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Brief reports

Trends in antimicrobial resistance of *Streptococcus pneumoniae* in children in a Turkish hospital

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The antimicrobial susceptibilities of 143 isolates of *Streptococcus pneumoniae* recovered from the sputa of children with lower respiratory tract infections in a Turkish university hospital were determined. Five isolates (3.5%) were resistant and 57 isolates (39.9%) intermediately resistant to penicillin. The most common serotype among these resistant isolates was serotype 23, followed by serotypes 19 and 14. The overall resistance rates were 31% for tetracyline, 11% for erythromycin and cefaclor, 4% for chloramphenicol, 2% for cefotaxime and 0% for vancomycin. The data highlight the need for surveillance of resistance and serotype distribution of *S. pneumoniae* in our geographical area.

Introduction

Clinical isolates of *Streptococcus pneumoniae* with decreased penicillin sensitivity and multiple antibiotic resistance have been reported increasingly worldwide, but the prevalance of penicillin-resistant pneumococci varies greatly from one part of the world to another.[1,2] Development of antimicrobial resistance by *S. pneumoniae* can result in increased mortality and may require a change in the antimicrobial agents used for the initial treatment of presumed pneumococcal disease. Although rare in the literature, there are also reports of penicillin-resistant pneumococci in Turkey,[3] but the serotype distribution of these isolates has not been previously evaluated in our country.

This study was undertaken to determine the in-vitro susceptibility of *S. pneumoniae*, isolated from children with lower respiratory tract infections, to various antibiotics and also to establish the serotype distribution of the penicillin-resistant strains in order to assess the coverage by the pneumococcal vaccine currently available.

Materials and methods

Bacterial isolates

One hundred and forty-three isolates of *S. pneumoniae* from the sputa of children with lower respiratory tract infections, such as acute pneumonia and exacerbations of cystic fibrosis or bronchiectasis, collected between January 1993 and December 1996, were included in the study. Numerous isolates recovered from the same patient were considered one isolate. The sputum samples were initially examined microscopically to evaluate the role of *S. pneumoniae* as the causative agent of the infection. Identification was based on colony morphology, Gram staining, bile solubility and optochin susceptibility.

Susceptibility testing

MICs were determined by the agar dilution method recommended by the National Committee for Clinical Laboratory Standards (NCCLS).[4] A suspension of organisms was prepared in Mueller–Hinton broth equivalent to 0.5 McFarland density and diluted 1 in 100 to give 10^6 cfu/mL; 10 μL from this dilution was used to inoculate Mueller–Hinton agar supplemented with 5% sheep blood containing increasing concentrations of the antimicrobial tested. Quality control strain *Enterococcus faecalis* ATCC 29212 was included in all runs. The following antimicrobial agents were tested in the range of 0.03–32 mg/L: penicillin G, erythromycin, cefaclor, cefotaxime, chloramphenicol, tetracycline and vancomycin. The results were interpreted according to the MIC breakpoints recommended by NCCLS,[4] as shown in Table I.

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Table I. Antimicrobial susceptibilities of clinical isolates of *S. pneumoniae* (n = 143)

Antimicrobial agent	MIC (mg/L)			MIC breakpoint		Percent of isolates	
	Range	MIC_{50}	MIC_{90}	I[a]	R[b]	I	R
Penicillin	≤0.03–4	0.06	0.5	0.1–1	≥2	39.9	3.5
Erythromycin	≤0.03–>32	0.06	0.5	0.5	≥1	1.4	9.8
Chloramphenicol	0.5–16	2	4		≥8		4.2
Tetracycline	≤0.03–>32	0.25	32	4	≥8	3.5	28.0
Cefaclor	0.06–>32	1	16				
Cefotaxime	≤0.03–2	0.06	0.5	1	≥2	1.4	0.7
Vancomycin	0.06–1	0.25	0.5		≥2	0	0

When no values are given, no NCCLS approved breakpoints were available.
[a]Intermediate.
[b]Resistant.

Table II. Multiply resistant *S. pneumoniae* by resistance patterns and serotype distributions

Resistance pattern	Number (%)	Serotype distribution (n)					
		NT[a]	6	9	14	19	23
Penicillin + erythromycin	10 (7.0)	3	1	1	4	–	1
Penicillin + tetracycline	17 (13.9)	–	–	1	–	6	10
Penicillin + chloramphenicol	3 (2.5)	1	1	–	–	1	–
Penicillin + cefotaxime	2 (1.6)	1	–	1	–	–	–
Penicillin + erythromycin + tetracycline	6 (4.9)	3				2	1
Penicillin + erythromycin + chloramphenicol	2 (1.6)	1				1	
Penicillin + erythromycin + tetracycline + chloramphenicol	1 (0.8)					1	

[a]Not typable with the available antisera.

Serotyping

Serotyping was performed by detection of the Quellung reaction with specific antisera from the Statens Serum-institut (Copenhagen, Denmark). Penicillin-resistant pneumococci only were typed with specific antisera against serotypes 3, 6, 9, 14, 18, 19 and 23.

Results

The susceptibilities of the 143 *S. pneumoniae* isolates to the seven antimicrobial agents tested are summarized in Table I. Five isolates (3.5%) were resistant (MIC > 1 mg/L) and 57 isolates (39.86%) were intermediate (MIC = 0.1–1 mg/L). Fourteen isolates were resistant to erythromycin (MICs 1–≥32 mg/L). Two isolates were intermediate and one isolate was resistant to cefotaxime. The cefotaxime-resistant isolate was also resistant to penicillin and cefaclor (MIC = 32 mg/L). One of the cefotaxime-intermediate isolates showed intermediate resistance to penicillin. Six isolates had MICs of ≥8 mg/L against chloramphenicol and 40 were resistant to tetracycline; 24 of these were also penicillin-resistant. All of the isolates were susceptible to vancomycin.

Table II shows the predominant antibiotic resistance patterns encountered among the 143 *S. pneumoniae* isolates. Forty-seven isolates showed resistance to more than one antibiotic and nine were multi-resistant, showing resistance to antibiotics of at least three different groups.[2] The most common serotypes among these multiresistant strains were serotypes 23 and 19.

Serotyping was performed on 53 of the 62 penicillin-resistant pneumococci and 44 were typed with the available antisera. Sixteen of the isolates (30.2%) were serotype 23, 11 (20.8%) were serotype 19, nine (17%) were serotype 9, six (11.3%) were serotype 14 and two (3.8%) were serotype 6.

Discussion

Antibiotic-resistant pneumococci, especially penicillin-resistant strains, are being isolated at an increasing rate

and they have become a significant problem in many countries.[1,2,5] These increasing resistance rates may cause serious difficulties in the treatment of pneumococcal infections.[5]

The isolates with reduced susceptibility to penicillin, noted in this study, document the presence of such strains in Turkey. Little information is available on pneumococcal resistance in our geographical area. Although this report is from a single hospital, the Hacettepe University Children's Hospital is a reference centre, and these results may indicate the antibiotic susceptibility status of pneumococci in our country. In this report, unless indicated otherwise, the term 'resistant' covers both intermediate and highly resistant strains. Although high-level penicillin resistance was rare, 39.9% of pneumococci were intermediately resistant to penicillin. This high incidence may result from the uncontrolled and frequent use of penicillin and its derivatives and/or non-compliance of patients with respect to antibiotic dosage and duration. High resistance rates from Hungary and Spain also indicate the role of selective antibiotic pressure on the changes in the antibiotic susceptibility of pneumococci.[6,7] The high incidence noted in this study may also be because the isolates were respiratory tract isolates which have been found to be more resistant than isolates from other sources in previous studies.[6,7]

There is considerable geographical variation in penicillin susceptibility among pneumococci. The prevalence of penicillin-resistant pneumococci varies between 0 and 52% in different countries, with Spain and Hungary having the highest and the USA the lowest rates of resistance.[1,2,6–9]

Penicillin resistance in pneumococci results from altered penicillin-binding proteins that have a decreased affinity for β-lactam antimicrobial agents.[10] Therefore strains of intermediate or high resistance to penicillin also exhibit reduced susceptibility to other β-lactam agents. This was also observed in our study. Twelve of the penicillin-resistant strains had cefaclor MICs of $\geqslant$32 mg/L, so the merit of cefaclor as an empirical treatment for suspected pneumococcal infections seems questionable. For such strains the MIC_{90} of cefotaxime was below the susceptibility breakpoint, except for two isolates.

Strains with reduced susceptibility to penicillin are usually cross-resistant to other antibiotics.[1,2,5] In this study it was observed that some penicillin-resistant isolates were also resistant to one or more of tetracycline, erythromycin and chloramphenicol. The majority of the isolates in the present study remained susceptible to chloramphenicol, whereas about 10% were resistant to erythromycin and about 30% were resistant to tetracycline. These resistance rates make erythromycin and tetracycline inappropriate for empirical therapy. Resistance to erythromycin probably results from the frequent use of macrolides for the treatment of upper respiratory tract infections in children in our country. Similar results were found and similar conclusions reached in France, where the resistance rate to erythromycin is about 30%.[8]

All strains were susceptible to vancomycin, in agreement with recent reviews,[1,7] so vancomycin may be an important alternative for use in the treatment of infections caused by pneumococci resistant to penicillin and other antimicrobial agents.

Serotyping indicated the predominance of serotype 23, followed by serotypes 19 and 9 among the penicillin-resistant *S. pneumoniae* isolates. Determination of the serotype distributions among pneumococci is very important in the formulation of vaccine for a given target population and may vary with age, time and place. Therefore it was thought important to augment the limited data available in our region. Of the 84 pneumococcal serotypes, it is known that serotypes 6, 9, 14, 19 and 23 are most often associated with penicillin resistance.[5] Our results are in concordance with those from Spain and France where serotype 23 was the most common one among penicillin-resistant strains.[7,8] The serotypes detected among the penicillin-resistant pneumococci in our study are among the serotypes covered by the currently available 23-valent pneumococcal vaccine, so use of the vaccine is quite valuable in children with underlying respiratory tract disease, such as cystic fibrosis and bronchiectasis, as well as in children with immune deficiencies.

The trend towards decreased sensitivity to antibiotics among pneumococci must be taken into consideration by clinicians prescribing therapy for infections caused by these organisms. This trend also mandates a routine and global surveillance programme for pneumococcal resistance so that empirical regimens, such as those used for otitis media or acute lower respiratory infections, can be most rationally selected.

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