

## ORIGINAL ARTICLE

# DRUG SUSCEPTIBILITY OF *STREPTOCOCCUS PNEUMONIAE* STRAINS ISOLATED IN TEHRAN, IRAN

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**Background** – The emergence and spread of penicillin and multidrug resistance in *Streptococcus pneumoniae* has become a major concern worldwide. Consequently, clinical laboratories should consider screening-selected isolates to determine whether they are susceptible to cefotaxime as well as to penicillin. The aim of this study was to survey drug resistance among clinically important isolates of *S. pneumoniae* recovered from patients in Tehran.

**Methods** – The drug susceptibility of 130 isolates of *S. pneumoniae* cultured from severely infected patients from 1998 to 2000 was determined using both agar disk diffusion and macro broth dilution tests. Isolates were grown from clinical specimens including blood (60%) and cerebrospinal fluid (20%) from patients with pneumonia, bacteremia or meningitis. The remaining isolates were recovered from sputa (10%) and sinus exudates. Bacterial cultures were sent to Pasteur Institute for conformational and drug susceptibility tests.

**Results** – Sixty-eight percent of isolates were resistant to penicillin, 10% to erythromycin, 52% to cotrimoxazole, and 56% to tetracycline. Of the 25% of the organisms that were found to be resistant to cefotaxime, 1% were highly resistant. None of the isolates were resistant to vancomycin. The minimum inhibitory concentrations (MICs) of cefotaxime corresponded to the MICs of penicillin (being the same or different by only 1 or 2 dilutions).

**Conclusion** – Resistance to penicillin and cefotaxime increased over the study period. Penicillin-resistant strains of *S. pneumoniae* cultured from patients in Tehran were also more likely to be resistant to other beta-lactams such as cefotaxime. The increasing trend of antibiotic resistance among strains of *S. pneumoniae* in Iran is alarming, and the treatment of infections with this organism will be more difficult in the future.

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**Keywords** • drug resistance • pneumococcus • *Streptococcus pneumoniae*

### Introduction

*Streptococcus pneumoniae* is responsible for many cases of community-acquired pneumonia and septicemia. It is one of the most common pathogens in bacterial meningitis, and the most prevalent agent in cases of otitis media.<sup>1, 2</sup> At the beginning of the antibiotic era, clinical isolates of *S. pneumoniae* exhibited a uniformly high sensitivity to antibiotics including benzylpenicillin, which had an extremely low (5 – 10 ng/mL) minimum inhibitory concentration (MIC) against these strains. Therefore, penicillin

was generally recommended as the antibiotic of choice in suspected or verified pneumococcal infections.<sup>3</sup> The emergence of drug-resistant *S. pneumoniae* poses new difficult challenges for treatment of these infections. The first appearance of clinically significant penicillin-resistant pneumococci occurred in South Africa in 1977 and 1978, and penicillin-resistant isolates have since been reported worldwide.<sup>4</sup>

The emergence and spread of penicillin and multidrug resistance among *S. pneumoniae* has become a major concern worldwide and is seriously challenging current treatment strategies.<sup>1, 5</sup> Consequently, clinical laboratories should consider screening-selected isolates to determine whether they are susceptible to cefotaxime as well

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as to penicillin.<sup>6</sup>

Since disk diffusion test does not provide acceptable accuracy as a screening method in drug susceptibility, routine determination of MICs of penicillin and selected cephalosporins has been recommended for treatment of serious pneumococcal infections.<sup>7</sup> The recognition of susceptibility to antimicrobial agents among *S. pneumoniae* strains in a given location is essential to guide treatment decisions.<sup>8</sup> The purpose of the present study was to obtain information on the antimicrobial susceptibilities of *S. pneumoniae* strains especially to penicillin and cefotaxime in Tehran.

## Materials and Methods

A total of 130 clinical isolates of *S. pneumoniae* were collected from eight medical centers in Tehran including Emam Khomeini Hospital, Children's Medical Center, Sina Hospital, and Bahar Clinical Laboratory between 1998 and 2000. Most isolates were from blood (n = 78, 60%), cerebrospinal fluid (n = 26, 20%), sputum (n = 14, 11%), and sinus exudates (n = 12, 9%).

All isolates were cultured in 5% sheep's blood agar, and incubated for 18 – 24 hours at 37°C in 5% CO<sub>2</sub>. Isolates were identified as *S. pneumoniae* based on colony morphology, susceptibility to optochin, and sodium deoxycholate solubility.

Disks containing antibiotics were purchased from Difco (Detroit, Michigan, USA). Initially, all clinical isolates were screened for resistance to penicillin using disks containing 1 µg oxacillin. Susceptibility of strains to erythromycin (19 µg), cotrimoxazole (trimethoprim/sulfamethoxazole 23.75/1.25 µg), chloramphenicol (30 µg), tetracycline (30 µg), ampicillin (10 µg), cefazolin (30 µg), oxacillin (1 µg), cefamandole (30 µg), and vancomycin (30 µg) were determined using the Kirby-Bauer disk diffusion method on Mueller-Hinton blood agar (Difco) according to National Committee for Clinical Laboratory Standards recommendations.<sup>9</sup> The MICs to penicillin and cefotaxime were confirmed by broth macrodilution with cation-adjusted Mueller-Hinton broth according to standard methods.<sup>9</sup>

The resistance of strains to penicillin was considered intermediate when MICs were 0.5 – 1 µg/mL, and high when MICs were at least 2 µg/mL.<sup>9</sup> Isolates were considered highly resistant

**Table 1.** Rate of susceptibility and resistance to various antibiotics obtained using the disk diffusion method.

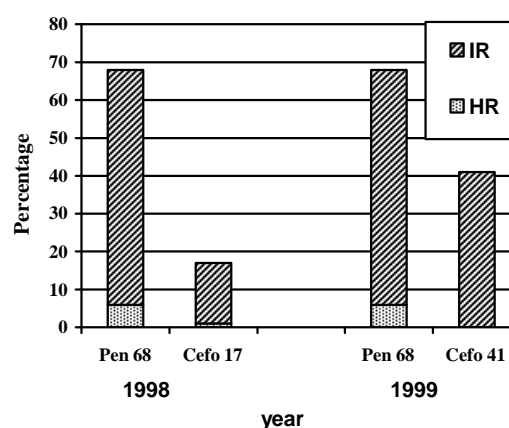
Antibiotics	Sensitivity (%)	Intermediate resistance (%)	High resistance (%)
Erythromycin	90	2	8
Cotrimoxazole	48	8	44
Chloramphenicol	78	0	22
Tetracycline	44	20	36
Ampicillin	100	0	0
Cefazolin	100	0	0
Oxacillin	12	42	46
Cefamandole	100	0	0
Vancomycin	100	0	0

to cefotaxime when the MIC was more than 4 µg/mL. Parametric methods (*t*-test) were used for statistical analysis of the data obtained from drug susceptibility testing.

## Results

Table 1 shows the results of disk diffusion susceptibility testing. When the isolates were tested by MIC to penicillin (Figure), only 32% of them were susceptible to penicillin (MIC ≤ 0.06 µg/mL). Sixty-eight percent of isolates were resistant to penicillin, of which eight (6%) were highly resistant (MIC 2.0 µg/mL). The remaining isolates (62%) showed intermediate resistance (MIC 0.1 – 1 µg/mL), of which 16% were relatively resistant to cefotaxime (MIC 0.5 – 1.0 µg/mL), and one was highly resistant to cefotaxime with an MIC > 4 µg/mL (Figure).

During the second year of the study (January 1999 to April 2000), there was no increase in the rate of penicillin resistance (67%), but resistance to cefotaxime steadily increased, and 41% of



**Figure.** Rate of resistance to penicillin, and cefotaxime among Iranian strains of *Streptococcus pneumoniae*. IR: intermediate resistance; HR: high resistance.

## Drug Susceptibility of *S. pneumoniae* Strains Isolated in Tehran, Iran

**Table 2.** Correlations between minimum inhibitory concentrations ( $\mu\text{g/mL}$ ) of penicillin G and cefotaxime.

Penicillin	0.1	0.2	0.4	0.8	1.6	3.2
Cefotaxime	0.01	0.2	0.4	0.8	1	1.6
Correlation (%)	91	60	79	91	88.7	0

isolates were relatively resistant with MIC between 0.5 – 1.0  $\mu\text{g/mL}$  (Figure).

MICs of cefotaxime correlated well with those of penicillin (Table 2).

Screening of isolates for susceptibility to penicillin with oxacillin disks (1  $\mu\text{g}$ ) showed that 88% were penicillin-resistant. Determination of MICs of penicillin demonstrated that the actual rate of resistance among these isolates was 12% lower than the result obtained by the disk diffusion test.

### Discussion

Pneumococci have remained important human pathogens despite the introduction of penicillin and the new generation of antibiotics. Our study showed that the incidence of penicillin-resistant strains among Iranian clinical isolates is alarmingly high. The rate of resistance to penicillin in our isolates was higher than the resistance rates reported from other countries,<sup>10</sup> underlining the necessity of more attention to the importance of antibiotic therapy for pneumococcal infections. A detailed study from South Spain indicated that the prevalence of resistant strains increased during the two years of the study period according to analysis of MICs.<sup>3</sup> Penicillin resistance is also a matter of concern in Spain where the rate of resistance to penicillin has been reported to be more than 50%.<sup>11-13</sup> In our study, the rate of resistance to cefotaxime was lower than resistance rates to penicillin. When the MIC of penicillin increased, the resistance to cefotaxime increased too (Table 2). This means that a similar mechanism is probably involved in the resistance of *S. pneumoniae* to both antibiotics. There was a significant increase in the rate of resistance to cefotaxime (24%) among strains isolated in the second year of our study. Investigations from other countries have also documented an increase in the prevalence of resistance to penicillin and other agents among pneumococcal strains.<sup>11, 14, 15</sup> Several authors in other countries have pointed out that resistance to cefotaxime is becoming more common due to the widespread use of this antibiotic to treat infections of the upper respiratory tract.<sup>16</sup>

In reports from other parts of the world, treatment failures with penicillin and third-generation cephalosporins are well described in infections such as meningitis due to penicillin-resistant *S. pneumoniae* (PRSP). The outcome of pneumonia due to PRSP with intermediate resistance to penicillin is not significantly different if a penicillin or cephalosporin is used in treatment. Thus, the empirical treatment of meningitis and prescribing additional drugs such as vancomycin is based on the likelihood of penicillin resistance.<sup>16, 17</sup> For routine testing of penicillin susceptibility in *S. pneumoniae*, use of 1  $\mu\text{g}$  oxacillin disks is currently recommended. However, our results showed that using this method, 20% of isolates were resistant to penicillin, whereas determination of MICs showed that all of these strains were sensitive to penicillin. The prevalence of resistance to erythromycin in our study was low (10%), probably due to the infrequent use of erythromycin in Iran. Tetracycline (56%), cotrimoxazole (52%), and chloramphenicol resistance (22%) were common among the pneumococci studied. Other investigators have demonstrated the existence of strains that are resistant to cephalosporins, macrolides, and tetracyclines.<sup>18</sup> However, there is considerable geographic variation in isolated strains depending on antibiotic consumption in different countries.<sup>19-21</sup>

The results of our study showed that *S. pneumoniae* strains isolated in Tehran are highly resistant to penicillin while the resistance to cefotaxime looks to be a major concern too. Thus, prescription of cephalosporins as a first choice antibiotic in the treatment of *S. pneumoniae* infections should be avoided; a combination therapy policy is recommended.

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