment of *H. pylori*.

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| Aztreonam/ avibactam | ++ | ++ | ++ | + | ++ | + |
| Imipenem/ relebactam | ++ | ++ | ++ | - | - | + |
| Meropenem/ vaborbactam | ++ | ++ | ++ | - | - | + |
| Cefepime/ zidebactam | ++ | ++ | ++ | + | + | - |
| Cefiderocol | ++ | ++ | ++ | ++ | ++ | + |
| Eravacycline | ++ | ++ | ++ | ++ | ++ | + |
| Plazomicin | ++ | ++ | ++ | ++ | ++ | + |
| Omadacycline | + | + | + | + | + | + |
| Octapeptins | + | + | + | + | + | + |
| Novanfyn | - | - | - | - | - | + |
| Murepavadin | - | - | - | - | ++ | - |

*Carbapenemases: KPC, OXA-48, metallo-beta-lactamases (MBL)*
multi-drug resistant and polymyxin-resistant Gram-negative bacteria and less nephrotoxic than polymyxin B (Blaskovich M, et al. EP0398). Novarifin is a novel peptide with rapidly bactericidal activity against both Gram-positive and Gram-negative multi-drug resistant pathogens including MRSA and Acinetobacter baumannii (Katvars L, et al; P1361). Apramycin is a monosubstituted deoxystreptamine, different in chemical structure from other aminoglycosides, with low nephrotoxicity, active against multi-drug resistant Gram-negative organisms and maintaining activity in the presence of aminoglycoside modifying enzymes and rRNA methylation (Hobbe S, et al; EP0403).

New antimicrobials active against Gram-positive microorganisms include the fluoroquinolones delafloxacin and lascufloxacin, the fluoroacetolide solithromycin, and the semi-synthetic pleuromutilin lefamulin for oral and IV use, currently in phase 3 trials for the treatment of community-acquired pneumonia in adults (Paukner S, et al; P1331). We know that it is necessary to maintain strategies for a better use of antimicrobials with the objective of slowing the development of resistance, but meanwhile, it is also necessary continuing to develop new antimicrobial agents at a sufficient rate to keep ahead of the bacteria, and we are in the way.

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