

Original Article

Nasopharyngeal Carrier Rate of *Streptococcus pneumoniae* in Children: Serotype Distribution and Antimicrobial Resistance

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Abstract

Background: This study aimed to define the nasopharyngeal carrier rates, serotype distribution, and antimicrobial resistance patterns of *Streptococcus pneumoniae* in healthy children less than ten years-old in Tehran.

Methods: This was a cross-sectional study conducted from November 2008 until January 2009. Nasopharyngeal specimens were collected by trained investigators from 1300 healthy children recruited from 20 randomly selected day care centers and 50 elementary schools in Tehran and inoculated into blood agar. Positive cultures that grew alpha-hemolytic colonies were gram-stained and serotypes of the isolates identified by the Quellung reaction. All isolated pneumococci were tested for sensitivity to different antibiotics by the disc diffusion method.

Results: The carrier rate for *Streptococcus pneumoniae* was 44.1%. Serotypes 19, 6, 14, 17, 20, 23, and 21 were most common, found in decreasing order from 11.9% to 6.1%. Only 38.56% of isolates belonged to strains covered by the heptavalent pneumococcal vaccine. Most (69.4%) were resistant to one or more antimicrobial agents: tetracycline (69.85%), clarithromycin (57.2%), azithromycin (54.9%), cotrimoxazole (11.8%), penicillin (9.2%), and vancomycin (1.5%). All isolates were sensitive to rifampin and meropenem.

Conclusion: This study has revealed the nasopharyngeal carrier rate, serotype distribution and antibiotic sensitivities of *Streptococcus pneumoniae* strains in children from Tehran. Our findings may have implications on the type and efficacy of pneumococcal conjugate vaccines that should be used for prevention of pneumococcal invasive disease in Iranian children.

Keywords: Nasopharyngeal carrier, pneumococcal vaccine, serotype, *streptococcus pneumoniae*

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Introduction

Pneumococcus or *Streptococcus pneumoniae* is a major cause of meningitis, bacteremia and sepsis, and a leading cause of morbidity and mortality worldwide.^{1,2}

The resident microbial floras in the nasopharynx, including pneumococci, constitute a reservoir of pathogens implicated in respiratory tract infections and invasive diseases in children and adults.²⁻⁵ *Streptococcus pneumoniae* which is carried in the nasopharynx of healthy children may reflect the infection-causing strains currently circulating in the community; it has often been reported as a precursor of invasive disease and a major factor in the spread of infection.^{2-4,6} The rate of antibiotic-resistant pneumococci has steadily increased in recent years. Studies to determine the prevalent pneumococcal serotypes and their antibiotic sensitivities are necessary for planning rational strategies for preventing and treating invasive pneumococcal disease in different communities.^{2,3}

The aim of this study was to determine the nasopharyngeal carrier rate, serotype distribution, and antimicrobial resistance patterns of *Streptococcus pneumoniae* in healthy children less than ten years of age in Tehran.

Materials and Methods

This cross-sectional study was performed from November 2008 to January 2009. A multistage sampling was performed. At first, 20 daycare centers and 50 elementary schools were chosen from an official list by simple random sampling that included about 2% of all day care centers and elementary schools. Next, 20 children were selected from each center or school by systematic random sampling. Healthy children less than ten years of age were recruited for the study.

Children with respiratory tract infections, chronic diseases, congenital cranio-facial anomalies, or those who had received antibiotics within two weeks prior to study entry were excluded.

After obtaining parental consent, members of the study team examined each child and documented relevant data on a pre-designed questionnaire.

Nasopharyngeal specimens (one from each child) were collected by a trained investigator. An extra-thin flexible wire swab with its tip bent at an angle of 30° was inserted through the mouth and placed 1–2 cm into the nasopharynx without touching the uvula or the tongue and kept in place for at least five seconds. The nasopharyngeal cultures were injected into Stuart transport medium tubes, which were sent to the microbiology laboratory within three hours and immediately processed at 37°C in complex media that contained blood. On blood agar, colonies characteristically produced a zone of alpha (green color) hemolysis, which differentiates *Streptococcus pneumoniae* from group A (beta-hemolytic) streptococcus. Gram staining was performed on alpha-hemolytic colonies,

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Table 1. Pneumococcal serotypes isolated from nasopharyngeal samples ($n = 573$).

Serotype	Number	%	Serotype	Number	%
19	68	11.8	10	11	1.9
6	58	10.1	35	10	1.7
14	55	9.6	1	10	1.7
17	42	7.3	27	10	1.7
20	40	6.9	41	8	1.4
23	39	6.8	47	8	1.4
21	35	6.1	7	8	1.4
11	30	5.2	5	7	1.2
33	29	5	36	5	0.9
Not serotyped	28	4.9	8	1	0.2
22	27	4.7	9	1	0.2
16	23	4	60	1	0.2
25	19	3.3	—	—	—

Table 2. Pattern of antibiotic resistance in pneumococci isolated from the nasopharynx ($n = 573$).

Antibiotics	Number of resistant specimens	%
Tetracycline	377	65.7
Clarithromycin	328	57.2
Azithromycin	315	54.9
Cotrimoxazole	68	11.8
Penicillin	53	9.2
Chloramphenicol	34	5.9
Ceftriaxone	26	4.5
Amoxicillin	23	4.0
Cefotaxime	17	2.9
Cloxacillin	11	1.9
Vancomycin	9	1.5
Ciprofloxacin	9	1.5
Rifampin	0	0
Meropenem	0	0

which were observed under a microscope. Gram positive diplococci were further tested to isolate the *Streptococcus pneumoniae* serotyped by the Quellung reaction using the antisera provided by the Mast Assure™ *Streptococcus Pneumoniae* Typing Antisera Kit (Mast Diagnostics, UK). The disc diffusion test was used to assess antibiotic sensitivities of the isolated serotypes to tetracycline, clarithromycin, azithromycin, co-trimoxazole, penicillin, vancomycin, rifampin, and meropenem.

Statistical analysis

STATA 9.1 software was used for statistical analysis. Frequency and 95% confidence interval (CI) were calculated using the Binomial Wald method, adjusted for cluster sampling.

Results

We included 1300 children (53.7% male) between the ages of 0.8 to 10 years (mean: 7.0 ± 2.6 years) in our study. Pneumococci were detected in the nasopharynx of 573 healthy children (44.1%). There were 23 serotypes found; of these, the most common were serotypes 6, 14, 17, 19, 20, 21, and 23. The prevalence of positive cases for pneumococci in children who attended elementary school (1000 children) was 42.5% (95% CI: 38.9%–47.1%) and day care centers (300 children) was 49.3% (95% CI: 44.9%–53.8%). The isolated serotypes are shown in Table 1.

Pneumococci isolated from 221 (38.56%) specimens were similar to the serotypes found in the heptavalent pneumococcal conjugated vaccine.

Most isolates (69.4%) were resistant to one or more antimicrobial agents: tetracycline (69.85%), clarithromycin (57.2%), azithromycin (54.9%), co-trimoxazole (11.8%), penicillin (9.2%), and vancomycin (1.5%). All isolates were sensitive to rifampin and meropenem. The antibiotic resistance pattern is shown in Table 2.

Discussion

Pneumococcal serotypes that cause invasive disease vary by geographic region and over time.⁷ We have studied the carrier rate of *Streptococcus pneumoniae* in healthy young children in Tehran, Iran in order to estimate the prevalence of nasopharyngeal carriers of different serotypes of pneumococci and to determine their antibiotic sensitivities.

Our study showed a high nasopharyngeal carrier rate (44.1%) for pneumococci in children from Tehran. A study in northern Taiwan of 478 children between 1 month and 14 years of age with no evidence of infectious disease showed that 19.9% were nasopharyngeal carriers of the pneumococci.⁸ Researchers from Italy report a carrier rate of only 8.6% from a study done on 2779 asymptomatic infants and children.³ The higher carrier rate in our study could be due to the fact that our samples were collected during winter. An Australian study has reported that pneumococcal nasopharyngeal carriers are common in Fijian children; one report from Malawi has quoted a rate of 47.5% in children under 5 years of age, with the highest rates in infants 3–12 months old.^{9,10} In a study from Belgium, 21% of 467 children aged 3–36 months were reported to be nasopharyngeal carriers of *Streptococcus pneumoniae*.⁶ In

Gambian villagers, the pneumococcal nasopharyngeal carrier rate was reported to be > 70%, with a record figure of 97% in babies less than one year old, that decreased with age.¹¹

A Turkish study of carrier rates and serotype distribution of pneumococci in 564 healthy children less than two years old who did not attend daycare centers has reported the carrier rate to be 22.5%. The most frequently isolated serotypes were 11, 23, 19F, 22, 9, 19, and 23B, in decreasing order; 8.5% of the isolated pneumococci were intermediately resistant and 6.8% were highly resistant to penicillin.¹² Their observations were similar to our results as > 9% of the pneumococci isolated from our subjects were penicillin-resistant. Serotypes 3, 19F, 23F, 19A, 6B, and 14 were the most commonly isolated serotypes in an Italian study.³ In a study by Malfroot et al., the pneumococcal serotypes isolated from the nasopharynx of young children were: 19F (27.3%), 6B (20.2%), 23F (19.2%), 19A (10.1%), 6A (7.1%), 14 (5.1%), and others (11%).⁶ In another report, the five most common serotypes of *Streptococcus pneumoniae* isolated from sterile locations in children < 14 years old with invasive pneumococcal disease were 14, 23F, 6B, 19F, and 3. Over 75% were found to have intermediate or high-level penicillin resistance, 93% were resistant to erythromycin, 11.2% to cefotaxime, and 0.3% to levofloxacin. Overall, 85% of serotypes were similar to those present in the heptavalent pneumococcal conjugated vaccine.²

In the Lo study, the major prevalent serotypes were 23, 6, and 19; only 10.5% of the 95 strains were susceptible to penicillin.⁸ Serotypes 19 and 23 were common in our study as well, however, < 10% of our isolates were resistant to penicillin and > 90% were susceptible to the third generation of cephalosporins. In a study from Italy, 52% of the isolated pneumococci were resistant to macrolides and 9.1% to penicillin.³ In a large study by Hsu et al. on 1379 cases (adults and children) with pneumococcal meningitis, 27.8% of the isolates were nonsusceptible to penicillin, 16.6% to meropenem, and 11.8% to cefotaxime. All isolates in that study were susceptible to vancomycin, and > 99% to levofloxacin and rifampin.¹³

We believe that pneumococcal isolates with reduced susceptibility to antibiotics and non-susceptible strains are increasing.⁴ Penicillin resistance has been documented for the first time in Fiji and as a result the first line treatment for meningitis has been altered.⁹ This increase in the prevalence of drug-resistant pneumococci has resulted from the frequent and unnecessary use of antimicrobial drugs⁴; thus prevention of pneumococcal infection through timely vaccination remains the only other reasonable alternative.

Although the 23-valent polysaccharide vaccine is generally considered to be immunogenic and effective in adults and older children, it may not produce sufficient immunity in children under two years of age who are at the greatest risk for invasive pneumococcal infections.⁸ Pneumococcal conjugate vaccines are effective in the prevention of invasive pneumococcal diseases in young children.^{2,14} In countries that have widespread use of the heptavalent pneumococcal conjugate vaccine (PCV7), which provides coverage against serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F, a major decline has occurred in the rates of invasive pneumococcal diseases in children. Furthermore, there has been a reduction in the rates of invasive pneumococcal diseases in adults and immunocompromised hosts through herd immunity.^{5,13,15,16} PCV7 is included in the routine immunization protocol of all young children < 24 months of age in many industrialized countries.

In the Marchisio study, 63.2% of the pneumococci were from

strains covered by the heptavalent pneumococcal vaccine³; their results contrasted ours, as < 39% of our isolates belonged to strains found in the heptavalent vaccine. Recent studies have raised concerns over the changing patterns of pneumococcal nasopharyngeal carriers from vaccine-susceptible to penicillin-resistant non-vaccine serotypes, especially 19A and 35B.^{5,13,15} These observations emphasize the need for continued surveillance of the pneumococcal strains present in the nasopharynx of young children.

The findings of this study reveal that more than 40% of young children in Tehran carry pneumococci in their nasopharynx. The study also defines the serotype distribution and the antibiotic sensitivities of the isolated strains.

Our findings have implications on the type and efficacy of pneumococcal conjugate vaccines that should be used for the prevention of pneumococcal invasive disease in Iranian children, and also on the selection of appropriate antibiotics for treatment of pneumococcal infection.

Ethical Approval

This research project was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences.

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A view of the origin of Zayanderud(life giver river),which is located near Shahr-e Kord inChaharmahal and Bakhtiari Province (photo by: M.H. Azizi MD, 2012)