

## Note

# Resistance Patterns of *Streptococcus pneumoniae* from Children in Central Italy

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**Abstract** Nasopharyngeal swabs were collected from children aged 3–5 years in central Italy who were attending day-care centres or hospital outpatient clinics. One hundred and twenty-one strains of *Streptococcus pneumoniae* isolated were tested for susceptibility to penicillin, cefotaxime, erythromycin, clindamycin, tetracycline, chloramphenicol and cotrimoxazole. A high prevalence of penicillin-resistant (14%), erythromycin-resistant (60%) and multiply resistant strains (53%) were found. An unusual finding was that 49 of the 64 (76.6%) multiply resistant strains were penicillin-susceptible, 28 serogroup 6 strains also being resistant to the other antibiotics tested. Such strains have not previously been reported from Italy but have the same features as strains recently found in child carriers in the eastern Mediterranean area.

## Introduction

*Streptococcus pneumoniae*, the most common cause of bacterial community-acquired pneumonia in adults and children, is responsible for 30–50% of episodes of otitis media [1] and is the leading cause of childhood pneumonia and meningitis.

After the first report of decreased penicillin susceptibility in *Streptococcus pneumoniae* in 1967 in Papua New Guinea [2], penicillin-resistant *Streptococcus pneumoniae* have become increasingly prevalent worldwide [3]. Several studies conducted in Europe also

show a high prevalence of penicillin-resistant *Streptococcus pneumoniae*, ranging from 29–44% in Spain [4] and Greece [5] to 36.1–92.9% in eastern and central Europe (Bulgaria, Romania and the Slovak Republic) [6]. In addition, penicillin-resistance has been accompanied by erythromycin-resistance in many countries, including Spain (10–20%) [4], Greece (19%) [5] and France (32%) [7].

Relatively few studies have addressed the problem of antimicrobial resistance to *Streptococcus pneumoniae* in Italy. In a previous survey of nasopharyngeal carriers among children in central Italy, conducted in 1995, we found that 20.7% of the *Streptococcus pneumoniae* strains isolated had decreased susceptibility to penicillin, most showing intermediate susceptibility. Sixty-four percent of these strains were erythromycin-resistant and 30% multiply resistant [8].

To confirm and extend our findings, we conducted a second survey of nasopharyngeal carriage of pneumococci in children in central Italy during the winter season of 1996–1997.

## Materials and Methods

A total of 1146 asymptomatic children aged 3–5 years attending the outpatient service of the 2nd Pediatric Department of the Policlinico Umberto I, Rome ( $n=514$ ), or attending day-care centres in three central Italian cities (Cassino,  $n=186$ ; Ciampino,  $n=165$ ; L'Aquila,  $n=281$ ) were included in this study. Exclusion criteria were the presence of upper or lower respiratory tract symptoms or treatment with antibiotics within the previous week. Nasopharyngeal specimens were collected between January and April 1997. Local public health authorities gave ethical approval for the study, and informed written consent was obtained from the parents of the children. Nasopharyngeal specimens were obtained perinasally using sterile swabs on flexible aluminium shafts. Specimens were transported within 3 h to the microbiology laboratory of the 2nd Pediatric Department of the Policlinico Umberto I, Rome, and inoculated onto Columbia agar plates supplemented with 5% sheep blood with or without 5 µg/ml of gentamicin (Becton Dickinson, Italy). Pneumococci were identified by means of the optochin inhibition test, the bile solubility

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test and detection of the pneumolysin gene by the PCR technique [9]. Susceptibility was determined on Mueller-Hinton agar plates supplemented with 5% sheep blood agar (Becton Dickinson, USA). All isolates were screened for penicillin resistance using 1 µg oxacillin disks, and penicillin (P) and cefotaxime MICs were determined by the E test applying oxacillin inhibition zone limits of <20 mm. Chloramphenicol (Ch) (30 µg disks), tetracycline (T) (30 µg disks), erythromycin (E) (15 µg disks), clindamycin (Cl) (2 µg disks) and cotrimoxazole (Co) (25 µg disks) were also tested. Plates were incubated for 24 h in 5% CO<sub>2</sub> at 37 °C and zone diameters were then read and interpreted according to NCCLS criteria [10]. Isolates resistant to three or more agents were considered multiply resistant. Strains were serogrouped using commercially available antisera (Statensserum Institute, Denmark).

## Results and Discussion

A total of 1146 nasopharyngeal swabs were collected as follows: 514 from Rome, 186 from two day-care centres in Cassino (100 km south of Rome), 165 from two day-care centres in Ciampino (20 km south of Rome), and 281 from two day-care centres in L'Aquila (100 km north-east of Rome). One hundred and twenty-one *Streptococcus pneumoniae* strains were isolated (10.6%). Carriage rates did not differ significantly between centres.

Thirteen (10%) strains were susceptible to all antimicrobial agents tested (Table 1). Susceptibility to penicillin was decreased in 17 (14%) strains, 16 showing intermediate susceptibility (MICs 0.12–1 µg/ml) and one being fully resistant (MIC 2 µg/ml); the latter was also the only strain (0.8%) with intermediate susceptibility to cefotaxime (MIC 1 µg/ml), all others being fully susceptible. Strains with decreased susceptibility to penicillin were more frequently resistant to the other agents tested. Thus, penicillin-resistant strains were more often erythromycin-resistant than penicillin-susceptible strains (88.2% vs. 55.8%;  $P < 0.03$ ) and more often multiply resistant (88.2% vs. 47.1%;  $P < 0.005$ ). Of the 121 strains, 89 (73.5%) were resistant to cotrimoxazole, 66 (54.5%) to tetracycline and 41 (33.9%) to chloramphenicol. Erythromycin resistance was found in 73 (60.3%) strains; 63 (86.3%) of which were also resistant to clindamycin.

**Table 1** Susceptibility to antimicrobial agents of *Streptococcus pneumoniae* strains isolated from the nasopharynx in children

Antibiotics	Number of strains		
	Susceptible	Intermediate	Resistant
Penicillin	104 (86%)	16 (13.2%)	1 (0.8%)
Cefotaxime	120 (99.2%)	1 (0.8%)	none
Erythromycin	48 (39.7%)	none	73 (60.3%)
Clindamycin	58 (47.9%)	none	63 (52.1%)
Cotrimoxazole	32 (26.5%)	none	89 (73.5%)
Tetracycline	55 (45.5%)	none	66 (54.5%)
Chloramphenicol	80 (66.1%)	none	41 (33.9%)

The most common patterns of resistance were Co (24.8%) and ChTECICo (23.1%). The Co pattern was found more frequently in strains isolated in Rome while ChTECICo was more prevalent outside Rome. The Co pattern was found in 90.9% of serotype 9 strains.

All 28 strains with the ChTECICo resistance pattern were found to be serotype 6. Clusters of six PChTECICo resistant strains and four TECICo resistant strains were serogroup 19. Eleven of the 17 strains resistant to penicillin were serotype 19, there being a far higher rate of penicillin resistance in this serotype than among non-type 19 strains (64.7% vs. 6.7%;  $P < 10^{-6}$ ). Serotypes 6 and 19 had far higher rates of resistance to erythromycin than the other serotypes tested (82.8% vs. 29.4%;  $P < 10^{-6}$ ).

While most erythromycin-resistant strains were clindamycin-resistant, ten of them were clindamycin-susceptible; six of these strains were serogroup 6 and also resistant to cotrimoxazole, only one of these being penicillin-resistant.

The main finding of this study was the high prevalence of resistance to penicillin (14%), erythromycin (60.3%) and multiple antibiotics (52.9%) in the *Streptococcus pneumoniae* strains isolated from Italian children. An additional unusual finding was the fact that 49 of the 64 (76.6%) multiply resistant strains were penicillin-susceptible, 28 of these strains being serogroup 6 and ChTECICo resistant (Table 2). These 28 strains had the same features as strains recently isolated from carriers in southwest Greece [5]; such strains have not been identified in Italy previously [8]. Penicillin resistance was found in 14% of strains, a higher rate than that reported in other parts of Italy in studies conducted in 1994 (6.5%) [7] and in 1995 (5.5%) [11], but lower than that found in our previous study (20%) [8]. However, this rate is still much lower than that found in France in 1994 (35% and 46–50%) [7, 12], in Greece in 1996 (29%) [5] and in Spain in 1993 (48%) [13], but much higher than that found in Finland in 1990 (1.7%) [14] and in the United Kingdom in 1993 (4%) [13].

The rate of macrolide resistance (60%) found in the present study confirms our previous finding in the same geographical setting (64%) [8]. This rate is considerably higher than that reported in other parts of Italy of 2.3% and 23% in 1993 and in 1996, respectively [11–15] and 24.3% (22.5% hospitalized children) in 1996–1997 (G. Cornaglia et al., 4th ICMASK, 1998, abstract no. 3.18). It is almost twice as high as the highest rate of resistance to macrolides reported in previous European studies (France 1993, 35% [12]; Greece 1996, 19% [5]; Spain 1993, 10–20% [13]; Bulgaria 1994, 34% [6]). The only higher rate of erythromycin resistance was reported in Romania [6]. The high rates of penicillin- and erythromycin-resistant strains found in our study

**Table 2** Serotype distribution and resistance patterns of *Streptococcus pneumoniae* strains isolated from the nasopharynx in children

Resistance patterns	Capsular serotypes of pneumococci							Total
	6	19	14	9	23	Other	Non-typable	
ChT							1	1
ChTECI	1	1					1	3
ChTECICo	28							28
E			1	1	1			3
Eco	6							6
P			1					1
PChECICo	1							1
PChTECI		1						1
PChTECICo		6			1			7
PECo							1	1
PT							1	1
PTECI	1	2						3
PTECICo		2						2
co	8	2		10	3	1 (B)	6	30
T	1							1
TECI	4						1	5
TECICo	1	4	1			1 (11)	6	13
TCo	1							1
Susceptible						3 (A, D, G)	10	13
Total	52	18	3	11	5	5	27	121

P, Penicillin; Ch, Chloramphenicol; T, Tetracycline; E, Erythromycin; Cl, Clindamycin; Co, Cotrimoxazole.

could at least in part be attributed to the fact that our strains were isolated from children aged less than 5 years, many of whom were attending day-care centres [16].

Of the 73 erythromycin-resistant strains, 63 (86.3%) were also resistant to clindamycin (MLS<sub>B</sub> phenotype), indicating the probable presence of an *erm* gene [17]. Only 13.7% of the erythromycin-resistant strains were susceptible to clindamycin, a phenotype of resistance associated with an energy-dependent efflux system in the presence of the *mefE* gene (M phenotype) [17]. Our study confirms that the MLS<sub>B</sub> resistance phenotype is more frequent than the M phenotype; however, in the USA [17] and in Israel [18] different results were obtained.

This study documents a high prevalence of antimicrobial resistance in child carriers of *Streptococcus pneumoniae* in central Italy. It also reveals the presence of strains with several unique resistance patterns, including multiply resistant but penicillin-susceptible serotype 6 strains previously only reported in Greece [5]. In view of these findings further studies are warranted, particularly in children, to determine the resistance patterns in other parts of Italy and to assess the effect of resistance on the outcome of infections.

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