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Antibiotic Resistance of Clinical Isolates of *Streptococcus pneumoniae* in Greece

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The antibiotic susceptibilities of 1,002 *Streptococcus pneumoniae* clinical isolates from patients with community-acquired pneumonia were determined over an 18-month period. Resistance rates were 14% for penicillin, 20% for erythromycin, 26% for tetracycline, and 1% for chloramphenicol. Resistance to non-β-lactam antibiotics was associated with penicillin resistance at statistically significant levels.

Streptococcus pneumoniae, the leading cause of community-acquired pneumonia, is the most common cause of bacterial meningitis in countries which immunize children aged 2 months or older with the *Haemophilus influenzae* type b conjugate vaccine, a major cause of acute otitis media in children, and the third most common significant isolate from blood cultures (5, 17). The mortality rate associated with pneumococcal infections is enhanced at the extremes of life, in patients with underlying disease or immunological abnormalities, and when *S. pneumoniae* is resistant to antimicrobial agents.

Penicillin has been the mainstay of treatment of pneumococcal infections for nearly 50 years (15). However, over the last two decades, clinical isolates of *S. pneumoniae* that have been found to have decreased susceptibilities to penicillin and, in many cases, to be associated with multiple antibiotic resistance traits have been reported from many countries (4, 9, 15, 17).

Clinical isolates of *S. pneumoniae* resistant to penicillin and other antibiotics such as erythromycin and tetracycline are known to be present in Greece. However, data describing their prevalence and patterns of resistance have not been published. In that context, we routinely studied all clinical isolates recovered from adult patients with community-acquired pneumonia for a period of 18 months.

A total of 1,002 isolates of *S. pneumoniae* recovered from sputum samples from outpatients with community-acquired pneumonia were tested. In all cases the criteria for bacterial pneumonia (fever, leukocytosis, positive radiographic findings, and purulent sputum) were fulfilled (7, 15). The causative role of *S. pneumoniae* was suggested by the demonstration of large numbers of gram-positive diplococci and polymorphonuclear cells (>25 per low-power field) in quality controlled gram-stained smears (epithelial cells, <10 per low-power field).

Pneumococci were identified by their susceptibility to ethylhydrocuprein and bile solubility. One isolate per patient was identified and tested.

Susceptibilities to chloramphenicol, erythromycin, and tetracycline were determined by the Kirby-Bauer disk diffusion method on Mueller-Hinton blood agar (Oxoid Limited, Hampshire, United Kingdom) incubated in a 5% CO_2 atmosphere. Disks were provided by a commercial source (Diagnostics Pasteur, Marnes-la-Coquette, France). Penicillin susceptibility was determined with oxacillin disks (1 μg) with a 20-mm cutoff (12). In all tests five pneumococcal strains from our culture collection with known susceptibilities to all antibiotics studied were included as controls.

Zones of inhibition (in millimeters) were determined in accordance with the interpretive standards outlined by the National Committee for Clinical Laboratory Standards (16). In this report the term "resistant" refers to relatively and highly resistant pneumococci.

Table 1 lists the susceptibilities of *S. pneumoniae* strains to penicillin, erythromycin, tetracycline, and chloramphenicol. Overall, of the 1,002 isolates tested, 138 (14%) were resistant to penicillin, 197 (20%) were resistant to erythromycin, 255 (26%) were resistant to tetracycline, and only 12 (1%) were resistant to chloramphenicol.

Remarkably, a higher proportion of the penicillin-resistant isolates expressed resistance to the non-β-lactam antibiotics (erythromycin, 56%; tetracycline, 46%; chloramphenicol, 4% in comparison with the proportion of their penicillin-susceptible counterparts that did so (erythromycin, 14%; tetracycline, 23%; chloramphenicol, 1%. The differences were statistically significant (erythromycin, $\chi^2 = 69.9$, $P < 0.001$; tetracycline, $\chi^2 = 17.2$, $P < 0.001$; chloramphenicol, $\chi^2 = 5.48$, $P < 0.05$).

In total, 370 isolates (37%) were resistant to one or more drugs (Table 2). Eleven different patterns of resistance were found; 18% of the isolates were resistant to a single drug, 16% were resistant to two drugs, and 3% were resistant to three drugs.

Interestingly, 24% of the penicillin-resistant isolates were resistant to multiple drugs in the sense that they were also resistant to two more drugs, erythromycin and tetracycline or chloramphenicol. It is worth mentioning that this pattern was not observed among penicillin-susceptible isolates.

The increasing number of penicillin-resistant *S. pneumoniae* isolates has become a worrisome problem worldwide (8). In some countries such as South Africa (9), the United States (New Mexico) (9), Hungary (13), and Spain (4) penicillin resistance is greater than 44%, the highest rate reported so far. Also, resistant strains have been isolated as part of the normal nasopharyngeal flora of healthy people, especially children (3, 13).

The results of the present study describe the problem of pneumococcal resistance in Greece, a part of the world not previously surveyed for this problem. Although for oxacillin-resistant pneumococci penicillin MICs are required to discriminate between relatively and highly resistant strains, the rela-

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TABLE 1. Susceptibilities of *S. pneumoniae* isolates to non-β-lactam antibiotics as related to penicillin resistance

Penicillin susceptibility, no. (%) of isolates	No. (%) of isolates resistant to:		
	Erythromycin	Tetracycline	Chloramphenicol
Susceptible, 864 (86)	120 (14)	192 (23)	7 (1)
Resistant, 138 (14)	77 (56)[a]	63 (46)[a]	5 (4)[b]
Total, 1002 (100)	197 (20)	255 (26)	12 (1)

[a] $P < 0.001$ compared with penicillin-susceptible isolates.
[b] $P < 0.05$ compared with penicillin-susceptible isolates.

tively low resistance rate to the drug, combined with the large number of strains tested, may reflect the level of penicillin resistance among isolates of *S. pneumoniae* from adults with pulmonary infections. In that respect, it should be considered that our strains were from a rather homogeneous population (adults) that, it is known, is less likely than children to be carriers of resistant serogroups (8). Nevertheless, our findings raise the question, voiced by many, of the therapeutic management of infected patients.

This consideration is all the more important because penicillin resistance is apparently not an isolated phenomenon in the susceptibility spectrum of pneumococci (13). Accordingly, we showed that a significantly higher rate of resistance to non-β-lactam antibiotics characterized the penicillin-resistant isolates. Furthermore, most of the isolates (63%) resistant to one or more non-β-lactam antibiotics were susceptible to penicillin (Table 2). Pneumococci with similar susceptibility patterns have been isolated in South Africa as well as Italy, France, and Belgiums, and they have been related to severe infections such as bacteremia and meningitis (8).

Bacteria found in the Mediterranean area have a rather homogeneous resistance rate that is much different from that for bacteria from northern countries (2). On the basis of the observations presented above, a similar pneumococcal resistance pattern in Greece and Spain would be expected. However, this is not the case, in that pneumococcal antibiotic resistance in Greece, except to erythromycin, is much lower than that in Spain. In order to highlight the epidemiology of pneumococcal resistance, work is in progress on a national basis for the study of pneumococcal resistance, with parallel DNA fingerprinting of the strains (10).

The mechanism of resistance is associated with alterations in penicillin-binding proteins (PBPs) (1, 6, 11). Moreover, pneumococci have been shown to be both the donors of altered PBP DNA sequences to other streptococcal species and the recipients of such sequences from other streptococcal species (3). The horizontal transfer of PBP genes has also been demonstrated among natural populations of pneumococci, and penicillin-resistant clones have even been shown to have taken transcontinental journeys (14). Mutation to high-grade resistance is thought to involve the acquisition of a genetic package, most likely the conjugative transposable element Tn*1545* (6, 15). This could be the resistance mechanism of our multiply resistant strains, in that Tn*1545* sequences confer resistance to non-β-lactam antibiotics such as erythromycin, tetracycline, and chloramphenicol.

TABLE 2. Distribution of main resistance phenotypes of *S. pneumoniae*

Antibiotics[a]	No. (%) of strains resistant
T	119 (12)
T, E	72 (7)
E, P	44 (4)
E	34 (3)
T, P	35 (3)
T, E, P	28 (3)
P	26 (3)
E, P, C	5
E, C	4
C	2
T, C	1
Total	370 (37)

[a] T, tetracycline; E, erythromycin; P, penicillin; C, chloramphenicol.

Pneumococci can no longer be considered pathogens with uniformly predictable susceptibilities to penicillin and other antimicrobial agents. The increasing frequency of pneumococcal resistance is especially worrisome in light of the trend toward higher levels of resistance to vancomycin among enterococci, since the horizontal genetic transfer of resistance to vancomycin to pneumococci seems likely (18). The present study serves to reinforce the importance of continued surveillance for the purpose of generating accurate local antimicrobial susceptibility data and studying alternative antibiotic therapies. Furthermore, routine testing of pneumococci and the implementation of an antibiotic treatment policy for patients with serious infections, including outpatients with infections, should be considered as an approach to reducing antibiotic resistance.

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