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Comparative Susceptibility of *Haemophilus* Species to Cefaclor, Cefamandole, and Five Other Cephalosporins and Ampicillin, Chloramphenicol, and Tetracycline

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The minimal inhibitory concentration of cefaclor, cephalexin, cephradine, cefamandole, cephalothin, cephapirin, cefazolin, ampicillin, chloramphenicol, and tetracycline for inhibition of 198 freshly isolated clinical strains of *Haemophilus* species (23 *H. influenzae* type b, 157 *H. influenzae* non-type b, 14 *H. parainfluenzae*, and 4 *H. aphrophilus*) was determined simultaneously by a slightly modified WHO-ICS agar dilution method. Nine strains were resistant to ampicillin. There was no correlation between ampicillin resistance and minimal inhibitory concentration of other antibiotics. All strains were susceptible to chloramphenicol, and all except five were susceptible to tetracycline. Cefaclor was the most active oral cephalosporin, and cefamandole was the most active parenteral cephalosporin. Among the seven cephalosporins tested, cefamandole was the most effective compound. All but two strains were inhibited by cefamandole at 2 μg or less per ml.

Ampicillin-resistant strains of *Haemophilus influenzae* are now widespread. Chloramphenicol is currently the recommended drug of choice in the empirical therapy of serious *Haemophilus* infections before the in vitro susceptibility of the infecting organism to ampicillin is known (4). *H. influenzae* resistant to chloramphenicol have also been reported (6). Alternative antibiotics with less potential toxicity than that of chloramphenicol are needed. Preliminary studies of two new cephalosporins, i.e., cefamandole for parenteral administration and cefaclor for oral administration, revealed that these two compounds had good in vitro activity against *H. influenzae* (1-3, 7, 8, 12). To put the in vitro activity of cefamandole and cefaclor against *Haemophilus* in perspective, we compared the in vitro activity of these two new cephalosporins with five other cephalosporins and with ampicillin, chloramphenicol, and tetracycline.

Strains of *Haemophilus* isolated from clinical materials during 1978 were obtained from clinical microbiology laboratories of hospitals in the Cincinnati area (the majority of isolates were obtained from Children's Hospital). The isolation and identification of *Haemophilus* were done according to standard bacteriological techniques (13). The identity of the clinical isolates was reconfirmed before antimicrobial susceptibility testing. Only one isolate per patient was studied. The following were tested: 23 strains of *H. influenzae* type b, 157 strains of *H. influenzae* non-type b, 14 strains of *H. parainfluenzae*, and 4 strains of *H. aphrophilus*.

A slightly modified WHO-ICS agar dilution method of antimicrobial susceptibility testing (12) was used to determine the minimal inhibitory concentration (MIC). For a given strain, the MIC of cefaclor, cephalexin, cephradine, cefamandole, cephalothin, cephapirin, cefazolin, ampicillin, chloramphenicol, and tetracycline was determined concurrently, using the same inoculum.

Haemophilus was grown on chocolate agar supplemented with 1% IsoVitalex (Baltimore Biological Laboratories) for 24 h at 37°C under 5% CO_2. Colonies were removed and suspended in 0.9% NaCl solution, and the turbidity was adjusted to a 0.5 McFarland barium sulfate standard. Colony counts performed on several strains showed that this suspension contained 10^7 to 10^8 colony-forming units per ml. A 10^{-2} dilution of this suspension was used as the inoculum, 0.002 ml delivered by a Steers' replicator (9). Mueller Hinton agar supplemented with 5% Fildes reagent (Difco Laboratories) was incorporated with the antibiotic to be tested in serial twofold dilutions ranging from 32 to 0.06 μg/ml. An agar plate with no antibiotic served as a control. The MIC was determined after 24 h of incubation at 37°C under 5% CO_2, as the lowest concentration of antibiotic that completely inhibited growth.

Nine strains were ampicillin resistant (MIC

≥ 4 μg/ml): 2 of 23 *H. influenzae* type b; 6 of 157 *H. influenzae* non-type b; 1 of 14 *H. parainfluenzae;* and none of 4 *H. aphrophilus.* Because of the lack of correlation between the in vitro susceptibility of other antibiotics and ampicillin resistance, the results of ampicillin-susceptible and ampicillin-resistant strains were not separated.

Tables 1 to 3 list the cumulative percent susceptibility of *H. influenzae* type b, *H. influenzae* non-type b, and *H. parainfluenzae* to the 10 antibiotics tested, respectively. Due to space limitation, the results of *H. aphrophilus* are not presented in the tabular form. Among the three oral cephalosporins, cefaclor was the most active compound. At 4 μg/ml, cefaclor inhibited 98.3, 88.5, 100, and 100% of *H. influenzae* type b, *H. influenzae* non-type b, *H. parainfluenzae,* and *H. aphrophilus,* respectively, whereas cephalexin at the same concentration inhibited only 0, 4.5, 0, and 25%, respectively. None of the 198 strains of *Haemophilus* were inhibited by cephradine at 4 μg/ml. At 8 μg/ml, cefaclor inhibited 95.7 and 96.8% of *H. influenzae* type b and *H. influenzae* non-type b, respectively.

Cefamandole was the most active parenteral cephalosporin. At 2 μg/ml, cefamandole inhibited 100 and 98.7% of *H. influenzae* type b and *H. influenzae* non-type b, respectively. All *H. parainfluenzae* strains were inhibited by cef-

TABLE 1. *Cumulative percent of H. influenzae type b (23 strains) susceptible to 10 antibiotics*

Antibiotic	% of strains susceptible to drugs at the following concn (μg/ml):									
	≤0.06	0.12	0.25	0.5	1	2	4	8	16	32
Cefaclor		4.3	4.3	21.7	26.1	65.2	78.3	95.7	100	
Cephalexin								8.7	21.7	60.9
Cephradine									17.4	47.8
Cefamandole	4.3	13.0	65.2	91.3	96.7	100				
Cephalothin			4.3	8.7	30.4	56.5	69.6	95.7	100	
Cephapirin			8.7	17.4	34.8	47.8	69.6	100		
Cefazolin	4.3	4.3	4.3	4.3	8.7	13.0	13.0	30.4	69.6	100
Ampicillin	8.7	26.1	87.0	87.0	87.0	91.3	100			
Chloramphenicol			21.7	87.0	100					
Tetracycline		4.3	8.7	65.2	91.3	95.7	95.7	100		

TABLE 2. *Cumulative percent of H. influenzae non-type b (157 strains) susceptible to 10 antibiotics*

Antibiotic	% of strains susceptible to drugs at the following concn (μg/ml):									
	≤0.06	0.12	0.25	0.5	1	2	4	8	16	32
Cefaclor		1.9	5.7	17.2	36.3	63.1	88.5	96.8	99.4	99.4
Cephalexin						0.6	4.5	21.0	40.8	60.5
Cephradine								8.9	35.7	56.1
Cefamandole	3.2	18.5	61.1	84.1	94.3	98.7	99.4	99.4	100	
Cephalothin		1.9	12.1	20.4	38.2	49.0	74.5	94.3	99.4	99.4
Cephapirin	1.3	2.5	8.3	23.6	38.2	52.9	78.3	98.1	99.4	99.4
Cefazolin	0.6	1.3	2.5	7.0	17.2	21.0	29.3	47.8	72.0	98.7
Ampicillin	5.1	29.3	87.3	92.4	93.6	96.2	98.1	99.4	100	
Chloramphenicol		5.1	35.0	91.1	98.1	99.4	100			
Tetracycline		1.3	19.1	63.7	96.8	96.8	97.5	99.4	100	

TABLE 3. *Cumulative percent of H. parainfluenzae (14 strains) susceptible to 10 antibiotics*

Antibiotic	% of strains susceptible to drugs at the following concn (μg/ml):									
	≤0.06	0.12	0.25	0.5	1	2	4	8	16	32
Cefaclor				14.3	50.0	78.6	100			
Cephalexin								7.1	21.4	35.7
Cephradine									21.4	50.0
Cefamandole			42.8	64.3	100					
Cephalothin				14.3	28.6	57.1	71.4	92.9	100	
Cephapirin				14.3	35.7	57.1	75.6	100		
Cefazolin					14.3	21.4	28.6	64.3	85.7	92.9
Ampicillin		21.4	85.7	92.9	92.9	92.9	100			
Chloramphenicol		7.1	35.7	100						
Tetracycline			7.1	85.7	100					

amandole at 1 μg/ml, and all *H. aphrophilus* strains were inhibited by 0.5 μg/ml. The activity of cephalothin and cephapirin was comparable; at 2 μg/ml only about half of the strains were inhibited. Cefazolin was the least active parenteral cephlosporin. At 2 μg/ml, cefazolin inhibited 13, 21, 21.4, and 25% of *H. influenzae* type b, *H. influenzae* non-type b, *H. parainfluenzae,* and *H. aphrophilus,* respectively.

All strains tested were susceptible to chloramphenicol. All *H. influenzae* type b strains were inhibited by chloramphenicol at 1 μg/ml; *H. influenzae* non-type b strains were inhibited by 4 μg/ml; and *H. parainfluenzae* and *H. aphrophilus* strains were inhibited by 0.5 μg/ml. Tetracycline was also an effective antibiotic; 95.7% of *H. influenzae* type b and 96.8% of *H. influenzae* non-type b were inhibited by tetracycline at 2 μg/ml. All *H. parainfluenzae* and *H. aphrophilus* were inhibited by tetracycline at 1 μg/ml.

In comparison with other cephalosporins, cefaclor was the most active oral cephalosporin, and cefamandole was the most active parenteral cephalosporin against *H. influenzae* type b, *H. influenzae* non-type b, *H. parainfluenzae,* and *H. aphrophilus.* If clinical trials substantiate the in vitro efficacy of these in vitro results, cefaclor and cefamandole may be the cephalosporins of choice for *Haemophilus* infections such as otitis media and chronic bronchitis in patients who are allergic to ampicillin, or where the infecting organism is resistant to ampicillin. However, cephalosporins are generally not recommended in the treatment of meningitis and other central nervous system infections, due to unpredictable penetration of cephalosporins into the cerebrospinal fluid compartment. Preliminary clinical trials of cefamandole in the therapy of meningitis revealed mixed results (5, 10), and further clinical trials are needed before cefamandole can be recommended for the therapy of central nervous system infections.

In our geographic area, there appears to be a decrease in the prevalence of ampicillin-resistant *H. influenzae* from 10% of the strains studied in 1976 (12) to 4.4% in the current study. In a recent U.S. national survey, the prevalence of ampicillin-resistant *H. influenzae* was 4.5% (11). We have not found any chloramphenicol-resistant *Haemophilus* in our area, and none were found in the recent U.S. national survey (11). Tetracycline remains a fairly effective compound against *Haemophilus.*

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