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NOTES

Isolation of an Ampicillin-Resistant, Non-β-Lactamase-Producing Strain of *Haemophilus influenzae*

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A 79-year-old female developed endocarditis and meningitis due to an ampicillin-resistant, non-β-lactamase-producing strain of *Haemophilus influenzae*. Carbenicillin and gentamicin therapy resulted in bacteriological and clinical cure. The mechanism of resistance of ampicillin-resistant, non-β-lactamase-producing strains of *H. influenzae* is unknown.

Recent reviews have documented the increasing frequency of serious infections due to *Haemophilus influenzae* in adults (8, 14). Optimal treatment of such infections in adults is controversial because of the emergence of strains of *H. influenzae* resistant to ampicillin (13). Most of these strains elaborate a β-lactamase (10) whose production is mediated by genes located on a plasmid (6, 19). Occasionally, ampicillin-resistant strains of *H. influenzae* have been isolated and found not to produce β-lactamase (11, 20, 22). Described herein is an elderly female patient with aortic valve endocarditis and meningitis due to an ampicillin-resistant, non-β-lactamase-producing strain of *H. influenzae* type b. Studies were undertaken to determine the most efficacious alternative antimicrobial regimen and the mechanism of ampicillin resistance.

A 79-year-old female was admitted to the Medical College of Virginia Hospitals on 29 March 1977 with a 6-h history of headache, fever, progressive confusion, and lethargy. Physical examination on admission revealed a toxic-appearing, confused, black female with a temperature of 39.2°C. There was marked nuchal rigidity with positive Kernig and Brudzinski signs. A soft, apical systolic murmur was heard. Initial laboratory studies included a leukocyte count of 22,900/mm^3, with 84% polymorphonuclear leukocytes, 10% band forms, and 6% lymphocytes. X ray of the chest was normal. Cerebrospinal fluid obtained on admission contained 11,600 leukocytes/mm^3, with 99% polymorphonuclear leukocytes, 1% lymphocytes, a protein of 1,500 mg/dl, and a glucose of less than 5 mg/dl. Gram stain of a centrifuged specimen of cerebrospinal fluid contained numerous polymorphonuclear leukocytes and pleomorphic gram-negative bacilli. The patient's hospital course is outlined in Fig. 1. Initially she received 1 g of ampicillin intravenously every 2 h. *H. influenzae* type b was grown from subsequent blood and cerebrospinal fluid cultures. The ampicillin therapy was continued when it was determined that the organism did not produce β-lactamase; however, the patient showed no improvement and continued to be febrile. Subsequently, broth dilution susceptibility tests revealed the organism to be resistant to ampicillin (see Table 1), and the patient was given chloramphenicol, with subsidence of the signs and symptoms of meningitis. After 4 days of chloramphenicol therapy a grade III/VI aortic diastolic murmur was heard. Fever recurred and blood cultures (previously negative on chloramphenicol) again yielded *H. influenzae* type b. Carbenicillin therapy was begun at a dose of 30 g/day intravenously. Again, the fever abated and the patient exhibited moderate improvement. On the regimen of chloramphenicol and carbenicillin, the serum bactericidal titer was <1:2. Gentamicin therapy was begun at a dose of 1.5 mg/kg intravenously every 8 h. The serum inhibitory titer and serum bactericidal titer improved markedly. Because of dose-dependent bone marrow toxicity, chloramphenicol therapy was discontinued after 14 days. Subsequent serum inhibitory titers and serum bactericidal titers showed further increases (Fig. 1). The remainder of the patient's hopitalization was unremarkable. Follow-up blood cultures taken after the cessation of antimicrobial therapy were negative, and the patient was well 3 months after discharge.

The strains isolated from the patient's cerebrospinal fluid and blood were identified as *H. influenzae* type b by standard methods. Susceptibility testing was carried out in Mueller-Hinton broth (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 5% Fildes reagent

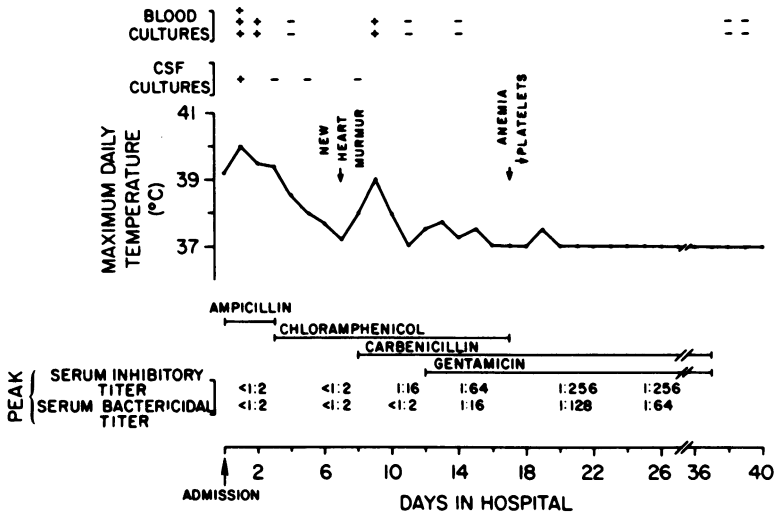

FIG. 1. *Clinical course of the present patient with regard to antimicrobial therapy and serum inhibitory and serum bactericidal titers. CSF, Cerebrospinal fluid.*

by the macrobroth dilution method described by the International Collaborative Study of the World Health Organization (7). The inoculum was prepared by suspension in supplemented Mueller-Hinton broth growth from an overnight chocolate agar plate, adjustment to a 0.5 McFarland standard, and dilution to contain 10^4 colony-forming units per ml. Known resistant (β-lactamase-positive) and susceptible (β-lactamase-negative) strains were processed in parallel. The minimal inhibitory concentration (MIC) was the lowest concentration of antimicrobial agent yielding no macroscopic growth. The minimal bactericidal concentration was the lowest concentration of antimicrobial agent yielding no macroscopic growth when 0.5 ml from each clear tube was subcultured onto antibiotic-free Mueller-Hinton agar supplemented with 1% IsoVitaleX (BBL) and 1% hemoglobin and incubated for 18 h at 35°C. Serum was obtained from the patient at the expected peak and nadir of serum antibiotic activity. Twofold dilutions of serum were made in 0.5-ml volumes of Mueller-Hinton broth supplemented with 5% Fildes reagent. An inoculum prepared as described above was added to each tube (except for one tube without antibiotic) to yield a final concentration of 5×10^3 colony-forming units per ml. The highest dilution yielding no visible growth after incubation for 18 h at 35°C was the serum inhibitory titer. Tubes showing no visible growth were subcultured in 0.1-ml volumes onto Mueller-Hinton agar supplemented with 5% Fildes reagent without antimicrobial agents and reincubated for 18 h at 35°C. The highest dilution yielding no growth was the serum bactericidal titer. β-Lactamase production was determined in duplicate by each of the following three methods with whole and sonicated cells: (i) a modification of the capillary test tube method of Thornsberry and Kirven (22); (ii) the chromogenic cephalosporin method of O'Callaghan et al. (18); and (iii) the rapid starch-iodine test as described by Catlin (3). Induction of β-lactamase was attempted by growing the *H. influenzae* on Mueller-Hinton agar containing 5% Fildes reagent and ampicillin or penicillin G in concentrations of 1 μg/ml.

Conjugal mating studies were performed in Mueller-Hinton broth supplemented with 5% Fildes reagent, with the test strain of *H. influenzae* as donor and *Escherichia coli* K-12 (*str rif*) as recipient. Donor and recipient were mixed in an Erlenmeyer flask at a ratio of 1:10, incubated for 2 h at 37°C, and plated at various dilutions on MHA containing ampicillin (5 μg/ml) and streptomycin (25 μg/ml). Appropriate control plates streaked with either donor or recipient were processed in parallel. In an attempt to isolate plasmid deoxyribonucleic acid, we prepared cleared lysates by a modification of the method of Clewell and Helinski (5) and analyzed them on agarose gels by the method of Meyers et al. (17).

Antimicrobial susceptibility of the test strain of *H. influenzae* is shown in Table 1. Whereas the MIC of ampicillin was 6.25 μg/ml, carbenicillin and gentamicin were the most active agents, with MICs of <0.049 μg/ml and 0.098 μg/ml, respectively. Among the three cephalosporin antimicrobial agents tested, cefamandole was the most active agent. Of note is the resistance of the organism to trimethoprim-sulfamethoxazole. Peak serum inhibitory titers and serum bactericidal titers are shown in Fig. 1. Serum inhibitory titers and serum bactericidal ti-

TABLE 1. *Antibiotic susceptibility pattern of the present strain of H. influenzae type b*

Antibiotic	MIC (μg/ml)	Minimal bactericidal concentration (μg/ml)
Ampicillin	6.25	12.5
Carbenicillin	<0.049	0.098
Cephalothin	0.78	3.125
Cefazolin	1.56	6.25
Cefamandole	0.196	0.196
Chloramphenicol	1.56	12.5
Trimethoprim-sulfamethoxazole	8/152	>8/152
Gentamicin	0.098	0.098
Streptomycin	3.12	6.25

ters were <1:2 while the patient was receiving chloramphenicol (6 g/day) intravenously. Serum obtained from the patient while she was receiving ampicillin showed a serum inhibitory titer similar to those obtained for chloramphenicol. With the addition of carbenicillin, the serum inhibitory titer and serum bactericidal titer became 1:16 and <1:2, respectively. A marked change in serum activity occurred with the addition of gentamicin, and the serum inhibitory titer and serum bactericidal titer rose to 1:64 and 1:16, respectively. Further increases were noted when chloramphenicol therapy was discontinued because of bone marrow suppression (Fig. 1).

β-Lactamase activity was not detected qualitatively by any of the methods employed, whether whole or disrupted cells were used under inducible or noninducible conditions. Conjugal transfer of ampicillin resistance was not observed. In addition, agarose gel electrophoresis of a cleared lysate of the test strain of *H. influenzae* failed to reveal the presence of extrachromosomal deoxyribonucleic acid, whereas plasmid deoxyribonucleic acid was apparent when known plasmid-carrying strains were analyzed.

In recent years, *H. influenzae* has emerged as a cause of serious infections in adults (8, 14). The strain of *H. influenzae* isolated from the blood and cerebrospinal fluid of the present patient was ampicillin resistant, but was a non-β-lactamase producer. Such isolates have been recognized infrequently. Thornsberry and Kirven reported that 2 of 20 strains of ampicillin-resistant *H. influenzae* gave negative results for β-lactamase by the rapid acidometric capillary tube method (22). Neither of these strains was a type b organism. Jorgensen and Alexander described a similar strain but did not report the serotype (11). Because of the paucity of strains isolated to date and the lack of description of the circumstances surrounding their isolation, the pathogenic potential of such strains has not been defined. However, the infection described herein suggests that such organisms do possess the ability to produce invasive disease. The mechanism of resistance in the present strain and those described above remain conjectural, though a cell envelope modification leading to decreased permeability and a target site alteration are attractive hypotheses. Walker and Smith (Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 18th, Atlanta, Ga., abstr. no. 290, 1978) have presented preliminary data on an ampicillin-resistant, β-lactamase-negative strain of *H. parainfluenzae* suggesting the latter mechanism. Of note is the fact that the non-β-lactamase-producing strains previously described have had MICs of 8 μg of ampicillin per ml (20, 22), similar to the MIC of the strain described herein. As a rule, β-lactamase-producing strains of *H. influenzae* have MICs of 16 μg or more of ampicillin per ml. The data regarding the activity of other antimicrobial agents against ampicillin-resistant, non-β-lactamase-producing strains of *H. influenzae* are meager. Jorgensen and Alexander evaluated the activity of various cephalosporins and found that, contrary to the data in Table 1, the MIC of cefamandole was 64 μg/ml (11). Carbenicillin and ticarcillin have been shown to be the most active penicillins against β-lactamase-producing strains of *H. influenzae* (9), in part because carbenicillin is a relatively poor substrate for the TEM type of β-lactamase (2) and in part because *H. influenzae* is more freely permeable to carbenicillin than to other β-lactam antibiotics (16). Few data are available on the activity of gentamicin against *H. influenzae*. In one study, gentamicin (2 μg or less per ml) inhibited all 25 strains of *H. influenzae* (21). None were as susceptible as the present strain (Table 1). The MIC (1.56 μg/ml) of chloramphenicol for the present strain is similar to levels reported previously (15). However, the minimal bactericidal concentration (12.5 μg/ml) would suggest a limited role for this drug in the treatment of infective endocarditis, a disease for which readily achievable bactericidal concentrations of antimicrobial agents are desirable (2). Unlike ampicillin-resistant, β-lactamase-positive strains of *H. influenzae*, the present strain was not susceptible to trimethoprim-sulfamethoxazole (15).

The patient described in this report improved on carbenicillin and gentamicin, although the efficacy of combination therapy was difficult to assess. The effect of chloramphenicol on the bactericidal capacity of the patient's serum (Fig. 1) suggests, but in no way proves, an antagonistic effect by chloramphenicol. Not only has such an

adverse effect with chloramphenicol been reported to alter unfavorably the course of *H. influenzae* meningitis (24), but it clearly was detrimental to the recovery of three patients with *H. parainfluenzae* endocarditis, as described by Chunn et al. (4). Experience with the patient described herein underscores the belief that so-called bacteriostatic agents such as chloramphenicol should be used with caution to treat patients with serious invasive infections such as endocarditis.

The emergence of ampicillin-resistant *H. influenzae* has prompted a change in the therapy of serious infections due to *H. influenzae* (1). Chloramphenicol remains the drug of choice in such instances; however, as with the patient described herein, drug toxicity and antimicrobial antagonism may cause problems. In addition, chloramphenicol-resistant strains of *H. influenzae* have been isolated (23). Carefully performed broth or agar dilution testing for antimicrobial susceptibility is mandatory when choosing other, less frequently used forms of therapy, whereas serum inhibitory titers and serum bactericidal titers are valuable in judging the efficacy of therapy. Susceptibility testing of additional strains of ampicillin-resistant, non-β-lactamase-producing strains of *H. influenzae* are needed to establish therapeutic guidelines for infections caused by such organisms.

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