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Original article

Serotype distribution and antimicrobial resistance of *Streptococcus pneumoniae* causing noninvasive diseases in a Children's Hospital, Shanghai

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ABSTRACT

Background: *Streptococcus pneumoniae*, which cause noninvasive pneumococcal diseases, severely impair children's health. This study analyzed serotype distribution and antimicrobial resistance of *S. pneumoniae* from January 2012 to December 2012 in a Children's Hospital, Shanghai.

Methods: A total of 328 pneumococcal isolates were serotyped by multiplex sequential PCR and/or capsule-quellung reaction. The minimum inhibitory concentrations for 11 antimicrobial agents were determined by broth microdilution method.

Results: Among 328 strains, 19F (36.3%), 19A (13.4%), 6A (11.9%), 23F (11.0%), 14 (5.8%), 6B (5.2%), and 15B/C (4.3%) were the most common serotypes. The coverage rates of 7-, 10-, and 13-valent conjugate vaccines (PCV7, PCV10, and PCV13) were 58.2%, 58.2%, and 84.1%, respectively. Out of the isolates, 26 (7.9%) strains were penicillin resistant. Most of the strains displayed high resistance rate to macrolides (98.5% to erythromycin, 97.9% to azithromycin, and 97.0% to clindamycin).

Conclusions: The potential coverage of PCV13 is higher than PCV7 and PCV10 because of the emergence of 19A and there should be long-term and systematic surveillance for non-vaccine serotypes.

Introduction

Streptococcus pneumoniae remains a major pathogen of pneumonia, meningitis and acute otitis in children, with high morbidity and mortality. According to a survey conducted by the World Health Organization (WHO), 50–60% of the 1.6 million deaths caused by pneumococcal infections in 2005 were in children younger than five years old, especially in developing countries.[1]

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Heptavalent pneumococcal conjugate vaccine (PCV7), targeting seven of more than 90 serotypes of *S. pneumoniae* (serotypes 4, 6B, 9V, 14, 18C, 19F, 23F), has reduced the burden of invasive pneumococcal diseases (IPD) and nasopharyngeal carriage rate of these serotypes in vaccinated children in developed countries.[2,3] Moreover, several reports indicate that in some countries PCV7 is also effective against noninvasive pneumococcal disease (NIPD) like pneumonia.[4,5] In USA, Grijalva et al.[4] reported that the all-cause pneumonia admission rates had declined by 39% for children younger than two years following licensure of PCV7. However, after the introduction of PCV7 the incidence of pneumococcal diseases caused by non-PCV7 serotypes such as 6A and 19A increased.[6,7] The Food and Drug Administration (FDA) approved two more vaccines: the 10-valent pneumococcal conjugate vaccine (PCV10) including serotypes 1, 5, and 7F in addition to PCV7 serotypes and the 13-valent pneumococcal conjugate vaccine (PCV13) including serotypes 3, 6A and 19A in addition to PCV10 serotypes. Both proved to be more effective than PCV7.[8–9]

In Mainland China, PCV7 has not yet been introduced into childhood immunization program despite being available in the market since 2008. Information about the use of PCV10 and PCV13 in Mainland China has also been unclear. It has been proposed that the choice of *S. pneumoniae* vaccine should be based on the distribution of serotypes, which vary with age, geographic region and time.[10] Therefore, systematic and accurate assessment of the serotype distribution of *S. pneumoniae* is necessary for choosing an optimal vaccine.

Interestingly, NIPD accounts for a major proportion of all pneumococcal infections. O'Brien et al.[11] reported that out of 14.5 million pneumococcal cases, 95.6% were cases of NIPD while only 4.4% were IPD. However, data about *S. pneumoniae* isolated from children with NIPD is rare. Moreover, continued surveillance of the distribution of pneumococcal serogroup isolated from specimens of non-sterile sites may help determining which serogroups are important in the development of invasive disease.[5] In this study, we investigated *S. pneumoniae* isolated from children with NIPD in 2012. The purpose of this surveillance study was to determine the serotype distribution and antimicrobial resistance pattern of *S. pneumoniae* strains causing NIPD in Shanghai, a metropolitan city located in the east of China. Such information could provide guidance for further clinical and epidemiologic studies, rational administration of antimicrobial agents, and selection of proper vaccines.

Material and methods

Study design and data collection

This surveillance was conducted between January 2012 and December 2012 in Shanghai Children's Hospital, which is one of the largest pediatric hospitals in Shanghai. The hospital serves 1.13 million outpatients and 20,000 inpatient admissions annually. All specimens were collected from children (0–14 years of age) who were hospitalized and diagnosed with NIPD. NIPD was defined as *S. pneumoniae* strains causing infection detected in ear, eye, nasopharynx, or tracheal aspirate specimens and in which no invasive (sterile sites) isolates were collected from the same patient.[12] Furthermore, the clinical data of enrolled patients including demographics, admission and/or discharge diagnosis, time of sampling, recent use of antimicrobial agents, vaccination history were obtained from the medical records. If two isolates expressing the same serotype from the same patient, only one isolate was included with duplicates being discarded.

Clinical isolates and microbiological tests

All specimens were processed in a microbiology laboratory according to the "National Guide to Clinical Laboratory Procedures".[13] A total of 328 non-duplicated strains were included. The samples were sent to the microbiology laboratory and inoculated on 5% sheep blood agar plates. The plates were incubated at 37 °C in a 5% CO_2 incubator and examined after 24–48 h. Typical colonies of *S. pneumoniae* were subcultured and identified by using the optochin disk (Oxoid, Hampshire, UK) and bile solubility test.[14] All strains were stored at −80 °C in 40% glycerol broth medium until further analysis. Pneumococcal isolates were serotyped by multiplex sequential PCR[15] and/or capsule-quellung reaction[16] with a set of antisera from the Statens Serum Institute (Copenhagen, Denmark). The minimum inhibitory concentrations (MICs) for penicillin (PEN), cefuroxime (CXM), ceftriaxone (CRO), erythromycin (ERY), azithromycin (AZM), clindamycin (CLI), levofloxacin (LEV), moxifloxacin (MXF), vancomycin (VAN) and trimethoprim-sulfamethoxazole (SXT) were determined by broth microdilution method according to the 2013 guidelines of the Clinical and Laboratory Standards Institute (CLSI M100-S23). *S. pneumoniae* ATCC 49619 was used as the control strain for the susceptibility test.

Statistical analysis

Whonet 5.6 software[17] and SPSS 16.0 (Statistical Package for Social Science, USA) were used for susceptibility statistics and analysis. The χ^2 test or Fisher's exact test of program were used for comparing categorical data. Two-tailed p-values <0.05 were considered statistically significant.

Results

Demographic data of patients

A total of 328 isolates of *S. pneumoniae* were included being 61.3% (201/328) detected in male patients and 38.7% (127/328) in females. Patients aged between 0 and 14 years old were divided into four groups: infant (<1 year, 37.8%), toddler (1–2 years, 28.4%), preschooler (3–5 years, 27.7%) and schooler (6–14 years, 6.1%). Upper respiratory tract infections were diagnosed in 243 children (74.1%) and 35 (10.7%) had a history of contact with other infected patients. All of these patients had history of prior hospitalization or recent physician visit and therapy with antibiotics. Only four children (1.2%) had received PCV-7 vaccination.