Serotypes and Penicillin Susceptibility of Pneumococci Isolated from Blood

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To learn the prevalence of penicillin-resistant pneumococci and the distribution of serotypes in proved pneumococcal infections, we studied 98 pneumococci recovered from blood over a 4-year period. Penicillin susceptibility was determined by the agar dilution method. Serotyping was done by the capsular swelling (quellung) test. Only one strain showed diminished susceptibility to penicillin (minimal inhibitory concentration, 0.12 $\mu\text{g/ml}$). Twenty-three different serotypes were identified; types 3, 4, 6, 8, 9, 14, and 19 were the most frequent. Type 4 was the most common serotype. Sixty-two percent of strains were serotypes included in a recently licensed 14-type pneumococcal polysaccharide vaccine (Pneumovax, Merck), and an additional 16% were antigenically related serotypes. Even though penicillin-resistant strains of pneumococci may rarely cause bacteremic pneumococcal infection, we suggest that isolates from cerebrospinal fluid, blood, and other normally sterile body fluids be tested for penicillin susceptibility. Inclusion of additional serotypes in future pneumococcal polysaccharide vaccines should be based on the current distribution of serotypes in large series of proved pneumococcal infections.

Pneumococci no longer can be assumed to be uniformly highly susceptible to penicillin. Since the first report in 1967 (13), strains of pneumococci moderately resistant to penicillin (minimal inhibitory concentrations [MICs] of 0.1 to 0.9 $\mu\text{g/ml}$) have been isolated from clinical specimens from many parts of the world (1). Some patients with meningitis caused by these strains have failed to respond to large doses of parenteral penicillin (1, 2, 10, 16, 17, 22, 24, 26). Strains markedly resistant to penicillin (MICs ≥ 1.0 $\mu\text{g/ml}$) have been recovered from patients and carriers in South Africa during a recent outbreak caused by pneumococci resistant to multiple antibiotics (18) and from the blood of a child with immunodeficiency disease in Minnesota (5). The prevalence of these resistant pneumococci in pneumococcal disease is not clear, because many studies have examined primarily sputum isolates of doubtful clinical significance (3) or nasopharyngeal or throat isolates from carriers (7, 11). To learn the prevalence of penicillin-resistant pneumococci and the distribution of serotypes in proved pneumococcal infections, we studied pneumococci recovered from blood cultures at the University of Colorado and Denver Veterans Administration Hospitals (Denver, Colo.) between September 1975 and March 1979.

MATERIALS AND METHODS

Pneumococci were identified by colonial characteristics, Gram stain, and ethylhydrocupreine suscepti-

bility. Penicillin susceptibility tests were performed by the agar dilution technique, using Mueller-Hinton agar with 5% defibrinated sheep blood containing serial twofold dilutions of a standard powder of potassium benzylpenicillin with final concentrations ranging from 0.008 to 4.0 $\mu\text{g/ml}$. The inocula were prepared from pneumococci grown overnight (18 h) on 5% sheep blood agar plates, suspended in Mueller-Hinton broth, and adjusted photometrically to 10^8 colony-forming units per ml with Mueller-Hinton broth. The inocula were applied with a multipoint inoculator (10^4 colony-forming units per spot). Agar plates were incubated at 35°C in a 5% CO_2 atmosphere for 18 to 24 h. The MIC was defined as the lowest concentration of antibiotic that prevented visible growth. Control organisms were five penicillin-resistant pneumococci with known MICs, kindly supplied by Clyde Thornesberry of the Center for Disease Control, Atlanta, Ga. All susceptibility tests were performed in duplicate. Serotyping was done by the capsular swelling (quellung) test, using Danish antisera (State Serum Institute, Copenhagen, Denmark). Subtyping of selected strains, using monotypic antisera, was done by Richard Facklam of the Center for Disease Control in order to recognize the exact subtypes included in Pneumovax (Merck).

RESULTS

Ninety-eight blood culture isolates were examined. Fifteen isolates were from children less than 10 years old, 28 were from adults 21 to 50 years old, 49 were from patients over 50 years old, and 6 were from patients of unknown age. Bacteremia was associated with pneumonia (74), meningitis (5), peritonitis (1), upper respiratory infection (3), or an occult focus (7); 8 hospital

charts were unavailable. The penicillin MICs by serotype are shown in Table 1. All strains except one (99%) were susceptible to $\leq 0.06 \mu\text{g}$ of penicillin G per ml. The sole moderately resistant strain (MIC, $0.12 \mu\text{g}/\text{ml}$) was recovered in 1976 from a 61-year-old man and was serotype 4. MICs of type 4 strains tended to be higher than those of other serotypes; otherwise, there was no correlation between serotype and penicillin MIC. Twenty-three different serotypes were identified; types 3, 4, 6, 8, 9, 14, and 19 were the most frequent. The higher serotypes, except for types 19, 22, 32, and 38, were uncommon. Type 4 was the most common serotype overall. Type 6 was the most common serotype recovered from children; five of six type 6 isolates were from children.

DISCUSSION

Our results show that in the Denver area pneumococci resistant or moderately resistant to penicillin rarely are responsible for bacteremic infections. Ahronheim et al. recently summarized the published surveys of pneumococcal penicillin susceptibility (1). The prevalence of pneumococci exhibiting MICs of $\geq 0.1 \mu\text{g}/\text{ml}$

varies worldwide from 0 to 35%. The prevalence seems to be greatest in parts of New Guinea (14). In North America the prevalence in the 1970s generally has been 0 to 3% (7).

Most clinical and bacteriological failures with penicillin treatment have occurred with pneumococcal meningitis. This is because the penetration of penicillin into cerebrospinal fluid is poor even when the meninges are inflamed. Cerebrospinal fluid penicillin concentrations in meningitis often are 0.1 to $1.0 \mu\text{g}/\text{ml}$ (15); these concentrations may not be sufficient to sterilize the cerebrospinal fluid when the MIC of the pneumococcus is so close to the achieved concentrations. Treatment failure in pneumonia due to resistant pneumococci appears to be less of a problem, probably because levels of penicillin in the lungs are higher than in cerebrospinal fluid (29). However, the preference of many physicians to treat pneumococcal pneumonia with low-dose penicillin may lead to more frequent treatment failures in pneumonia as well as in meningitis (4). Serious pneumococcal infections should not be treated with small doses of penicillin (8).

Even though penicillin-resistant strains are uncommon at this time, it seems prudent to test selected clinically significant isolates for penicillin susceptibility. Pneumococci, particularly from cerebrospinal fluid but also from blood and other normally sterile body fluids, such as joint, middle ear, or pleural effusions, should be screened for penicillin susceptibility (8). Because of the difficulty in interpreting the significance of pneumococci isolated from sputum and throat or nasopharyngeal specimens (3), routine susceptibility testing of pneumococci from these specimens is not warranted.

Standardized procedures exist for agar dilution MIC testing of pneumococci, but many laboratories do not have the technical capability to determine MICs. The disk diffusion (Kirby-Bauer) test, using an oxacillin ($1 \mu\text{g}$) or methicillin ($5 \mu\text{g}$) disk, is the most convenient method to screen pneumococci for penicillin susceptibility (6, 7, 19); however, the method for testing pneumococci needs better standardization of the agar medium, inoculum, incubation conditions, disk content, and definition of breakpoints. No widely accepted standardized method exists at present.

In this study 23 different serotypes were responsible for bacteremia. The distribution of serotypes was similar to those in other recent reports (9, 12, 20, 23, 25, 28). Only 62% of the strains were antigenically identical to serotypes included in a 14-type pneumococcal polysaccharide vaccine (Pneumovax-Merck; Merck & Co., Inc., Rahway, N.J.) recently licensed for use in

TABLE 1. Penicillin G MICs and serotypes of pneumococci from 98 patients with pneumococcal bacteremia

Serotype ^a (Danish system)	No. of strains with MIC ($\mu\text{g}/\text{ml}$) of:				
	0.01	0.03	0.06	0.12	Total
1	1	2			3
3	9				9
4	6	7	2	1	16
6 [6A(2); 6B(4)]	4	2			6
7 [7F(2)]	1	1			2
8	4	4			8
9 [9N(1); 9V(6)]	2	5			7
11	1	1			2
12 [12F(2)]	1	1			2
14	5	4			9
19 [19A(5); 19F(5)]	7	3			10
22	3	1			4
23 [23A(1); 23F(3)]	2	2			4
32		2	1		3
38	2	2			4
Other types ^b	3	6			9
Cumulative % inhibited	52	95.9	99.0	100	(98)

^a Serotypes included in commercially available pneumococcal vaccine (Pneumovax, Merck) (27) are in boldface type.

^b One each of types 2, 5, 10, 13, 16, 17, 29, and 35 and one nontypable strain.

the United States (27). Sixteen percent were antigenically related serotypes against which the vaccine may offer some protection. Types in the vaccine are 1, 2, 3, 4, 6A, 8, 9N, 12F, 14, 19F, 23F, 25, 7F, and 18C (Danish typing system). If the prevalence of types 22, 32, and 38 in our study is corroborated by other studies, inclusion of these types in future polyvalent pneumococcal vaccines should be considered. Others also have emphasized the possible limitations of the current vaccine as well as the need for updating the included serotypes based on surveillance of blood and other clinically significant isolates of pneumococci (8, 9, 23).

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