RESEARCH NOTE

Serotypes and antimicrobial susceptibilities of 1033 pneumococci isolated from children in Greece during 2001–2004

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ABSTRACT

Pneumococci (n = 1033) isolated in the major paediatric hospitals of Athens during 2001–2004 from children with invasive infections (n = 186), non-invasive infections (n = 641) and healthy carriers (n = 206) were studied. The most prevalent serotypes were serotypes 14 (44.6%), 19F (43.5%) and 6B (22.8%) in invasive, non-invasive and carriage isolates, respectively. Among invasive isolates, the potential coverage by the seven-valent conjugate vaccine was 75.3%. Resistance rates to penicillin, amoxycillin, cefotaxime, erythromycin, co-trimoxazole, clindamycin, tetracycline and chloramphenicol were 44.6%, 2.7%, 1.2%, 43.6%, 43.5%, 12.4%, 34.7% and 5.9%, respectively. The M-phenotype accounted for 68.0% of the erythromycin-resistant isolates. All isolates were susceptible to ofloxacin.

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Streptococcus pneumoniae is a common cause of infections in childhood. Recent studies have documented a worldwide increase in the prevalence of isolates resistant to antibiotics [1–3]. In Greece, the spread of penicillin- and multidrug-resistant strains has been observed since the early 1990s [4]. Since then, occasional studies have indicated a continuous increase in the frequency of antibiotic-resistant pneumococci [5–8]. In the present study, the serotypes and the antibiotic susceptibilities of pneumococci isolated in Greece were studied for a 4-year period in order to clarify current resistance trends and to provide baseline data before the commencement of systematic use of the seven-valent conjugate vaccine in 2004.

Consecutive, non-duplicate pneumococci from infections in children (aged ≤14 years) treated at the three major paediatric hospitals in Athens (Aghia Sofia, Penteli and P. & A. Kyriakou) during 2001–2004 were studied. Additionally, nasopharyngeal carriage of pneumococci was studied in healthy children (aged 3–6 years) in ten day care centres during January–March 2003. Exclusion criteria in the carriage study were antibiotic treatment within the preceding 3 months and the presence of respiratory tract infection or chronic illness.

Species identification was performed using standard methods. Serogrouping was performed with the Quellung reaction using pooled and selected factor antisera (Statens Seruminstitut, Copenhagen, Denmark). Susceptibility to erythromycin (ERY), clindamycin (CLI), co-trimoxazole (SXT), tetracycline (TET), chloramphenicol (CHL) and rifampicin was determined using the disk-diffusion method [9]. MICs of penicillin (PEN), amoxycillin (AMX), cefotaxime and ofloxacin (OFX) were determined with Etests (AB Biodisk, Solna, Sweden). Results were interpreted using the current CLSI (NCCLS) criteria [9]. *S. pneumoniae* ATCC 49619 was used as a reference strain. Phenotypic characterisation of macrolide resistance was performed as described previously [10].

Statistical analysis was performed using the chi-square test.

In total, 827 *S pneumoniae* isolates causing infection were obtained from 448 male (54.2%) and 379 female (45.8%) patients. Of these, 186 were implicated in invasive pneumococcal disease (IPD) and were recovered from blood (n = 161), cerebrospinal fluid (n = 20) or other clinical specimens (n = 5). The age of IPD patients ranged from 22 days to 14 years; 103 (55.4%) of these patients were aged ≤2 years. The remaining 641 isolates were recovered from non-invasive pneumococcal disease (NIPD), mainly acute otitis media (n = 438), but also eye (n = 106) and lower respiratory tract infections (n = 97). In the carriage study, 206 isolates of *S. pneumoniae* were obtained from 873 children (23.6% carriage rate).

The serotype distribution differed among the IPD, NIPD and carriage isolates (Table 1). The most prevalent serotype in IPD was serotype 14 (44.6%), followed by 6B, 9V and 19F, together accounting for 69.9% of IPD isolates. The potential coverage by the seven-valent conjugate vaccine was 75.3%. Nevertheless, vaccine serotypes were encountered more often among IPD isolates from patients aged ≤2 years than from older children (87.4% vs. 60.2%; $p < 0.001$). The most frequent serotype in the NIPD group was serotype 19F (43.5%), with an additional 32.1% of the NIPD isolates belonging to serotypes 14, 23F, 6B and 9V. Among carriage isolates, 6B was the predominant serotype (22.8%), with another 55.3% of isolates belonging to serotypes 19F, 14, 23F and 9V.

Antibiotic resistance rates were higher among the NIPD and carriage isolates compared with the IPD isolates (Table 2). Among IPD isolates, 17.2% were non-susceptible to PEN, compared with 53% of NIPD isolates and 43.2% of carriage isolates ($p < 0.001$). Only 0.5% of IPD isolates were fully resistant to PEN, while the respective rates for NIPD and carriage isolates were significantly greater (Table 2). Additionally, isolates with decreased susceptibility to AMX, while absent in IPD, occurred sporadically among the NIPD and carriage groups.

Significant rates of resistance to ERY were observed in all three groups, with a resistance rate of 49.8% among NIPD isolates. The M phenotype was predominant among the ERY-resistant pneumococci. Relatively high resistance rates were found for SXT and TET. All isolates were susceptible to OFX. Resistance to three or more drugs (multiresistance) was common among the NIPD and carriage groups (44.0% and 36.8%, respectively; Table 2) and was observed more often among IPD isolates from younger children (≤ 2 years; 71.4%).

Table 1. Serogroup-serotype distribution of 1033 pneumococcal isolates from various clinical samples

	Number of isolates (%) obtained from:			
Serotype/ serogroup	IPD n = 186	NIPD n = 641	Carriers n = 206	Total n = 1033
1	5 (2.7)	7 (1.1)	1 (0.5)	13 (1.3)
2	–	1 (0.2)	1 (0.5)	2 (0.2)
3	1 (0.5)	22 (3.4)	5 (2.4)	28 (2.7)
4	2 (1.1)	2 (0.3)	1 (0.5)	5 (0.5)
6B	21 (11.3)	45 (7.0)	47 (22.8)	123 (11.9)
6-non-B	5 (2.7)	20 (3.1)	4 (1.9)	29 (2.8)
7F	5 (2.7)	3 (0.5)	1 (0.5)	9 (0.9)
8	–	3 (0.5)	2 (1.0)	5 (0.5)
9 V	14 (7.5)	39 (6.1)	26 (12.6)	79 (7.6)
9-non-V	1 (0.5)	2 (0.3)	–	3 (0.3)
10	–	6 (0.9)	–	6 (0.6)
11	–	11 (1.7)	5 (2.4)	16 (1.5)
12	1 (0.5)	1 (0.2)	–	2 (0.2)
14	83 (44.6)	66 (10.3)	21 (10.2)	170 (16.4)
15	1 (0.5)	4 (0.6)	4 (1.9)	9 (0.9)
17	–	1 (0.2)	–	1 (0.1)
18C	3 (1.6)	15 (2.3)	2 (1.0)	20 (1.9)
18-non-C	4 (2.2)	3 (0.5)	2 (1.0)	9 (0.9)
19F	12 (6.5)	279 (43.5)	37 (18.0)	328 (31.7)
19-non-F	10 (5.4)	23 (3.6)	2 (1.0)	35 (3.4)
20	1 (0.5)	2 (0.3)	1 (0.5)	4 (0.4)
22	–	–	1 (0.5)	1 (0.1)
23F	5 (2.7)	56 (8.7)	30 (14.6)	91 (8.8)
23-non-F	4 (2.2)	3 (0.5)	1 (0.5)	8 (0.8)
33	1 (0.5)	–	1 (0.5)	2 (0.2)
Non-typable	7 (3.8)	27 (4.2)	11 (5.3)	45 (4.3)

IPD, invasive pneumococcal disease; NIPD, non-invasive pneumococcal disease.

Table 2. Antibiotic resistance rates of 1033 pneumococcal isolates from various clinical samples

	Number (%) of non-susceptible isolates derived from:			
Antibiotic	IPD n = 186	NIPD n = 641	Carriers n = 206	Total n = 1033
Penicillin[a]	32 (17.2)	340 (53.0)	89 (43.2)	461 (44.6)
I	31 (16.6)	234 (36.5)	63 (30.6)	328 (31.8)
R	1 (0.5)	106 (16.5)	26 (12.6)	133 (12.9)
Amoxycillin[a]	0	22 (3.4)	6 (2.9)	28 (2.7)
I	0	22 (3.4)	6 (2.9)	28 (2.7)
Cefotaxime[a]	2 (1.1)	9 (1.4)	1 (0.5)	12 (1.2)
I	2 (1.1)	8 (1.2)	0	10 (1.0)
R	0	1 (0.2)	1 (0.5)	2 (0.2)
Erythromycin	56 (30.1)	319 (49.8)	75 (36.4)	450 (43.6)
M	48 (25.8)	225 (35.1)	33 (16.0)	306 (29.6)
CR	6 (3.2)	87 (13.6)	35 (17.0)	128 (12.4)
IR	2 (1.1)	7 (1.1)	7 (3.4)	16 (1.5)
Co-trimoxazole	44 (23.7)	314 (49.0)	91 (44.2)	449 (43.5)
Chloramphenicol	3 (1.6)	36 (5.6)	22 (10.6)	61 (5.9)
Tetracycline	19 (10.2)	267 (41.7)	72 (35.0)	358 (34.7)
Clindamycin	6 (3.2)	87 (13.6)	35 (17.0)	128 (12.4)
Rifampicin	0	2 (0.3)	0	2 (0.2)
Multiresistant[b]	21 (11.3)	282 (44.0)	75 (36.8)	378 (36.6)

IPD, invasive pneumococcal disease; NIPD, non-invasive pneumococcal disease; I, intermediately-susceptible isolates; R, resistant isolates; M, M-phenotype macrolide resistance; CR, constitutive macrolide resistance; IR, inducible macrolide resistance.
[a]Sum of non-susceptible isolates.
[b]Resistance to three or more drug classes.