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Rate of Carriage, Serotype Distribution and Penicillin Resistance of Streptococcus pneumoniae in Healthy Children

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Abstract: The purpose of this study was to define the carriage rates for Streptococcus pneumoniae in a given population in Ankara and also to determine the serotypes and penicilin resistance of these strains. Oropharyngeal swabs were taken from a total of 661 children between 0 and 11 years of age living in a province of Ankara between January 1995 and January 1997. Serotyping was performed by detection of the Quellung reaction. Penicillin susceptibilities of the isolates were screened by agar dilution method according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS). The total rate of pneumococcal carriage in the study population was 23.90% and the isolation rate was found to be statistically associated with the age, being higher in small children. Of the 158 S. pneumoniae isolates, the most prevalent serotypes in order of frequency were 6, 19, 9, 23, 3 and 14. Penicillin susceptibility tests were performed in 120 of the isolates. Fifty-five (45.83%) were susceptible, 53 (44.17%) were intermediate and 12 (10.0%) were highly resistant to penicilin. Evaluation of the results showed that serotypes 6, 14 and 23 are those most associated with penicillin resistance. The significant rate of isolation of penicilin resistant pneumococci in healthy carriers indicated the importance of active immunization in risk groups and also the importance of the rational use of antibiotics to limit the spread of resistant strains.

Key Words: Streptococcus pneumoniae, carriage, serotype, penicillin susceptibility.

Introduction

Streptococcus pneumoniae is one of the most common causes of bacterial pneumonia, bacterial meningitis and otitis media, and also a common significant isolate from blood cultures (1). Incidence of penicilin-resistant pneumococci have been reported increasingly worldwide, and the control of penicillin-resistant pneumococci has become one of the most serious therapeutic challenges for clinicians today (1, 2).

It is well-known that the pneumoccal strains carried in the nasopharynx can cause pneumonia, meningitis, otitis media and several other infections when they reach the normally sterile sites adjacent to the nasopharynx (3). The nasopharyngeal flora has therefore mainly been analyzed as a reservoir for S. pneumoniae. Furthermore, colonized children can transmit pneumococci to other subjects (3, 4). Upper respiratory tract carriage studies are also important in monitoring the resistance patterns of S. pneumoniae against the antimicrobial agents in clinical use.

Of the 84 pneumococcal serotypes, penicillin resistance is mostly associated with serotypes 6, 9, 14, 19 and 23. Epidemiological studies have shown that these five serotypes may account for over 70% of pneumococci isolated from children and that they are associated with prolonged carriage and rapid reacquisition (2). Adults acquire these resistant organisms, commonly through exposure to colonized children (5).

As far as the data on pneumococcal carriage in our country is concerned, a few is known about the carriage rates and serotypes. The purpose of this study is to define the carriage rates for pneumococci in a given population in Ankara and also to determine the serotypes and penicillin resistance of these strains to assess the coverage by the currently available 23-valent pneumococcal vaccine.

Materials and Methods

Study population: A total of 661 children between 0–11 years of age living in the Cebeci district of Ankara were enrolled between 15 Jan 1995–15 Jan 1997. Verbal informed consent was obtained from the parents or teachers of all children and a questionnaire was administered to provide information about the sex, age, socioeconomic status and size of the family of the subject and the history of any recent respiratory tract infection, antibiotic treatment in the previous 3 weeks, or hospitalization during the previous month. Children were excluded if they had an acute respiratory tract infection or were using antimicrobial agents.

The enrollment procedure consisted of two primary schools, two day–care centers and one mother and child health–care center chosen randomly in the Cebeci district.

Collection of specimens: Oropharyngeal swabs were obtained with cotton–tipped wooden swabs. The swabs were transported to the laboratory in modified Stuart's medium and inoculated onto agar plates within 4 hours of sampling.

Isolation of S. pneumoniae: The oropharyngeal swab specimens were inoculated onto trypticase soy agar plate containing 5% sheep blood and also onto trypticase soy agar containing 5% sheep blood and 5μg/ml gentamicin for selective isolation of S. pneumoniae. The agar plates were incubated in 5–10% CO_2 at 37°C. Alpha–hemolytic colonies with morphology suggestive of S. pneumoniae were selected for identification.

Identification of S. pneumoniae: Pneumococci were identified by typical appearance, alpha–hemolysis, optochin susceptibility and bile solubility (6).

Serotyping of S. pneumoniae: Serotyping was performed by detection of the Quellung reaction with specific antisera from the Statens Seruminstitut (Copenhagen, Denmark), according to the guidelines of the manufacturer. The antisera used included the serotypes that most commonly cause infection in children and also some of the types that are represented in the current 23–valent pneumococcal polysaccharide vaccine, namely, the serotypes 1, 2, 3, 4, 5, 6, 7, 8, 9, 14, 18, 19 and 23.

Penicillin–susceptibility testing of S. pneumoniae: Isolates were screened for resistance to penicillin by agar dilution method according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) (7). A suspension of organisms to be tested was prepared in Mueller–Hinton broth equivalent to 0.5 McFarland density and diluted 1:100 to give 10^6 cfu/ml, and 10 μl of this dilution was used to inoculate Mueller–Hinton agar supplemented with 5% sheep blood that contained a series of increasing twofold concentrations of penicillin varying from 0.03 to 32 μg/ml. Quality control strain Enterococcus faecalis ATCC 29212 was included in all runs. The results were interpreted according to the MIC breakpoints recommended by NCCLS, with MIC ≥ 0.1–1 μg/ml as intermediate and MIC ≥ 2 μg/ml as resistant.

Statistics: The results were analyzed by Chi–square and Kolmogorov–Smirnov tests.

Results

A total of 661 healthy children below age 11 were included in the study, 305 (46.14%) were girls and 356 were (53.86%) boys. The mean age of the study population was 6.57±0.08 years. Of the 661 subjects, 248 belonged to the 0–5 year age group and 413 belonged to the 6–11 year age group. The distribution of the S. pneumoniae isolated from the related age groups is shown in Table 1. Statistical evaluation showed that the rate of pneumococcal carriage was related to age (x^2=6.52, p=0.088).

Table 1. The rate of pneumococcal carriage in relation to age groups.

Age groups (years of age)	S. pneumoniae (+) n	%	Total
0–2	33	30.28	109
3–5	38	27.34	139
6–8	44	23.78	185
9–11	43	18.85	228

(x^2=6.52, p=0.088)

Pneumococci were recovered from 158 (23.90%) of the subjects; 71 from the 0–5 year age group (n=248) and 87 from the 6–11 year age group (n=413). The mean age of the pneumococcal carriers was 5.84±0.12 years. As a general observation, the recovery of S. pneumoniae was higher in the plates with gentamicin than in the simple blood agar plates. Serotyping was performed on 155 of the 158 S. pneumoniae isolates. Table 2 shows the serotype distribution of these isolates in the two age groups. Fifty–one of the isolates failed to be serotyped by the available antisera. The most prevalent serotypes in the entire study population in order of frequency were serotypes 6, 19, 9, 23, 3 and

Table 2. Serotype distribution of the S. pneumoniae isolates from the two age groups (n=155).

Serotype	Age group 0–5 years		Age group 6–11 years		Total	
	n	%	n	%	n	%
3	5	7.35	6	6.90	11	7.10
6	10	14.70	16	18.39	26	16.78
7	0	0.0	1	1.15	1	0.64
9	6	8.83	12	13.79	18	11.61
14	4	5.88	3	3.45	7	4.51
18	1	1.47	4	4.60	5	3.23
19	11	16.18	12	13.79	23	14.84
23	5	7.35	8	9.20	13	8.39
NT[a]	26	38.24	25	28.74	51	32.90
Total	68	100.0	87	100.0	155	100.0

[a]: The isolates that failed to be serotyped with the available antisera.

14, accounting for about 63% of all the isolates. Serotype 19 was the most prevalent type in the 0–5 years age group and serotype 6 in the 6–11 years age group years age group. Serotypes 1, 2, 4, 5 and 8 were not encountered in any of the S. pneumoniae isolates tested.

The results of penicillin susceptibility testing performed in 120 of the isolates revealed that 55 (45.83%) were susceptible, 53 (44.17%) were intermediate and 12 (10.00%) were resistant to penicilin. Penicilin MIC values of resistant strains (intermediate and resistant) varied from 0.125 to 4 µg/ml. The MIC_{50} and MIC_{90} values were 0.125 and 1 µg/ml, respectively. The serotype distribution of the intermediate and resistant strains are shown in Table 3.

Penicillin susceptibility was found to be associated with serotype of the S. pneumoniae tested (Contingency coefficient=0.41; p=0.007) (Table 3). However, because of the small number of isolates tested in each serotype, it could not be statistically determined which serotypes were highly associated with penicillin resistance. Rough evaluation of the results in Table 3 shows that serotypes 6, 14 and 23 seem to be more resistant to penicillin than the other serotypes and the non–serotypable strains.

Discussion

It is well known that most penicillin resistance among pneumococci is associated with serotypes 6, 9, 14, 19 and 23, the serotypes isolated most frequently from the pneumococcal infections seen in children (2). Most types of S. pneumoniae that commonly cause disease appear to be those usually found in healthy carriers (3, 5). Therefore, the knowledge about the serotype distribution and the penicillin resistance of the S. pneumoniae strains isolated in a certain carrier population will provide information about the strains from the diseased cases.

The frequency of pneumococcal carriership in the upper respiratory tract varies between 2 and 35% in the general population, with the highest isolation rate in young children (3, 4, 8, 9). The total rate of pneumococcal carriage in our study population was 23.90%, and the isolation rate was found to be statistically associated with the age of the subject, being higher in young children than in older ones (Table 1). In the United States, Arnold et al (4) found pneumococcal carriage in children below age 6 to be 47%, Hendley et al. (8) found this rate to be 35% below age 6 and 29% in grammar school children. A multicenter study in eastern and central Europe showed that 31.2% of children carried pneumococci (10). The carriage rate was found to be 60% at day–care centers in İstanbul, Turkey (11). These results show that considerable variation in carriage rates can be observed for pneumococci in different countries. These different rates are most probably due to the differences in the ages of the populations sampled, sampling methods and cultivation techniques. During the study, we also observed that inoculation of throat swabs onto standard sheep blood agar plates alone failed to detect a large proportion of carriers of S. pneumoniae. Incorporation of gentamicin into the medium increases the sensitivity of direct plate inoculation by reducing the population pressure due to other organisms.

Table 3. The serotype distribution of the penicillin susceptibility-tested strains (n=120).

Serotypes	Susceptible		Intermediate		Resistant	
	n	%	n	%	n	%
3	6	10.90	2	3.77	0	0.0
6	4	7.27	13	24.53	4	33.34
7	0	0.0	1	1.89	0	0.0
9	7	12.73	4	7.55	1	8.33
14	2	3.64	4	7.55	1	8.33
18	4	7.27	0	0.0	0	0.0
19	9	16.37	8	15.09	1	8.33
23	1	1.82	7	13.20	4	33.34
NT[a]	22	40.0	14	26.42	1	8.33
Total	55	100.0	53	100.0	12	100.0

[a]: The isolates that failed to be serotyped with the available antisera (Contingency coefficient=0.41, p=0.007).

The capsular serotypes encountered most commonly among these healthy children were types 6, 19, 9, 23 and 3, in order of decreasing frequency. Since the financial supports of this study were limited, it was not possible to obtain all of the serotypes included in the current pneumococcal vaccine. Therefore, about 35% of the isolates could not be serotyped with the available antisera. Although the results of this study do not supply complete information about the distribution of all pneumococcal serotypes in Turkey, it provides information about distribution of the most common serotypes encountered in the world. Our data indicate that the typable ones are among the serotypes that are frequently associated with infections caused by S. pneumoniae, as indicated in the literature (1, 9, 12, 13). In a previous study held in our hospital, it was observed that the most prevalent serotypes in order of frequency were 23, 19, 9, 14 and 6 among the S. pneumoniae strains isolated from children with lower respiratory tract infections (14). Therefore, it can be concluded that most types of S. pneumoniae that commonly cause disease are those found in healthy carriers. This finding is compatible with the concept that the types of S. pneumoniae that cause disease in man are usually derived from normal human flora (3, 5, 8). Mastro et al. (15) found that in healthy children in Pakistan, group 19 was the most prevalent of the S. pneumoniae nasopharyngeal isolates. In Finland, Takala et al. (13) determined the most common pneumococcal serotypes among the carriers to be serotypes 6, 14, 19 and 23, similar to those found in United States (4, 5).

In has been previously shown that serotypes 6, 9, 14, 19 and 23 are those most often associated with antimicrobial resistance (12). The data obtained from this study are consistent with the results of the previous studies. We found that serogroups 6, 14 and 23 in particular are significantly associated with resistance to penicilin. As a whole, about 54% of the isolates tested were intermediate or resistant to penicilin. This high incidence of penicillin resistant pneumococci in healthy carriers is most probably due to the frequent use of this antibiotic in our population due to financial reasons. Penicillin is the principal drug administered widely for the treatment of upper respiratory tract infections in Turkey. Similar results were also achieved in Hungary, Spain and South Africa, where antibiotic resistant pneumococci are commonly isolated (2, 9).

The increasing occurence of penicillin-resistant pneumococcal strains and their isolation from healthy carriers in the community provides additional reason for consideration of active immunization against infection with those pneumococcal types most frequently responsible for infection, particularly in children under risk of pneumococcal infection. Limited data suggest that immunization with a conjugate pneumococcal vaccine results in reduced carriage rate of pneumococcal serotypes included in the vaccine (4).

Since pneumococcal vaccines in current use include serotypes most commonly associated with resistance (6, 9, 14, 19, 23), these vaccines may be extremely useful for preventing transmission and infections with drug-resistant pneumococcal strains. In addition, the results of this study also indicate the importance of the rational use of antibiotics, particularly in general practice, in order to limit the spread of resistant strains. In view of increasing rates of resistance among pneumococci in

many regions of the world, larger surveillance studies for resistance are necessary to guide the rational choice of antimicrobial agents.

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