Penicillin-Resistant *Streptococcus pneumoniae* in Iran

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Key Words

Streptococcus pneumoniae • Penicillin-nonsusceptible
S. pneumoniae • Penicillin, minimum inhibitory concentration

Abstract

Objective: To determine the prevalence of penicillin-resistant *Streptococcus pneumoniae* isolated from patients with community-acquired pneumococcal infections. **Materials and Methods:** A broth dilution method was used to determine the minimum inhibitory concentration (MIC) of penicillin and other commonly used antibiotics. 115 heavy growth or pure culture of *S. pneumoniae* strains were isolated from: blood 10, cerebrospinal fluid (CSF) 15, ear 5, eye 12, purulent rhinosinusitis 48, sputum 22, and pleural fluid 3. **Results:** Of the 115 pneumococcal isolates, 76 (66.1%) were sensitive to penicillin while the remaining 39 (33.9%) were nonsusceptible (15.6% resistant and 18.3% intermediately resistant). Among the 25 pneumococcal isolates from sterile sites (blood 15, CSF 10), 15 (60%) were penicillin-resistant whereas among the 90 isolates from nonsterile sites, 24 (26.7%) were resistant to penicillin (<0.004). The MIC values of antibiotics tested for *S. pneumoniae* were: penicillin 0.008–4 µg/ml, chloramphenicol 0.25–32 µg/ml, erythromycin 0.008–128 µg/ml, tetracycline 0.06–64 µg/ml, vancomycin 0.03–0.5 µg/ml, azithromycin 0.016–128 µg/ml, ciprofloxacin 0.006–8 µg/ml, cefotaxime 0.007–2 µg/ml, and ceftriaxone 0.016–12 µg/ml. **Conclusion:** Approximately one third of *S. pneumoniae* isolated from the clinical specimens were nonsusceptible to penicillin in this region.

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Introduction

Streptococcus pneumoniae are major bacterial pathogens that cause mucosal infections such as pneumonia, sinusitis, meningitis, otitis media and bronchitis among children and predisposed adults [1]. For many years these bacteria were fully susceptible to benzyl penicillin with minimum inhibitory concentration (MIC) values of 5–10 ng/ml. Penicillin-resistant *S. pneumoniae* (PRSP) from clinical infection was first reported in Australia in 1967 [2]. During the past three decades a changing sensitivity pattern of the bacteria to penicillin, other β-lactams and non-β-lactam agents has emerged, notably in developing countries and other parts of the world [3–6]. Highly PRSP (MIC ≥ 2 µg/ml) are more likely to be resistant to multiple antibiotics than are those with intermediate susceptibility (MIC = 0.1–1 µg/ml) [7, 8]. The nonsusceptible strains of *S. pneumoniae* have penicillin MICs of ≥ 1 µg/ml which are often resistant to several other antibiotics [7, 8]. Development of resistant strains of *S. pneumoniae* to chloramphenicol, trimethoprim-sulfamethoxazole, and tetracycline limits the therapeutic effects of these antibiotics. Erythromycin-resistant strains among patients with pneumonia in some regions of European and Asian countries and treatment failure of pneumococcal meningitis with extended spectrum cephalosporins, ceftriaxone and cefotaxime in Europe and the USA [9–11] have further complicated the treatment of pneumococcal infections such that the current initial empirical treatment of infections need to be altered. Although there are various reports on the PRSP from Asian countries [4, 6, 12–

14], there is no authentic data on this issue from Iran. In Iran, pneumococcal infections are especially prevalent among children and elderly [15]. Moreover, antibiotic consumption is high and in some instances without prescription, therefore multiple drug-resistant isolates should be expected in this area.

The present study was conducted in order to determine the prevalence of PRSP isolated from patients with community-acquired pneumococcal infections and also to investigate the multidrug resistance strains of these bacteria to the commonly used antibiotics.

Materials and Methods

Specimen Collection and Isolation of S. pneumoniae

Clinical specimens including cerebrospinal fluid (CSF), blood, sputum and nasal secretion from patients (mean age 28.9, range 1–70 years; male/female ratio 1.34) who were admitted either to our University Hospitals or to the clinics, were sent to the Department of Microbiology, Shiraz Medical School, for isolation and characterization of *S. pneumoniae*. A direct smear from CSF, sputum and nasal swab was prepared, Gram-stained and examined microscopically for the presence of Gram-positive diplococci and polymorphonuclear cells. These specimens were then cultured on sheep blood agar (SBA) and chocolate agar plates, prepared by blood agar base (Merck KGaA, Darmstadt, Germany) and 5% defibrinated sheep blood, and incubated at 35°C in the presence of 5% CO₂ for 48 h. Blood samples from patients were cultured on trypticase soy broth supplemented with lysed horse blood and then subcultured on chocolate and SBA plates. *S. pneumoniae* were characterized by the typical greenish (α) type hemolysis on SBA, bile solubility test and sensitivity to ethylhydrocupreine (optochin test) with the zone of inhibition ≥ 14 mm in diameter.

A total number of 115 heavy growth or pure culture of *S. pneumoniae* strains, isolated from blood 10, CSF 15, ear 5, eye 12, purulent rhinosinusitis 48, sputum 22, and pleural fluid 3, were included in this study. Any repeat isolate from the same patient obtained more than once and *S. pneumoniae* isolated from patients on antibiotic therapy were excluded from this study.

Oxacillin Disk Diffusion Assay

The 1- μ g oxacillin disk diffusion method was carried out on all *S. pneumoniae* isolates on SBA. The diameter of the zone of inhibition was measured after 24 h of incubation at 35°C, in the presence of 5% CO₂. The inhibition zone of ≥ 20 mm was considered penicillin-susceptible, while those with a diameter ≤ 19 mm were considered resistant as recommended by CLSI [16].

MIC Determination

The microbroth dilution method was used for determination of the MICs of antimicrobial agents for each isolate of *S. pneumoniae* [17]. The following concentrations of antimicrobial agents (expressed as μ g/ml) were used: penicillin 0.015–16, chloramphenicol 1–32, tetracycline 0.25–32, erythromycin and azithromycin 0.06–8, vancomycin 0.5–8, cefotaxime 0.015–8, and ciprofloxacin 0.125–4. Each pneumococcal isolate was grown on tryptic

soy agar with 5% defibrinated sheep blood in the presence of 5% CO₂ at 35°C for 18 h. Pneumococcal cell suspension equal to that of a 0.5 McFarland turbidity standard was prepared in Mueller-Hinton broth and diluted 1:10 to give 10⁷ colony-forming units/ml. This dilution of bacteria was used to inoculate cation-adjusted Mueller-Hinton broth supplemented with 5% lysed horse blood which contained a series of increasing concentrations of antimicrobial agents. All cultures were incubated at 35°C in 5% CO₂ for 24 h. The MIC was taken as the lowest concentration of antibiotics which inhibited the visible growth of bacteria. A penicillin-resistant strain of *S. pneumoniae* ATCC 49619 with a MIC of 0.25–0.5 μ g/ml was used as control in each susceptibility test.

Statistical Analysis

The significant differences of antibiotic resistance patterns were determined by the χ^2 and Fisher exact tests. SPSS, version 11.5, was used to perform statistical analysis.

Results

The susceptibilities of the pneumococcal isolates from the different sources are summarized in table 1. Of the 115 isolates, 76 (66.1%) were penicillin-sensitive while the remaining 39 (33.9%) were penicillin-nonsusceptible (21 isolates, 18.3%, intermediately resistant and 18, 15.6%, fully resistant). Among the 25 pneumococcal isolates from sterile sites (blood 15, CSF 10), 15 (60%) were penicillin-nonsusceptible whereas among the 90 isolates from nonsterile sites 24 (26.7%) were nonsusceptible to penicillin ($p < 0.004$). The lowest resistance to penicillin was observed among *S. pneumoniae* isolated from eye infection (16.7%), while bacteremia (70%) had the highest resistance.

The activities of the tested drugs against the 115 clinical isolates categorized by penicillin susceptibility are summarized in table 2. Generally, penicillin-susceptible isolates of *S. pneumoniae* were also susceptible to the other tested antibiotics, whereas penicillin-resistant isolates were more likely to be resistant to other antibiotics. Full resistances were found to penicillin (15.6%) chloramphenicol (7.8%), erythromycin (18.3%), tetracycline (24.3%), azithromycin (13%), ciprofloxacin (7.8%), cefotaxime (4.4%), and ceftriaxone (6.1%). None of the pneumococcal isolates were resistant to vancomycin. Of the 18 isolates that were highly resistant to penicillin, 13 (72.2%) also had decreased sensitivity of chloramphenicol, 13 (72.2%) to erythromycin, 9 (50%) to tetracycline, 11 (61.1%) to azithromycin, 11 (61.1%) to ciprofloxacin, 8 (44.4%) to cefotaxime, and 4 (22.2%) to ceftriaxone. The distribution of the 115 *S. pneumoniae* isolates based on the penicillin MIC findings compared to the 1- μ g oxacillin disk diffusion inhibition zone size showed agreement

Table 1. Distribution of *S. pneumoniae* isolates by source and susceptibility to penicillin based on MIC breakpoints

Specimen	PS (%)	PI (%)	PR (%)	Total number of isolates (%)
Blood	3 (30)	1 (10)	6 (60)	10 (8.7)
CSF	7 (46.7)	1 (6.6)	7 (46.7)	15 (13)
Eye discharge	10 (8.33)	2 (16.7)	0 (0)	12 (10.4)
Ear discharge	4 (80)	1 (20)	0 (0)	5 (4.3)
Sputum	16 (72.7)	4 (18.2)	2 (9.1)	22 (19.1)
Pleural fluid	2 (66.7)	0 (0)	1 (33.3)	3 (2.6)
Swab from rhinosinusitis	34 (70.8)	12 (25)	2 (4.2)	48 (41.7)
Total (%)	76 (66.1)	21 (18.3)	18 (15.6)	115 (100)

The MIC breakpoints were based on the recommendation of National Committee for Clinical Laboratory Standards [16].

PS = Penicillin-susceptible ≤ 0.1 $\mu\text{g/ml}$; PI = penicillin intermediately resistant 0.1 – 1.0 $\mu\text{g/ml}$; PR = penicillin-resistant ≥ 2 $\mu\text{g/ml}$; PN = penicillin nonsusceptible.

Table 2. Sensitivity of clinical isolates of *S. pneumoniae* from Fars Province, Iran

Antibiotics	Total number of cases (n = 115)			Penicillin sensitivity									MIC range μg/ml
				sensitive (n = 76)			intermediate (n = 21)			resistant (n = 18)			
	S	I	R	S	I	R	S	I	R	S	I	R	
Penicillin	76 (66.1)	21 (18.3)	18 (15.6)										<0.008–4
Chloramphenicol	75 (65.2)	31 (27)	9 (7.8)	70 (92.1)	4 (5.3)	2 (2.6)	10 (47.6)	7 (33.3)	4 (19.1)	5 (27.8)	5 (27.8)	8 (44.4)	<0.25–32
Erythromycin	76 (66.1)	18 (15.6)	21 (18.3)	68 (89.5)	4 (5.25)	4 (5.25)	9 (42.8)	6 (28.6)	6 (28.6)	5 (27.8)	8 (44.4)	5 (27.8)	<0.008–128
Tetracycline	67 (58.3)	20 (17.4)	28 (24.3)	60 (79)	10 (13.1)	6 (7.9)	5 (23.8)	10 (47.6)	6 (28.6)	9 (50)	5 (27.7)	4 (22.3)	<0.06–64
Vancomycin	115 (100)	0	0	76 (100)	0 (0)	0 (0)	21 (100)	0	0	18 (100)	0	0	<0.03–0.5
Azithromycin	80 (69.6)	20 (17.4)	15 (13)	66 (86.8)	6 (7.9)	4 (5.3)	9 (42.8)	6 (28.6)	6 (28.6)	7 (38.9)	6 (33.3)	5 (27.8)	<0.016–128
Ciprofloxacin	95 (82.6)	11 (9.6)	9 (7.8)	72 (94.7)	3 (3.9)	1 (1.4)	8 (38.1)	8 (38.1)	5 (23.8)	7 (38.9)	5 (27.8)	6 (33.3)	0.06–8
Cefotaxime	94 (81.7)	16 (13.9)	5 (4.4)	70 (92.1)	4 (5.3)	2 (2.6)	14 (66.7)	4 (19)	3 (14.3)	10 (55.5)	5 (27.8)	3 (16.7)	0.007–2
Ceftriaxone	95 (82.6)	13 (11.3)	7 (6.1)	72 (94.7)	4 (5.3)	0 (0)	16 (76.2)	4 (19)	1 (4.8)	14 (77.8)	2 (11.1)	2 (11.1)	<0.016–>128

Figures in parentheses are percentages.

S = Sensitive; I = intermediate; R = resistant. Break point of antibiotics ($\mu\text{g/ml}$): penicillin = 0.1; chloramphenicol = 16; erythromycin = 4; tetracycline = 8; vancomycin = 16; azithromycin = 5; ciprofloxacin = 5; cefotaxime = 32; and ceftriaxone = 30.

between the two methods for the susceptible and resistant strains. However, among 21 pneumococcal isolates with penicillin intermediate resistance (MIC = 0.1–1 µg), 14 and 7 isolates showed oxacillin inhibition zones of 16–19 and ≤16 mm respectively.

Discussion

In the present study the overall prevalence of penicillin resistance (15.6%) and penicillin intermediate resistance (18.3%) to *S. pneumoniae* is similar to values reported worldwide ranging from 1.4 to 71% [4, 7, 18, 19]. Although this study was performed only in Shiraz, Fars Province, it reflects the overall picture since patients were referred from various parts of the country to our medical center. In Iran, antibiotic consumption is high and in some cases without prescription, thereby leading to pneumococci with reduced susceptibility. Data obtained from some European countries, South Africa and the USA showed that half of the pneumococcal isolates from clinical specimens were not susceptible to penicillin [20, 21]. Recent data collected by the Asian Network for Surveillance of Resistant Pathogen (ANSORP) with respect to pneumococcal isolates from clinical specimens, documented a high prevalence of penicillin and multidrug resistance in some Asian countries [4, 22]. Penicillin-nonsusceptible *S. pneumoniae* in Korea, Japan and Vietnam were estimated to be >50% compared to 33.9% of the present study. Significant differences of penicillin MIC was noted among pneumococcal isolates from patients with pneumococcal bacteremia and meningitis, when compared with the isolates from patients with respiratory tract infections ($p < 0.005$) similar to previously reported observations [12, 23]. It seems that certain serotypes are involved in systemic pneumococcal infections.

Macrolides are used as an alternative to β -lactams for treatment of respiratory tract infections, however, recent surveillance data showed an increasing prevalence of macrolide-resistant *S. pneumoniae* in many parts of the world [24, 25]. In our study, resistance to erythromycin was observed among both penicillin-resistant (27.8%) and penicillin-susceptible strains (5.25%), similar to reports from other Asian countries [6, 26]. Although erythromycin has been suggested as an alternative oral therapy for pneumococcal infection and penicillin-sensitive individuals, our results do not support this recommendation as 27.8% of penicillin-resistant isolates were also nonsusceptible to erythromycin. A high consumption, inappropriate and overdose use of erythromycin and other mac-

rolides for treatment of pneumococcal infections may be the main contributor to the increased prevalence of macrolide resistance in Iran and elsewhere.

Of special concern is the occurrence of *S. pneumoniae* resistance to ceftriaxone, a broad-spectrum cephalosporin which is frequently used in septicemic infections. Most of the pneumococcal isolates that were resistant to penicillin have also lowered susceptibility to ceftriaxone. In the present study, among the 39 penicillin-nonsusceptible isolates (resistant 18, intermediately resistant, 21) 23% were nonsusceptible to ceftriaxone, whereas only 5.3% of 76 *S. pneumoniae* isolates were penicillin-sensitive ($p < 0.009$). Kam et al. [7] in Hong Kong have found 76.6% resistance to ceftriaxone among 59 penicillin-resistant isolates. The extended-spectrum cephalosporins have been recommended for treatment of pneumococcal meningitis, however, treatment failures have been reported from the USA and Spain among patients with pneumococcal meningitis [10, 27, 28]. This failure is believed to be due to an increase in the MIC of ceftriaxone and cefotaxime against PRSP. The resistance to ceftriaxone and cefotaxime among pneumococcal isolates is alarming, as these are considered alternative drugs used in treating infections with PRSP.

Conclusion

Although a number of therapeutic guidelines recommended are for pneumococcal infections, the local pattern of resistance/susceptibility must be considered. Data presented in this article and related publications emphasize the desperate need to control the proper use of antibiotics to decrease the selective pressure for this and other organisms. Moreover, development of multiple antibiotic resistance to *S. pneumoniae* indicates that newer antibiotics have to be developed to combat drug-resistant pneumococcal infections.

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