

## ANTIMICROBIAL RESISTANCE

# High incidence of penicillin resistance amongst clinical isolates of *Streptococcus pneumoniae* in northern Palestine

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**One hundred and thirteen consecutive isolates of *Streptococcus pneumoniae* were collected in Nablus, Palestine between March and Aug. 1997 from children with acute lower respiratory tract infections. Resistance rates were: penicillin 88%, cefuroxime 85%, erythromycin 63%, tetracycline 45%, chloramphenicol 27% and ofloxacin 2%. Resistances to erythromycin and cefuroxime were significantly associated with penicillin resistance. Ofloxacin may be useful against pneumococci resistant to traditional antimicrobial agents. Factors associated with penicillin resistance included hospitalisation and previous use of  $\beta$ -lactam antibiotics.**

### Introduction

Penicillin has long been the drug of choice for the treatment of pneumococcal infections, but there has been a world-wide increase in reports of penicillin resistance in pneumococci since the 1960s. In many cases, penicillin resistance is associated with multiple resistances to other antibiotics [1, 2] and this situation is causing increased concern. The present study aimed to obtain a snapshot of pneumococcal resistance in northern Palestine, a part of the world not previously surveyed for this type of resistance.

### Materials and methods

#### *Subjects and study design*

This study was performed at An-Najah National University, Nablus, Palestine between March and Aug. 1997. Study participants were children <8 years of age from a private paediatric out-patient population and the paediatric in-patient ward at the Al-Watani Hospital, a referral centre for children in northern Palestine. The children were evaluated by one of two study physicians and selected if they had the symptoms of acute lower respiratory tract infection: i.e., cough, fever, tachypnoea (respiratory rate >50/min), leucocytosis, chest retraction or pulmonary infiltrates on chest X-ray [3, 4]. Information regarding underlying

illnesses, hospitalisation and previous antibiotic use (including over-the-counter drugs) was recorded on a standardised questionnaire completed with the help of the study physicians. Previous use of antibiotic included all antibiotics received within 1 month before the culture date.

#### *Sample collection and processing*

Nasal or oropharyngeal samples were obtained. A nasal specimen was obtained from a depth of 1 cm in the nostril with a cotton-tipped swab. Oropharyngeal swab specimens were obtained from the posterior wall of the oropharynx. Pneumococci were identified by their  $\alpha$ -haemolysis on sheep blood agar (Oxoid Blood Agar Base No. 2), colony morphology and susceptibility to an optochin disk (Oxoid). Their identity was further confirmed by the coagglutination test (Phadebact Pneumococcus Test; Pharmacia, Uppsala, Sweden).

#### *Antimicrobial susceptibility testing*

Isolates were screened for penicillin resistance with 1- $\mu$ g oxacillin disks (Oxoid) by the Kirby-Bauer method. Isolates giving inhibition zones of  $\geq 20$  mm were considered penicillin-susceptible, whereas those with smaller zones were considered penicillin-resistant [5]. These latter isolates giving a zone <20 mm were confirmed as penicillin-resistant by MIC tests on Mueller-Hinton Agar (Oxoid) supplemented with sheep blood 5% [6]. The penicillin MICs (mg/L) used to define susceptible, intermediately resistant and resistant isolates were <0.1, 0.1–1.0 and >1.0, respectively, as proposed by the National Committee for Clinical

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Laboratory Standards [7]. Throughout the rest of this report, the term 'penicillin-resistant' is used to refer collectively to both intermediately resistant and fully resistant isolates. Susceptibility to other antibiotics was tested by the Kirby-Bauer disk diffusion method on Mueller-Hinton agar supplemented with sheep blood 5% and incubated in an atmosphere containing CO<sub>2</sub> 5%. Disks were from Oxoid and the zones of inhibition were interpreted according to the recommendations of the National Committee for Clinical Laboratory Standards [8]. *S. pneumoniae* ATCC 6305 was used as the control strain.

### Statistical analyses

Statistical analysis was performed with the programme Statistical Package for Social Sciences (SPSS, Chicago, IL, USA). For risk factor analysis, relative risks for infection with penicillin-resistant pneumococci versus susceptible strains were calculated, along with their 95% confidence intervals by the  $\chi^2$  test. A p value <0.05 was considered statistically significant. Risk factors studied included the patients' gender, underlying disease, hospitalisation and previous antibiotic use.

### Results

Altogether, 510 children with symptoms of acute lower respiratory infection were enrolled in the study. Of these, 111 (21.8%) were admitted to Al-Watani Hospital for treatment. The median age was 36 months (range 10 days to 144 months); a definite infiltrate on the X-ray was seen in 22.5% of the children, as judged by radiologists. Diagnosis was based on the clinical signs or symptoms in the remaining children.

A total of 113 isolates of *S. pneumoniae* was identified (Table 1). Resistance to penicillin was seen in 88% of these, resistance to cefuroxime in 85%, to erythromycin in 63%, to tetracycline in 45%, to chloramphenicol in 27% and to ofloxacin in 2%. High-level penicillin resistance (MIC >1 mg/L) was seen in 77% of the penicillin-resistant isolates.

**Table 1.** Resistance to penicillin and its association with resistance to other antibiotics in 113 clinical isolates of *S. pneumoniae*

Penicillin susceptibility (number of isolates)	Number of isolates resistant to					Multiple antibiotics*
	Ery	Chl	Cxm	Tet	Ofi	
Susceptible (14)	5	1	7	4	0	2
Resistant (99)	66 <sup>†</sup>	29	89 <sup>†</sup>	47	2	48 <sup>†</sup>
Total (113)	71	30	96	51	2	50

Ery, erythromycin; Chl, chloramphenicol; Cxm, cefuroxime; Tet, tetracycline; Ofi, ofloxacin.

\*Isolates resistant to at least two of erythromycin, chloramphenicol, tetracycline and ofloxacin.

<sup>†</sup>Statistically significant differences compared with penicillin-susceptible pneumococci (p < 0.05 in  $\chi^2$  test).

The rates of resistance to erythromycin, chloramphenicol, cefuroxime and tetracycline were higher among penicillin-resistant than penicillin-susceptible isolates (Table 1) and these differences were statistically significant (p < 0.05) for erythromycin and cefuroxime. Likewise, multiresistance occurred significantly more frequently (p < 0.05) in penicillin-resistant than penicillin-susceptible isolates, and 49% of the penicillin-resistant isolates were resistant to at least two of erythromycin, chloramphenicol, tetracycline and ofloxacin.

Table 2 shows the predominant antibiotic resistance patterns encountered. Only five isolates (4%) were susceptible to all the antibiotics; the remaining 108 showed 21 different patterns of resistance.

Significant risk factors associated with penicillin-resistant pneumococci are listed in Table 3. Antibiotic use within the previous month was significantly associated with penicillin resistance: specifically, prior  $\beta$ -lactam use was a risk factor for penicillin resistance, whereas prior trimethoprim-sulphamethoxazole and erythromycin treatment were not risk factors. Of 36 children who had received antibiotics, 5 (14%) gave a history of use of over-the-counter  $\beta$ -lactams. Penicillin resistance was also associated with hospitalisation: among 31 hospitalised children there were 30 (97%) carriers of penicillin-resistant pneumococci. Ten of these carriers (33%) acquired the organism in hospital, the remaining 20 had the organism before hospitalisation and remained persistent carriers. Underlying disease and the patient's gender had no apparent association with penicillin-resistant pneumococci.

### Discussion

Antibiotic-resistant pneumococci – especially those with penicillin resistance – are increasingly isolated, and are a serious problem in many areas. Many strains are multiresistant: South Africa, New Mexico (USA)

**Table 2.** Resistance patterns among 113 pneumococcal isolates

Resistance pattern	Number of isolates (%)
Susceptible*	5 (4)
Pen, Tet, Ery, Cxm	23 (20)
Pen, Ery, Cxm	20 (18)
Pen, Chl, Ery, Cxm	5 (4)
Pen, Chl, Tet, Cxm	4 (4)
Pen, Chl, Tet, Ery, Cxm	14 (12)
Pen, Chl, Tet, Ery, Cxm, Ofi	2 (2)
Pen, Cxm	17 (15)
Pen	6 (5)
Cxm	3 (2)
Tet, Ery, Chl, Cxm	1 (1)
Tet, Ery, Cxm	1 (1)
Other 10 patterns	12 (11)
Total	113 (100)

Abbreviations as in Table 1.

\*Susceptible to all antibiotics tested.

**Table 3.** Risk factors associated with penicillin-resistant pneumococci

Factor	Penicillin susceptibility		p value	Odds ratio (CI)
	Resistant (n = 99)	Susceptible (n = 14)		
Age (months) (SD)	29 (31)	30.2 (18)		
Gender				
Male	60	10	NS	0.62 (0.18–2.10)
Female	39	4		
Hospitalisation				
Yes	30	1	0.042	5.65 (0.71–45.18)
No	69	13		
Underlying disease				
Yes	12	1	NS	1.79 (0.22–14.96)
No	87	13		
Previous antibiotic use*				
$\beta$ -Lactam	33	1	0.023	7.95 (0.84–53.48)
Trimethoprim/sulphamethoxazole	1	0	NS	0.99 (0.96–1.02)
Erythromycin	1	0	NS	0.99 (0.96–1.02)
None	64	13		

NS, not statistically significant ( $p > 0.05$ ).

\*Antibiotics received within 1 month before culture date.

[9], Hungary [10] and Spain [11] all have reported penicillin resistance rates nearing 50%, and even higher prevalence rates have been reported in the Far East [12]. Data from the present study indicate an extremely high rate (88%) for penicillin-resistant pneumococci in Palestine. This high rate is probably due, in part, to the selective pressure resulting from uncontrolled, inappropriate and frequent administration of penicillin and its derivatives. This is promoted by the lack of an antibiotic policy and the availability of antibiotics sold over-the-counter. The high rate of resistance has major therapeutic implications, insofar as penicillin resistance is associated with multiresistance [10, 13, 14]. Nearly half (49%) of the penicillin-resistant isolates (MIC  $> 1$  mg/L) were also resistant to at least two or more of erythromycin, chloramphenicol, tetracycline and ofloxacin (Table 2). Nevertheless, resistance to ofloxacin was not related to resistance to other agents. Therefore, ofloxacin may provide an alternative therapy for use in patient populations likely to be colonised with multi-resistant pneumococci [15].

Analysis showed that hospitalisation was associated with carriage of penicillin-resistant pneumococci. This finding is consistent with the close inter-personal contact and frequent antimicrobial drug usage that co-exist in hospitals [16, 17], favouring transmission and persistence of resistant bacteria. As found in previous reports [16, 18], children who had received  $\beta$ -lactam antibiotics in the present study were more likely than other children to carry penicillin-resistant pneumococci. Moreover, antimicrobial agents may eradicate sensitive organisms from the oropharynx and facilitate the establishment of a resistant strain.

In conclusion, the high frequency of penicillin resistance among *S. pneumoniae* has clearly emerged as a serious problem in Palestine. It is necessary to

carry out continuous surveillance of this problem to generate accurate local data and identify alternative therapies.

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