



Serotyping, Antibiotic Susceptibility and Related Risk Factors Aspects of Nasopharyngeal Carriage of *Streptococcus pneumoniae* in Healthy School Students

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Abstract

Background: *Streptococcus pneumoniae* is an important problem worldwide and nasopharyngeal colonization plays significant role in pneumococcal infections. The aims of this study were to determine the nasopharyngeal colonization rate, serotyping, antibiotics susceptibility and study the risk factors for nasopharyngeal colonization with *S. pneumoniae* in students in Kashan, Iran.

Methods: A cross-sectional study was conducted on children aged 7 to 19 years from December 2011 to November 2012. Nasopharyngeal swabs were plated onto brain heart infusion agar plates with 5% sheep blood and 4µg/ml of gentamycin. Antimicrobial susceptibility profiles were determined on Mueller-Hinton agar in accordance with CLSI. *S. pneumoniae* strains were investigated for the presence of the most common pneumococcal serotypes using a multiplex polymerase chain reaction.

Results: 13.9% were found to be carriers. The most prevalent serogroups were 19F (30%), 6A/B (18.9%), 15A (16.5%), 11 (11.3%), 23F (8.2%), 1 (6.2%), 19A (3.4%), and 35B (2.4%). Nine strains (3.1%) were non-typeable. The carrier rate was significantly higher in 12 to 15 year old age group. Upper respiratory tract infections within the last month (OR=1.5, $P<0.011$), previous hospitalization (OR=1.6, $P<0.001$), previous antibiotic usage last two weeks (OR=1.89, $P<0.001$), rhinorea (OR=1.9 $P<0.001$), male sex (OR=3.5 $P<0.001$) and passive smoking (OR=1.56, $P<0.001$) have been determined to be risk factors for *S. pneumoniae* carriage. The highest pneumococcal resistance was to tetracycline (25.4%). All strains were susceptible to linezolid and levofloxacin.

Conclusion: Our information leads to an important source to screen the future impact of pneumococcal vaccination on bacterial colonization.

Keywords: *Streptococcus pneumoniae*, Nasopharyngeal, Serogroup, Antibiotic resistance

Introduction

Streptococcus pneumoniae is a gram-positive, alpha-hemolytic, fastidious bacterium. The surface capsular polysaccharide of *S. pneumoniae* initiates a type-specific protective immune response and provides the basis for serotyping of this organism. Currently, more than 90 different pneumococcal

serotypes have been identified. Among the various serotypes and according to 60–80 percent of the worldwide reported infections, the 6, 14, 18, 19, and 23 are the most prevalent ones (1). Though, the distribution of serotypes is sequential and varies according to geographic location. Nasopharyn-

geal colonization of *S. pneumoniae* varies according to age and health status (2). In the industrial world, the expected annual rate of pneumonia is about 14.5 per 10,000 in children under age 16 years old (3). The mortality rate due to *S. pneumoniae* in developed countries is low (4). On the contrary, in developing countries, respiratory tract infections are more common and more severe, accounting for more than 2 million deaths annually; pneumonia infections are the number one killer of children in these societies(5). Lower socioeconomic groups have a higher prevalence of lower respiratory tract infection, which correlates best with family size, as a reflection of environmental crowding (6). In spite of the availability of a vaccine covering 23 different serotypes, *S. pneumoniae* is currently the major invasive pathogen of children and adults and is a principal cause of otitis media, pneumonia, bacteremia, and meningitis(7). Information about the distribution of pneumococcal serotypes and demographic characteristics related with *S. pneumoniae* colonization, are essential for the proposing of strategies to prevent and control these infections (7). The distribution of serotypes also varies between carriage isolates and invasive disease and antibiotic resistance is most common in pneumococcal serotypes (types 6, 9, 14, 19, and 23) that are carried by children (8). On the other hand, the pneumococcal vaccination is not presently a part of the childhood immunization plan in Iran, and data about the serotype distribution and prevalence of *S. pneumoniae* colonization, antibiotic susceptibility and risk factors of nasopharyngeal colonization of *S. pneumoniae* in healthy school-age children is scarce.

The objectives of this study were to determine the nasopharyngeal colonization rate, serotyping, antibiotics susceptibility and risk factors for nasopharyngeal colonization with *S. pneumoniae* from students in Kashan, Iran.

Materials & Methods

Overall, 2100 students enrolled in this study sampled from December 2011 to November 2012 in 22 schools in Kashan, Iran. We excluded children

showing symptoms or signs of respiratory infection, as well as those who received at least one dose of any antibiotic treatment during the previous 15 days. One of the children's parents signed informed consent and provided clinical and demographic information, such as age, sex, crowding (two or more people sleeping in the same room), smokers at home, and socioeconomic status. Detailed medical information such as previous diagnosis of asthma, allergic rhinitis and history of hospitalization in previous 6 months, and antibiotic treatment in previous 3 months were recorded. The study was approved by the ethical committees of Kashan University of Medical sciences. Specimens from nasopharynx of the students were obtained by using a sterile cotton-tipped swab. Swabs were plated immediately onto brain heart infusion agar plates with 5% sheep blood and 4µg/ml of gentamycin. *S. pneumoniae* isolates were identified by gram staining, colony characteristics, susceptibility to optochin, and bile solubility. *S. pneumoniae* strains were investigated for the presence of the most common pneumococcal serotypes using a multiplex polymerase chain reaction (PCR) assay as described previously(9).

The susceptibility pattern of *S. pneumoniae* strains to rifampin (5 µg), erythromycin (15 µg), oxacillin (1 µg), linezolid (30 µg), clindamycin (2 µg), levofloxacin (5 µg), vancomycin (30 µg), tigecycline (15 µg), and tetracycline (30 µg) were determined by disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines(10). *S. pneumoniae*_ATCC 49619 was used as standard control strain.

Results

The study population included 2100 healthy school-age children aged 7 to 19 years (13.08 ± 3.284 years), of which (1275) 60.7% were male. The distribution of age was as follows: 7-11 years: 730 (34.8%) students; 12-15 years: 909(43.2%) students; and 16-19 years: 461(22%) students. The demographic and clinical characteristics of the students enrolled in this study are listed in Table1. *S.*

pneumoniae was isolated from 291(13.9%) students. The percentage of *S. pneumoniae* colonization showed a peak (144 out of 909) at the age of 12-15 years old (15.8%) ($P < 0.009$) and a low prevalence of 9.8% was observed in 16-19 years old students. As mentioned above, the highest rates of *S. pneumoniae* colonization were observed among children with age 12-15 years old (15.8%), male sex (18.8%), rhinorrhea (21.4%) with previous history of respiratory infections (18.9%). The occurrence of *S. pneumoniae* colonization was not statistically differs between children with asthma, frequent respiratory infection, and crowding (Table 1). The *S. pneumoniae* strains isolated in this study showed 8 different pneumococcal serotypes, of which 19F (30%), 6A/B (18.9%), 15A (16.5%), 11 (11.3%) 23F (8.2%), 1(6.2%), 19A (3.4%) and

35B (2.4%) were the most common ones. Nine (3.1 %) of the strains were non-typeable. The most resistance rate among isolated *S. pneumoniae* strains was tetracycline (Table 2). The various observed serotypes among the 43 penicillin-non susceptible *S. pneumoniae* strains were as follow: 6A/B (25.6%), 11(18.6%), 19F (18.6%), 15A (14%), 1(9.3%), 23F (4.6%), and non-typeable (9.3%). The most resistance rate to oxacillin observed in serotypes 11(24.2%), 1 (22.2%), and 6A/B (20%). The serotypes observed among the 74 tetracycline resistance *S. pneumoniae* strains were 19F (27%), 15A (18.9%), 6A/B (17.6%), 1(9.4%), 11(9.4%), 23F (8.1%), 19A (5.4%), 35B (1.4%) and non-typeable (2.7%). The most resistance rate to tetracycline detected in serotype 19A (40%), 1(38.8%), 15A (29.2%), 23F (25%), 6A/B (23.6%), 19F (23%), 11(21.2%) and 35B (14.3%).

Table 1: The risk factors for pneumococcal carriage among students in Kashan, Iran

Risk factor	pneumococcal carriage NO. (%)	P value	Odds Ratio (95% CI)
Age group/ 7-11 yr (730)	102(14)	0.009	
12-15 yr (909)	144(15.8)		
16-19 yr (461)	45(9.8)		
Sex/ Boy(1275)	240(18.8)	< 0.001	3.5(2.5 - 4.8)
Girl(825)	51(6.2)		
Crowding/ 2-3(373)	50(13.4)	0.125	
4-5(1418)	209(14.7)		
>5(309)	32(10.3)		
Smoker at home(609)	109(17.9)	0.001	1.6(1.2 – 2.03)
Asthma(174)	27(15.5)	0.508	1.15(0.7 – 1.7)
Rhinitis (401)	86(21.4)	< 0.001	1.9(1.5 – 2.6)
Previous respiratory infection(264)	50(18.9)	0.011	1.5(1.1 – 2.1)
Frequent respiratory infection(166)	31(18.7)	0.06	1.47(0.98 – 2.2)
Previously hospitalized(364)	70(19.2)	< 0.001	1.6(1.2 – 2.1)
Previous antibiotic use(314)	67(21.3)	< 0.001	1.89(1.3 – 2.5)

Table 2: Frequency rates of antimicrobial resistance among 291 *S. pneumoniae* strains isolated from students in Kashan, Iran according to serotypes

Antibiotics	<i>S. pneumoniae</i> strains			19F	6A	15A	11	23	1
	Sensitive n (%)	Intermediate n(%)	Resistance n (%)	No. 87 n(%)	No. 55 n (%)	No. 48 n (%)	No. 33 n (%)	No.24 n(%)	No.18 n (%)
Rifampin(5 µg)	288(99)	3(1)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Erythromycin(15µg)	267(91.8)	14(4.8)	10(3.4)	4(4.6)	1(1.8)	2(4.2)	1(5.6)	0(0)	1(5.6)
Clindamycin (2µg)	281(96.6)	5(1.7)	5(1.7)	0(0)	0(0)	0(0)	0(0)	1(4.2)	0(0)
oxacillin (1µg)	244(83.8)	4(1.4)	43(14.8)	8(9.2)	11(20)	6(12.5)	8(24.2)	2 (8.3)	4(22.2)
levofloxacin(5µg)	291(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
vancomycin (30µg)	287(98.6)	4(1.4)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
linezolid(30µg)	291(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
tetracycline(30µg)	201(69.1)	16(5.5)	74(25.4)	20(23)	13(23.6)	14(29.2)	7(21.2)	6(25)	7(38.9)
Tigecycline(15 µg)	242(83.2)	11(3.8)	38(13.1)	15(17.2)	6(10.9)	5(10.4)	4(12.1)	2(8.3)	4(22.2)

Discussion

In the present study, we observed a pneumococcal colonization prevalence of 13.9% in Kashan, Iran. The prevalence of *S. pneumoniae* colonization described in different parts of the world has changed widely. The distribution pattern of *S. pneumoniae* serotypes varies by geographic locations. A variety of demographic and clinical characteristics have been explained to be related with an increase in *S. pneumoniae* colonization, for example age, society, family size, crowding, siblings, smoking at home, recent antibiotic use, and socioeconomic status of study population regarding variations in sampling and isolation techniques (2, 11, 12). In our study previous history of upper respiratory tract infection within the last month, hospitalization, antibiotic usage during last two weeks, rhinorea, male sex and passive smoking have been determined to be risk factors for *S. pneumoniae* carriage. Cigarette smoking compromises natural pulmonary defense mechanisms by disrupting both muco-ciliary function and macrophage activity. Exposure to cigarette smoke, especially in smoking mothers increases the risk of pneumonia in infants younger than one year of age. We did not observe statistical differences in the prevalence of *S. pneumoniae* colonization between children with asthma and children without respiratory distresses. *S. pneumoniae* colonizes at the upper respiratory tract and plays role as normal flora of healthy individuals. A particular serotype can be carried for many months before being eradicated or replaced by a different serotype. Carriage increases in the first few months of life and the highest asymptomatic colonization rates (>40%) observed in young children. Factors associated with increased carriage including winter season, day care crowding, and living in crowded conditions. Carriage in adults is approximately 10–20% and the duration of carriage is generally shorter. The distribution of serotypes also varies between carriage isolates and invasive diseases. Antibiotic resistant serotypes most frequently related to pneumococcal strains which carried by children (types/groups 6, 9, 14, 19, and 23) (8). Resistance to tetracycline was observed in

25.4% of the strains. We did not find any *S. pneumoniae* strains showing resistance to levofloxacin, linezolid, and vancomycin. The probable reason is frequent using of antibiotic therapy in small children. Therefore, exposure of these serotypes to antimicrobial drugs, may leads to a selective advantage of resistant mutants(13). There are multiple risk factors for acquisition of infection with antibiotic-resistant pneumococci. Most of these factors have a unity in exposure to the drugs that select the resistance. Macrolide resistance is also a function of exposure, particularly for a long-acting drug such as azithromycin (13). The macrolides have been used extensively to treat community-acquired respiratory tract infections around the world, and in recent years macrolides resistance in *S. pneumoniae* has been raised considerably. Macrolide-resistant *S. pneumoniae* are now more common than penicillin-resistant *S. pneumoniae* in many parts of the world(14).

The selection of resistant strains is complicated by multiple resistances where macrolides appear to be better selectors of multi-resistant strains than β -lactam drugs do (15). Fluoroquinolone resistance in *S. pneumoniae* relatively remains low around the world (less than 1%), but it tends increasing, particularly in some countries. Studies carried out over the past decade recommended that the prevalence of levofloxacin-resistant strains in the North America remains less than 2% (16-18). Upper rates have been reported in Spain (7%), Sri Lanka (9.5%), the Philippines (9.1%), Korea (6.5%) and Hong Kong (15.2%)(19-22). The prevalence of rifampin resistance among pneumococcal isolates is low at the present time, and reported rates vary between 0.1 and 1.5% (23). In our study, we observed high prevalence rates of oxacilin (14.8%) resistant strains. The prevalence of penicillin resistance among *S. pneumoniae* isolates ranged from 18.2 to 22.1%(24, 25). Quintero et al. reported the prevalence of antimicrobial resistance among *S. pneumoniae* strains to erythromycin 38%, penicillin 34% (intermediate 20% and high level resistance 14%), tetracycline 34%, and clindamycin 29%.(1). Resistance to penicillin is associated with some degree of non-susceptibility to all β -lactam antibiotics. Resistance rates re-

ported for amoxicillin are relatively low (less than 5%) (26, 27). Tigecycline is the prototype compound of a new class of antimicrobial agents known as glycylicylines. This derivative of minocycline provides clinicians with a novel, expanded broad-spectrum antibiotic with activity against difficult-to-treat pathogens. Worldwide, serotypes 6B, 6A, 9V, 14, 15A, 19F, 19A, and 23F have shown the highest rates of resistance to penicillin and erythromycin, with the highest rates in respiratory isolates reported in South Africa (74%), Asia (63%) and the Middle East (54%)(28). The data from molecular typing upon numerous studies has shown that there is considerable diversity among resistant strains within most serotypes(29). McGee et al. found 30.5% of erythromycin-resistant pneumococci and showed that 83% of these isolates were associated to a serotype 19F strain originating in Taiwan(30). Monitoring the changes in pneumococcal nasopharyngeal carriage in children is important, and may provide relevant information on the identification of serotypes that may significantly contribute to pneumococcal diseases. In this research 57% (166 out of 291) of the isolated serotypes were identified as 19F, 6A/B and 23F. Though there are at this time over 90 distinct serotypes, definite serotypes commonly account for the majority of *S. pneumoniae*_nasopharyngeal isolates, but the circulation of serotypes differs in relation to geographic position. Though serological determination by a quelling reaction of pneumococcal cells with anti-polysaccharide sera is the presently used method for pneumococcal serotyping the high cost of antiserum, the requirements for technical proficiency and bias in interpretation of results are major limitations of the procedure. Multiplex PCR has the likely to overcome these problems and has been reported to have higher sensitivity and specificity than conventional quelling reaction techniques (9, 31-33).The serotype distribution between carriage isolates is frequently used as an indicator for hypothetical vaccine coverage. Vaccination could potentially reduce the carriage rate of antibiotic-resistant pneumococci. At the present time the pneumococcal vaccination is not a part of the childhood immunization program in Iran.

The best to our knowledge, there are few data on nasopharyngeal pneumococcal colonization and serotyping among children in Iran.

Conclusion

A high prevalence of *S. pneumoniae* colonization in the nasopharynx in students was observed and a high rate of resistance to β -lactams and tetracycline of isolates was confirmed. We found that pneumococcal serotypes 19F, 6A/B were the most common carried serotypes in our studied population. For increase protection against *S. pneumoniae*, it is recommended to vaccinate infants and young children with pneumococcal vaccines.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors. The study protocol was approved by the Kashan University of Medical Sciences ethical committee.

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