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Antibiotic Resistance and Serotypes of *Streptococcus pneumoniae* from Patients with Community-Acquired Pneumococcal Disease

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From August 1978 to December 1981, 200 *Streptococcus pneumoniae* strains isolated from adult patients with pneumococcal disease were tested for susceptibility to penicillin G, erythromycin, clindamycin, tetracycline, and chloramphenicol by disk diffusion. Minimal inhibitory concentrations (MICs) were determined by agar dilution and broth dilution. The sources (numbers) of these isolates were blood (111), cerebrospinal fluid (30), sputum (26), pleural fluid (16), and miscellaneous (17). Of the 200 strains, 18 were partially resistant (MIC, 0.1 to 1 μg/ml) and 2 were resistant to penicillin. A total of 144 (72%) strains were tetracycline resistant, 87 of which had MICs of ≥64 μg/ml. Ninety (45%) isolates exhibited various degrees of chloramphenicol resistance, with MICs ranging from 16 to 64 μg/ml. Five strains were resistant to erythromycin and clindamycin. Eleven penicillin-resistant strains were also resistant to chloramphenicol and tetracycline. Twenty-one different serotypes were encountered among the 120 typed strains studied. The most prevalent serotypes, in order of frequency, were 3, 1, 5, 19, 8, 6, 9, and 4, representing approximately two-thirds of the total number of isolates serotyped. These findings clearly indicate the need to perform antibiotic susceptibility testing in all cerebrospinal fluid isolates and other clinical significant isolates.

Reports of pneumococci resistant to penicillin and other drugs, including tetracycline, erythromycin, clindamycin, and chloramphenicol, have appeared during the last 15 years. Organisms with much greater resistance to penicillin and other antibiotics were first discovered in South Africa and were later found in other countries (1, 2, 11). In 1978, we decided to screen all pneumococci in our hospital for resistance to penicillin and other antimicrobial agents commonly used in the treatment of pneumococcal disease. In a preliminary study (5), we recently reported a high prevalence of chloramphenicol-resistant pneumococci among our clinical isolates and the therapeutic dilemma posed by meningitis patients infected by these resistant strains.

In the present study, we describe the antibiotic susceptibility patterns and serotype distribution of 200 strains of pneumococci isolated in patients with community-acquired pneumococcal disease seen at our institution over a 30-month period from August 1978 to December 1981.

MATERIALS AND METHODS

The sources (numbers) of the isolates included blood (111), cerebrospinal fluid (30), sputum (26), pleural fluid (16), and miscellaneous sources (17). All sputa were from patients with acute lower respiratory tract infections clinically compatible with pneumococcal pneumonia. Organisms were identified by their typical colonial morphology, bile solubility, and optochin sensitivity. The last isolates were serotyped according to their specific capsular reaction to typing sera from Statens Seruminstitut, Copenhagen (performed by J. Casal, Centro Nacional de Microbiología, Majadahonda, Madrid).

The following antibiotics were used: penicillin G, chloramphenicol, erythromycin, clindamycin, and tetracycline. The antibiotic-impregnated disks (Difco Laboratories) included penicillin G (10 IU), oxacillin (1 μg), clindamycin (2 μg), erythromycin (15 μg), tetracycline (30 μg), and chloramphenicol (30 μg).

Disk diffusion tests were performed on blood agar plates by the modified Kirby-Bauer technique used by Jacobs et al. (8).

Agar dilution testing. Inocula were prepared by diluting an overnight culture in Mueller-Hinton broth to a final concentration of 10^7 CFU/ml. With the help of a Steers replicator, the organisms were applied to Mueller-Hinton agar plates supplemented with 5% defibrinated horse blood and appropriate concentrations of the antibiotics (14). Approximately 10^4 CFU of organisms were delivered onto each spot. The plates were incubated at 35°C for 18 h in candle jars. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of antibiotic preventing visible growth. Selected strains showing penicillin or chloramphenicol resistance on agar dilution were test-

ed by a tube dilution technique (8). An inoculum of approximately 10^5 CFU/ml was used. Tubes were incubated for 18 h at 35°C, and the highest dilution showing no visible growth was read as the MIC. In addition, the last 30 cerebrospinal fluid and blood isolates were tested by a microdilution technique as previously described (13). Strains showing decreased penicillin susceptibility were tested for β-lactamase production by the chromogenic cephalosporin method (10).

RESULTS

MICs are shown in Table 1. Of the 200 strains tested, 20 (10%) had decreased penicillin G susceptibilities; they were isolated (number) from blood (6), cerebrospinal fluid (4), sputum (5), pleural fluid (2), and miscellaneous sources (3). Eighteen strains were partially resistant to penicillin (MIC, 0.1 to 1 μg/ml), and two strains had MICs of 2 and 4 μg/ml, respectively. All were β-lactamase negative.

A total of 144 strains (72%) were resistant to tetracycline, 87 of which had MICs of ≥64 μg/ml. Ninety (45%) isolates exhibited various degrees of chloramphenicol resistance, with MICs ranging from 16 to 64 μg/ml. Only 5 strains (2.5%) were resistant to erythromycin and clindamycin. Eleven penicillin-resistant strains were also resistant to chloramphenicol and tetracycline.

Twenty-one different serotypes were encountered in the last 120 strains of pneumococci studied. The most prevalent serotypes, in order of frequency, were 3, 1, 5, 19, 8, 6, 9, and 4, representing approximately two-thirds of the total number of isolates serotyped. Table 2 shows the antibiotic resistance patterns and distribution frequency of the different serotypes of 120 strains studied.

TABLE 1. Susceptibility of 200 pneumococci to five antibiotics

MIC (μg/ml)	No. of strains inhibited by[a]:				
	Pen G	Clin	Eryth	Tet	Chlor
0.016	146	12	12		
0.032	24	119	125		
0.064	10	62	58		
0.125	4	1			
0.25	8	1		9	
0.5	5			33	
1	1			5	13
2	1			5	25
4	1			4	40
8		2	2	2	32
16				32	70
32				23	17
64		1	1	41	3
128		2	2	46	

[a] Pen G, Penicillin G; Clin, clindamycin; Eryth, erythromycin; Tet, tetracycline; Chlor, chloramphenicol.

TABLE 2. Resistance patterns and serotypes of strains studied

Resistance pattern[a]	No. of strains	Serotype[b]
S	52	1, 3, 4, 5, 6, 7, 8, 12, 16, 19, 20, 28, 34
P	3	11, 23
PT	6	6, 19, 23
PC	1	6
PTC	10	9, 11, 14, 15, 19, 23
T	49	1, 2, 3, 4, 5, 9, 15, 16, 22, 33
TC	74	1, 3, 4, 5, 6, 7, 8, 9, 19, 23, 31, 33
TCECl	5	15, 19

[a] S, Susceptible to all antibiotics tested; P, resistant to penicillin G; T, resistant to tetracycline; C, resistant to chloramphenicol; E, resistant to erythromycin; Cl, resistant to clindamycin.

[b] Only the last 120 strains were serotyped.

The MICs obtained by agar dilution, tube dilution, and microdilution were found to be equal or within one doubling dilution of each other. Early in the study, therefore, macrobroth testing was stopped and replaced by the more easily performed microbroth dilution technique.

The correlation between zone diameter and MIC for tetracycline, erythromycin, and clindamycin was excellent, in agreement with previous reports (9). For penicillin susceptibility, the 1-μg oxacillin disk correctly identified all strains with decreased susceptibility to this antibiotic (zone diameter, ≤20 mm).

Our findings for chloramphenicol were similar to those reported by Jacobs et al. (9). Thus, the separation of susceptible from resistant strains was more reliable by disk diffusion than by MIC determination. Eleven strains with chloramphenicol MICs of 8 μg/ml had inhibition zone diameters of <19 mm. The MIC obtained after incubation in a subinhibitory concentration of this drug was ≥32 μg/ml. This property is linked to the production of inducible chloramphenicol acetyltransferase (4, 12).

DISCUSSION

Since an earlier report of pneumococci with decreased penicillin susceptibility in 1967 (7), resistant strains and relatively resistant strains (MIC, 0.1 to 0.9 μg/ml) have been reported from many nations. The prevalence of such relatively resistant pneumococci ranges from 1 to 16% (3). The sources of these isolates have been, in many cases, children and carriers. Our finding of a 10% incidence of pneumococci with decreased penicillin susceptibility is particularly relevant in

that all strains were from hospitalized adults who had pneumococcal disease. Of note is the fact that four of these strains were from the cerebrospinal fluid of patients with meningitis and that in two instances the MICs were 2 and 4 μg/ml, respectively.

Most alarming is our finding of a 45% incidence of chloramphenicol resistance among our isolates. The percentage of resistant strains from carriers and patients reported goes from 0 to 14% (6). The unexpected number and degree of chloramphenicol resistance in our study is the highest recorded to date. This high degree of chloramphenicol resistance could be related in part to the widespread use of this drug in Spain over many years. The degree of resistance found has been moderate in many strains (MIC, 10 to 20 μg/ml). The clinical relevance of this finding is not clear because the critical point of resistance has not been well defined. The recently reported cases (1, 11) and our own experience (5) of pneumococcal meningitis that failed to respond to chloramphenicol are disturbing and indicate that therapeutically attainable levels of chloramphenicol can no longer be assumed for all pneumococci. Also, in contrast with our earlier experience (5), the last 11 penicillin-resistant strains were also resistant to chloramphenicol and tetracycline. The increasing number of reports of multiply resistant strains (3, 11) clearly indicates that the treatment of pneumococcal meningitis needs to be reevaluated and that clinical trials with other antimicrobial agents are urgently needed.

The high incidence of tetracycline resistance (72%) among our clinical isolates certainly precludes its use as an antipneumococcal agent. The majority of chloramphenicol-resistant strains were also resistant to tetracycline, as previously reported by others (4). Erythromycin and clindamycin are very active and inhibit the majority of strains at low concentrations.

It is clear nowadays that antibiotic susceptibility testing should be performed in all cerebrospinal fluid, blood, and other significant isolates. In our study, the modified Kirby-Bauer technique has been excellent as a screening procedure. Further testing of all penicillin-resistant isolates is indicated for accurate MIC determinations. We have found the microdilution method described by Tarpay (13) to be very reliable and recommend it as the most suitable method for clinical laboratories.

Different pneumococcal serotypes predominate in different parts of the world. Of the strains typed, 28% belonged to serotypes not included in the current vaccine formulation. Of note is the fact that serotype 5, the third-most-common serotype encountered in our study, is not included. Although the total number of isolates used was small and obtained from a single institution, these data indicate the need for broader epidemiological studies in different areas to determine the prevalence of different serotypes and, accordingly, to reformulate the currently available vaccine.

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