Antibiotic Resistance and Clonal Spread

Oral Poster Abstracts

ISPPD-0323 Antibiotic Resistance and Clonal Spread

EMERGING MULTIDRUG-RESISTANT CLONE OF STREPTOCOCCUS PNEUMONIAE SEROTYPE 24F/24A IN URUGUAY AFTER CONJUGATE VACCINES INTRODUCTION

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Background: Decline of IPD caused by drug-resistant Streptococcus pneumoniae is an expected effect of conjugate vaccine introduction. Historically, drug-resistance has been associated to a limited number of serotypes, most included in vaccine formulas. We evaluated antibiotic-nonsusceptible IPD trends before and after PCV7 and PCV13 implementation in Uruguay. Multidrug-resistant isolates were further analyzed to identify emerging clones.

Methods: Isolates from all IPD cases (2003-2012) were serotyped and tested for 9 antibiotics by disc-diffusion and for 4 by E-test. Predominant profiles were identified and PFGE and multilocus sequence typing (MLST) were used to explore genetic relationships.

Results: We included 1887 cases of IPD in patients <5 years (n=742) and >5 years (n=1145). Rise in the number of isolates with penicillin MIC >0.06 μg/mL occurred during the pre-PCV period (2003-2007) from 17.5% to 41.9% among children <5 years. A decline to 12.9% was observed until 2011. However, by 2012, 40% of IPD isolates had penicillin MIC >0.06 and most were also multidrug-resistant. Antibiotic resistance was lower among IPD patients >5 years old and no significant changes has been observed over the period.

Among multidrug-resistant isolates, one profile resistant to 5 antibiotics, was identified with increasing frequency (n=29), 20 were of serotype 24F and 9 of 24A. Preliminary results showed identical PFGE profiles for the 24F and 24A isolates (n=24), while 2 susceptible 24F isolates showed completely different profiles. Six isolates analyzed by MLST were ST230.

Conclusion: A multidrug-resistant clone, of serotypes 24F/24A has emerged probably as a result of serotype replacement after conjugate vaccines introduction.

No conflict of interest

ISPPD-0070 Antibiotic Resistance and Clonal Spread

IDENTIFICATION OF SINGLE NUCLEOTIDE POLYMORPHISMS ASSOCIATED WITH BETA-LACTAM RESISTANCE WITHIN PNEUMOCOCCAL MOSAIC GENES

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Beta-lactam antibiotics have been used as a treatment for infections caused by Streptococcus pneumoniae. Due to its highly recombinogenic nature, the bacterium can quickly incorporate DNA fragments encompassing altered sites that make the transformed strains beta-lactam resistant. By comparing a number of beta-lactam susceptible and non-susceptible genomes, several studies have identified the mosaic regions encompassing penicillin-binding proteins, which are introduced by recombination as a source of resistance. However, the mosaic window is relatively large and the studies so far have been focused on a few hundred samples. Here, we performed an association study and searched for particular alterations of amino acid sites that match beta-lactam non-susceptibility using 3,033 Thai [1] and 607 USA [2] pneumococcal isolates as a discovery and replicate dataset. The large sample size allows us to narrow the source of beta-lactam non-susceptibility from long recombinant fragments down to more discrete causative sites. With ongoing experimental validation, causal SNPs can be distinguished from hitchhiking sites. The majority of these sites appear to be universal, contributing equally to non-susceptibility in at least two classes of beta-lactam antibiotics. However, some play a larger role in resistance to certain antibiotics than others. All the identified alleles have a highly non-uniform distribution amongst vaccine-controlled and non-vaccine-controlled lineages, which may be clinically important. Identification of single nucleotide changes underlying resistance will be essential for future use of genome sequencing in clinical microbiology.

Reference: 1. Turner, P., et al. PloS one, 2012. 7(5): p. e38271. 2. Croucher, N.J., et al., Nature genetics, 2013.

No conflict of interest
EPIDEMIOLOGY OF SEROTYPE 6C CARRIED BY YOUNG PORTUGUESE CHILDREN BETWEEN 2009 AND 2012

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Background and Aim: Serotype 6C, which is structurally similar to 6A, was first described in 2007. Studies on the epidemiology of this serotype are important to evaluate whether the new 13-valent pneumococcal conjugate vaccine (which targets 6A), impacts on it. We previously described the epidemiology of this serotype in carriage, in the period 1996-2007 in Portugal. The aim of the current study was to update the previous study.

Methods: Nasopharyngeal swabs were obtained from 1,824 children (0-6 years old) in cross-sectional studies conducted between 2009 and 2012. Pneumococci were isolated by routine procedures and serotyped by PCR and/or Quellung reaction. Antibiotyping was performed by disc diffusion or Etest and genotyping was done by MLST.

Results: Pneumococcal carriage remained stable throughout the study (range 60.2%-63.8%). The prevalence of serotype 6C was 8.2%, 17.5%, 7.4% and 8.6% in consecutive years (2009-2012), being higher than previously reported (0.2%-5.8% between 1996-2007). The most common resistance pattern was non-susceptibility to penicillin, and resistance to macrolides, lincosamides, streptogramins and tetracycline, which was found in 3.3% (2009), 17.0% (2010), 28.6% (2011) and 0% (2012) of the isolates. MLST of 42 representative isolates showed that 41 isolates belonged to the clonal complexes (CC) - CC156, CC315 and CC395 - already identified in our previous study. Multiresistance was mainly associated to CC315.

Conclusion: Between 2009-2012, serotype 6C has remained in circulation among young children due to the maintenance of three lineages. Further studies are in progress to evaluate the evolution of this serotype after introduction of PCV13 in Portugal.

No conflict of interest

A DESIGNED SYNTHETIC ANTIMICROBIAL PEPTIDE EXHIBITING POTENT IN VITRO AND IN VIVO ANTIPNEUMOCOCCAL ACTIVITIES

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Background: Streptococcus pneumoniae is a major cause of life-threatening infections. Novel antibiotics with potent antimicrobial activity are highly demanding particularly with the continued expansion of antibiotic-resistant pneumococci.

Methods: Five synthetic antimicrobial peptides (DM1-5) designed via peptide-peptide hybridization strategy were tested for antipneumococcal minimum inhibitory concentration (MIC), pneumocidal kinetics, synergism with peptides and antibiotics, antibacterial spectrum, and cell morphological changes using transmission electron microscopy (TEM). Cell toxicity was determined using hemolytic assay and cell cytotoxicity against NL20 and A549 lung epithelial cell lines. In vivo toxicity was determined in mice and the therapeutic efficacy was evaluated using an in-house lethal mouse pneumococcal infection model.

Results: The five DMs showed potent antipneumococcal MICs irrespective of penicillin (PEN) susceptibility of the isolates (MIC = 7.81- 250 μg/ml), produced antipneumococcal synergism in combination with penicillin, and were broad-spectrum. The peptides showed higher pneumocidal rate than penicillin by 30 – 75% and induced multiple cellular damages leading to pneumococcal cell death. Hemolytic activity was low (HC50 > 250 μg/ml) while IC50 against lung cell lines ranged from 96 - >250 μg/ml. DM3 given at 40 mg/kg protected 50% of the mice from lethal pneumococcal systemic infection inoculated by a penicillin-resistant strain. The survival rates were significantly enhanced to 90% (DM3 20 mg/kg, PEN 10 mg/kg) and 100% (DM3 20 mg/kg, PEN 20 mg/kg) by combining DM3 and penicillin to produce therapeutic synergism.

Conclusion: DM3 represents a potential candidate to be developed as a standalone antimicrobial candidate or in formulation with conventional antibiotics to enhance treatment outcome especially cases involving antibiotic-resistant pneumococci.

No conflict of interest
RISK FACTORS FOR ANTIBIOTIC NON-SUSCEPTIBLE STREPTOCOCCUS PNEUMONIAE (NS-SP) CARRIAGE ISOLATES, MASSACHUSETTS, 2001-2011

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Background: Following introduction of the 7-valent pneumococcal conjugate vaccine, non-susceptible Streptococcus pneumoniae (NS-SP) among carriage isolates initially declined, subsequently NS-SP increased in Massachusetts.

Objective: Examine risk factors for carriage of SP non-susceptible to penicillin, erythromycin or ceftriaxone and describe trends over time.

Methods: Nasopharyngeal swabs were collected from children <7 in 9 Massachusetts communities during 5 fall and winter seasons between 2001 and 2011. SP was isolated and antibiotic susceptibility was performed. Classification of susceptibility used current breakpoints for non-meningeal isolates defined by the Clinical Laboratory Standards Institute. Generalized linear mixed models were constructed to examine trends in NS-SP colonization among all subjects, including carriers of susceptible SP and non-carriers, adjusting for known predictors of NS-SP carriage and clustering by community.

Results: In multivariate models, prevalence of NS-SP increased between 2004 and 2011 for penicillin (OR: 3.34, 95%CI: 1.11, 10.10), erythromycin (OR: 1.46, 95%CI: 1.00, 2.13) and ceftriaxone (OR: 8.48, 95%CI: 2.05, 35.07). Age and child care attendance were significantly associated with penicillin-NS, erythromycin-NS and ceftriaxone-NS SP carriage. Young siblings (1 sibling OR: 1.46, 95%CI: 1.12-1.89; 2+ siblings OR: 1.70, 95%CI: 1.13-2.55) was also associated with erythromycin-NS SP carriage. Carriage of ceftriaxone-NS SP was more common among children with respiratory tract infection at the time of sampling (OR: 1.95, 95%CI: 1.15-3.31).

Conclusion: Common risk factors for penicillin, erythromycin and ceftriaxone non-susceptible SP carriage remain similar to those found prior to 2004. Between 2004 and 2011 we found an increased likelihood of carrying SP that was non-susceptible to each antibiotic studied.

Conflict of interest

ANTIMICROBIAL RESISTANCE OF STREPTOCOCCUS PNEUMONIAE AMONG NAVAJO INVASIVE PNEUMOCOCCAL DISEASE (IPD) CASES: COMPARISON BETWEEN 2007-2009 AND 2011-2012

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Background: Following 7-valent pneumococcal conjugate vaccine (PCV7) introduction in 2000, Navajo children aged <5 years experienced increased rates of antimicrobial resistance (AMR) invasive pneumococcal disease (IPD). This was almost entirely attributable to increased rates of serotype 19A IPD. PCV13, which includes serotype 19A, replaced PCV7 in April 2010.

Methods: Active, laboratory, population-based IPD surveillance was conducted on the Navajo Nation. Invasive isolates were serotyped by Quellung; 6A and 6C were distinguished by PCR. Antimicrobial susceptibility was determined by broth microdilution using Clinical and Laboratory Standards Institute (CLSI) guidelines (2011). We compared incidence of AMR-IPD for the PCV7 era (2007-2009) and early-introduction-PCV13 (2011-2012) era.

Results: AMR testing was conducted on 74 (94%) 2007-2009 and 19 (95%) 2011-2012 isolates from children <5 years. Comparing 2007-2009 to 2011-2012 the IPD rate decreased from 110 to 20 cases/100,000 person-years (p = 0.0001). The overall rate of AMR (i.e. all serotypes and any antibiotic) decreased from 18 to 4.4 cases/100,000 person-years (p = 0.04). The rate of penicillin-resistant and trimethoprim sulfamethoxazole-resistant IPD decreased from 10 to 0 (p = 0.03) and 14 to 0 (p = 0.01) cases/100,000 person-years respectively. All cases of PCV13-type AMR-IPD were caused by serotype 19A. Rates of non-PCV13 resistant serotypes remained unchanged between 2007-2009 and 2011-2012 (4.2 to 4.4 cases/100,000 person-years (p = 0.95) respectively).

Conclusion: Following introduction of PCV13, AMR-IPD dramatically decreased in Navajo children aged <5 years. This change was driven by the decrease in serotype 19A IPD incidence. Ongoing surveillance to monitor for emergence of other AMR strains is important in this population at high risk of IPD.

No conflict of interest
ISPPD-0037  
Antibiotic Resistance and Clonal Spread

SEROTYPE DISTRIBUTION AND ANTIBIOTIC SUSCEPTIBILITIES OF STREPTOCOCCUS PNEUMONIAE ISOLATES IN ADULT PATIENTS WITH PNEUMOCOCCAL INFECTION OR CARRIAGE IN KOREA (2010-2013)

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Background and Aims: The objectives of this study were to investigate the serotypes and antimicrobial susceptibility of pneumococcal isolates from adult patients with pneumococcal infection or carriage during recent two years.

Methods: Between July 2010 and February 2013, Streptococcus pneumoniae isolates from clinical samples among adult patients (218 years) were identified in Korea University Anam Hospital. A retrospective chart review was performed. Serotyping of pneumococcal isolates were performed using a multiplexed immunoassay for capsular polysaccharides in lysates of pneumococcal cultures, identifying 27 serotypes: 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 7F, 8, 9N, 9V, 10A, 11A, 11E, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, and 33F. Antimicrobial susceptibility was determined using the MicroScan MicrOStREP plus panel.

Results: During the study period, a total of 186 patients with pneumococcal infection (n=130, 69.9%) or carriage (n=56, 30.1%) were analyzed. Median age was 69 [interquartile range, 60-75] years and 121 patients (65.1%) were ≥ 65 years. Fifteen patients (8.1%) presented pneumococcal bacteremia. Of 137 (73.7%) pneumococcal isolates that were able to determine serotypes, the major serotypes included 19A (20.4%), 3 (18.2%), 11A/D/F (16.1%), and 19F (13.1%). PCV7 and PCV13 serotypes were 23.4% (32/137) and 67.2% (92/137), respectively. The nonsusceptibility rates of 48 isolates were 41.7% for penicillin, 83.3% for erythromycin, 31.3% for cefotaxime, 10.4% for levofloxacin, 77.1% for meropenem and 0% for vancomycin.

Conclusion: In this study, non-PCV7 serotypes 3 and 19A that are included in PCV13 were prevalent in adult pneumococcal isolates.

Conflict of interest

ISPPD-0098  
Antibiotic Resistance and Clonal Spread

DRUG RESISTANT STREPTOCOCCAL PNEUMONIAE FROM ACUTE RESPIRATORY INFECTION AND HEALTHY CHILDREN IN CENTRAL VIETNAM

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Background: Nasopharyngeal colonization of Streptococcus pneumoniae plays an important role in development of invasive pneumococcal disease (IPD). Streptococcus pneumoniae drug resistance pattern and serotype distribution data among hospitalized acute respiratory infection (ARI) and healthy children of are crucial for appropriate clinical management of IPD and pneumococcal vaccine introduction in Vietnam.

Methods: S. pneumoniae isolated from pediatric ARI cases admitted to Khanh Hoa General Hospital from January 2008 through December 2008, and healthy children from the community (January and July 2008, 350 each) were collected. Drug resistance (MIC) and serotype were determined by conventional and molecular assays. Information on potential risk factors for S. pneumoniae carriage were collected and analyzed.

Results & Conclusion: S. pneumoniae isolates; 230 (38.85%) from 592 ARI children and 267 (38.14%) from 700 healthy children enrolled during the study period were studied. Carriage study among healthy children revealed that pneumococcal carriage in general or by resistance to penicillin or multidrug-resistant was independently associated with cool-wet season and day-care center attendance (p = <0.0001). High rate of b-lactams and quinolone resistance strains were detected. Majority (80%) of S. pneumoniae had high level resistance to macrolides and 32.9% had multidrug-resistant. Strikingly S. pneumoniae isolates from 28% of ARI and 22% of healthy children had a high level of Meropenem resistance. Majority of serotypes (6A/B, 19F, 14, 23F) from ARI (70%) and healthy children (60-65%) were covered by current available pneumococcal conjugate vaccine (PCV). The study findings will be valuable for clinical management and future PCV introduction in Vietnam.

No conflict of interest
BACTERIAL ETIOLOGY OF ACUTE OTITIS MEDIA PRIOR TO UNIVERSAL PNEUMOCOCCAL VACCINATION IN CHINESE CHILDREN

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Background and Aims: Acute Otitis Media (AOM) is one of the most common bacterial infections in children. Pneumococcal conjugate vaccine was introduced into China in 2008 and only some children were vaccinated. This study investigated the bacterial etiology of AOM and the antibiotic susceptibility, serotype distribution and clone spread of Streptococcus pneumoniae (Sp) in Suzhou, China.

Methods: Since 2011, a surveillance study was conducted in Soochow-University-Affiliated-Children’s-Hospital. All children with AOM and having middle-ear-effusion (MEF) were enrolled and cultured for bacterial pathogen, tested antibiotic susceptibility by E-test. Sp strains were identified serotype, macrolide-resistant genes and sequence types.

Results: From January 2011 to December 2012, among the 168 episodes, 113 (67.3%) samples were positive for bacterial pathogens. The leading cause was Sp (43.5%), followed by Staphylococcus aureus (14.9%) and Haemophilus influenzae (7.7%). The Sp isolation rate decreased with age (p < 0.01). The resistant rate to erythromycin, tetracycline was up to 98.6%, and 57.5% isolates were non-susceptible to penicillin. Moreover, all Sp isolates were resistant to ≥3 types of antibiotics with a major multidrug resistance pattern of erythromycin/co-trimoxazole/clindamycin/tetracycline. The most common serotypes were 19A (37.8%) and 19F (37.8%). The coverage rate of PCV7 serotypes was 51.4% and of PCV13 was 100%. The macrolide resistance was mainly mediated by both ermB and mefA genes (89.2%). CC271 was the only clone complex (CC) and belong to PMEN14 clone.

Conclusion: S. pneumoniae was a leading cause for AOM in children in Suzhou, China. Multidrug resistance among isolates was mainly due to the spread of PMEN14 CCs.

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No conflict of interest

RARE PNEUMOCOCCAL/HAEMOPHILUS BASED BACTERIAL MENINGITIS AMONG HOSPITALIZED CHILDREN IN A TEACHING HOSPITAL

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A study on the incidence of bacterial meningitis among hospitalized children in Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife was carried out using standard microbiological techniques. A sum of one hundred and thirty-five patients with the age range from 1 hour to 8 years was involved in the study and was placed in classes; the highest study class being 41.5 % ranged from 1 hour to 6 days, followed by age range 1 year to 4 years (25.9 %). Three of them (2.22%) showed positive presence of bacterial pathogens in their cerebral fluid (statistically non-significant (p ≥ 0.05) but medically important). These three positive patients were between the 1 month to <12 months (33.3%) and <4 years to 8 years (66.7%). Two isolates of Streptococcus pneumoniae recovered were resistant to ampicillin, chloramphenicol, streptomycin and gentamycin, cotrimoxazole but sensitive to ceftriazone, augmentin, cefixime and ofloxacin. At least one of the isolates was resistant to cloxacillin, cephalaxin and ceftazidine. The only Haemophilus influenzae recovered was resistant to ampicillin, cloxacillin, chloramphenicol, streptomycin, cotrimoxazole, cefixime and erythromycin. The sample size was small and therefore the data presented is suggestive of prior antibiotic abuse. The incidence of meningitis is unusual in the study area and suggests the need for proactive health control measures against rare infections.

No conflict of interest
IMPACT OF ANTIMICROBIAL USE ON NASOPHARYNGEAL COLONIZATION BY PENICILLIN-NON-SUSCEPTIBLE STREPTOCOCCUS PNEUMONIAE (PNSP) — ALASKA, 2000–2010

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Background and Aims: We evaluated the relative impact of PCV7 (introduced in 2001) and penicillin use on penicillin non-susceptible Streptococcus pneumoniae (PNSP) colonization in children.

Methods: We recruited and obtained nasopharyngeal swab specimens from a convenience sample of children aged <5 years at pediatric clinics annually during 2000–2004 and 2008–2010. Penicillin use <3 months before enrollment was determined by interview/medical records review. We completed pneumococcal identification/serotyping by standard methods, and penicillin (Pen) susceptibility testing by Etest. Isolates were classified as PenR (MICs >1 mcg/mL), PenI (MICs >0.064–1 mcg/mL), or PNSP (PenR/PenI).

Results: We recruited 3,496 children during the study period; the proportion of children age-appropriately vaccinated with PCV7 increased (0%–90%, p-value for trend [p] <0.01), PCV7-type carriage decreased (20% – <1%, p-value <0.01), and non-PCV7-type carriage increased (16%–38%, p <0.01). Proportion of children receiving penicillin/year was unchanged (mean: 24%, p=0.09). Proportion of children with PenR pneumococcal carriage decreased (23%–9%, p <0.01) and PenI pneumococcal carriage increased (13%–24%, p <0.01); overall PNSP carriage was unchanged. During the study period, penicillin nonsusceptibility among colonizing PCV7-type and non-PCV7-type pneumococci remained unchanged; a mean of 31% of PCV7-type and 10% of non-PCV7-type isolates were PenR, and 10% of PCV7 and 20% of non-PCV7-type isolates were PenI.

Conclusion: Penicillin nonsusceptibility did not increase among colonizing PCV7-type or among non-PCV7-type pneumococci despite stable penicillin use among children <5 years. However, population-level colonization by predominantly PenI non-PCV7 serotypes increased and predominantly PenR PCV7 serotypes decreased because of PCV7 vaccination, leaving overall PNSP colonization unchanged.

No conflict of interest

FLUOROQUINOLONE RESISTANCE AMONG INVASIVE PNEUMOCOCCAL DISEASE ISOLATES, SOUTH AFRICA, 2003-2012

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Aims: We previously described the emergence of fluoroquinolone (FQ) non-susceptible pneumococci (FQNSSP) causing invasive pneumococcal disease (IPD) among children in South Africa. The 7-valent pneumococcal conjugate vaccine (PCV7) was introduced in 2009, transitioning to PCV-13 in 2011. We aimed to review FQ resistance among IPD isolates.

Methods: IPD cases were reported to the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA) surveillance from 2003 through 2012. Serotypes were defined as ‘vaccine-type’ (VT) if in PCV13 or non-vaccine serotypes (NVT). MIC testing was performed. FQNSSP was defined as ofloxacin/levofloxacin MIC ≥4 µg/mL. Multi-drug resistant (MDR) isolates had resistance to 3 or more antimicrobial classes.

Results: 43,623 IPD cases were reported: 25 (0.1%) and 26 (0.2%) FQNSSP identified in adults (≥15 years) and children (<15 years), respectively. 15/25 cases in adults were VT and no FQNSSP increases were seen over the years. Of the 16,012 (37%) cases reported in children, 12,006 had viable isolates. FQNSSP cases were as follows: 2 (0.2%), 5 (0.3%), 4 (0.3%), 2 (0.1%), 7 (0.5%), 0 (0.1%), 1 (0.1%), 3 (0.5%), 1 (0.2%) for each year; respectively, 20/21 cases reported prior to vaccine introduction (<2010) were VT and MDR; 19F (n = 13, 62%), 14 (n = 5, 24%), 4 (n = 1, 5%), 19A (n = 1, 1%). One 35B reported in 2007 was also intermediately-resistant to trimethoprim-sulfamethoxazole. Post PCV (2010-2013), 5 FQNSSP cases were reported: 3 VT and MDR (19F (n = 2) and 14), and 2 NVT (13 and 15B), both resistant only to FQ and trimethoprim-sulfamethoxazole.

Conclusion: FQNSSP cases among children remain low. Reporting of 2 NVT FQNSSP strains is of concern.

No conflict of interest
A STUDY ON PREVALENCE AND ANTIBIOMIC OF PNEUMOCOCCAL PNEUMONIA

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Background and Aims: Streptococcus pneumoniae is the most common cause of community-acquired respiratory tract infections such as otitis media, sinusitis, and pneumonia. Globally, pneumococcal diseases account for 1 to 2 million deaths annually in both extremes of age. This study was planned to estimate prevalence of pneumococcal pneumonia in pediatric patients and drug resistance pattern of the isolates.

Methods: 250 nasopharyngeal aspirates and sputum samples were collected from clinically diagnosed cases on pneumonia. The samples were inoculated on sheep blood agar. The organism grown was identified with gram staining, hemolysis on sheep blood agar, bile solubility, and sensitivity to optochin. Demographic and clinical details such as age, sex, type of infection, underlying disease, and response to treatment, for all 250 patients were noted. Antibiotic testing was done for penicillin, tetracycline, erythromycin, ciprofloxacin, cotrimoxazole and results interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results: Of the 250 specimens, S. pneumoniae was isolated in 10 samples. 1 isolate was resistant to penicillin, 2 isolates were penicillin intermediate resistant and rest were sensitive to penicillin. 1 strain was MDR. 30 % resistance was seen with tetracycline and cotrimoxazole and 20% strains were erythromycin resistant. All patients responded to antimicrobial therapy and none of the patients died.

Conclusion: Increasing prevalence and emergence of antibiotic resistant among S. pneumoniae in the community set up is a matter of great concern and large scale studies are required to estimate prevalence, antibiotic resistance and serotypes of the isolates so that national guidelines can be formulated regarding prevention and treatment of pneumococcal pneumonia.

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No conflict of interest

ANTIMICROBIAL RESISTANCE AND CLONAL SPREAD OF STREPTOCOCCUS PNEUMONIAE ISOLATED FROM CHILDREN UNDER 5 YEARS OLD IN SUZHOU, CHINA

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Background and Aims: Dissemination of non-antimicrobial-susceptible clones was known as an important factor in the emergence and prevalence of resistance in pneumococcus. This study was to explore the molecular characteristics of pneumococci and the circulating clones in hospitalized children in Suzhou, China.

Methods: Nasopharyngeal aspirates were collected from children less than 5 years old admitted to Suzhou-University-Affiliated-Children's-Hospital being respiratory infections. Pneumococcal isolates were serotyped by multiplex PCR. Antimicrobial susceptibility was tested by E-test. We detected macrolide resistance genes and performed multi locus MLST on all isolates.

Results: From July 2012 to July 2013, all the 175 pneumococcal isolates were resistant to erythromycin and clindamycin, 94.3% resistant to tetracycline and 39.4% non-susceptible to penicillin. Overall, 172 (98.3%) isolates were resistant to ≥3 types of antibiotics. The most common serotypes were 19F (28.6%), 6B (20.0%), 23F (17.7%), 19A (17.2%) and 14 (5.1%). 126 (72.0%) were included in PCV7serotypes and 156 (89.1%) in PCV13 serotypes. MLST resolved 175 isolates into 44 sequence types (STs), and 45.1% of STs derived from CC271 (PMEN14CCs), 13.7% from CC81 (PMEN1CCs) and 4.0% from CC242 (PMEN15CCs). The PMENCCs isolates indicated a higher non-susceptibility rate in β-lactams antibiotic, chloramycetin and co-trimoxazole than the non-PMENCCs isolates (p < 0.01). In addition, there was 80.0% of the PMENCCs isolates both expressed ermB and mefA/E genes whereas 71.2% of the non-PMENCCs isolates only expressed mefA/E (p < 0.01).

Conclusion: The PMEN14 and PMEN1 clones played a predominant role in the emergency and dissemination of multidrug-resistant strains in Suzhou. Considering the potential serotype replacement (19A), the introduction of PCV13 in children maybe a promising way to control the increasing trend of international clonal spread.

No conflict of interest
ISPPD-0090
Antibiotic Resistance and Clonal Spread

SURVEILLANCE OF ANTIBIOTIC RESISTANCE IN STREPTOCOCCUS PNEUMONIAE AT A TERTIARY CARE CENTRE IN NEW DELHI
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Introduction: Streptococcus pneumoniae is the most frequent isolate from clinical samples of respiratory tract infection, including acute exacerbations of chronic bronchitis and community-acquired pneumonia. The emergence of multiple drug-resistance has complicated the empirical treatment of pneumococcal infection. S. pneumoniae is a major infectious disease with millions of cases diagnosed every year worldwide. In the present study we evaluated the antimicrobial resistance pattern in the S. pneumoniae isolates obtained from a tertiary care centre in Delhi.

Methodology: All the subjects, of all age groups and sex, presenting at a tertiary care centre in Delhi were included in the study. A total of 41 S. pneumoniae isolates were obtained and investigated. All were subjected to disc diffusion testing and MIC testing by E-Test.

Results: Susceptibility testing showed 100%, 97%, 95%, 97%, 100%, 68%, 49% sensitivity to penicillin, levofloxacin, erythromycin, clindamycin, chloramphenicol, cotrimoxazole and tetracycline, respectively. Maximum number of isolates have been obtained from blood (48.78%), followed by sputum (17.07%) and ET secretion (14.63%).

Conclusion: Initial data from our study shows that all the S. pneumoniae isolates are uniformly sensitive to penicillin and chloramphenicol, with a low level resistance to quinolones and macrolides. The isolates are maximally resistant to cotrimoxazole and tetracycline.

No conflict of interest

ISPPD-0307
Antibiotic Resistance and Clonal Spread

MACROLIDE RESISTANCE DETERMINANTS AMONG INVASIVE AND NON-INVASIVE STREPTOCOCCUS PNEUMONIAE ISOLATES IN SERBIA
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Background: The aim of this study was to determine the distribution of macrolide resistance phenotypes and genotypes among invasive and noninvasive erythromycin resistant S. pneumoniae isolates in Serbia.

Methods: During 2010, a total of 297 macrolide resistant pneumococcal strains (45 invasive and 252 noninvasive) isolated throughout country were analyzed. Macrolide resistant phenotypes were determined using double disk test, MIC values by E test, while resistance genes were examined by PCR. Serotyping was performed for invasive isolates.

Results: cMLS phenotype, characterized by high MICs values for both erythromycin (MIC≥256 µg/ml) and clindamycin (MIC≥256 µg/ml) was detected in 77% of all isolates, while the remaining 23% were assigned to the M phenotype, with lower level of resistance to erythromycin (MIC≤4 µg/ml) and susceptibility to clindamycin (MIC<0.06 µg/ml). All M phenotype had the mefA gene and all MLS phenotype strains had the ermA gene. Nine MLS isolates possessed both ermA and mefA genes. No difference was found in distribution of macrolide resistance phenotypes and genotypes between invasive and noninvasive isolates. Co-resistance to penicillin and erythromycin was detected in 25% and 47% of invasive and noninvasive strains, respectively. Of 12 different serotypes detected among invasive strains, the most numerous were: 19F (n=15), 14 (n=11), 6A (n=6), 6B (n=3) and 23F (n=3). All isolates expressing 19F and 14 serotypes possessed ermA gene, while all 6A strains were mefA positive.

Conclusion: Macrolide resistance among pneumococcal invasive and noninvasive isolates from Serbia is predominantly mediated by ermA genes. Nearly half of noninvasive isolates expressed co-resistance to penicillin and erythromycin.

No conflict of interest

ISPPD-0091
Antibiotic Resistance and Clonal Spread

MOLECULAR EPIDEMIOLOGY AND GENOTYPE REPLACEMENT OF SEROTYPE 14 STREPTOCOCCUS PNEUMONIAE ISOLATED FROM CHINA
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Objective: The aim of this study was to determine the genetic structure of 144 serotype 14 Streptococcus pneumoniae from children with acute respiratory infections collected from 1997-2012 in China.

Methods: PCR was used to detect the macrolide resistance genes ermA and mefA, as well as the sulfamethoxazole-trimethoprim resistance gene dhfr. Restriction fragment length polymorphism of the PBPs genes pbp1a, pbp2b and pbp2x and multilocus sequence typing (MLST) were used to investigate the relationship between
the serotype 14 strains prevalent in China.

Results: From 1997 to 2012, the percentage of serotype 14 increased. All the isolates were susceptible to amoxicillin-clavulanic acid, vancomycin and levofloxacin, 143 were resistant to erythromycin, all of which carried the ermB gene and 13 carried both mefA/E and ermB genes. The nonsusceptibility rate to cephalosporins increased from 1997-2012. All trimethoprim-resistant isolates contained the 1100-L mutation. There were 30 sequence types (STs), among which ST876 was the most prevalent ST, followed by ST875. From 1997 to 2012, the percentage of CC876 increased from 0% in 1997-2000 to 96.4% in 2010-2012, whereas CC875 decreased from 84.2% to 0%. CC876 showed high nonsusceptibility rate to β-lactam antibiotics than CC875.

Conclusion: The percentage of serotype 14 of S. pneumoniae increased from 1997-2012 in China. The increase of nonsusceptibility rate to β-lactam antibiotics is associated with the spread of resistance clone CC876 due to the selection of antibiotics.

No conflict of interest

**ISPPD-0046**

Antibiotic Resistance and Clonal Spread

CHILDHOOD PNEUMOCOCCAL MENINGITIS AND ANTIBIOTIC SUSCEPTIBILITY IN A SAVANNAH REGION OF NORTHERN NIGERIA: A 12 YEAR REVIEW

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Background and Aim: Streptococcus pneumoniae meningitis (SPM) accounted for majority (47%) of cases of meningitis in children <5 years between 2002 – 2008 in sub-Saharan Africa. This review describes pneumococcal meningitis in our centre.

Methodology: Cerebrospinal fluid (CSF) reports of children with suspected acute pyogenic meningitis from 2000 to 2012 were analysed in FMC Gombe, a 400 bed, 12 year old tertiary hospital.

Results: 10,891 children were admitted between 2000 -2012. 2,647 lumbar punctures yielded 116 (4.4%) positive CSF cultures. Streptococcus pneumoniae constituted 22 %(25) of positive cultures. 14 males and 10 females. There were 4 cases in 2001, 3 cases each in 2006 and 2007; 6 cases in 2008, 2 cases in 2011 and 4 cases in 2012. There were no cases of SPM in 2002, 2005, 2009 and 2010. Increased SPM incidence in 2006, 2007 and 2008 was followed by a meningococcal epidemic in 2009 in the sub region. 80% (20) of SPM occurred in dry months of December to May. 21 (86%) cases occurred in children <5 years. 48% (13/25) of CSF was turbid, 23 (92%) had WBC>10 phf.

Conclusion: Children under 5 years are at risk for SPM. Increased cases of SPM preceded meningococcal epidemic in 2009. Chloramphenicol and ampicillin are effective against SP and pre-LP treatment could be responsible for large SP negative CSF cultures.

No conflict of interest

**ISPPD-0029**

Antibiotic Resistance and Clonal Spread

PRE-VACCINE COTRIMOXAZOLE AND MACROLIDE RESISTANCE AMONG PENICILLIN NON-SUSCEPTIBLE PNEUMOCOCCI IN NIGERIA: MACROLIDE RESISTANCE GENES AND SEROTYPE AND GENOTYPE AFFILIATIONS

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Background and Aims: There is paucity of epidemiological and molecular information on cotrimoxazole and macrolide resistant (MR) pneumococci in West Africa, where penicillin non-susceptible pneumococci (PNSP) burden is high and treatment concerning. This study determined co-trimoxazole and erythromycin resistance rates and carriage of MR genes among PNSP in Nigeria.

Methods: Thirty-one viable pneumococci from 172 patients aged 8 mo - 62 years during the 2010 – 2012 hospital surveillance period were studied. The isolates were serotyped and their susceptibility to six essential anti-pneumococcal antibiotics plus Inducible clindamycin resistance phenotyping-iMLS was done using disk diffusion and E-test methods. Each isolate was genomically screened for virulence (ply + lty genes), ribosomal methylase-ermB and drug efflux-mefa genes by PCR, followed by genotyping by RAPD.

Results: A total of 23 (74.2%) virulent PNSP, comprising 11 of 14 (45.1%) and 17 out of 20 (64.5%) macrolide and cotrimoxazole resistant strains were recovered. Penicillin-cotrimoxazole-erythromycin co-resistance caused 80% of Multi-drug-resistance (48.4%) seen. The MR strains belonged to six serotypes (5, 6B, 6A, 9V, 14, 23F), causing diseases that included meningitis (100%) and severe pneumonia (66.7%). Seventeen (54.8%) and 3 (9.7%) isolates were mefA only positive and mefA + ermB negative. MR and iMLS phenotype (9.7%) were significantly (< 0.05) associated with dual carriage of ermB + mefA genes. RAPD revealed 7 distinct genetic types among the local MR strains. PCVs 7 and 13 coverage was 67.8% and 100% respectively.

Conclusion: Cotrimoxazole and macrolide resistance mediated by ermB and mefA genes and involving multiple serotypes, are common among PNSP in Nigeria.

No conflict of interest
STREPTOCOCCUS PNEUMONIAE: SEROTYPE PREVALENCE IN CARRIERS IN ROMANIA; ANTIBIOTIC RESISTANCE PATTERNS

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Background and Introduction: Typing of Streptococcus pneumoniae to determine the serotype prevalence has paved the way for polyvalent vaccines to prevent invasive pneumococcal infection. Variation of serotype prevalence in different geographical areas necessitates typing of strains from these areas for effective vaccine protection.

Objectives: In our study purpose was to find prevalence of pneumococcal serotypes in the Romanian children up to 5 years old with samples of nasopharyngeal exudates. The second objective was to describe the pneumococcal serotypes results obtained from local laboratory assays and to assess the antibiotic susceptibility of the isolates by each serotype.

Methods: We conducted a multi-centre observational study, from November 2011 to March 2013 on children aged between 0 – 5 years. The antimicrobial susceptibility testing was made according to CLSI with: Disk diffusion, MIC (E-test).

Results: There were a total of 2018 samples nationwide, from which 503 were tested positive, split into 443 identified samples by serotype and 60 nontypeable samples. From the total of 503 positive samples, 55.1% were of masculine sex, 49% in the age group 3 to 5 years old, 79.8% were from urban areas and 89.4% from collectivity.

Conclusion: Serotypes 19 (30.41%) and 6 (29.22%) were the most frequent isolated on the age group 3-5 years old, on boys from urban areas. Sub serotype 19 F was the most frequent isolated strain (60 %) amongst serotype 19 General PEN resistance was 16.10 % for oral penicillin and 1.67 % for parenteral penicillin. No strains were resistant to moxiflaxcin or vancomycin.

No conflict of interest

ISPPD-0033
Antibiotic Resistance and Clonal Spread

SEROTYPES AND ANTIMICROBIAL RESISTANCE OF STREPTOCOCCUS PNEUMONIAE ISOLATES FROM INVASIVE INFECTIONS OF KOREAN CHILDREN AFTER INTRODUCTION OF PCV7, 2006-2010

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Background: This is a multicenter study on the serotype distribution and antimicrobial resistance of pneumococcal isolates from invasive infections (IPD) in children between 2006 and 2010, when 7-valent pneumococcal conjugate vaccine (PCV7) had been used as an optional vaccine in Korea.

Methods: One hundred and forty isolates from IPD in children <18 years of age were collected in 8 centers. Serotype was determined by Quellung reaction and multiplex PCR, and antimicrobial susceptibility was tested by E-test.

Results: The clinical diagnoses were: bacteremia without focus (39.3%), pneumonia (35.7%), meningitis (20.0%), and other IPD associated with bacteremia (5.0%). Common serotypes identified were 19A (22.9%), 19F (12.1%), 68 (8.6%), and 23F (7.9%). In a trend analysis between 2006 and 2010, PCV7 serotypes had decreased from 62.5% to 21.4% (P = 0.002), whereas 3 PCV13-specific serotypes (3, 6A, and 19A) had increased from 18.8% to 42.9% (P = 0.016). Nonsusceptibility rates of penicillin, cefotaxime, and erythromycin (according to 2003 Clinical and Laboratory Standards Institute (CLSI) guidelines, non-meningitis breakpoints) were 88.6%, 23.6%, and 87.0%, respectively. Among 102 multidrug resistant isolates, proportion of PCV7 serotypes significantly decreased (from 65.2% in 2006 to 21.7% in 2010, ρ = 0.001) and 3 PCV13-specific serotypes (3, 6A, and 19A) increased over time (from 17.4% in 2006 to 47.8% in 2010, ρ = 0.008).

Conclusion: This study demonstrates the impact of optional PCV7 vaccination in Korea; proportion of PCV7 serotypes decreased while that of 3 PCV13-specific serotypes, especially 19A, had increased.

Conflict of interest
PNEUMOCOCCAL CONJUGATE VACCINATION IN STOCKHOLM LEAVES INVASIVE DISEASE INCIDENCE UNAFFECTED IN THE ELDERLY AND INCREASES SEROTYPE DIVERSITY

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Background: Pneumococcal conjugated vaccines (PCV) target a limited number of serotypes prevailing in the community. Vaccine introduction in several countries has shown a decreased incidence of invasive pneumococcal disease (IPD), however, also an increase of non-vaccine types. In Stockholm PCV7 was introduced in the childhood immunization program in October 2007 and PCV13 in January 2010.

Methods: We collected IPD isolates from the Stockholm county during 2005-2012 and carriage isolates before and after vaccine introduction, and characterized them with serotyping, molecular typing and antibiotic susceptibility. Serotype diversity was calculated using Simpson index for IPD and carriage. Clinical information was collected for children with IPD.

Results: We found a reduced incidence of IPD in vaccinated children, but an unaltered incidence in the elderly after vaccine introduction. There was a decreased incidence of septicaemia, and meningitis in children, however, the severity of disease was unchanged. Non-vaccine types increased in IPD in vaccinated and non-vaccinated populations. The serotype diversity increased in both IPD and childhood carriage.

Conclusion: Vaccine introduction has beneficial effects on the incidence of vaccine type disease in children, however, the selective pressure leads to an increase of non-vaccine types both in disease and carriage and an expansion of prevaccine clones also in non-vaccinated age groups such as the elderly. An increased diversity of pneumococcal serotypes post vaccination suggests the potential of new emerging clones, which has impacts on future vaccine strategies.

No conflict of interest

ANTIMICROBIAL SUSCEPTIBILITY TRENDS AMONG STREPTOCOCCUS PNEUMONIAE OVER AN 11-YEAR PERIOD IN AN IRANIAN REFERRAL CHILDREN HOSPITAL

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Background and Aims: The appearance of antibiotic resistance in Streptococcus pneumoniae has raised a global concern over the past three decades. This study was conducted to determine the antimicrobial susceptibility of S. pneumoniae isolated from patients in Children’s Medical Center Hospital during 2001 to 2011.

Methods: During the 11 years period, a total of 194 S. pneumoniae isolates were collected in CMC Hospital. Time series analysis of the different antibiotics was performed.

Results: Time series analysis of the 5 antibiotics of penicillin, erythromycin, chloramphenicol, ceftriaxone, and trimethoprim-sulfamethoxazole showed an overall decreasing trend for S. pneumoniae susceptibility during 2001 to 2011 and even forecasting prediction for 2016. The prevalence of susceptibility to penicillin decreased from 78% in 2001 to 32% in 2011. In the same time period, susceptibility to erythromycin declined from 75% (in 2001 to 35% in 2011 and susceptibility to chloramphenicol started to decrease from 94% to 55%. In addition, during these couple of years, susceptibility to ampicillin declined from 70% to 62%. Beside this, susceptibility to ceftriaxone started to fall from 100% to 87% and susceptibility to sulfamethoxazole went down from 57% to 40%. This study identifies unstable patterns of resistance to available antimicrobial drugs during 11 years.

Conclusion: Continued epidemiological surveillance appears to be prudent practice to guide effective chemotherapy. Moreover, it would be an important key to consider antimicrobial stewardship as an essential factor to prevent the development of antimicrobial resistance.

Conflict of interest
MOLECULAR EPIDEMIOLOGY OF STREPTOCOCCUS PNEUMONIAE (SP) SEROTYPES 6C AND 6D IN SOUTHERN ISRAEL

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Background and Aims: The capsular locus of Streptococcus pneumoniae (Sp) serotype 6C and Sp6D is similar to Sp6A and Sp6B, respectively, except for wciN<sub>6C,6D</sub>. The 7/13-valent pneumococcal conjugate vaccines (PCVs) were introduced into the Israeli immunization program in 2009/2010, respectively. This study aimed to identify Sp6C and Sp6D from previously characterized Sp6A and Sp6B, explore their molecular epidemiology and determine their circulation among two distinct subpopulations, Jewish and Bedouin children <5 years of age.

Methods: This study covers 14 years (1999-2012) pre- and post-PCV implementation. Sp were cultured from Blood/cerebrospinal fluid (CSF), MEF, conjunctiva and nasopharynx. PCR targeting wciN<sub>6C,6D</sub>, wciN<sub>6A</sub> and wciP<sub>6B</sub> was used to segregate Sp6C and Sp6D from Sp6A and Sp6B, respectively. Clonality was determined by PFGE and MLST.

Results: For the years 1999-2008 (Pre-PCV) Sp6C and Sp6D constituted 8.2% (106/1299) and 6.7% (107/1593) of presumed Sp6A and Sp6B, respectively. Penicillin non-susceptibility was 5.7% (6/106) in Sp6C compared to 72.0% (77/107) in Sp6D. Molecular analysis revealed the presence of 2 Sp6C major clones, which constituted 76.4% (81/106) of Sp6C, of which 91.4% (74/81) susceptible to all antibiotics tested. Sp6D isolates belonged to 3 major clones, which constituted 56.1% (60/107) of Sp6D, all resistant to ≥3 antibiotics. A striking clonal segregation was found among the two subpopulations. Data for the 4-year post-PCV7/13 implementation (2009-2012) are currently being analyzed and will be presented.

Conclusion: Sp6C and Sp6D are non-negligible among serogroup 6. The current analysis will show whether PCV7/13 were successful in reducing these minorities as was demonstrated for Sp6A and Sp6B.

No conflict of interest

ANTIBIOTIC SENSITIVITY PATTERN OF STREPTOCOCCUS PNEUMONIAE ISOLATED FROM THE NASOPHARYNX OF HEALTHY CHILDREN IN SOUTH INDIA

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Background: Invasive disease due to Streptococcus pneumoniae is one of the commonest causes of death among children under 5 years of age in developing countries. Nasopharyngeal colonization with S. pneumoniae precedes the invasive disease in some children and it will be clinically significant to determine the drug resistance pattern of serotypes colonised.

Aims: To determine the antibiotic susceptibility pattern of S. pneumoniae isolated from the nasopharynx of children under 5 years of age.

Methods: Antimicrobial susceptibility was tested for 45 isolates of pneumococcal isolates from the nasopharynx by Kirby Bauer method and results were interpreted as per Clinical and Laboratory Standards Institute guideline (CLSI) guidelines. Penicillin MIC was determined by “E” test for penicillin resistant strains.

Results: Of the 45 S. pneumoniae isolates, all were sensitive to vancomycin and linezolid (100 %), 23 were sensitive to erythromycin (51%), 26 were sensitive to tetracycline (58%), 44 were sensitive to Levofoxacin (98%), 29 were sensitive to clindamycin (66%), 14 were sensitive to co-trimoxazole (33%), 39 were sensitive to chloramphenicol (87%), 40 were sensitive to penicillin (89%). Five strains were penicillin resistant (three stains with intermediate resistance and two with high resistance).

Conclusion: Increase in penicillin resistance among S. pneumoniae is a disturbing trend. Further studies comparing the antimicrobial susceptibility pattern of invasive isolates and nasopharyngeal isolates of S. pneumoniae are required.

No conflict of interest
ISPPD-0221
Antibiotic Resistance and Clonal Spread

A STUDY ON DRUG RESISTANCE PATTERN OF INVASIVE STREPTOCOCUS PNEUMONIAE ISOLATES IN AN IRANIAN 1000-BED TERTIARY CARE HOSPITAL

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1
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Background and Objective: Streptococcus pneumoniae is one of the most leading causes of serious problems such as bacterial meningitis, sepsis and community acquired pneumonia. Invasive pneumococcal disease has a high rate of mortality and morbidity especially in children, due to emerging of resistance to antibiotics. The aim of the study was to determine the antimicrobial susceptibility pattern of invasive pneumococcal isolates.

Methods: All clinical specimens submitted to microbiology laboratory of Milad Hospital, In an Iranian 1000-bed tertiary care hospital were subject for isolation of S. pneumoniae. All isolates were identified by routine microbiological methods. Susceptibility testing were performed using E-test MIC Method. CLSI guideline was used for interpretation of results.

Results: From December 2010 to January of 2013 a total of 50 strains of S. pneumoniae isolated from patients admitted to Milad Hospital. Septicemia (44%) and pneumonia (46%) were the most common clinical conditions associated with invasive pneumococcal disease. We observe a high rate of resistance to penicillin. In our study of 35 (705) isolates were resistant to penicillin. The rate of resistance to other antibiotics including erythromycin, clindamycin, azithromycin, co-trimoxazole, tetracycline and chloramphenicol was 60%, 64%, 56%, 50%, 54% and 24% respectively. All isolates were susceptible to vancomycin and only one strain was resistant to levofloxacin.

Conclusion: Invasive pneumococcal disease is the serious problem in Iran and is associated with high case fatality in spite of treatment in the hospital setting. Penicillin resistance is currently is very high in our country. Using of polysaccharide pneumococcal vaccine is recommended for prevention of disease in high risk patients.

No conflict of interest

ISPPD-0191
Antibiotic Resistance and Clonal Spread

INCREASED PENICILLIN-NONSUSCEPTIBLE NON-VACCINE INVASIVE PNEUMOCOCCAL ISOLATES IN THE POST-PCV13 VACCINE ERA IN ALASKA

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Background: Introduction of conjugate vaccines (PCV7 - 2001; PCV13 - April 2010) in Alaska resulted in reduced rates of vaccine-type invasive pneumococcal disease (IPD). We evaluated the impact of conjugate vaccines on penicillin-nonsusceptible (PNS) IPD isolates from 2001 - 2012.

Methods: IPD isolates collected from 2001 - 2012 through statewide laboratory surveillance were confirmed and serotyped by standard methods. Antimicrobial susceptibility testing was performed by broth microdilution; isolates with a MIC of ≥ 0.12 µg/ml were classified as PNS.

Results: Of 1351 IPD isolates recovered from 2001 - 2012, 237 (17.5%) were PNS. The proportion of PNS IPD isolates did not change from the pre-PCV13 period (2001 - March 2010) to the post-PCV13 period (April 2010 - 2012). The rate of PNS IPD also did not change (2.93 to 2.87/100,000/y; p = 0.89). The proportion of PNS vaccine serotypes decreased from 85% [pre] to 40% [post] (p < 0.001) while the proportion of PNS non-vaccine serotypes increased (15% [pre] to 60% [post]; p < 0.001). These non-vaccines serotypes included 6C, 15A, 15B, 15C, 23A, 23B, 29 and 35B. While the rate of PNS PCV13-type IPD declined significantly (2.5/100,000 to 1.2/100,000; p < 0.001) from the pre-PCV13 to the post-PCV13 period, the rate of PNS non-vaccine type IPD increased (0.45 to 1.7/100,000/y; p < 0.001).

Conclusion: The proportion of PNS IPD isolates has changed little since the introduction of conjugate vaccines. However, PNS IPD due to vaccine types has declined while PNS IPD due to non-vaccine types has increased. Continued surveillance is warranted to monitor changes in antimicrobial resistance and serotypes causing IPD in the conjugate vaccine era.

No conflict of interest
ISPPD-0192
Antibiotic Resistance and Clonal Spread

POPULATION STRUCTURE OF INVASIVE PNEUMOCOCCAL ISOLATES AMONG ALASKAN CHILDREN IN THE CONJUGATE VACCINE ERA, 2001-2012

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**Background:** Introduction of conjugate vaccines (PCV7 - 2001; PCV13 - April 2010) has led to changes in the epidemiology of invasive pneumococcal disease (IPD) in Alaska. We examined the population structure of IPD isolates recovered from children <5 years old during 2001 - 2012.

**Methods:** IPD isolates received through statewide laboratory surveillance were confirmed and serotyped by standard methods. Multilocus sequence typing (MLST) was performed to identify sequence types (ST); clonal complexes (CC) were assigned using the eBURST algorithm. ST/CC diversity was calculated using Simpson’s diversity index.

**Results:** Of 273 (18.4%) IPD isolates recovered from children <5 years old, 254 were available for testing. The most common serotypes (19A, 7F, 22F, 33F and 12F) made up 57% of the isolates. We identified 69 STs; 8 were new to the MLST database. Predominant STs included 199 (15%), 172 (11%), 191 (8%) and 2469 (5%). Sequence types which increased significantly over time included ST320 and ST63. The ST composition of the 2007-09 data set (0.92) was less diverse than the 2001-03 (0.96) and 2004-06 (0.94) data sets due to an increase in the number of isolates associated with CC191, CC172, and CC320 (p=0.03). This trend continued into the 2010 - 2012 data set (0.93) with emergence of ST63. Most 15B/C isolates were ST199; however, some isolates had STs not previously associated with that serotype (ST644, ST3934).

**Conclusions:** The population structure of IPD isolates recovered from children in Alaska has become less diverse over time. Further surveillance is necessary to continue monitoring the effects of conjugate vaccines.

No conflict of interest

ISPPD-0007
Antibiotic Resistance and Clonal Spread

THE SUBTYPES AND ANTIBIOTIC RESISTANCE OF SEROTYPE 6 STREPTOCOCCUS PNEUMONIAE ISOLATED FROM CHILDREN IN CHINA

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**Background and Aims:** This study aimed to define the distribution of serotypes, antimicrobial resistances and genotypes of serogroup 6 Streptococcus pneumoniae collected in China.

**Methods:** The serotypes of the isolates were identified by the Quellung reaction and the serotype-specific PCR on 225 S. pneumoniae strains isolated from 1997-2011. All of the isolates were tested for sensitivity to 11 antibiotics by E-test method or disc diffusion. The sequence types (STs) and cap-locus gene subtypes were analyzed using the MLST procedure and wzy and rmlA gene sequencing, respectively.

**Results:** Serotypes were determined as follows: 6A, 105 (46.7%); 6B, 102 (45.3%); 6C, 14 (6.2%) and 6D, 4 (1.8%). All of the isolates were susceptible to amoxicillin–clavulanic acid, ceftriaxone, vancomycin and levofloxacin. No isolate resistant against parenteral penicillin was found, whereas the intermediate and resistant rate against oral penicillin reached 28.4% and 4.0%. The most common STs were ST982 (52 isolates, 23.1%), ST90 (33, 14.7%), ST4542 (17, 7.6%) and ST2912 (11, 4.9%). The serotype 6B isolates could be subtyped as 6B-I (28, 27.5%), 6B-II (20, 19.6%) and 6B-III (49, 48.0%). Other new subtypes (5, 4.9%) were found in the present study. Isolates belonged to subtype 6B-III were more resistant significantly to beta-lactam antibiotics than other subtypes and serotypes, and ST90 was the common ST in the 6B-III isolates.

**Conclusions:** This study revealed that all recognized types and subtypes of serogroup 6 could be found in China. Serotype 6B isolates, especially subtype 6B-III with ST90, were more resistant than others in the group 6S. pneumoniae.

No conflict of interest
ANTIMICROBIAL RESISTANCE OF INVASIVE PNEUMOCOCCI IN FINLAND AFTER INTRODUCTION OF 10-VALENT PNEUMOCOCCAL CONJUGATE VACCINE (PCV10) INTO THE NATIONAL VACCINATION PROGRAMME

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Background and Aims: Antimicrobial non-susceptibility among invasive has increased over the past decade in Finland. PCV10 (2+1) was introduced into the national infant vaccination programme in September, 2010. We studied non-susceptibility trends of PCV10 and non-PCV10 serotypes in 2010-2012.

Methods: Invasive pneumococcal disease is nationally notifiable and the isolates are sent to the THL reference laboratory for characterisation. For this study, 2,391 isolates from 2010-2012 were serotyped by multiplex-PCR and/or the Quellung reaction. Their antimicrobial susceptibility was tested by the agar dilution method.

Results: Overall, the proportion of penicillin non-susceptible isolates increased from 23% (192/826) to 28% (213/768), while that of erythromycin non-susceptible decreased from 29% (237/826) to 23% (174/768). The PCV10 serotypes accounted for 73-92% and 76-88% of the penicillin and erythromycin non-susceptible isolates, respectively. Serotypes 19F, 19A, and 23F accounted for the highly penicillin-resistant (MIC≥8 mg/L) isolates (n=5). Among isolates from 0-2-year-olds, there was a reduction especially in the number of penicillin or erythromycin non-susceptible PCV10 serotype isolates. In 2010, the proportion of non-susceptible isolates from this age-group was 41% to penicillin and 58% to erythromycin, while in 2012, it was 24% and 33%, respectively. The proportion of penicillin non-susceptible isolates increased among ≥65-year-olds, accompanied with an increase in non-susceptible non-PCV10 serotypes, i.e. 6C and 19A.

Conclusions: During the first two years after routine infant vaccination, penicillin non-susceptibility seemed to increase slightly, while erythromycin non-susceptibility seemed to decrease. Ongoing changes in the pneumococcal population emphasise the need for future surveillance to determine the impact of PCV10 on antimicrobial resistance.

No conflict of interest

RAPID GROWTH OF ANTIBIOTIC-RESISTANT STREPTOCOCCUS PNEUMONIAE IN MONGOLIAN POPULATION

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Background: Due to extreme weather and poor air quality, Mongolia is facing a huge problem of respiratory disease. Unfortunately antibiotic-resistant Streptococcus pneumoniae is increasing dramatically in recent years, therefore we conducted the study to identify the cause and serotype distribution. No data was available previously.

Method: We collected samples and questionnaire of 115 patients under 5 years with respiratory tract infection, from 3 hospitals cross Mongolia since 2011. An antibiotics susceptibility was tested by the disk diffusion method.

Results: A total 96 (84%) were positive for S. pneumoniae and 25 serotypes were detected. Among the isolates, serotype 19A was the commonest, followed by 1, 23B, 6B, 13. The resistance levels to penicillin, erythromycin, tetracycline, trimethoprim-sulfamethoxazole and ciprofloxacin were 85%, 79%, 68%, 31%, and 6.8%, respectively. Multidrug resistance was detected in 29% of them and the most prevalent serotypes were 19A, 19F, 13 and 6B. Surprisingly, 76% of total patient’s parents were answered that before coming to hospital they have already tried antibiotic treatment according to a pharmacist choice.

Conclusion: A high prevalence of penicillin-resistance is found, and it is no longer considered a drug of choice. Other alternative antibiotics such as erythromycin, ciprofloxacin, and tetracycline also showed resistance against the isolates. Antibiotic misuse and inappropriate dosage might facilitate the spread of multidrug-resistant strains. We urgently need an antibiotic restriction law to restraint the indiscrete use of antibiotics and limit the selling without prescription. Most important, is the need to provide a better understanding of drug-resistant infections in the community

No conflict of interest
PNEUMOCOCCAL CONJUGATE VACCINE (13-VALENT) PROTECTION AGAINST PNEUMOCOCCI IN ADULT PATIENTS

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Background and Aims: The Food and Drug Administration (USA) approved of the use of PCV-13 to prevent IPD in adults aged ≥50 years. To predict the potential benefit of PCV-13, we evaluated for serotype coverage over pneumococci.

Methods: 157 isolates from normally sterile sites were obtained from adult patients aged 50-89 years from 16 hospitals during 2005-2012. They were serotyped by Quellung reaction, using group and factor sera of Pneumotest kits. 88 isolates were obtained during 2009-2012.

Results: The rates of PCV-13 serotype coverage for pneumococci from sterile sites of the elderly Thai patients were 53.7% (95% CI, 38.4% to 68.9%), 58.5% (95% CI, 43.5% to 73.6%), 53.3% (95% CI, 38.8% to 67.9%) and 70% (95% CI, 53.6% to 86.4%) for patients aged 50-60, 61-70, 71-80 and >80 years, respectively. The overall coverage rate of PCV-13 was 58% (95% CI, 50.2% to 65.7%). The five most common serotypes, accounting for 46.5% of all, were serotypes 6B (16.6%), 19A (8.9%), 18C (5.1%), 23F (4.5%) and 3, 4, 7F (3.8% each). For isolates during 2009-2012, the overall coverage rate of PCV-13 was also 58% and covered 52%, 63%, 52% and 72% for the patients aged 50-69, 61-70, 71-80 and >80 years, respectively. The most common serotypes were 6B (28%), 19A (16%), 18C (12%), and 23F (10%). Interestingly, patients aged >80 years had the highest coverage of PCV-13.

Conclusion: Our results help to evaluate the effectiveness of PCV-13 and to monitor changes in serotypes in Thailand.

No conflict of interest

ANTIBIOTIC SUSCEPTIBILITY PATTERN OF BLOOD ISOLATES WITH SPECIAL REFERENCE TO STREPTOCOCCUS PNEUMONIAE INFECTION

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Background and Aims: Septicemia remains a serious cause of morbidity and mortality in critically ill patients worldwide. We studied antibiotic susceptibility pattern of bacterial isolates including Streptococcus pneumoniae in patients admitted with community acquired septicemia and those who developed nosocomial septicemia during their stay in the hospital.

Methods: Venous blood, 5 ml from adults and 3 ml from children was collected aseptically for blood culture. Organisms were identified and subjected to antibiotic susceptibility testing to relevant antibiotics.

Results: A total of 7147 adults and children were studied. Among these 1040 patients had positive blood culture. Gram negative bacteria were responsible for the majority of infections. Community acquired septicaemia was seen in 556 (53.4%) patients while septicaemia of nosocomial origin was recorded in 484 (46.5%). Blood culture was positive in 26 (2.5%) patients -19 (73%) children and 7 (27%) adults for S. pneumoniae infection. S. pneumoniae was sensitive to penicillin and erythromycin in 19 (73%) patients while cloxacillin, gentamicin and netilmicin in 23 (88.4%) and cefotaxime in 26 (100%) patients.

Conclusions: This study highlights the rising level of drug resistance amongst the bacterial isolates including S. pneumoniae from blood and hence the need to update and formulate newer drug policies. There is need for controlling of spread of these resistant strains before they reach an alarming level in the region.

No conflict of interest
ANTIMICROBIAL SUSCEPTIBILITIES OF STREPTOCOCCUS PNEUMONIAE ISOLATED FROM INVASIVE PNEUMOCOCCAL DISEASE PATIENTS IN CHINA

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Background and Aims: Invasive pneumococcal disease (IPD) is a leading cause of morbidity and mortality worldwide, especially in China. The epidemiology data of IPD in China are very limited. Therefore, this study aimed to assess the antimicrobial susceptibilities of Streptococcus pneumoniae isolated from IPD patients in both children and adults in China.

Methods: From 2010 to 2013, S. pneumoniae isolated from sterile sites in patients with clinical signs and symptoms of IPD were collected. All isolates were sent to the clinical microbiology lab of Peking Union Medical College Hospital for testing of antimicrobial susceptibilities of 16 antibiotics against all the strains using Etest®.

Results: A total of 114 isolates were collected from 18 hospitals in China, with 55 isolates (48.2%) from children (<18 years) and 59 isolates from adults (≥18 years). Among these isolates, 78 (68.4%) were collected from blood, 18 (15.8%) from cerebrospinal fluid, 15 (13.2%) from pleural fluid, 2 (1.6%) from joint fluid and 1 (0.9%) from lung tissue. In the 18 isolates from meningitis patients, only 11.7% were penicillin-susceptible S. pneumoniae (PSSP), whereas in the 96 isolates of non-meningitis isolates, 91.7% were PSSP. The most susceptible agents against all the isolates were moxifloxacin, linezolid and vancomycin (100% susceptible), followed by ertapenem and levofloxacin (99% susceptible). Erythromycin, azithromycin and clindamycin show poor activities (>94% resistant) against S. pneumoniae.

Conclusion: S. pneumoniae isolated from meningitis patients were highly resistant to β-lactams, but isolates from bacteremia, pneumonia and arthritis patients were still frequently susceptible to penicillin in China.

Conflict of interest

ANTIMICROBIAL RESISTANCE IN COLONISING PNEUMOCOCCI IN A COHORT OF INFANTS AND THEIR MOTHERS LIVING ON THE THAILAND-MYANMAR BORDER

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Background and Aims: The prevalence of, and risk factors for, colonisation by antimicrobial resistant pneumococci were explored as part of a longitudinal study of Streptococcus pneumoniae colonisation in a cohort of refugees from the Thailand-Myanmar border.

Methods: 234 mother-infant pairs were followed from birth for 24 months. Nasopharyngeal swabs were taken at monthly visits and cultured following the WHO pneumococcal carriage protocol. Antimicrobial susceptibilities were determined by disk diffusion +/- Etest (CLSI breakpoints). Pneumococci were termed multi-drug resistant (MDR) if resistant to ≥3 of chloramphenicol, erythromycin, penicillin (MIC ≥0.12 µg/mL), tetracycline, co-trimoxazole. MLST genotyping was done for a proportion of isolates.

Results: 2,050 pneumococci were analysed (one isolate/serotype carriage episode). 40.0% of infant isolates were penicillin non-susceptible (PNS) and 34.6% were MDR. PCV13 serotypes were more likely to be MDR (p < 0.001). However, 80.5% of non-typeable isolates were PNS and 38.5% were MDR. The proportion of antimicrobial resistant isolates was greater in infants compared to mothers (MDR: 34.6% vs. 26.9%, p = 0.001; PNS 40.0% vs. 34.3%, p = 0.02). The presence of other young children in the house was associated with a greater risk of acquisition of both MDR and PNS pneumococci in infants (p < 0.001). Acquisition of another pneumococcal serotype at the preceding NPS was protective against acquisition of a drug resistant pneumococcus (p < 0.001).

Conclusion: Antimicrobial resistant pneumococci were frequently carried by infants and their mothers in this rural Asian refugee population: PCV13 serotypes predominated. Non-typeable pneumococci were also frequently resistant and these isolates may provide a persistent reservoir of resistance genes in the population.

No conflict of interest
A SURVEILLANCE OF PNEUMOCOCCAL INFECTIONS IN ADULTS, >50 YEARS OF AGE, IN A TERTIARY CARE HOSPITAL IN NEW DELHI (INDIA)

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Introduction: Streptococcus pneumoniae is a major cause of mortality and morbidity in elderly patients. In the present study we evaluated antimicrobial susceptibilities and serotypes of pneumococcal isolates recovered in New Delhi, India.

Methodology: All subjects ≥ 50 years of age presenting with presumed diagnosis of community acquired pneumonia were included in the study. A total of 20 clinical isolates of S. pneumoniae were isolated and investigated. They were subjected to disk diffusion susceptibility testing, MIC testing by E-test and serotyping by Quellung reaction.

Results: Susceptibility testing showed that 100%, 95%, 90%, 95%, 40% and 100% sensitivity to penicillin, levofloxacin, erythromycin, clindamycin, cotrimoxazole, and chloramphenicol, respectively. Serotypes 9, 6, 8, 19F were more common and formed 40% of serotypes. Serotypes 3, 19A, 23F, 17 and 5 each accounted for 6.25% of isolates. Most importantly 2 (20%) of serotypes were from non-vaccine (23-valent polysaccharide) group.

Conclusion: Preliminary data of our study identifies no resistance of S. pneumoniae to penicillin and a low rate of resistance to quinolones or macrolides and high rate of resistance to cotrimoxazole. This study also identifies prevalence of non-vaccine serotypes in India, which impresses the need to re-evaluate the efficacy of the current vaccine.

Conflict of interest

DETECTION OF ANTIBIOTICS RESISTANCE OF BACTERIAL AGENTS ISOLATED FROM CHILDREN WITH PNEUMONIAE IN HAMADAN, WEST IRAN

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Background and Aims: Bacterial pneumonia is still one of the most dangerous infection diseases and causes serious complications and mortalities in children. The aim of the present study was to identify the most common of bacterial agents causing pneumonia in children less than 12 years old and detection of their resistance to current antibiotics in Hamadan.

Methods: Overall 542 children suspected to pneumonia were investigated for results of pleural fluid cultures and antibiogram patterns. Frequency of age, sex and seasons of patients were also studied from 1999 to 2003. The data were gathered through a questionnaire and analysed using Epi6 system. The species were identified by biochemical and serological methods. Antibiogram tests were also performed by Kirby-Bauer method.

Results: Out of 542 children suspected to have pneumonia, 72 cases (13.2%) had positive bacterial culture that 54.4% were Gram negative and 43.6% were also Gram positive bacteria. The most common species were: Staphylococcus aureus (18.6%), Streptococcus pneumoniae (16.9%), Klebsiella ozaenae (12.3%), Pseudomonas aeruginosa (11.80%), Haemophilus influenzae (9.4%). The results of antibiogram showed that the most effective antibiotics were azithromycin, ceftriaxone, gentamycin and ciprofloxacin for both Gram positive and Gram negative bacteria, but they showed high resistance to erythromycin, cefixime tetracyclin, amoxicillin and ampicillin.

Conclusion: The present study showed that some Gram positive bacteria in particular, S. aureus and S. pneumoniae are predominant causes of bacterial pneumonia in children less than 12 years old in these regions. Most species showed high resistance to routine antibiotics such as tetracycline, amoxicillin and ampicillin.

Conflict of interest