Antimicrobial susceptibility of *Streptococcus pneumoniae* from children attending day-care centers in a central Italian city

Clin Microbiol Infect 1998; 4: 622–626

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Objective: To undertake a survey of nasopharyngeal carriage of *Streptococcus pneumoniae*, which reflects strains causing infection, in 100 children under 3 years of age attending day-care centers in Frosinone, a city near Rome.

Methods: Fifty-three unique isolates of *S. pneumoniae*, isolated from 41 of the children tested, were tested for antimicrobial susceptibility to penicillin, cefotaxime, erythromycin, clindamycin, tetracycline, chloramphenicol and trimethoprim–sulfamethoxazole.

Results: Resistance rates were as follows: penicillin, 20.7% (15% intermediate; 5.7% resistant); trimethoprim–sulfamethoxazole, 64.2%; erythromycin, 64.2%; clindamycin, 30.2%; tetracycline, 32.1%; and chloramphenicol, 3.8%. Except for three intermediate strains, all strains were susceptible to cefotaxime. Only five strains were susceptible to all of the antibiotics tested. An unusual finding of this study was that 23 of the 34 erythromycin-resistant strains were penicillin susceptible, whereas erythromycin-resistant strains found in other countries are predominantly penicillin resistant as well. In addition, 18 of the 34 erythromycin-resistant strains were susceptible to clindamycin. Serogroups 6, 14, 19 and 23 accounted for 84.9% of the isolates.

Conclusions: These data show that carriage of antibiotic-resistant pneumococci in children under 3 years of age is high in Frosinone, Italy. Information on resistance rates in pneumococcal disease in different age groups and on prevalence of drug resistance in other parts of the country is urgently needed.

INTRODUCTION

Streptococcus pneumoniae is a major cause of mortality and morbidity worldwide. It is the most common cause of community-acquired bacterial respiratory tract infections and bacterial meningitis in adults and the leading cause of death in children under 2 years of age in developing countries, causing about 1.2 million deaths annually in this age group [1,2]. Children below 2 years of age, the elderly, and patients with impaired defense mechanisms as a result of, for example, HIV infection, diabetes mellitus, malnutrition, sickle cell anemia, splenectomy, agammaglobulinemia, multiple myeloma, chronic lymphocytic leukemia, hepatic cirrhosis, chronic pulmonary disease and prior viral infection, have the highest infection rates. *S. pneumoniae* is the commonest cause of acute otitis media worldwide, being responsible for 30–50% of episodes [3,4], as well as the leading cause of bacteremia and bacterial meningitis [5,6]. At highest risk for pneumococcal disease are children in the first 2 years of life and the elderly [7].

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Accepted 7 May 1998

While *S. pneumoniae* was initially fully susceptible to penicillin (MICs ≤0.03 mg/mL), decreased susceptibility was first documented in Boston in 1965 [8]. Subsequently, this phenomenon was reported from Australia in 1967 [9] and South Africa in 1977 [10]. Since these early reports, antibiotic-resistant pneumococci have been reported around the world. However, information on antibiotic susceptibility patterns in many countries is not available. While nasopharyngeal carriage of *S. pneumoniae* without disease occurs in all age groups, carriage rates differ with age, being highest in children [4]. More than 95% of children have had an episode of nasopharyngeal pneumococcal colonization by 2 years of age [1], while 30–60% are nasopharyngeal carriers of pneumococci at any point in time. A given serotype is carried from a month to a year, with an average of 6 weeks [11]. As pneumococcal disease follows nasopharyngeal carriage [12], it is not surprising that studies done in South Africa and Pakistan showed that the antimicrobial susceptibility profile of nasopharyngeal strains reflects that of invasive strains in the same age group [13–15]. Therefore, surveillance of pneumococcal resistance in nasopharyngeal isolates is a practical way to determine the prevalence of resistant strains in children and is considered a reasonable predictor of resistance in disease.

Mediterranean countries such as Spain and France are currently known to have high prevalence rates of penicillin-resistant pneumococci, but information on the prevalence of penicillin-resistant pneumococci, the patterns of antibiotic resistance and the serogroups/serotypes of resistant strains is lacking in Italy. The best data from Italy have been published by Marchese et al: they tested about 300 strains from the north of Italy and found the percentage of penicillin-non-susceptible pneumococci to be 5.5%, including equal numbers of penicillin-intermediate and -resistant strains [16]. In 1996, as part of the Alexander Project, data were presented which confirmed the very low rate of penicillin-resistant pneumococci in northern Italy, with 8% of strains being penicillin intermediate [17].

MATERIALS AND METHODS

Children aged 8–36 months attending three different day-care centers were included in this study. The day-care centers were in Frosinone, a city 80 km from Rome, with a population of 100 000. Nasopharyngeal carriage of *S. pneumoniae* was determined on three different occasions at the day-care centers: December 1995, March 1996 and April 1996. The local public-health authorities gave the ethical approval of the study and an informed written consent was obtained from the parents of the children.

Nasopharyngeal secretions were collected pernasally with calcium alginate swabs on flexible aluminum shafts. Swabs were placed in 0.85% saline and transported after a maximum of 3 h from collection to the Pediatric Microbiology Laboratory of Policlinico Umberto I in Rome. Specimens were plated onto Columbia agar plates supplemented with 5% sheep blood agar with and without 5 mg of gentamicin per liter (Becton Dickinson, Milan, Italy). An optochin disk was placed on each plate and the plates were incubated overnight at 35°C in a 5% CO_2 atmosphere. Isolates of *S. pneumoniae* were identified by a zone of inhibition around the optochin disk and by a positive bile solubility test.

Strains were subcultured and overnight growth was suspended in serum glycerol freezing medium and stored at −70°C. Strains were transported by air to Case Western Reserve University in accordance with local and international shipment regulations. The identification of strains was confirmed in Cleveland by positive bile solubility tests, and susceptibility testing was performed on Mueller–Hinton agar supplemented with 5% sheep blood agar (Becton Dickson Microbiology Systems, Cockeysville, MD). Susceptibility to the following agents was determined by disk diffusion: chloramphenicol (30-μg disks), tetracycline (30-μg disks), erythromycin (15-μg disks), clindamycin (2-μg disks), and trimethoprim–sulfamethoxazole (25-μg disks). Plates were incubated overnight in 5% CO_2 and zones of inhibition read and interpreted according to National Committee for Clinical Laboratory Standards criteria [18]. All isolates were also screened for penicillin resistance using disks containing 1 μg of oxacillin. MICs to penicillin and cefotaxime were determined by the E-test method (AB Biodisk, Solna, Sweden) on strains with oxacillin zones of <20 mm [18,19]. Strains resistant to three or more classes of antimicrobial agents were considered multiply resistant.

Strains were serogrouped by the capsular swelling method using commercially available antisera (Statens-serum Institute, Copenhagen, Denmark).

RESULTS

One hundred children attending the three day-care centers were enrolled in the study. The mean age of the children was 25 months (range 8–36 months), and 51% were male. There were no significant differences between age and sex distributions among the three centers. During the investigation period, 52 children were tested three times, 26 children twice, and 22 children once, with a total of 230 swabs being obtained. Strains with the same susceptibility pattern and serogroup from the same carrier were only included once in data analysis.

Overall, 41% of the 100 children carried 53 strains of *S. pneumoniae* on one or more occasions. Thirty children were only tested once (N=22) or carried the same strain on retesting. Ten children carried a different strain on retesting, and one child carried three different strains on the three occasions. The carriage rate was 65.2% in center 2 (N=23), 39.6% in center 1 (N=48), and 24.1% in center 3 (N=29).

Susceptibility testing of the 53 strains from 41 children showed that only five strains were susceptible to all agents tested, while 48 strains were resistant to one or more agents (Tables 1 and 2). Eleven strains (20.7%) had decreased susceptibility to penicillin, with eight being intermediate (MICs ranging from 0.12 to 0.5 mg/L) and three resistant (MICs 2 mg/L); nine of these strains were multiply resistant. The three penicillin-resistant strains were all resistant to tetracycline, erythromycin, clindamycin and trimethoprim–sulfamethoxazole, and one strain was also resistant to chloramphenicol (Table 2). The three penicillin-resistant strains had intermediate susceptibility to cefotaxime (MICs 1 mg/L), with all other strains being susceptible to this agent. Erythromycin and trimethoprim–sulfamethoxazole resistance was found in 34 strains (64.2%), while 16 strains (30.2%) were resistant to clindamycin. Seventeen strains (32.1%) were resistant to tetracycline, and two strains (3.8%) to chloramphenicol.

Of the 53 strains isolated, 25 (47.2%) belonged to serogroup 6, nine (17%) to serogroup 19, seven (13.2%) to serogroup 23, five (9.4%) to serogroup 33, four to serotype 14, and three to other serogroups. Correlations between susceptibility patterns and serogroups are shown in Table 2. The commonest pattern was serogroup 6, resistant to erythromycin and trimethoprim–sulfamethoxazole, with 15 strains (11 from center 2, three from center 1 and one from center 3). Six serogroup 6 strains resistant only to trimethoprim–sulfamethoxazole were recovered from carriers in center 1. Only five strains were susceptible to all agents tested, and all belonged to serogroup 33; these strains were found in centers 1 and 3. Different patterns were found in the three day-care centers, with erythromycin and trimethoprim–sulfamethoxazole-resistant serogroup 6 strains predominating in center 2 and trimethoprim–sulfamethoxazole-resistant serogroups 6 and 23 in center 1.

Table 1 Susceptibility of *S. pneumoniae* to agents tested

	No. (percentage) of strains		
	Susceptible	Intermediate	Resistant
Penicillin	42 (79.2)	8 (15.8)	3 (5.7)
Cefotaxime	50 (94.3)	3 (5.7)	–
Erythromycin	19 (35.8)	–	34 (64.2)
Clindamycin	37 (69.8)	–	16 (30.2)
Trimethoprim–sulfamethoxazole	19 (35.8)	–	34 (64.2)
Tetracycline	36 (67.9)	–	17 (32.1)
Chloramphenicol	51 (96.2)	–	2 (3.8)

Table 2 Resistance patterns and serotypes of *S. pneumoniae* isolates

Resistance pattern	No. of strains of serogroup						
	6	14	19	23	33	Other	Total
None					5		5
P						2[a]	2
ES	15						15
S	6			4			10
TEC′S				3			3
PTEC′S	2[b]	1[a]					3
PS						1[a]	1
PTEC′			3[a]				3
TEC′			5				5
T			1				1
E		3					3
PCTEC′S	2[c]						2
Total	25	4	9	7	5	3	53

P, penicillin; E, erythromycin; C′, clindamycin; S, trimethoprim–sulfamethoxazole; T, tetracycline; C, chloramphenicol.
[a]Penicillin intermediate; [b]penicillin resistant; [c]one penicillin intermediate and one penicillin resistant.

DISCUSSION

The rising prevalence of antibiotic-resistant pneumococci worldwide mandates selective susceptibility testing, epidemiologic surveillance, rational use of antimicrobial agents, and re-evaluation of indications for therapeutic and prophylactic use of antimicrobial agents.

The clinical implications of infections with drug-resistant pneumococci have not been fully established. Retrospective studies have shown that penicillin is inadequate for treatment of meningitis due to penicillin-resistant pneumococci [19–22]. The impact of in vitro drug resistance on the clinical outcome of non-meningeal pneumococcal infections such as bacteremia, pneumonia, otitis media and sinusitis is yet to be clarified [1,19,20]. As the degree of resistance of pneumococci to β-lactams is variable, the level of drug achievable at sites of infection may greatly influence the outcome of infections, especially with orally administered agents [21]. Consequently, the usefulness of a β-lactam agent in treating pneumococcal infections depends on the site and nature of the infection, the

route of drug administration, the bioavailability of the drug, and the MIC of the agent against the organism. This is a particular problem in otitis media, where the penetration of orally administered antibiotics into middle ear fluid is poor and levels are often below MICs of penicillin-resistant pneumococci. As a result of decreased activity of many oral β-lactams against penicillin-resistant strains, such strains have been recognized as causes of treatment failure in acute otitis media and mastoiditis and as causes of chronic sinusitis and otitis media [22–25]. Of the oral β-lactams, amoxycillin has the best pharmacodynamic parameters and good bioavailability in the middle ear, and this agent remains the recommended initial empirical agent for treatment of otitis media even in areas of high prevalence of penicillin-intermediate and -resistant pneumococci [20,21]. Myringotomy or tympanocentesis should be done to permit identification of causative organisms and tests of susceptibility to antibiotics in patients who do not respond to antibiotic therapy. Agents to which the organisms are susceptible can then be used.

This study showed that the majority of pneumococci carried by children attending three day-care centers in a central Italian city were resistant to one or more classes of agents, with 66.7% being multiply resistant. Of particular concern is the finding of 34 strains (64.2%) resistant to erythromycin (and therefore other macrolides) and to trimethoprim–sulfamethoxazole, 17 strains (32.1%) resistant to clindamycin, and 11 strains (20.8%) intermediate or resistant to penicillin. An important and unusual finding of this study was that 23 of the 34 erythromycin-resistant strains were penicillin susceptible, whereas erythromycin-resistant strains found in other countries are predominantly penicillin resistant as well. In addition, 18 of the 34 erythromycin-resistant strains were susceptible to clindamycin, a feature frequently seen in US strains due to the presence of a macrolide-efflux pump [26]. In a recent study from Greece, all erythromycin-resistant strains were resistant to clindamycin [27], as was also reported in erythromycin-resistant *S. pyogenes* in Italy [28].

β-Lactam resistance in pneumococci, due to multiple PBP changes, can often be overcome if adequate drug levels can be attained at the site of infection. Resistance to macrolides and trimethoprim–sulfamethoxazole is absolute and not able to be overcome by pharmacologically achievable levels of these agents [20]. The findings of this study therefore suggest that macrolides and trimethoprim–sulfamethoxazole are unsuitable for empirical use in the communities where these children live. As most of the penicillin-non-susceptible strains were in fact penicillin intermediate, use of the more active oral β-lactams such as amoxycillin and cefuroxime axetil, which are active against penicillin-intermediate strains, should be considered for empirical use in otitis media, sinusitis and bronchitis [20–25].

As found throughout the world [1–5], serogroups 6, 14, 19 and 23 accounted for the majority (84.9%) of isolates. They are the commonest serogroups in children and the types most often associated with resistance. Although the 23-valent vaccine contains serotypes representative of these serogroups, it is ineffective in children below 2 years [12], and development of conjugate vaccines that are immunogenic in this age group is an important goal to reduce carriage of these serotypes and thus the spread of these strains as well as infections with these strains.

It is difficult to compare data from the present study with the few published data on antibiotic susceptibility of *S. pneumoniae* in Italy, because the published data were obtained from the north of Italy from a predominantly adult population. This may explain why in a different part of the country (central–south) the present study found a substantially higher prevalence of penicillin-intermediate and -resistant pneumococci in children <36 months old. However, the data from Marchese et al [16] showed that penicillin-resistant pneumococci were often multiply resistant, as was found in the present study. In contrast to two Italian studies, where erythromycin resistance rates were 3–4% [16,17], the rate of erythromycin resistance was considerably higher (64%) in the present study. In addition, erythromycin resistance was found in both penicillin-susceptible (61.9%) and -resistant (72.7%) strains, whereas macrolide resistance was predominantly found in penicillin-resistant strains in these published studies.

In conclusion, this study has shown that considerable antimicrobial resistance is present in *S. pneumoniae* strains carried by children attending day-care centers in south–central Italy. Further surveillance is necessary to extend these findings to other parts of the country.

Acknowledgments

We are grateful to Dr Giuseppe Lo Russo, Dr Francesco Guglielmi and Dr Lucio Annibaldi for their assistance and support.

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