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Susceptibility of *Haemophilus influenzae* Isolates from Blood and Cerebrospinal Fluid to Ampicillin, Chloramphenicol, and Trimethoprim-Sulfamethoxazole

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Susceptibility to ampicillin and chloramphenicol in vitro has been determined for *Haemophilus influenzae* strains isolated from blood and/or cerebrospinal fluid cultures of patients admitted to two Atlanta hospitals from 1 January 1974 to 31 March 1975. Since the appearance of ampicillin-resistant strains of this organism in early 1974, chloramphenicol has been used in these hospitals as initial therapy for severe infection due to *H. influenzae*. Strains from five of 94 patients were resistant to ampicillin (minimum inhibitory concentration [MIC] $\geq$ 12.5 μg/ml), but all strains were susceptible to chloramphenicol (MIC $<$ 2 μg/ml). The first 35 strains studied, including three resistant to ampicillin, were also tested for in vitro susceptibility to trimethoprim-sulfamethoxazole; all were highly susceptible (MIC $\leq$ 0.0312 μg of trimethoprim and 0.625 μg of sulfamethoxazole per ml).

In January and February, 1974, ampicillin-resistant *Haemophilus influenzae* type b organisms were recovered from blood and/or cerebrospinal fluid of three patients at this hospital; epidemiological studies at that time suggested that these patients had a common contact or association (17). Since that time, we have tested the in vitro susceptibility to ampicillin and chloramphenicol of all *H. influenzae* strains isolated from cultures of blood, spinal fluid, or joint fluid obtained from patients at Grady Memorial Hospital and the Henrietta Egleston Hospital for Children in Atlanta. In addition, epidemiological and clinical features were reviewed for cases in which ampicillin-resistant strains were isolated in an attempt to find some association among patients. Since it has been found that strains resistant to ampicillin may be susceptible to trimethoprim-sulfamethoxazole (11), some of the strains also were tested in vitro for susceptibility to this combination.

MATERIALS AND METHODS

From 1 January 1974 to 31 March 1975, a total of 139 cultures of blood and cerebrospinal fluid at the two hospitals yielded *H. influenzae* organisms. Of these, we obtained isolates for antimicrobial susceptibility testing from 61 patients at Grady Memorial Hospital and from 33 patients at the Henrietta Egleston Hospital for Children. Organisms were isolated from blood cultures alone in 35 cases, from cerebrospinal fluid alone in 29 patients, and from both blood and spinal fluid in 30 additional patients. Thus, there were 124 specimens from 94 patients available for testing. In 92 of the 94 cases, the organism isolated was *H. influenzae* type b. *H. influenzae* type d was isolated from the cerebrospinal fluid of one child with meningitis, and a nontypable strain was isolated from the blood of one child with cellulitis.

Each strain was identified as *H. influenzae* on the basis of requirements for both hemin (X-factor) and nicotinamide adenine dinucleotide (V-factor) and by microscopic and colonial morphology on chocolate agar and blood agar. For all except the one nontypable strain, the identity was confirmed on the basis of agglutination with commercial type-specific antisera. Susceptibility to ampicillin and chloramphenicol was determined by tube dilution assay using Trypticase soy broth to which 10% Fildes reagent was added and an inoculum of 10^5 organisms/ml (17). Results were determined after 18 h of incubation. Ampicillin-resistant organisms were defined as those for which the minimum inhibitory concentration (MIC) of ampicillin was greater than or equal to 3.12 μg/ml, and chloramphenicol-resistant strains were defined as those for which the MIC was greater than or equal to 1.56 μg/ml.

The first 35 strains studied (including three resistant to ampicillin) were also tested for susceptibility to trimethoprim and sulfamethoxazole in a 1:20 ratio. This testing was done by an agar-dilution method using the Steers replicator (15) and thymidine-deficient Mueller-Hinton agar plus 5% lysed horse blood plus 2.5 μg of reduced nicotinamide adenine dinucleotide per ml (11). The inoculum of 10^5 organisms/ml was monitored by plate count, and results were determined after 18 h of incubation.

RESULTS

The isolation of five ampicillin-resistant strains from three patients (one with bacteremia, two with meningitis) in January and February 1974 has previously been described (17). From March 1974 through January 1975, we tested 87 strains from 62 patients and found them susceptible to ampicillin (Table 1). During February and March 1975, 17 strains from 13 patients were also susceptible to ampicillin. However, during this period an additional two patients with ampicillin-resistant organisms were identified. Strains of *H. influenzae* type b isolated from cultures of blood and spinal fluid of a 2.5-year-old girl with meningitis were found to have ampicillin MICs of 12.5 and 6.25 μg/ml, respectively. This child had been treated with chloramphenicol and recovered uneventfully. A resistant strain (ampicillin MIC, 12.5 μg/ml) was also recovered from the blood of a 3-month-old child with cellulitis and septic arthritis of the left elbow. The child recovered uneventfully after incision and drainage of the septic joint and treatment with parenteral ampicillin. The cases were not related epidemiologically.

All tested isolates were susceptible to chloramphenicol (Table 1). In addition, all of the 35 strains tested against trimethoprim-sulfamethoxazole, including three resistant to ampicillin, were found to be susceptible to less than 0.0312 μg of trimethoprim per ml combined with 0.625 μg of sulfamethoxazole per ml.

DISCUSSION

Since the initial reports of serious clinical illness due to ampicillin-resistant strains of *H. influenzae* (10, 14, 17), organisms resistant to this drug have been encountered in many areas of the world and in association with a number of different clinical presentations (4, 9). In testing of the strains isolated from one of our patients (17), the mechanism of resistance appeared to be a beta-lactamase similar to that found in *Klebsiella pneumoniae* (8), and not a change in the permeability of the cell (12). The structural gene for this beta-lactamase has been shown to reside in a plasmid (7).

The appearance of resistant organisms has occasioned much discussion about appropriate treatment of severe disease caused by *H. influenzae* (13). Since a number of rapid presumptive tests for ampicillin resistance in *H. influenzae* have recently been described (2, 16), many current recommendations suggest that choice of therapy be predicated on local experience with ampicillin-resistant strains and also upon whether facilities are available to detect these strains (6). With the appearance of ampicillin-resistant strains in our community in early 1974 (17), chloramphenicol became the drug of choice for initial treatment of severe infections caused by *H. influenzae*. This policy was continued for the 11-month period in which no strains of this organism were found to be resistant to ampicillin (March 1974–January 1975). Although this finding provided some support for returning to the use of ampicillin for initial therapy in serious *Haemophilus* infections, the subsequent finding of two patients with ampicillin-resistant strains in February–March 1975 has led us to continue to use chloramphenicol as initial therapy in our area. Indeed, it has even been suggested that in situations in which the causative organism is *H. influenzae* or unknown, it would be reasonable "anywhere in North America to include intravenous chloramphenicol 100 mg/kg/day for initial treatment of bacterial meningitis in children more than 2 months old" (1).

TABLE 1. *Distribution of MICs of ampicillin (AMP) and chloramphenicol (CHL) for H. influenzae strains from blood and spinal fluid, 1 January 1974 through 31 March 1975*

Period	Agent	No. of strains for which MIC (μg/ml) was:									
		≤0.09	0.19	0.39	0.78	1.56	3.12	6.25	12.5	25	50
1/74–2/74	AMP	3	9	5						1[a]	4[a]
(17 patients)	CHL[b]			1	13	7					
3/74–1/75	AMP	30	49	7	1						
(62 patients)	CHL[c]		3	5	35	27					
2/75–3/75	AMP	5	9	3				1[d]	2[d]		
(15 patients)	CHL			1	12	7					

[a] Five strains from three patients described in reference 17.
[b] One strain not tested.
[c] MICs were not determined for 17 strains from 14 patients, but all were inhibited by 2 μg of CHL per ml.
[d] Three strains from two patients described in the text.

One of the ampicillin-resistant strains was obtained from a child with septic arthritis who was cured of her infection after surgical drainage and therapy with ampicillin. This strain was found positive for beta-lactamase production by the test of Thornsberry and Kirven (16). The relative contributions of surgical drainage and antibiotic therapy to the patient's recovery cannot be assessed. However, recovery after treatment with ampicillin alone has been reported for a patient with meningitis whose cerebrospinal fluid yielded a strain of *H. influenzae* with an MIC of 16 μg/ml (5). These two cases suggest that the correlation between the in vitro susceptibility test and in vivo response is not necessarily complete.

During the period of surveillance, all of the strains for which the MIC to chloramphenicol was determined were susceptible to 1.56 μg/ml or less. The possible occurrence of strains resistant to this drug has been suggested (3), but no resistant strains have yet been reported from centers that use chloramphenicol instead of ampicillin for treatment of severe infections due to *H. influenzae*.

All of the 35 strains tested against trimethoprim-sulfamethoxazole were very susceptible to this combination, and this was true both for ampicillin-susceptible and for resistant strains. These in vitro data suggest further study of this antimicrobial combination for treatment of serious illness due to *H. influenzae* type b, since the potential for serious toxicity appears to be less than that of chloramphenicol.

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