Chloramphenicol and Penicillin Resistance in Pneumococci Isolated from Blood and Cerebrospinal Fluid: a Prevalence Study in Metropolitan Denver

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From January through October 1981, we screened blood and cerebrospinal fluid pneumococcal isolates from 101 patients in the Denver, Colorado area. Isolates from seven patients (6.9%) showed relative resistance to penicillin, with minimal inhibitory concentrations ranging from 0.12 to 1.0 μg/ml. Two isolates (2.0%) were resistant to chloramphenicol, both with a minimal inhibitory concentration of 16 μg/ml. One of these was multiply resistant (to penicillin, chloramphenicol, and tetracycline). All isolates were susceptible to erythromycin, sulfamethoxazole-trimethoprim, and rifampin. On the basis of penicillin susceptibilities performed by participating hospitals on the isolates from 215 patients in the Denver area (101 included in the survey, 114 not included), we estimated the rate of relative resistance to penicillin to be approximately 4.3%. Compared with resistance rates reported in a previous study in Denver, these penicillin and chloramphenicol resistance rates may represent a trend of increasing resistance to these antibiotics in the Denver area. We recommend screening all isolates from invasive pneumococcal infections for penicillin and chloramphenicol susceptibility.

Pneumococci are no longer uniformly susceptible to penicillin. Since Hansman first described a pneumococcus relatively resistant to penicillin (minimal inhibitory concentration [MIC], 0.6 μg/ml) in 1967 (10), others have reported the prevalence of relative resistance to penicillin (0.1 to 1.0 μg/ml) to be as high as 15.5% in a metropolitan area in the United States (19) and 29% in two hospitals in South Africa (12). Generally, however, 0 to 3% of clinical pneumococcal isolates in the United States are relatively resistant to penicillin (1).

Chloramphenicol is frequently chosen in the treatment of pneumococcal infections in penicillin-allergic patients or in infections with penicillin-resistant strains (3). Pneumococci resistant to chloramphenicol, first reported in 1970 (4), have recently been reported in the United States (18). Most studies on the prevalence of antibiotic resistance in the United States, however, have centered on penicillin susceptibility and have not reported findings on chloramphenicol susceptibility. In other parts of the world, chloramphenicol resistance has been reported more frequently (8, 9, 17).

The recent report of a multiply resistant pneumococcus from Denver (18) prompted us to carry out this study to learn the prevalence of chloramphenicol, penicillin, and multiple resistance among pneumococci causing invasive infections in the Denver area. We studied pneumococci recovered from blood and cerebrospinal fluid (CSF) cultures from the Denver metropolitan area from January through October 1981. A previous study of invasive pneumococcal isolates from a large hospital in Denver (14) allowed us to compare the 1981 rates of resistance in our study with that reported in this pre-1980 data.

MATERIALS AND METHODS

During the study period, the 21 acute-care hospitals in the Denver area, serving an immediate population of 1.63 × 106 and a larger referral area, submitted pneumococcal isolates recovered from blood and CSF to their laboratories. In addition, these laboratories submitted pneumococcal isolates from other sites (including respiratory sites) during the first 3 months of the study. Pneumococci were confirmed with optochin disk susceptibility and bile solubility at the laboratory of the Colorado Department of Health, Denver, and screened for antimicrobial susceptibility by the modified Kirby-Bauer disk diffusion method on Mueller-Hinton agar plates supplemented with 5% sheep blood. An inoculum of approximately 1.5 × 108 orga-
PNEUMOCOCCAL RESISTANCE

The number of colonies per ml was obtained by incubating four to six colonies in brain heart infusion broth to the desired turbidity as compared to a 0.5 MacFarland barium sulfate standard. Plates were inoculated by using a cotton swab and incubated aerobically overnight at 37°C without added carbon dioxide. Disks used (Difco Laboratories, Detroit, Mich.) included oxacillin (1 μg), chloramphenicol (30 μg), tetracycline (30 μg), erythromycin (15 μg), rifampin (5 μg), and sulfamethoxazole-trimethoprim (23.25-1.25 μg). Zone diameters were read with calipers after 24 h of incubation. Zone size breakpoints used for resistance were those recommended by Jacobs (13) and Thornsberry (22) as follows: oxacillin, chloramphenicol, and tetracycline, ≤19 mm; erythromycin and rifampin, ≥20 mm; sulfamethoxazole-trimethoprim, ≤15 mm.

Strains showing resistance to any agent by disk diffusion screening were sent to the Antimicrobics and Infection Mechanisms Branch, Centers for Disease Control, Atlanta, Ga., for verification and determination of MICs. These determinations were carried out with an inoculum size of 10^5 CFU/ml, using Mueller-Hinton broth with 5% lysed horse blood, and a microdilution technique (20).

To determine the total number of blood and CSF pneumococcal isolates from patients in participating hospitals, laboratory records from each hospital were reviewed for all such cultures reported as positive for the pneumococcus and results of antimicrobial susceptibilities, if performed. Names of patients were compared with those on the list received during the study.

RESULTS

We screened a total of 104 blood and CSF pneumococcal isolates from 101 patients. Isolates from eight patients showed zone sizes of ≤19 mm for oxacillin, and MIC testing of these isolates showed seven (6.9%) to be relatively resistant to penicillin. The two (1.9%) isolates with zone sizes of <19 mm for chloramphenicol both had an MIC of 16 μg/ml (Table 1). One of the chloramphenicol-resistant strains was also resistant to penicillin and tetracycline (MICs, 1.0 and 16 μg/ml, respectively) and was serotype 6B. The other was susceptible to penicillin, had a tetracycline MIC of 16 μg/ml, and was not typeable. Both strains were from people with bacteremic pneumonia. Neither patient had received chloramphenicol in the previous 3 months.

Screening and MIC testing showed all blood and CSF isolates to be susceptible to erythromycin, sulfamethoxazole-trimethoprim, and rifampin (≤0.12, ≤9.5-0.5, and ≤0.12 μg/ml, respectively). Five (4.8%) showed tetracycline resistance. Of these isolates, 98, 1, 2, and 3 had tetracycline MICs of ≤4, 8, 16, and >16, respectively; 99, 5, and 0 had sulfamethoxazole-trimethoprim MICs of ≤4.8-0.25, 9.5-0.5, and 19-1, respectively; and 100, 4, and 0 had rifampin MICs of ≤0.06, 0.12, and 0.24, respectively. The lowest MICs were defined by zone size breakpoints in some cases.

During the first 3 months of the study, we screened an additional 119 pneumococcal isolates from sites other than blood or CSF. These included pneumococci from sputum (65 isolates), middle ear (6 isolates), eye (16 isolates), lung (8 isolates), and other miscellaneous sites (24 isolates). Six (5.1%) isolates from these sources were relatively resistant to penicillin by MIC testing. None showed resistance to chloramphenicol.

The review of laboratory records revealed that 215 patients had had blood or CSF cultures positive for pneumococci. A total of 180 patients (83.7%) had been tested for penicillin susceptibility at hospital laboratories, 168 (93.3%) with oxacillin disks and 12 (6.7%) with penicillin disks. Five cultures (2.7%) were interpreted as resistant and 175 as susceptible, and 35 were not tested. All five cultures interpreted as resistant were submitted to our screening program, and all had MICs of >0.1 μg/ml. Of the 210 isolates interpreted as susceptible or not tested by hospitals, 99 were submitted to our program; 2 (2.0%) of these were found to be relatively resistant by screening and MIC testing in our survey. Extrapolating from these totals (2% resistance among isolates interpreted as susceptible or not tested; 100% resistance among isolates interpreted as resistant), we estimate that 4.3% [(2.0% of 210 isolates) + (100% of 5 isolates)/215 total isolates] of pneumococci in the Denver area during the study period showed relative resistance to penicillin. Certainly, the minimum penicillin resistance rate is 3.3% (7 of 215).

TABLE 1. Chloramphenicol and penicillin susceptibility of pneumococci isolated from blood and CSF in Denver from January through October 1981

<table>
<thead>
<tr>
<th>Source</th>
<th>No. of isolates with indicated MIC (μg/ml) of:</th>
<th>Chloramphenicol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Penicillin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≤0.03*</td>
<td>0.06</td>
</tr>
<tr>
<td>Blood</td>
<td>85*</td>
<td>1</td>
</tr>
<tr>
<td>CSF</td>
<td>11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> The MIC was defined by zone size breakpoint in some cases (see text).

<sup>b</sup> Three susceptible isolates were duplicate isolates from three patients.

<sup>c</sup> One isolate was resistant to penicillin, chloramphenicol, and tetracycline.
DISCUSSION

Our results show a significant rate of relative resistance to penicillin among invasive (blood and CSF) pneumococcal isolates in the Denver area. Resistance to chloramphenicol, although less common, is also present in this area. The penicillin resistance rate of 4.3% is considerably higher than that found in a previous survey in the Denver area (1%) (14) and somewhat higher than the general rate in North America (0 to 3%) (6) and may represent a trend of increasing resistance in the Denver area. The chloramphenicol resistance rate (2.0%) is higher than that found in previous surveys done in Wisconsin (15) and Oklahoma (A. J. Saah, personal communication), where no chloramphenicol resistance was seen.

The single multiply resistant strain found in the survey was the same serotype (6B) as that seen in a recently reported multiply resistant strain in the Denver area (18), the isolate which prompted this study. We know of no other multiply resistant strains reported in the United States, but such strains have been found frequently in South Africa (12) and less frequently in other parts of Africa (5), Great Britain (17), and Australia (16).

Most clinical and bacteriological failures with penicillin treatment for pneumococcal infections have occurred in patients with meningitis (14), where CSF peak levels of penicillin may be less than 1 μg/ml (11). In our survey, the one patient with meningitis due to a relatively penicillin-resistant pneumococcus responded to intravenous ampicillin and chloramphenicol. Although achievable levels of penicillin in blood and sputum are higher than those in CSF (21), the use of low-dose penicillin therapy for pneumonia may also lead to more frequent treatment failure for pneumonia as well as meningitis (2). It seems prudent to never treat serious pneumococcal infections with small doses of penicillin (7).

Chloramphenicol is often used in penicillin-allergic patients with serious pneumococcal infections or in patients with penicillin-resistant strains not responding to penicillin therapy (3). Garau et al. described a patient with pneumococcal meningitis who did not respond to chloramphenicol therapy (8); this strain had a chloramphenicol MIC of 12.5 μg/ml. In our survey, one of seven relatively penicillin-resistant strains was also resistant to chloramphenicol (MIC, 16 μg/ml). It is likely that patients with infections due to penicillin-resistant strains will receive chloramphenicol as an alternative to penicillin, making it imperative to screen these isolates for chloramphenicol susceptibility.

Disk diffusion (Kirby-Bauer) testing is the most convenient method to screen pneumococcal isolates for antimicrobial susceptibility. Studies have shown that the use of a 1-μg oxacillin disk to discriminate between penicillin-susceptible and penicillin-resistant pneumococci is more reliable than the use of a penicillin disk (6, 13, 22). Laboratories in the Denver area have found the method to be efficient and practical. Standards for oxacillin disk diffusion testing are established (22). Jacobs et al. (13) have also established breakpoints for chloramphenicol and other antimicrobial agents. We suggest screening all invasive pneumococcal isolates for penicillin and chloramphenicol susceptibility.

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LITERATURE CITED


