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Original Article

Serotype Distribution and Antibiotic Susceptibilities of Clinical *Streptococcus pneumoniae* Strains

Aykut Kurt[1], Nevriye Gönüllü[1]

[1]İstanbul University Cerrahpaşa Medical Faculty, Medical Microbiology, İstanbul, Turkey

Address for Correspondence: Aykut Kurt, **E-mail:** aykutkurt@windowslive.com
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ABSTRACT

Purpose: *Streptococcus pneumoniae* is a pathogen that cause significant mortality and morbidity in humans. In particular, bacteremia, pneumonia, meningitis and middle ear inflammation. *S.pneumoniae* can cause many diseases in children and adults. Recently, *S.pneumoniae* is the most important cause of acute respiratory tract diseases in developing countries. Increasing antibiotic resistance in *S.pneumoniae* strains has recently gained importance. The aim of our study was to investigate the antimicrobial susceptibility and distribution of pneumococcal serotypes of *S.pneumoniae* strains isolated in our University Hospital.

Methods: In this study we evaluated 80 *S.pneumoniae* strains isolated from adults and children between September 2012 and September 2013. The isolates were obtained from sputum (n=56), nasal secretions (n=4), throat swabs (n=1), blood (n=9), tracheal aspirat (n=2), conjunctival smear (n=1), bronchoalveolar lavage (n=6), and biopsy (n=1). Seventy-six isolates were from adults and four were from children. Antibiotic susceptibility testing to penicillin were performed on Mueller-Hinton agar supplemented with 5% sheep blood by the E-test method (bioMerieux, France); erythromycin, clindamycin, vancomycin, chloramphenicol, tetracycline, ofloxacine, levofloxacin, trimethoprim/sulfamethoxazole, rifampin, linezolid, and oxacillin susceptibilities performed by disc diffusion method and 7-10-13-valent Pneumotest-Latex (Statens Serum Institude, Denmark) kit was used for serotyping.

Results: A total of 39/80 isolates (48.75%) were intermediate, and 5/80 (6.25%) were resistant to penicillin according to Clinical & Laboratory Standards Institude (CLSI) guidelines for oral penicillin. Penicillin resistance was not found for parenteral penicillin. Resistance rates of erythromycin, clindamycin, vancomycin, chloramphenicol, tetracycline, ofloxacine, levofloxacin, trimethoprim/sulfamethoxazole, rifampin, and linezolid were as 31.25%, 21.25%, 0%, 5%, 28.75%, 15%, 2.5%, 45%, 0%, and 0% respectively. The most common *S.pneumoniae* serotypes were determined as serotypes 19, 6, and 23. Serotyping showed serotype 19 to be the leading serotype among the macrolide-resistant isolates, and serotype coverage of 7-10-13-valent pneumococcal vaccine was 68.75%.

Conclusion: The increase in intermediate penicillin resistance in *S.pneumoniae* in our hospital should be monitored carefully and the distribution of pneumococcal serotypes is similar to countries where the Pneumococcal Conjugate Vaccine (PCV) has been introduced.

Keywords: Streptococcus pneumoniae, serotyping, antibiotic resistance, e-test.

INTRODUCTION

S.pneumoniae is one of the most important cause of morbidity and mortality among children under 2 years of age, and elderly patients especially in developing countries. Community-acquired pneumonia can be fatal especially in adults over 60 years of age. *S.pneumoniae* is a member of upper respiratory tract microbiome. It causes many diseases such as meningitis, otitis media, sinusitis and bacteremia, as well as pneumonia (1).

S.pneumoniae have been serotyped according to their capsule polysaccharides and more than 90 distinct pneumococcal serotypes have been identified. Serotype distribution and antibiotic resistance varies by age, geographic regions and socio-economic status. The most common 23 serotypes that cause infections have been found in polysaccharide pneumococcal vaccine. However, this vaccine is not effective for children under 2 years of age. Conjugated vaccines covering 7 serotypes have been developed for children under 2 years of age and being used since 2005. The first choice of therapy in *S.pneumoniae* infections is penicillin but an obvious penicillin resistance was observed in recent years, especially resistance to erythromycin is crucial in macrolides, and similar increase has been observed as in erythromycin resistance too. Moreover, resistance to trimethoprim/sulfamethoxazole is

also in high levels. Some *S.pneumoniae* strains of some serotypes have shown higher antibiotic resistance and 80% of penicillin resistant strains consist of the serotypes that are included in conjugated vaccine (1, 2).

In our study, we aimed to determine antibiotic resistance and serogroups/serotypes of *S.pneumoniae* strains isolated in our hospital, to determine compatibility levels of them with vaccine serotypes and to compare these results with previous studies.

METHODS

Bacterial strains

In this study, 80 *S.pneumoniae* strains were isolated from various samples of children and adult patients referred to the Department of Microbiology between 2012–2013.

The isolates were obtained from sputum (56 strains), blood cultures (nine strains), nasal secretions (four strains), throat swabs (four strains), tracheal aspirates (two strains), bronchoalveolar lavages (six strains), biopsy (one strain) and conjunctival smear (one strain). Seventy-six strains were isolated from adults and four from children. Twenty-eight strains were isolated from inpatients and 52 from outpatients.

S.pneumoniae strains were stored in 10% glycerol at-70°C. After thawing the stored strains were cultured by direct-plating to 5% sheep blood agar. S. pneumoniae was identified according to its Gram stain smears morphology, catalase negative reaction, bile solubility and its susceptibility to optochin. Serogroups/serotypes of *S.pneumoniae* strains were determined by latex agglutination method (7-10-13-valent Pneumotest-Latex kit, Statens Serum Institude, Denmark) by using ready-to-use solutions containing latex particles coated by antiserums against polysaccharides of *S.pneumoniae* capsule acquired from rabbit and these serogroup/serotypes were examined. Moreover, sensitivity tests of strains against various antibiotics were performed by disc diffusion and E-test methods.

Antimicrobial Agents

Antibiotics used in this study were penicillin erythromycin, clindamycine, vancomycine, tetracycline, ofloxacin, levofloxacin, trimethoprim/sulfamethoxazole, chloramphenicol, rifampicin and linezolid.

Susceptibility Testing

Penicillin minimum inhibitory concentration (MIC) values were measured using E-test method (bioMerieux, France) on Mueller-Hinton agar containing 5% sheep blood; oxacillin, erythromycin, clindamycine, vancomycine, tetracycline, ofloxacin, levofloxacin, trimethoprim/sulfamethoxazole, chloramphenicol, rifampicin and linezolid susceptibilities were measured using disk diffusion technique on the same agar according to CLSI guidelines. The control strain was *S.pneumoniae* ATCC 49619.

RESULTS

Our study involved 80 *S.pneumoniae* strains isolated from various clinical cases accepted tothe laboratory of Medical Microbiology between September 2012-September 2013.56 of *S.pneumoniae* strains were sputum samples (70%), four (5%) were nasal swab samples, one (1.25%) was throat swab sample, one (1.25%) was eye swab sample, nine (11.25%) were blood samples, six (7.5%) were bronchoalveolar lavage (BAL) samples, two (2.5%) were endotracheal aspirate (ETA) samples and one (1.25%) was biopsy sample.

In our study, of 11 serogroup/serotypes serogroup 19 (17 strains), serogroup 6 (7 strains), serogroup 23 (6 strains), serotype 14 (6 strains), serogroup 18 (5 strains), serotype 3 (4 strains), serogroup 7 (3 strains), serogroup 9 (3 strains), serotype 5 (2 strains), serotype 1 (1 strains), serotype 4 (1 strain) were found, respectively. Of the 80 strains 55 (68.75%) were serogrouped/serotyped, whereas 25 (31.25%) could not (Table 1). Three of four strains isolated from juvenile patients were serotyped; two of them were sputum samples and other was throat swab sample; their serogroups were determined as serogroup 6, serogroup 23 and serogroup 19, respectively. *S.pneumoniae* strains which could not be serotyped/serogrouped were mostly sputum samples (four strains), BAL samples (two strains), ETA sample (one strain) and conjunctival smear samples (3), respectively. Only one strain isolated from sputum sample which is one of four juvenile patients could not be typed.

Table 1. Serogroups and Serotypes distributions in 80 *S.pneumoniae* strains

Serogroup/Serotype	N/%
1	1/1.25
19F, 19A, 19B, 19C	17/21.25
7F, 7A, 7B, 7C	3/3.75
14	6/7.50
18F, 18A, 18B, 18C	5/6.25
6A, 6B, 6C	7/8.75
23F, 23A, 23B	6/7.50
4	1/1.25
3	4/5
5	2/2.50
9A, 9L, 9N, 9V	3/3.75
Can not be serotyped	25/31.25
Total	80

In sputum samples, the most common serogroups were 19, 6 and 18. Three sputum samples of juvenile patients were found serogroup 19, 23 and 6 respectively. Four of five blood samples were found serogroup 19. While forty-four of eighty strains were found to be resistant to oxacillin, thirteen of these strains were found to be serogroup 19. According to E-test findings, 39 (48.75%) strains were resistant to oral penicillin at low level and 5 (6.25%) strains were resistant to penicillin at high level.

Due to results of penicillin E-test, distribution of serotype/serogroups, 13 strains having low-level resistance were determined to be in serogroup 19. Three strains of five high-level resistant strains were found as serogroup 23 (Table 2).

Table 2. The distribution of oral penicillin resistant strains according to Serogroup and Serotypes

Serogroup/Serotype	Low-level oral penicillin resistance (N)	High level oral penicillin resistance (N)
1	-	-
19F, 19A, 19B, 19C	13	-
7F, 7A, 7B, 7C	1	-
14	3	-
18F, 18A, 18B, 18C	4	-
6A, 6B, 6C	5	1
23F, 23A, 23B	2	3
4	-	-
3	1	-
5	2	-
9A, 9L, 9N, 9V	3	-
Can not be serotyped	5	1

The highest resistance was observed in trimethoprim/sulfamethoxazole as 45%; this was followed with 31.25% in erythromycin. All strains were susceptible to vancomycin, linezolid and rifampicin. According to the results of macrolide phenotypes, the study showed that 17 strains (21.25%) have had constitutive Macrolide, Lincosamide, and Streptogramin B (cMLSB) phenotype and eight strains (10%) have had M phenotype. Inducible Macrolide, Lincosamide, and Streptogramin B (iMLSB) + M phenotype was not encountered. Serogroups 19, 6, 23 and 18 were found more resistant than the other groups. The highest resistance level of nearly all antibiotics was found in serogroup 19. There was no resistance found in serotype 1 (Table 3 and 4).

DISCUSSION

S.pneumoniae is the most important cause of diseases which can cause acute respiratory tract infections in developing countries. *S.pneumoniae* strains recently gained importance because of the increasing resistance to antibiotics. In recent years, due to penicillin resistance in therapy, antibiotics such as macrolides, sulfamethoxazole/trimethoprim, tetracycline, chloramphenicol and quinolones which are supposed to be alternative also have been observed to increase resistance. Strains showing resistance to antibiotics are frequently resistant to more than one antibiotic group and some specific serotypes of these strains have shown clonal distribution in the world (3).

According to various studies of penicillin resistance in various countries of the world; one study in USA detected that 32% of 1647 strains showed penicillin resistance (4). In an Asian study, penicillin resistance rates and CLSI parenteral penicillin resistance rates of 2184 strains were calculated as 0.7% and 57.5%, respectively (5). In a review about resistance profiles, penicillin resistance rates in Spain, France, Greece and Israel were determined between 25–50% ; in Portugal, Ireland, Finland and Turkey were between 10–25% ; in Italy 5–10%; in England, Germany, Austria, Norway and Sweden it was in between 1–5% (2).

Table 3. The distribution of antibiotic resistant strains according to Serogroup and Serotypes

	Serogroups/Serotypes											
Antibiotics	**1**	**19G**	**7G**	**14**	**18G**	**6G**	**23G**	**4**	**3**	**5**	**9G**	**C**
Oxacillin	-	12	1	3	4	6	5	-	1	2	3	7
Eritromycin	-	7	-	3	3	4	4	-	1	-	-	3
Tetracycline	-	7	-	2	1	3	4	-	1	-	-	5
Levofloxacin	-	1	-	-	-	-	-	-	-	-	-	1
Ofloxacin	-	2	(1)	(1)	-	2+ (1)	1	(1)	2	-	-	3
SXT	-	11	-	3	2	5	2+ (1)	-	1	2	3	3+ (4)
Chloramphenicol	-	-	-	-	2	2	-	-	-	-	-	-
Clindamycin	-	6	-	2	3	3	-	-	1	-	-	2

G: Serogroup, **C:** Can not be serotyped, **(n):** Intermediate resistance, **SXT:** Sulfamethoxazole-Trimethoprim

Table 4. Antibiotic resistance of *S.pneumoniae* strains

Antibiotics	Sensitive (N/%)	Intermediate (N/%)	Resistant (N/%)
Eritromycin	55/68.75	-	25/31.25
Clindamycin	63/78.75	-	17/21.25
Vancomycin	80/100	-	-
Tetracycline	57/71.25	-	23/28.75
Ofloxacin	64/80	4/5	12/15
Levofloxacin	78/97.50	-	2/2.50
SXT	39/48.75	5/6.25	36/45
Chloramphenicol	76/95	-	4/5
Rifampicin	80/100	-	-
Linezolid	80/100	-	-
Oxacillin	41/51.25	-	39/48.75

SXT: Sulfamethoxazole-Trimethoprim

According to studies about penicillin resistance, one study performed in Istanbul between 2008–2010 has shown that 61 pneumococcus strains have had 54% low-level resistance to penicillin and 8.2% high-level resistance (6). In a study performed in our hospital in 2008, 97 *S.pneumoniae* strains were detected to have low-level resistance as 28% to penicillin and high level resistance as 3.1% (7).

In our study, 44 *S.pneumonia*e strains (55%) were found to be resistant to oxacilin. According to oral penicillin MIC values, 39 pneumococcus strains (48.75) showed low-level resistance to penicillin. Five pneumococcus strains (6.25%) were shown to have high-level resistance to penicillin. According to parental penicillin criteria, there were no resistance. Low-level resistance to penicillin was found higher than the other studies have shown. These findings emphasize a current increase in penicillin resistance.

The highest resistance, was observed for trimethoprim/sulfamethoxazole. In a study from Taiwan, 837 strains were resistant to trimethoprim/sulfamethoxazole in a rate of 81% (8). Between 1988–1992, 195 strains in Brazil were shown to be resistant to trimethoprim/sulfamethoxazole in a rate of 29.2% (9).

A study in Turkey has indicated that trimethoprim/sulfamethoxazole and erythromycin resistance rates of 61 pneumococcus strains isolated between 2008–2010 were 67.2% and 55.8%, respectively (5). Our study also demonstrated that the resistance rate against trimethoprim/sulfamethoxazole was 45% while the intermediate resistance rate was 6.2%. In our study, serogroup 19 was determined to have the highest resistance rate against trimethoprim/sulfamethoxazole and erythromycin. Tetracycline resistance was also high (28.75%).

In various studies, serogroups 19, 6, 1, 18 have been reported to have multiple antibiotic resistance (10). In our study, the highest antibiotic resistance was detected in serogroup 19, serogroup 6, serogroup 18 and serogroup 23, respectively. Resistance levels of serogroup 7, serogroup 9, serotype 4 and serotype 3 were low and untyped 25 strains were observed to have high-level resistance. 21 strains were detected to have multiple drug resistance and these strains were serogroups 19, 6, 23, 18, 14 and 3. Three strains of multiple drug resistant strains belong to unserotyped group. There was no resistance for linezolid, vancomycin and rifampicin.

Nowadays, vaccine applications as well as antibiotic usage have been reported to be effective on prevalence of pneumococcus serotypes having antibiotic resistance. Beginning with the vaccine application, it was reported that many countries have decreasing numbers of resistant pneumococcus serotypes while antibiotic usage decreased in pneumococcus infections. Many countries applied pneumococcus vaccines to their vaccination programs and by providing protection by vaccines, some serotypes decreased. Decreased serotypes were generally reported to be resistant serotypes (1).

In the studies reported from many countries in the world, after vaccination serotype 19A was reported as both to be the common and to be the serotype which has the highest possibility of resistance (11).

It was considered to be the result of vaccination programs that the prevalence of serotypes which could not be avoided by vaccination is expected to be increased in time. Variation in serotype distribution has occurred in time and geographically. New clones derived through clonal propagation and capsular changes are crucial for antibiotic resistance.

According to the results of our study, the number of non-serotyped strains was calculated as 25 (31.25%). Of serotyped strains, 17 strains (21.25%) which showed the highest resistance rate were determined as serogroup 19 as similar in other studies (3, 5, 8, 9). Other serotypes which are frequently compared except serogroup 19 were found as seven strains in serogroup 6 (8.75%); 6 strains in serotype 14 (7.50%); six strains in serogroup 23 (7.50%) and five strains in serogroup 18 (6.25%).

Although, the pneumococcal vaccine decreases nasopharyngeal carriage and obtain herd immunity in sensitive populations, to our knowledge there is not enough data about pneumococus serotypes, serogroups and antimicrobial susceptibility in Turkey. Soysal et al. (2016) reported that, 45% of *S.pneumoniae* strains isolated were non-vaccine type and 55% were vaccine type (PCV7 and PCV 13). They also reported that frequently isolated serotypes were 6A/B/C (n = 22, 16.5%), 19F (n = 18, 13.5%), 23F (n = 15, 11.2%), serotype 9V/A (n = 10, 7.5%), 12F (n = 5, 4.5%), 15A/F (n = 7, 4.5%) and 22 A/F (n = 6, 4.5%). According to the results, which based on the minimum inhibitory concentration (MIC) values, S. pneumoniae strains found penicillin resistant with rate of 62%, and 13% of the strains were ceftriaxone resistant. Erythromycin and clindamycin resistance were 43% and 31%, respectively (12). Correlatively with these results, a study by Arvas et al (2017) also determined that, vaccine type serotypes were detected in 12.6% of children who received full-dose PCV13 vaccine and those strains were found penicillin susceptible with the rates of 85.7% (13). In our study, similar to other studies from Turkey, serogroup 19 and 6 were detected frequently. S. pneumoniae isolates resistance rates were 31.25% for erythromycin and for clindamycin% 21.25. Serogroups 19, 6, 23 and 18 were found more resistant than the other serogroups. The highest resistance rates to almost all antibiotics were found in serogroup 19.

These findings demonstrated that non-serotyped strains consisted of other serotypes which are not covered by the vaccine and in the light of this information, importance of the non-serotyped strains can be understood. Many of recently used pneumococcus vaccines has been known to be protective against the serotypes which have a high possibility to develop resistance; however, as stated in other studies, it can be discussed that protection by vaccination against serotypes which have high resistance possibility can increase the prevalence of other serotypes which the vaccine cannot be protective and this may provide the occurrence of an infection effect. Vaccine usage and its affect on

serotype distribution and resistance rate of these serotypes should be monitored and more studies should be performed.

The results emphasize the global problem of resistant pneumococcus strains and the need for protection through multiple strategies. These strategies can be methods such as vaccination programs, antibiotics control programs and surveillance.

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Board of our university. Ethical approval: B.30.2.İST.0.30.90.00/29603, 08 October 2012

Informed Consent: All patients gave their informed consent before participating in the study.

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Conflict of Interest: The authors declare that there are no conflicts of interest.

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