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Serotypes and antibiotic resistance of non-invasive *Streptococcus pneumoniae* circulating in pediatric hospitals in Moscow, Russia

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SUMMARY

Background: Pneumococcal infections remain a major medical problem associated with high morbidity and mortality. Moreover, the resistance of *Streptococcus pneumoniae* to conventional antibiotics is constantly growing. The implementation of pneumococcal conjugate vaccines (PCVs) in the last decade has dramatically reduced the incidence of the vaccine type-associated invasive pneumococcal diseases in many countries. However, information on the seroepidemiology of *S. pneumoniae* in Russia is limited.

Methods: We report the results of serotyping and antibiotic susceptibility testing performed on 863 non-invasive pneumococcal isolates collected prospectively in 2009–2013 from children (median age 3.5 years) who sought medical care at five pediatric hospitals in Moscow. The isolates were recovered from the nasopharynx (71.2%), middle ear fluid (14.3%), and lower respiratory tract specimens (13.6%).

Results: In total, we identified 45 different serotypes. The six leading serotypes (prevalence >5%) included 19F (21.7%), 6B (12.8%), 23F (10.1%), 14 (9.0%), 6A (8.4%), and 3 (7.5%). Serotype 19A isolates had a prevalence of 2.3%. The proportion of PCV-13 serotypes was 78%; the coverage by PCV-7 was 58.2% and was similar to that of PCV-10 (59.8%). The rate of multidrug-resistant pneumococci (i.e., resistant to ≥3 antimicrobials) was 22%. The majority of the multidrug-resistant isolates were serotype 6B, 14, 19A, and 19F. Penicillin non-susceptibility was displayed by 28% of the isolates. The resistance rate to erythromycin was 26%. Among the examined erythromycin-resistant strains, 54% had the *erm(B)* gene and 13% had the *mef* gene as a single resistance determinant, whereas both determinants were found in 31% of these strains.

Conclusions: Our data predict a good coverage of the circulating *S. pneumoniae* by the PCVs and could be useful for evaluating the serotype distribution in support of the introduction of PCV in Russia. In addition, the antimicrobial resistance rate of *S. pneumoniae* in Russia is substantial, and the emergence of pneumococcal strains with a dual macrolide resistance mechanism is alarming.

1. Introduction

Streptococcus pneumoniae (pneumococcus) is a common bacterial pathogen responsible for various infections, especially in children. The severity of pneumococcal illness varies from self-limiting mucosal infections to life-threatening invasive diseases like bacteremia and meningitis. *S. pneumoniae* is the leading cause of pediatric community-acquired pneumonia and acute otitis media (AOM), which have a substantial burden on healthcare resources worldwide.[1–4] Pneumococcal infections contribute to a large number of medical care visits and antibiotic prescriptions in children. This coincides with the growing antibacterial resistance of *S. pneumoniae* to a wide range of antibiotics that are used to treat pneumococcal infections.[5–7]

The real burden of pediatric pneumococcal infections in the Russian Federation is difficult to estimate precisely because the surveillance system is underdeveloped. A low rate of blood and

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cerebrospinal fluid (CSF) culture in conjunction with the common practice of parenteral antibiotic administration in children suspected to have bacteremia or meningitis before any laboratory investigations, preclude the assessment of the true incidence of invasive pneumococcal disease (IPD) in these patients. In addition, only a few laboratories in the country are able to isolate, identify, and serotype *S. pneumoniae* reliably. Thus, figures on the incidence of IPD in the Russian Federation are mainly based on expert evaluations. These data estimate the following incidence of IPD per 100 000 children under 6 years of age per year: pneumococcal meningitis, 0.22–0.53; pneumococcal bacteremia, 58.5–130; pneumococcal community-acquired pneumonia, 490–1300.[1,8–10] In addition, the incidence of severe hospitalized pneumococcal AOM has been assessed as being 97.4–122.1 per 100 000.[8]

Pneumococcal capsular polysaccharide represents the principal virulence factor that protects the bacteria from phagocytosis and generates the specific antibody immune response. More than 90 variants of capsular polysaccharide, i.e. pneumococcal serotypes, have been described so far. Not all serotypes are equally pathogenic, and the majority of pneumococcal infection is associated with a limited number of serotypes.[11] A subset of capsular polysaccharides from clinically important serotypes is included in the pneumococcal polysaccharide conjugate vaccines (PCV). The introduction of PCVs into national immunization programs has been shown to substantially decrease the incidence of IPD caused by vaccine-type pneumococci in many countries.[2,12–16]

Three PCVs (7-, 10-, and 13-valent) are licensed in the Russian Federation, but none has yet been implemented in the national immunization program. To estimate the prophylactic potential of the PCVs on pneumococcal infections, it is important to know how close they match the set of circulating serotypes. The actual local data on pneumococcal serotypes are limited and fragmented, and only a few reports from Russia have been published in the international literature in the last decade.[17–19] In the present article, we report serotyping and antibiotic susceptibility testing data for a large collection of *S. pneumoniae* clinical non-invasive isolates obtained prospectively in a number of pediatric hospitals in Moscow. The vast majority of isolates were recovered from non-sterile respiratory specimens and middle ear fluid (MEF) from children who sought medical care suffering primarily from acute febrile respiratory infections and AOM.

2. Materials and methods

This prospective study included all isolates of *S. pneumoniae* that were recovered from specimens collected at five pediatric hospitals located in Moscow during March 2009 to April 2013. The pneumococci were isolated from various biological materials collected from patients (median age 3.5 years, interquartile range 0.08–6.0 years) with fever and symptoms of an acute respiratory infection presumably of bacterial etiology, with AOM, and with chronic lung disease.

S. pneumoniae was isolated from several specimen types, including nasopharyngeal swabs, lower respiratory tract specimens (sputum, tracheal, or bronchial aspirates), MEF obtained from children with AOM by tympanocentesis or by swabbing the ear discharge in the case of spontaneous draining, and some others. Biological material was collected using an eSWAB kit (Copan, Italy). Signed informed consent was obtained from the parents or legal representatives of enrolled children before sampling.

All specimens were delivered to the laboratory of the Scientific Center for Children's Health (Moscow) and processed there within 24 h after sampling. The specimens were plated on blood agar medium with 5% sheep blood and 2% horse serum and incubated at 37 °C with 5% CO_2 for 24–48 h. *S. pneumoniae* was identified by optochin test and latex agglutination with the Slidex pneumo-kit (bioMérieux, France). Serotyping was performed by pool antisera for latex agglutination and factor/type antisera in the Quellung reaction using Staten Serum Institut products (SSI, Copenhagen, Denmark). Isolates that agglutinated none of the pool sera (A to I and P to T) were considered non-typeable.

Antibiotic susceptibility testing was done using the disk diffusion method with disks from Bio-Rad (USA). Oxacillin-resistant isolates were further tested for penicillin susceptibility by E-test strips (Oxoid, UK). The penicillin minimum inhibitory concentration (MIC) category interpretations were based on updated standards (European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2012). Intermediate and resistant isolates were collectively grouped as non-susceptible. A PCR was used to detect the *erm(B)* and *mef* determinants of macrolide resistance, as described previously.[19]

The statistical analysis was performed using IBM SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, NY, USA). Contingency table analysis for comparing serotype distributions was done by Chi-square or Fisher's exact test, where appropriate. Proportions of serotypes were compared by means of the *Z*-criterion. The tests were considered statistically significant at $p < 0.05$.

3. Results

In total, 863 pneumococcal isolates were collected. The majority of strains were obtained from nasopharyngeal swabs (n = 617, 71.2%). One hundred eighteen isolates (13.6%) were recovered from sputum and tracheal/bronchial aspirates (lower respiratory tract specimens), and MEF provided 124 (14.3%) isolates. In addition, four strains were isolated from other loci (three from the vagina and one from the eye).

3.1. Serotype distribution

Serotyping was performed for 835 isolates; 10 (1.2%) isolates were not preserved for typing and 18 (2.1%) strains were non-typeable. Thus, the serotype was determined in 835 (96.8%) pneumococcal isolates.

In total, 45 different serotypes were identified. Six major serotypes accounted for 69.5% of the distribution and included serotype 19F (21.7%), 6B (12.8%), 23F (10.1%), 14 (9.0%), 6A (8.4%), and 3 (7.5%) isolates (Table 1).

Among the nasopharyngeal specimens, 44 different serotypes were recovered, whereas only 18 different serotypes were isolated from the MEF specimens. Serotype prevalence in nasopharyngeal, lower respiratory tract, and MEF specimens compared by the Chi-square test was different (Chi-square = 118, p = 0.018), however a paired statistical analysis showed that the proportion of serotypes varied significantly only between nasopharyngeal and MEF specimens (Chi-square = 64, p = 0.015). Serotype 3 (12.3% vs. 6.8%, Z = 2.13, p = 0.033) and 19A (6.6% vs. 1.6%, Z = 3.42, p = 0.001) strains were more prevalent among MEF isolates than among the other specimen types. In contrast, no serotype 11A isolates were recovered from MEF, and the proportion of this serotype was significantly higher in the nasopharyngeal and lower respiratory tract specimens (0% vs. 3.5%, Z = −2.11, p = 0.039). The prevalence of all remaining serotypes was not statistically different between the specimen sources.

The four *S. pneumoniae* strains recovered from the other loci had serotypes 11A, 19F, 23F (vaginal isolates) and 6A (a conjunctival isolate).

Considering marginal differences in the serotype distribution, the pneumococci from all sources were collectively designated as 'non-invasive isolates' for further analysis.