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Distribution of capsular types and drug resistance patterns of invasive pediatric *Streptococcus pneumoniae* isolates in Teheran, Iran

Hamidreza Houri[a], Sedigheh Rafiei Tabatabaei[b,*], Yasaman Saee[c], Fatemeh Fallah[a,*], Mohammad Rahbar[d], Abdollah Karimi[b]

[a] Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[b] Department of Clinical Pharmacy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[c] Pediatric Infections Research Center, Mofid Children's Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[d] Health Reference Laboratories, Research Center, Ministry of Health and Medical Education, Tehran, Iran

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SUMMARY

Objectives: To explore the serotype distribution and drug resistance patterns of invasive pneumococcal isolates from children under 5 years of age.
Methods: During a 32-month period, 585 clinical samples (including blood, cerebrospinal fluid (CSF), and synovial fluid) from children suspected of having meningitis, sepsis, pneumonia, or septic arthritis were analyzed using the BACTEC culture system. Positive cultures were examined using biochemical tests and *lytA* amplification for the identification of pneumococcal strains. The confirmed pneumococcal isolates were examined to determine capsular types using a modified sequential multiplex PCR and susceptibility to antimicrobial agents.
Results: Fifty-three pneumococcal isolates were detected in the 585 clinical samples: 21 (39.6%) blood samples and 32 (60.4%) CSF samples. The most frequent serotype was 23F (24.5%), followed by serotypes 19F (18.9%), 19A (7.5%), and 9V (7.5%). Twenty-one percent of pneumococcal isolates were penicillin-non-susceptible and serotype 19A was significantly associated with resistance to penicillin.
Conclusions: This study indicated that the 13-valent pneumococcal conjugate vaccine (PCV13) could cover the majority of the invasive pneumococcal isolates. Drug-resistant and multidrug-resistant *Streptococcus pneumoniae* strains are circulating in Iran. Therefore, public immunization of infants using PCV13 is recommended to reduce the incidence of pneumococcal disease and pneumococcal-resistant strains in Teheran.

Introduction

Streptococcus pneumoniae, often referred to as the pneumococcus, can colonize the human nasopharynx asymptomatically and sometimes causes a wide range of adverse health effects, ranging from non-invasive infections (including acute otitis media and sinusitis) to serious and lethal diseases (such as meningitis, invasive pneumonia, and bacteremia).[1,2] Invasive pneumococcal disease (IPD) is determined by the infection of any sterile body site and is recognized as a major cause of morbidity and mortality worldwide.[3] Despite the availability of effective antibiotics and vaccines, the World Health Organization (WHO) reported that IPD was responsible for approximately 1.6 million deaths around the world in 2008.[4] Infants and young children under 5 years of age are most susceptible to IPD due to the lack of a mature immune system and frequent contact with and colonization by pneumococcal strains.[5] The infant mortality rate due to IPD is particularly significant in disadvantaged and low-income countries, where it causes approximately a quarter of all preventable deaths in children under 5 years of age and more than 1.2 million infant deaths annually.[6,7] Therefore, early identification followed by immediate antibiotic therapy is vital to achieving promising treatment outcomes in IPD.[8] The diagnosis of IPD requires the isolation of pneumococcus from a normally sterile site, such as blood, cerebrospinal fluid (CSF), and pleural or ascitic fluid.[9]

Pneumococci are divided into more than 90 capsular types based on the structural characterization of the antigenic capsular polysaccharide.[10] The polysaccharide capsule, which delivers resistance to phagocytosis in the absence of type-specific antibody

* Corresponding authors. Tel./fax: +98 21 22226941.
E-mail addresses: srtabatabaei@sbmu.ac.ir (S.R. Tabatabaei), fafallah@sbmu.ac.ir (F. Fallah).

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and helps the bacteria to evade the host's defenses, plays a major role in the invasion of pneumococcus into the systemic blood system.[11] Serotype-specific pneumococcal polysaccharide vaccines (PPVs) and pneumococcal conjugate vaccines (PCVs) have been designed according to the pathogenic mechanism of *S. pneumoniae*, which combine the capsular polysaccharides of the most prevalent invasive serotypes.[12] The WHO recommends universal immunization against this pathogen as the best preventive strategy against pneumococcal disease. The issue has become more important with the growing emergence of multidrug-resistant pneumococcal strains during recent decades.[13] Because the pneumococcal capsule plays a significant role in pathogenesis and the distributions of the invasive serotypes vary geographically, knowledge of the serotype-specific epidemiology is required for an appropriate vaccine schedule.

Since data on the epidemiology of invasive pneumococcal serotypes in Iran are inadequate, the present study investigated the prevalence, serotype distribution, and drug resistance patterns of invasive pneumococcal isolates from children with suspected IPD under the age of 5 years.

Methods

Patients and bacterial isolation

This study involved 585 children aged <5 years with suspected IPD admitted to several teaching and university medical centers, community hospitals, and two children's hospitals in Teheran, Iran, from July 2013 to March 2016. A case of IPD was defined by the isolation of pneumococci from a normally sterile body site.[3] In this study, 381 blood samples were collected from children with suspected sepsis, pneumonia, or septic arthritis, 200 CSF samples from children with suspected infectious meningitis, and four synovial fluid samples from children with suspected septic arthritis; these samples were immediately inoculated into BACTEC vials for the BACTEC system (BD BACTEC Peds Plus/F Culture Vials). Samples were only taken from one site per patient. When cultures were found to be radiometrically positive, subcultures were streaked on Columbia agar (Merck Co., Germany) with 5% sheep blood supplemented with 5 μg/ml gentamicin. These were incubated for 24 h at 35 °C in the presence of 5% CO_2.[14]

Bacterial identification

Phenotypic and conventional biochemical tests were performed on radiometrically positive samples. Gram staining, observed alpha hemolysis on blood agar, and catalase and optochin susceptibility testing were performed to confirm the isolates as *S. pneumoniae*. When necessary, a bile (sodium deoxycholate) solubility test was also performed.[15] In order to confirm the pneumococcal diagnosis, *lytA* amplification was performed as described previously.[16]

Antimicrobial susceptibility testing

Antibiotic susceptibility testing was performed using the broth microdilution method in accordance with the Clinical Laboratory and Standards Institute guidelines (CLSI-2014). The medium used for this test was cation-adjusted Mueller–Hinton broth (BD Co., USA) with 5% lysed horse blood. Minimum inhibitory concentrations (MICs) of penicillin, cefotaxime, ceftriaxone, levofloxacin, vancomycin, linezolid, trimethoprim–sulfamethoxazole, erythromycin, and tetracycline (Sigma-Aldrich Co., USA) were determined (erythromycin, trimethoprim–sulfamethoxazole, and tetracycline were tested only on non-meningeal isolates). *S. pneumoniae* ATCC 49619 was used as the standard strain. The results were interpreted according to current CLSI breakpoints for meningeal and non-meningeal isolates, individually.

DNA extraction

Bacterial cells were suspended in 250 μl of phosphate buffered saline, followed by DNA extraction using the High Pure PCR Template Preparation Kit (Roche Co., Germany) according to the manufacturer's instructions. Eluted DNA was stored at −20 °C for later analysis.

Molecular capsular typing by multiplex PCR

First, all of the confirmed pneumococcal isolates were examined for amplification of *cpsA*. A multiplex PCR was then performed with specific primer sequences in seven reactions, as per Pai et al., with minor modifications designed to cover most of the predominant serotypes reported in Asia and Africa.[17] Accordingly, each reaction was designed to contain four primer pairs targeting serotype-specific genes of four individual serotypes and included an internal positive control targeting all known pneumococcal *cps* loci (Supplementary Material (Table S1)). Specifications of the primers used in each reaction in this study are summarized in the **Supplementary Material** (Table S1). The PCR products were analyzed by gel electrophoresis on 2% NuSieve agarose gels in 1 × TAE buffer (Tris base, acetic acid, and ethylenediaminetetraacetic acid) at 120 V for 45 min. The size of the PCR products was determined by comparison with a molecular size standard.

Statistical analysis

IBM SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA) was applied for the statistical analysis. The Chi-square test was used for the comparison between serotypes and resistance to penicillin. A p-value of <0.05 was considered to be statistically significant.

Results

Identification of pneumococcal isolates

During the 32-month period, 585 blood and CSF samples were collected from children under 5 years of age (mean age 2.7 ± 0.5 years) with clinical signs of IPD. Of the 585 samples, 189 (32.3%) were radiometrically positive according to the BACTEC system and confirmed for the existence of live bacteria. The instrument also gave three (1.6%) false-positive signals. Out of the 189 organisms isolated, *S. pneumoniae* was detected and identified in 53 (28%) specimens by conventional diagnostic methods and amplification of the *lytA* gene. Twenty-one (39.6%) pneumococcal strains were isolated from blood samples and 32 (60.4%) from CSF samples. No *S. pneumoniae* was isolated from any synovial fluid sample. The majority of the pneumococcal invasive strains (47.2%) were isolated from children aged between 4 and 24 months.

Antimicrobial resistance patterns in pneumococcal isolates

Tables 1 and 2 show the antibiotic susceptibility of the pneumococcal isolates. Eleven (20.8%) pneumococcal isolates were penicillin-non-susceptible, including nine (17%) penicillin-resistant strains (MIC ≥8 μg/ml for non-meningeal isolates and MIC ≥0.12 μg/ml for meningeal isolates) and two (3.8%) intermediate penicillin-resistant strains (MIC = 4 μg/ml). MIC_{50} and MIC_{90} values of penicillin were 1 μg/ml and 8 μg/ml, respectively, for non-meningeal isolates, and 0.06 μg/ml and 0.12 μg/ml,