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Limited In Vitro Activity of Cefamandole Against 100 Beta-Lactamase- and Non-Beta-Lactamase-Producing *Haemophilus influenzae* Strains: Comparison of Moxalactam, Chloramphenicol, and Ampicillin

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In the present study, the minimal inhibitory concentrations and minimal bactericidal concentrations of moxalactam, cefamandole lithium, ampicillin, and chloramphenicol were determined, both in broth and on solid medium, against 75 non-beta-lactamase-producing and 25 beta-lactamase-producing strains of *Haemophilus influenzae*. Most of the 75 strains were inhibited or killed by 2 μg or less of ampicillin, chloramphenicol, or moxalactam per ml, but cefamandole exhibited poor bactericidal activity against 11 non-beta-lactamase-producing strains, of which 9 were non-type B *H. influenzae*. Most of the 25 beta-lactamase-producing *H. influenzae* were resistant to 128 μg of ampicillin per ml. Both moxalactam and chloramphenicol, which had minimal inhibitory concentrations of less than 0.25 and 2 μg/ml, respectively, were more active than cefamandole, which had a minimal inhibitory concentration ranging from 2 to ≥128 μg/ml.

The widening spectrum of *Haemophilus influenzae* infections affecting both young children (8) and healthy adults (23) and the emergence of strains resistant to ampicillin (14, 21, 27) have stimulated investigators to study the in vitro efficacy of other antimicrobial agents against this microorganism (4, 11, 12, 20, 30).

On the basis of these observations, cefamandole has been suggested as a useful cephalosporin for *H. influenzae* infections other than those affecting the central nervous system (3, 6, 9, 25, 26), but its clinical efficacy in infections due to ampicillin- or chloramphenicol-resistant strains has yet to be confirmed on a large scale.

In the present study, we report the comparative in vitro activity of moxalactam, a new semisynthetic 1-oxa-beta-lactam (28, 31), cefamandole lithium, ampicillin, and chloramphenicol.

MATERIALS AND METHODS

Organisms. Clinical isolates (75 ampicillin susceptible, 25 ampicillin resistant) were collected at the Centre Hospitalier de l'Université Laval between 1975 and 1979. Of 54 strains, 17 were beta-lactamase producers of type B, and 46 were non-type B *H. influenzae*. The *H. influenzae* strains included 26 from ocular specimens, 19 from throat and expectoration cultures, 16 from ears, 16 from cerebrospinal fluid (3 from patients with concomitant septicemia), 16 from blood, 3 from nasal or sinus discharge, 3 from synovial fluid, and 1 from pleural fluid. They were characterized as *H. influenzae* by demonstrating requirements for both X and V factors. Xylose fermentation (1, 16, 17, 22), indole production (1, 16, 17, 22), 5% human erythrocyte hemagglutination (16), gas production (16), horse blood hemolysis (16, 22), H_2S production (16), and the porphyrin (15, 18) test allowed us to differentiate *H. influenzae* from *Haemophilus hemolyticus*, *H. aegypticus*, and *H. parainfluenzae*. All of the strains of *H. influenzae* were biotyped by the method of Kilian (15, 16). The presence of beta-lactamase was assessed by the phenol red test (5). All strains were stored and frozen at −70°C in glycerol broth before testing.

Antibiotics. The antibiotic powders were kindly provided by the following pharmaceutical companies: moxalactam (LY127935) and cefamandole by Eli Lilly & Co., Indianapolis, Ind., and ampicillin and chloramphenicol by Ayerst Laboratories and Parke-Davis, Montreal, Canada, respectively.

In vitro testing. Susceptibility testing was determined both in brain heart infusion (BHI) broth (BBL Microbiology Systems, Cockeysville, Md.) and in BHI agar (Difco Laboratories, Detroit, Mich.). Both media were supplemented with 1% IsoVitaleX (BBL) and 1% hemin extract (Eastman Kodak Co., Rochester, N.Y.). Chocolate agar was provided by the Institut Armand Frappier, Montreal.

Broth studies. Minimal inhibitory concentrations (MICs) were determined by using a microdilution technique in which the appropriately diluted antibiotics were distributed in U-shaped microliter plates (Cooke Engineering Co.) with 100-μl calibrated pipettes. The final antibiotic concentrations ranged from 0.06 to 128 μg/ml. The bacteria were regenerated on chocolate agar medium until growth was uniform. The organisms were suspended in BHI-supplemented broth and incubated for 18 h at 37°C. The overnight cultures were diluted 1:100 in the same medium, and 100 μl of this dilution was added to each well to obtain a final concentration of 10^6 colony-forming units per

ml. The plates were then incubated overnight, and the MICs were determined the next day. For the minimal bactericidal concentrations (MBCs), broth from the holes where no visible growth was observed was sampled with a 4-mm loop and plated on chocolate agar dishes which were incubated at 37°C for 18 h in a candle jar. The lowest concentration of antibiotic yielding less than five colonies was defined as the MBC.

Agar studies. The diluted antibiotics were added to the liquid agar at 50°C in petri dishes, allowed to solidify, and stored at 4°C for no more than 3 days. The final concentrations of antibiotics in the plates ranged from 0.06 to 128 μg/ml. On the day of the experiments, a loopful of organisms taken from an 18-h culture of *H. influenzae* grown on chocolate agar was suspended in 0.5 ml of BHI-supplemented broth and incubated at 37°C for 3 h. These solutions, containing 10^6 colony-forming units per ml as evaluated by colony counts, were inoculated on BHI-supplemented agar with the use of a Steers replicator (12, 24). The plates were then incubated for 18 h at 37°C in a candle jar. The MIC was defined as the lowest concentration of antibiotic at which there was no growth detectable with the naked eye.

RESULTS

The results of in vitro susceptibility tests comparing moxalactam, cefamandole lithium, ampicillin, and chloramphenicol against 75 non-beta-lactamase-producing strains of *H. influenzae* are shown in Table 1. Most strains were inhibited by concentrations equal to or less than 2 μg of either antibiotic per ml. The MBCs of cefamandole for 11 strains of *H. influenzae* were much higher than the MICs, whereas there was no such difference (no more than one or two tube dilutions) between the MIC and MBC for the other 64 strains. As shown in Table 2, no correlation could be found between the MIC or MBC, the biotype, and the sources of these 11 strains, but 9 of the 11 strains were non-type B *H. influenzae*. No such difference between the MIC and MBC of the other drugs was noted. The MICs of ampicillin, chloramphenicol, and cefamandole observed on solid media were slightly higher (one tube dilution) than those observed in liquid media.

Table 3 shows the results obtained when the four drugs were tested against 25 ampicillin-resistant strains of *H. influenzae*. Most microorganisms were resistant to 128 μg of ampicillin per ml, whereas both chloramphenicol and moxalactam exhibited good activity. The last compound appeared to be the most active antimicrobial agent. In contrast, the MIC and MBC of cefamandole were much higher. In fact, for 20 out of 25 strains, the MBC was equal to or above 32 μg/ml. There was no difference between the MIC determined in liquid or solid medium. The 25 ampicillin-resistant and the 20 relatively resistant strains of *H. influenzae*, as estimated by the MBC of cefamandole, were distributed into the five biotypes and came from different clinical specimens (Table 4).

DISCUSSION

In the present investigation, the use of either solid or liquid medium did not modify significantly the in vitro activity of either moxalactam, cefamandole, ampicillin, or chloramphenicol against *H. influenzae*.

The MICs and MBCs of both ampicillin and chloramphenicol against the 100 strains of *H. influenzae* were comparable to those obtained by other investigators (11, 12, 20, 25, 26). There was no more than a one- (rarely two-) tube dilution difference between the MIC and the MBC.

TABLE 1. *MICs in liquid and solid media and MBCs of four antibiotics against 75 non-beta-lactamase-producing strains of H. influenzae*

Antibiotic	Determination (medium)	Absolute no. of strains inhibited or killed at antibiotic concn (μg/ml):											
		≤0.06	0.12	0.25	0.5	1.0	2	4	8	16	32	64	128	>128
Moxalactam	MIC (liquid)	49	25		1									
	MBC	35	27	12			1							
	MIC (solid)	50	24	1										
Cefamandole lithium	MIC (liquid)			29	32	9	4	1						
	MBC			25	8	16	9	4	9	2	1		1	
	MIC (solid)				59	9	7							
Ampicillin	MIC (liquid)		4	40	26	5								
	MBC		2	23	32	13	2	3						
	MIC (solid)			7	50	16	2							
Chloramphenicol	MIC (liquid)		3	11	36	20	5							
	MBC		2	5	18	38	8	3	1					
	MIC (solid)		1	3	27	42	2							

With respect to cefamandole, we noted that this antibiotic had limited bactericidal activity against 11 of our 75 ampicillin-susceptible strains. In fact, with these strains we have observed more than a twofold dilution between the MIC and MBC. Most investigators have observed the MIC of cefamandole by using the agar dilution method (2, 3, 6, 9, 10, 14). Few of them have reported the MBCs of cefamandole against ampicillin-susceptible strains of *H. influenzae*: Meyers et al. reported on 20 isolates (19), Yourassowski et al. reported on 6 strains (32), and Flemming and Fierer reported on 10 strains (7). Although we have no explanation for this observation, which was reproduced in three successive experiments, it may represent an anomaly inherent to serotype differences, 9 of the 11 non-beta-lactamase-producing strains being non-type B *H. influenzae*. There does not seem to be a good correlation between the biotype and the susceptibility pattern of the *H. influenzae*.

TABLE 2. *Sources and biotypes of 11 ampicillin-susceptible strains of H. influenzae relatively resistant to the bactericidal activity of cefamandole.*

Strain	Source	Serotype	Biotype	MIC (μg/ml)	MBC (μg/ml)
60	Eye	Non B	I	0.5	16
88	Sputum	Non B	I	1.0	16
166	Eye	Non B	I	0.5	8.0
169	Eye	B	III	1.0	128
171	Eye	B	V	0.5	8
173	Eye	Non B	V	0.5	8
224	Blood	Non B	I	0.5	32
235	Ear	Non B	I	1.0	8
239	Pleural fluid	Non B	IV	0.25	4
247	Ear	Non B	I	0.5	8
303	Eye	Non B	III	0.5	8

We do not believe that the inoculum (7, 9, 25), the medium, a misreading of the plates (7), or a combination of these was responsible for such a disparity between the MICs and MBCs of cefamandole. The composition of our medium (pH 7.24) respected the norms depicted by Turner et al. (29) and did not accelerate the hydrolysis of the antibiotic. Furthermore, an unfavorable environment or an inoculum effect should have modified the activity of the tested drug against all of the strains studied.

The MICs and MBCs of cefamandole against the 25 beta-lactamase-positive strains was in accordance with those of other investigators (7, 9, 13, 25), who have already observed its poor inhibiting and killing power. This event is even more striking with a large inoculum (7), as used in the present report.

Moxalactam exhibited an excellent inhibiting and killing power against all 100 strains of *H. influenzae*. Other investigators have made similar observations, but with a very limited number of strains of *H. influenzae*. In view of our present in vitro observation, moxalactam appears to be an acceptable antibiotic against both beta-lactamase- and non-beta-lactamase-producing strains of *H. influenzae*, whereas cefamandole appears to have an unpredictable bactericidal activity against both type B and non-type B *H. influenzae*. Of the 31 strains of *H. influenzae* which exhibited resistance to the bactericidal activity of cefamandole (MBC ≥ 32 μg/ml), 8 were associated with severe infections (four cases of septicemia, one case of meningitis, one case of pneumonia, one case of septic arthritis, and one case of epiglottitis).

TABLE 3. *MICs in liquid and solid media and MBCs of four antibiotics against 25 beta-lactamase-producing strains of H. influenzae*

Antibiotic	Determination (medium)	Absolute no. of strains inhibited or killed at antibiotic concn (μg/ml):												
		≥0.06	0.12	0.25	0.5	1.0	2	4	8	16	32	64	128	>128
Moxalactam	MIC (liquid)	20	3	2										
	MBC	17	5	3										
	MIC (solid)	16	5	4										
Cefamandole lithium	MIC (liquid)						2	4	3	2	7	2		5
	MBC							2	3		1	8	2	9
	MIC (solid)							1	9	5	5	4	1	
Ampicillin	MIC (liquid)										1	1	2	21
	MBC												2	23
	MIC (solid)												1	24
Chloramphenicol	MIC (liquid)				11	11	3							
	MBC				1	7	7	9	1					
	MIC (solid)				9	16								

TABLE 4. *Sources and biotypes of 25 ampicillin-resistant strains of H. influenzae of which 20 were resistant to the bactericidal activity of cefamandole*

Strain	Source	Serotype	Biotype	MIC (μg/ml)	MBC (μg/ml)
43	Pharynx	B	V	>128	>128
49	Pharynx	B	V	32	>128
53	Blood	B	IV	>128	>128
87	Sputum	B	I	64	128
105	Blood	B	III	16	>128
153	Eye	B	V	32	>128
158	Cerebrospinal fluid	B	V	32	>128
164	Ear	B	IV	4	8
165	Ear	Non B	IV	8	32
172	Eye	B	III	8	64
177	Eye	B	III	8	64
178	Pharynx	Non B	III	2	4
179	Eye	Non B	I	32	64
180	Eye	Non B	I	64	128
182	Eye	B	I	32	64
188	Ear	B	III	32	64
191	Ear	B	III	4	8
193	Synovial fluid	B	II	>128	>128
195	Ear	Non B	III	2	4
203	Eye	Non B	I	32	64
206	Ear	B	III	4	8
214	Blood	B	I	>128	>128
215	Epiglottis	B	V	>128	>128
222	Eye	B	I	4	64
251	Ear	Non B	III	16	64

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