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Antimicrobial Susceptibility of *Streptococcus pneumoniae*: Serotype Distribution of Penicillin-Resistant Strains in Spain

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This study examined the resistance to penicillin, tetracycline, erythromycin, and chloramphenicol of 318 pneumococcal strains isolated in Spanish hospitals from blood or cerebrospinal fluid of patients during 1979 to 1981. The serotypes of these strains were determined to discover whether a correlation between serotype and patterns of antibiotic resistance could be found. Seven and nine patterns of resistance were found in strains isolated from blood and cerebrospinal fluid, respectively; tetracycline was the most frequent pattern, followed by tetracycline associated with chloramphenicol. A random distribution of serotypes which was similar to the general distribution of serotypes was found for resistance to tetracycline and chloramphenicol, but penicillin-resistant strains were confined to seven serotypes. Thirty-six strains of penicillin-resistant pneumococci isolated from sources other than blood or cerebrospinal fluid were also serotyped. They represented the same serotypes, suggesting that serotype distribution among penicillin-resistant strains could be a manifestation of local epidemiological factors.

Pneumococci have long been considered as naturally penicillin-susceptible microorganisms, such as group A hemolytic streptococci or meningococci. However, since 1965 (9), strains with a degree of resistance to penicillin have been isolated in many parts of the world (2, 5, 12); in some instances, these strains became resistant in the clinic (3, 11). The resistance to penicillin was frequently associated with resistance to other antibiotics, presenting new problems for clinicians. The South African outbreak in 1979 (1), where multiresistant strains were responsible for many cases of empyema and bacteremia together with pneumonia or meningitis, is perhaps the best example of these problems.

In this paper, I examined the resistance to antibiotics of 318 pneumococcal strains isolated in Spanish hospitals from blood or cerebrospinal fluid (CSF) of patients during 1979 to 1981. The serotypes of these strains were identified to determine whether a correlation between serotype and the patterns of antibiotic resistance could be found.

MATERIALS AND METHODS

Pneumococcal strains. A total of 318 pneumococcal strains were studied: 272 isolated from the blood of patients with symptoms of pneumonia and 46 from the blood or CSF of patients with meningitis. These strains were sent to my laboratory from 20 hospitals throughout Spain. Thirty-six pneumococcal strains isolated from other sources (pleural fluid and exudates from otitis, appendicitis, conjunctiva, or the pharynx) were also studied. When these strains arrived in the laboratory, they were immediately subcultured on Mueller-Hinton medium plates (Difco Laboratories, Detroit, Mich.) supplemented with 5% sheep blood and incubated at 37°C for 18 h in a 5% CO_2 atmosphere. An optochin disk was placed in the upper part of the streaked area.

Susceptibility testing. A disk agar diffusion test and an agar dilution assay were both performed in Mueller-Hinton medium supplemented with 5% sheep blood.

(i) Inoculum preparation. For both methods, the inoculum was prepared by suspending the growth of an overnight culture so as to obtain a density equivalent to a no. 5 McFarland standard. This suspension was then diluted 1:100 for a final concentration of 10^7 bacteria per ml.

(ii) Disk agar diffusion. The inoculum was spread onto the agar by streaking in four directions with a sterile cotton swab previously soaked in the inoculum. After the agar surface was dry, antimicrobial disks were applied, and the plates were incubated aerobically at 37°C for 18 h. The following disks (Difco Laboratories) containing antibiotics were used: penicillin, 2 U; tetracycline, 30 μg; erythromycin, 15 μg; and chloramphenicol, 30 μg. The limits of the diameter of the halo for considering a strain susceptible were: penicillin, 30 mm; tetracycline, 20 mm; erythromycin, 20 mm; and chloramphenicol, 19 mm (7, 8). When the sizes of the inhibiting halos were lower, determinations of the minimal inhibitory concentrations (MICs) were made for these antibiotics.

(iii) Agar dilution method (15). For the determination of the MIC, antibiotics were added to the medium in

doubling dilution to give final concentrations ranging from 0.05 to 1.6 μg/ml for penicillin and from 3.1 to 100 μg/ml for the other antibiotics. The organisms were applied with a loop (3 mm diameter), inoculating up to 12 strains on the same plate. Inoculated plates were incubated for 18 h in aerobic conditions at 37°C.

Strains were considered susceptible to penicillin if inhibited by concentrations of <0.1 μg/ml, intermediate if inhibited by 0.1 to 1 μg/ml, and resistant if inhibited by >1 μg/ml (7). Strains were considered susceptible to tetracycline if inhibited by concentrations of <12 μg/ml and resistant if inhibited by ≥12 μg/ml. Strains were considered susceptible to chloramphenicol if inhibited by concentrations of <25 μg/ml and resistant if inhibited by ≥25 μg/ml. Strains were considered susceptible to erythromycin if inhibited by concentrations of <6.2 μg/ml and resistant if inhibited by ≥6.2 μg/ml (7, 8).

Penicillin, tetracycline, erythromycin, and chloramphenicol were supplied as basic powders from Antibioticos S.A., Madrid, Spain.

Serotyping. The strains were typed by the capsular reaction with standard antisera (Statens Seruminstitut, Copenhagen, Denmark).

RESULTS

Antibiotic susceptibility. A total of 28 strains (8.7%) showed resistance to penicillin (Table 1), 0.2 and 0.4 μg/ml being the most frequent MICs. A total of 214 strains (67.1%) were resistant to tetracycline and 65 (20.4%) to chloramphenicol, most of the strains having an MIC of 25 μg/ml against these two antibiotics. Only six strains (1.8%) presented resistance to erythromycin, with prevalent MICs of ≥100 μg/ml.

Eight patterns of resistance were found in strains isolated from blood, and 10 patterns were found in those from CSF (Table 2). Resistance to tetracycline was the most frequent in both cases, followed by tetracycline in association with chloramphenicol. Of the total number of strains tested, 69% from blood and 74% from CSF presented resistance to one or more antibiotics.

Serotype distribution. A total of 27 different serotypes were found in strains from blood (Table 3), serotypes 1, 3, 5, 8, and 14 accounting for about 50% of them. Strains from CSF were distributed among 17 different serotypes, serotypes 6 and 23 being the most frequently encountered.

TABLE 2. Antibiotic resistance patterns of pneumococci

Resistance[a]	No. (%) of resistant strains isolated from:	
	Blood	CSF
P	5 (1.8)	1 (2.2)
T	126 (46.3)	12 (26.1)
C	1 (0.3)	1 (2.2)
E	0	0
P and T	8 (2.9)	5 (10.8)
T and C	43 (15.8)	7 (15.2)
T and E	0	1 (2.2)
P, T, and C	4 (1.5)	3 (6.5)
T, C, and E	0	3 (6.5)
P, T, C, and E	1 (0.3)	1 (2.2)

[a] Strains resistant to penicillin (P), tetracycline (T), chloramphenicol (C), and erythromycin (E).

Serotype distribution in penicillin-resistant strains. All penicillin-resistant strains, from both blood and CSF, were distributed among seven serotypes: 6, 9, 11, 14, 15, 21, and 23 (Table 3). Serotype 6 was the most common from both sources. In addition, among all of the pneumococcal strains belonging to serotype 6, 38% of those from blood and 85% from CSF were resistant to penicillin (Table 3). Penicillin-resistant strains from other sources were found to belong to six different serotypes that were the same as those from blood or CSF (Table 3).

DISCUSSION

We have found that 8.7% of the strains present resistance to penicillin; however, MICs appear relatively low in all strains (except for one strain isolated from blood having an MIC of 1.6 μg/ml). This percentage is difficult to compare with those from different geographical areas. Ward (14) reviewed the prevalence of strains of pneumococci resistant to penicillin in various parts of the world, showing that in most

TABLE 1. Susceptibility of the 318 pneumococcal isolates to penicillin, tetracycline, chloramphenicol, and erythromycin

Antibiotic	No. (%) of isolates inhibited by following concentration (μg/ml)[a]									
	≤0.05	0.2	0.4	0.8	1.6	<12.5	12.5	25	50	≥100
Penicillin	290 (91.1)	10 (3.1)	11 (3.4)	6 (1.9)	1 (0.3)					
Tetracycline						104 (32.7)	49 (15.4)	68 (21.3)	63 (19.8)	34 (10.6)
Chloramphenicol						143 (45.0)	110 (34.6)	64 (20.1)	1 (0.3)	
Erythromycin						312 (98.8)		1 (0.3)	1 (0.3)	4 (1.2)

[a] Data are presented only for those concentrations tested.

TABLE 3. Serotypes of 318 pneumococci by source

Serotype	Blood (272)[a]		CSF (46)[a]		Other sources (36)[a,b]
	Total	Penicillin resistant	Total	Penicillin resistant	Penicillin resistant
1	37	—[c]	1	—	—
2	18	—	2	—	—
3	34	—	4	—	—
4	12	—	—	—	—
5	29	—	—	—	—
6	18	7	7	6	4
7	17	—	4	—	—
8	21	—	2	—	—
9	7	1	—	—	—
10	1	—	3	—	—
11	3	3	1	1	14
12	10	—	1	—	—
13	1	—	—	—	—
14	19	2	3	—	2
15	3	1	4	—	1
16	6	—	—	—	—
17	—	—	—	—	—
18	7	—	2	—	—
19	14	—	3	—	—
20	2	—	2	—	—
21	2	2	1	1	5
22	1	—	—	—	—
23	3	2	5	2	10
24	—	—	—	—	—
25	1	—	1	—	—
26	—	—	—	—	—
27	—	—	—	—	—
28	1	—	—	—	—
29	—	—	—	—	—
30	—	—	—	—	—
31	1	—	—	—	—
32	—	—	—	—	—
33	4	—	—	—	—
34	—	—	—	—	—
35	1	—	—	—	—

[a] Number in parentheses is number of serotypes.
[b] Only penicillin-resistant strains were studied.
[c] —, None detected.

of the European countries it varies, from 0.1% in England to 3.5% in Poland; however, higher percentages have been found in South Africa (8.4%) and New Guinea (22.0%). Resistance to tetracycline and chloramphenicol is higher in Spain than in other countries (4, 6, 14). This situation can be explained by the extensive use of these two antibiotics in Spain.

When the antibiotic resistance and the serotype of each strain were correlated, a random distribution of serotypes similar to that of Table 3 was found for tetracycline and chloramphenicol, but penicillin-resistant strains were confined to only seven serotypes in strains from blood and four of these same seven types in strains from CSF. Fortunately, none of the more frequent serotypes (i.e., serotypes 1, 3, 5, and 8) manifested resistance to penicillin.

The same serotypes that we found associated with penicillin resistance have been described by other authors, but other serotypes have been found among penicillin-resistant strains (10, 13) that were not present in this study. The reason for these findings may be related to the use of antibiotics and the local epidemiology of pneumococcal types. To pursue this idea, a sample of 36 strains of penicillin-resistant pneumococci isolated from sources other than blood or CSF were serotyped. Our findings show that these penicillin-resistant strains were of the same serotypes as those isolated from blood or CSF (Table 3), indicating that serotype distribution of

penicillin-resistant strains is probably not fortuitous.

The vaccine against pneumococci is constituted by 14 serotypes; these serotypes represent 76.7% of the 20 different serotypes that we have identified. Thus, immunization of high-risk groups in Spain would give protection against around 75% of the pathogenic pneumococci.

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LITERATURE CITED

1. **Appelbaum, P. C., A. Bhamjee, J. N. Scragg, A. F. Hallett, A. A. Bowen, and R. Cooper.** 1977. *Streptococcus pneumoniae* resistant to penicillin and chloramphenicol. Lancet **ii**:995–997.
2. **Finland, M.** 1978. And the walls come tumbling down. More antibiotic resistance and new pneumococci (Editorial). N. Engl. J. Med. **299**:770–771.
3. **Garau, J., J. Liñares, and C. Dominguez.** 1981. Letter to the editor. Lancet **ii**:147–148.
4. **Gopalaskrishana, K. V., and P. I. Lerner.** 1973. Tetracycline-resistant pneumococci, increasing incidence and cross-resistance to newer tetracyclines. Am. Rev. Respir. Dis. **108**:1007–1010.
5. **Hansman, D., H. Glasgow, I. Sturt, L. Devitt, and R. Douglas.** 1971. Increased resistance to penicillin of pneumococci isolated from man. N. Engl. J. Med. **284**:175–177.
6. **Howard, A. J., C. J. Hince, and J. D. Williams.** 1978. Antibiotic resistance in *Streptococcus pneumoniae* and *Haemophilus influenzae*. Br. Med. J. **ii**:1657–1660.
7. **Jacobs, M. R., H. S. Koornhof, R. M. Robins-Browne, C. M. Stevenson, Z. A. Vermaak, I. Freiman, G. B. Miller, M. A. Witcomb, M. Isaacson, J. Ward, and R. Austrian.** 1978. Emergence of multiply resistant pneumococci. N. Engl. J. Med. **299**:735–740.
8. **Jacobs, M. R., Y. Mithal, R. M. Robins-Browne, M. N. Gaspar, and H. J. Koornhof.** 1977. Antimicrobial susceptibility testing of pneumococci: determination of Kirby-Bauer breakpoints for penicillin G, erythromycin, clindamycin, tetracycline, chloramphenicol, and rifampin. Antimicrob. Agents Chemother. **16**:190–197.
9. **Kislak, J. W., L. M. B. Razavi, A. K. Daly, and M. Finland.** 1965. Susceptibility of pneumococci to nine antibiotics. Am. J. Med. Sci. **250**:261–268.
10. **Mufson, M. A.** 1979. Clinical aspects and importance of pneumococcal infections. Pathol. Biol. **27**:519–523.
11. **Radetsky, M. S., G. R. Istre, T. L. Johansen, S. W. Parmelee, B. A. Lauer, A. M. Wiesenthal, and M. P. Glode.** 1981. Multiply resistant pneumococcus causing meningitis: its epidemiology within a day-care center. Lancet **ii**:771–773.
12. **Tarpay, M.** 1978. Importance of antimicrobial susceptibility testing of *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **14**:628–629.
13. **Tempest, B., J. P. Carney, and B. Eberle.** 1974. Distribution of the sensitivities to penicillin of types of *Diplococcus pneumoniae* in an American Indian population. J. Infect. Dis. **130**:67–69.
14. **Ward, J.** 1981. Antibiotic-resistant *Streptococcus pneumoniae*: clinical and epidemiological aspect. Rev. Infect. Dis. **3**:254–266.
15. **Washington, J. A., II, and V. L. Sutter.** 1980. Dilution susceptibility test: agar and macro-broth dilution procedures, p. 453–458. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.), Manual of clinical microbiology. American Society for Microbiology, Washington, D.C.