Distribution of serotypes and antimicrobial resistance of Streptococcus pneumoniae in a children's hospital in Turkey

Meral Özalp[1], Güler Kanra[2], Deniz Gür[3]

[1]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, [2]Department of Pediatrics, Faculty of Medicine and [3]İhsan Doğramacı Children's Hospital, Clinical Microbiology Laboratory, Hacettepe University, Ankara, Turkey

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Streptococcus pneumoniae isolates resistant to penicillin and other antibiotics have been increasing in many parts of the world. The aim of this study was to evaluate the antimicrobial susceptibilities to penicillin and other commonly used agents in 98 isolates recovered between 1997 and 1998 from clinical specimens from children, and to determine the serotypes/serogroups related to resistance. Susceptibility to penicillin was determined by E-test and disk diffusion tests were used for the other antimicrobials. Serotyping was performed on all the isolates by the quelling reaction. The rates of intermediate- and high-level resistance to penicillin were 29.6% and 2%, respectively. Overall resistance to trimethoprim-sulfamethoxazole was high (46%), of which 21% coexisted with penicillin resistance. Resistance rates to erythromycin and chloramphenicol were 5% and 1%, respectively. Five isolates were multi-drug resistant. The most frequent serotypes associated with penicillin resistance were serotypes 19, 23, 6, 9 and 15.

Key words: Streptococcus pneumoniae, penicillin resistance, serotypes.

Streptococcus pneumoniae is the most commonly identified bacterial cause of pneumonia, otitis media, meningitis and systemic infections in children[1,2]. Penicillin has been used effectively in the treatment of these infections for many years; however, in recent years strains with decreased susceptibilities to penicillin and other β-lactam antibiotics have been increasing in many parts of the world. Most of the penicillin-resistant strains are associated with serotypes 6, 9, 14, 19 and 23. Penicillin-resistant isolates are also reported to be more resistant to other groups of antimicrobials which are used alternatively in the treatment of S. pneumoniae infections[3].

Although penicillin-resistant S. pneumoniae (PRSP) strains have been reported from different centers in Turkey, there are only a few studies on the distribution of the serotypes of resistant isolates[4,5].

The aim of this study was to evaluate the antimicrobial susceptibilities to penicillin and other commonly used agents, and to determine the serotypes/serogroups related to resistance.

Material and Methods

Bacterial Isolates

A total of 98 strains isolated consecutively from clinically significant specimens from children between 1997 and 1998 in Hacettepe University, Faculty of Medicine, İhsan Doğramacı Children's Hospital were included in this study. Only one isolate per patient was included. Distribution of the specimens are shown in Table I.

Table I. Distribution of the Isolates According to the Specimens

Specimen	Number
Sputum	53
Pus	17
Eye	10
Blood	7
Middle ear fluid	5
Cerebrospinal fluid	3
Tracheal aspirate	2
Urine	1
Total	98

Culture and identification

All the specimens were inoculated into 5% sheep blood agar and incubated in 5% CO_2 atmosphere at 35°C for 24 hours. Sputum samples which had polymorphonuclear leukocytes >25 and epithelial cells <10 per field in Gram's stain were included. S. pneumoniae was identified by Gram's stain, colony morphology, optochin sensitivity and bile solubility tests[6].

Antimicrobial agents and susceptibility tests

The following antimicrobial agents were tested against all isolates: Penicillin, erythromycin, cefotaxime, chloramphenicol and trimethoprim-sulfamethoxazole (TMP/SMX). The in vitro susceptibilities of the isolates to penicillin were determined by the E-test (AB-Biodisk, Sweden) using Mueller Hinton agar supplemented with 5% sheep blood. Isolates were classified as susceptible [minimum inhibitory concentration (MIC) ≤0.064 mg/L], intermediately resistant (MIC 0.12-1 mg/L) and resistant (MIC ≥2 mg/L).

For the remaining antimicrobials, disk diffusion test was employed following National Committee for Clinical Laboratory Standards (NCCLS) guidelines[7]. ATCC 49619 S. pneumoniae was used as the quality control strain.

Serotyping

Serotypes were determined by the capsular typing test using the Pneumotest Kit (Statens Serum Institut, Denmark) with 12 pooled antisera.

Results

During the study period, 98 isolates were recovered from clinical specimens, most frequently from sputum, followed by pus samples (Table I).

According to their MIC's, 67 (68.4%) of the isolates were susceptible to penicillin, 29 (29.6%) were intermediately and 2 (2%) were highly resistant.

The majority of the resistant isolates were from sputum (80.6%): two were from middle ear fluid, two were from cerebrospinal fluid, one was from blood and one was from an eye exudates. Highly resistant isolates were from sputum samples.

Among the PRSP, 26 (83.8%) isolates were also resistant to one or more antimicrobial agents. The most frequent resistance pattern was resistance to penicillin and TMP/SMX. Resistance rates and patterns are shown in Tables II and III, respectively.

Serotyping was performed on all of the isolates, with results given in the descending order of frequency: type 19 (12.2%), 6 (10.2%), 15 (9.2%), 9 (9.2%), 23 (7.1%), 11 (4.1%), 8 (3%), types 1, 4, 14 and 18 (2% each) and types 5, 7, 17, 20, and 22 (1% each). Eight strains (8.2%) were identified as non-vaccine serotypes. Twenty-three isolates (23.5%) could not be serotyped with the available antisera. Isolates resistant to penicillin were more frequently associated with serotypes 19, 23, 6, 9 and 15. Among multi-drug resistant isolates, two were serotype 19, and the others were serotypes 6, 1 and 18. There was no resistance to penicillin among the non-vaccine type isolates. Table IV shows the distribution of the serotypes according to penicillin resistance.

Table II. Resistance to Antimicrobial Agents in S. penumoniae (n:98)

Antimicrobial agent	Number	%
Penicillin	31	31.6
TMP/SMX	45	45.9
Chlorampenicol	2	2
Erythromycin	5	5.1
Cefotaxime	0	0

TMP/SMX: trimethoprim-sulfamethoxazole.

Table III. Resistance Profiles in Penicillin-resistant S. pneumoniae

Resistance pattern	Number	%
Penicillin+TMP/SMX	21	21.4
Penicillin+TMP/SMX+Erythromycin	4	4
Penicillin+TMP/SMX+Erythromycin+Chloramphenicol	1	1

TMP/SMX: trimethoprim-sulfamethoxazole.

Table IV. Distribution of the Serotypes According to Penicillin Resistance

Serotype/serogroup	Intermediately resistant	High-level resistant
19	6	–
23	5	–
6	5	–
9	4	1
15	3	1
11	2	–
14	2	–
1	1	–
18	1	–
Total	29	2

Discussion

Resistance to penicillin and other antimicrobials among S. pneumoniae isolates is increasing worldwide. Resistance to penicillin shows geographical variations: high rates of resistance are reported in countries such as Spain, Hungary, and Greece, whereas in other European countries such as Belgium, Germany and Italy, the rates are low[8-13]. In a recent multicenter study from Turkey, the overall penicillin resistance among isolates from children was 35.7%, and most of these isolates were from respiratory specimens[14]. This rate of resistance to penicillin is similar to our findings.

In recent years, pneumococci resistant to other classes of drugs along with penicillin has begun to emerge in many countries and is a potential problem in the therapy of infections due to these bacteria[3,9].

Resistance to macrolides is reported to be associated with penicillin resistance[3]. In European countries such as Spain, Italy and Hungary, resistance to erythromycin among PRSP was between 22.2-53%[8,9,13]. In our study, although the overall resistance to erythromycin was lower (5%), al isolates were resistant to penicillin.

Rate of resistance to TMP/SMX varies by country and can be as high as 65.9% as in Hungary, and as low as 4.5%, as in Israel[9,15]. It was frequent in this study, reaching 46%. In 26% of TMP-SMX-resistant isolates, resistance coexisted with resistance to penicillin, two of them being highly resistant. These resistance rates are very similar to the results of the multicenter study in Turkey, where the overall rate of resistance to TMP/SMX was 47.4% in children, reaching 52.5% in Hacettepe University Children's Hospital isolates, which suggests that the level of resistance to this agent may be increasing in our hospital[14].

Overall resistance to chloramphenicol was 2% in our isolates. This rate is lower compared to some countries, and may be explained by the low use of this agent in our hospital[10].

Resistance to antibiotics of at least three different groups has been defined as multiple resistance[3]. Multidrug resistant pneumococci are common and reported to be increasing worldwide[16-18]. In this study, five isolates (5.1%) were multi-drug resistant and belonged to different serotypes which were covered in the 23 valent vaccine.

Determining the serotypes of S. pneumoniae from different clinical samples is important as the vaccine production is based on the most common serotypes. Ninety serotypes of S. pneumoniae are currently recognized based on capsular polysaccharides. Twenty-three capsular polysaccharides from the most commonly isolated serotypes are used in a polyvalent vaccine. The majority of the PRSP strains found worldwide belong to serogroups 6, 9, 14, 19, and 2 and are included in the polyvalent vaccine and 7, 9 and 11 valent protein conjugate vaccines[3,6,9]. These five serotypes are the most common serotypes in PRSP in countries such as Spain, Hungary, Israel and South Africa[8,9,15,16]. In Greece, in addition to these serotypes, serotype 15 has been reported[10]. In two recent studies from Turkey, serotypes 19 and 23 were the most prevalent in PRSP[4,5]. In a previous study, seroypes 6 and 9 were the most common serotypes in PRSP from adults[19]. In our study, two isolates which were highly resistant to penicillin were serotypes 9 and 15,

and serotypes 19, 23 and 6 were the most common serotypes in intermediately resistant isolates. In a recent multicenter molecular study from Turkey, the same serotypes were common; however, there was no correlation between antibiotic susceptibilities, serotypes and clones[20]. Our results are similar to those from other parts of the world with regard to serotypes; however, it is not possible to suggest whether there is a clonal spread of these isolates in Turkey with the data at hand.

Studies investigating resistance rates to penicillin and serotypes of resistant isolates should be performed periodically in order to be aware of the changes in resistance patterns and serotypes in our country.

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