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In Vitro Activities and Targets of Three Cephem Antibiotics against *Haemophilus influenzae*

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The antimicrobial activities of cefixime, cefpodoxime, and ceftibuten were determined with 18 ampicillin-susceptible (Amp^s), 13 ampicillin-resistant β-lactamase-producing (Amp^{r}BLP), and 7 ampicillin-resistant non-β-lactamase-producing (Amp^{r}NBLP) strains of *Haemophilus influenzae*. An effect of inoculum density on apparent MIC, the bactericidal activity of these agents, and the targets of the three cephems were determined. The MICs of cefixime, cefpodoxime, and ceftibuten for 90% of the Amp^s and Amp^{r}BLP isolates were 0.04, 0.08, and 0.08 μg/ml, respectively. In contrast, the MICs for 90% of the Amp^{r}NBLP strains were 0.96, 1.92, and 7.68 μg/ml. No significant inoculum effect was observed for any group of strains comparing inocula of 10^3 and 10^5 CFU, whereas only the Amp^{r}NBLP isolates showed a marked effect at an inoculum of 10^6 CFU. Although bactericidal levels were achieved for the Amp^s and Amp^{r}BLP strains, tolerance to cefixime and ceftibuten was observed. The bactericidal activity for the Amp^{r}NBLP strains was limited, with cefixime showing the highest activity of the three cephems. Penicillin-binding proteins 2, 4, and 5 revealed high affinity, with 50% inhibitory concentration levels below the MIC for all three cephems, suggesting that these are important targets of these agents in *H. influenzae*. We conclude that the cephems are highly active in vitro against Amp^s and Amp^{r}BLP strains of *H. influenzae*, but less so against Amp^{r}NBLP isolates.

The parenterally administered extended-spectrum cephalosporins have had a major impact on treatment of certain infections since their availability in the early 1980s. More recently, three orally administered cephalosporins, the cephems, have been developed, but await licensure. Although all three cephems, ceftibuten, cefixime, and cefpodoxime, have been shown to have good in vitro activity against the majority of *Haemophilus influenzae* strains (1, 4–7, 9, 11, 12, 18), a comparative study with all three compounds has not been performed, and the in vitro data with ampicillin-resistant non-β-lactamase-producing (Amp^{r}NBLP) isolates have been limited. In addition, the major targets of these β-lactams in *H. influenzae* remain unknown.

The objective of this study was to compare the antimicrobial activities of the three cephems against 38 isolates of *H. influenzae* representing three different phenotypes: ampicillin-susceptible (Amp^s), ampicillin-resistant β-lactamase-producing (Amp^{r}BLP), and Amp^{r}NBLP isolates. We also sought to determine the bactericidal activity of these and comparative agents and questioned whether there was an effect of inoculum density on apparent MIC. To define the targets of these agents, we determined the penicillin-binding protein (PBP) profile of the three cephems in comparison to cefotaxime.

MATERIALS AND METHODS

Antimicrobial agents. The antibiotics used in this study were as follows: ceftibuten (Schering Corp., Bloomfield, Ill.), cefpodoxime (The Upjohn Co., Kalamazoo, Mich.), cefixime (American Cyanamid Co., Pearl River, N.Y.), cefmetazole (Upjohn), ceftriaxone (Hoffmann-La Roche Inc., Nutley, N.J.), cefotaxime (Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J.), cefuroxime (Glaxo Group Research, Ltd., Greenford, England), cefaclor (Eli Lilly & Co., Indianapolis, Ind.), amoxicillin (Sigma Chemical Co., St. Louis, Mo.), and amoxicillin-clavulanate (Beecham Laboratories, Bristol, Tenn.).

Bacterial strains. All 38 strains required β-NAD (V factor) and hemin (X factor) for growth when incubated at 37°C in 5% CO_2. Each strain was examined for iridescence on translucent media and tested for agglutination with typing sera (Difco Laboratories, Detroit, Mich.); type b and polyvalent sera were used. β-Lactamase production was determined by incubating suspensions of each strain with β-lactamase substrate (chromogenic cephalosporin; Calbiochem-Behring, La Jolla, Calif.) at room temperature for 30 min and visually assessing color development. The Amp^r NBLP strains were identified previously (15, 16), with ampicillin MICs of ≥1.5 μg/ml in comparison to Amp^s isolates, for which MICs were ≤0.5 μg/ml. All 18 susceptible strains were nontypable, with the site of isolation as follows: 10 respiratory tract, 5 eye, 1 ear, 1 ATCC 33391, and 1 site unknown. The 13 Amp^{r}BLP strains consisted of 10 nontypable strains: 6 respiratory, 3 ear, and 1 ATCC 33929 isolate and 3 type b (2 blood and 1 site unknown). The seven Amp^{r}NBLP strains were all nontypable: five respiratory, one cerebrospinal fluid, and one from an unknown site.

Media. Brain heart infusion (BHI) broth supplemented with 10 μg of hemin (X factor), 10 μg of L-histidine, and 10 μg of β-NAD (V factor) per ml (sBHI) was used for growth of organisms to the logarithmic phase. The media used for agar dilution MIC, broth dilution MIC and MBC, and the rate of bactericidal determination activity was haemophilus test media (HTM) (8). Phosphate-buffered saline (PBS) was used for dilutions, and sBHI agar plates were used for determination of bacterial density.

Agar dilution MIC. All 38 strains were tested in duplicate with the following 10 antibiotics: amoxicillin, amoxicillin-

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clavulanate, cefaclor, cefuroxime, cefmetazole, cefotaxime, ceftriaxone, cefixime, cefpodoxime, and ceftibuten. The strains were inoculated onto fresh sBHI agar plates from vials of skim milk stored at −70°C and were incubated overnight in 5% CO_2 at 37°C. Cells were harvested from sBHI agar plates after overnight growth and diluted in PBS to give final inocula of 10^3 and 10^5 CFU when plated with a Steers replicator on HTM agar containing graded antibiotic concentrations. The MIC was defined as the lowest concentration of antibiotic which inhibited visible growth of the inoculum in comparison with growth on antibiotic-free media. Plates were examined after 18 to 24 h of incubation in 5% CO_2 at 37°C. The concentrations tested for the three cephems, cefotaxime, and ceftriaxone were twofold dilutions from 0.005 to 30.72 μg/ml, and those for amoxicillin, amoxicillin-clavulanate, cefaclor, cefmetazole, and cefuroxime were twofold from 0.125 to 64 μg/ml. To assess the effect on MIC with the higher inoculum of 10^6 CFU, we tested five Amp^s, four Amp^{r}BLP, and seven Amp^{r}NBLP strains with six cephalosporins (the three cephems, cefotaxime, ceftriaxone, and cefuroxine) and compared the geometric mean titers of the MICs at inocula of 10^3 and 10^6 CFU.

Broth dilution MIC and MBC. Two strains from each subset (Amp^s, Amp^{r}BLP, and Amp^{r}NBLP), which includes the Amp^s isolate ATCC 33391 and the Amp^{r}BLP strain ATCC 33929, were selected for testing in duplicate with the following seven antibiotics: ampicillin, cefaclor, cefuroxime, ceftriaxone, cefixime, cefpodoxime, and ceftibuten. The strains were grown to mid-log phase (A_{660} of 0.6) in sBHI broth and diluted in PBS to give a final inoculum of 10^5 CFU/ml after addition of 100 μl of bacterial suspension to 1.9 ml of HTM broth containing antibiotic present in twofold increasing concentrations. The concentrations tested for the three cephems were 0.01, 0.02, 0.04, 0.08, 0.156, 0.313, 0.63, 1.25, 2.5, 5.0, 7.5, 10, 15, and 30 μg/ml. Ceftriaxone concentrations tested were twofold from 0.002 to 2.0 μg/ml, whereas concentrations of amoxicillin, cefaclor, and cefuroxime were twofold from 0.469 to 30 μg/ml. Actual inoculum was determined by plating on sBHI agar. The MIC was defined as the lowest concentration of antibiotic that completely inhibited visual growth after 18 to 24 h at 37°C in 5% CO_2. From each nonturbid tube 0.01 ml was then inoculated onto sBHI agar plates, which were incubated for 18 to 24 h at 37°C in 5% CO_2. The $MBC_{99.9}$ was determined as the lowest antibiotic concentration which killed at least 99.9% of the initial inoculum.

Killing kinetics. One strain of each subset, including the Amp^s isolate ATCC 33391 and the Amp^{r}BLP isolate ATCC 33929, was selected for testing with each of the following antibiotics: cefaclor, cefuroxime, and ceftibuten. The strains were grown to mid-log phase (A_{600} of 0.6) in sBHI broth and diluted to give a final inoculum of 10^5 CFU/ml in HTM broth. Three multiples of the MIC of the antibiotic for each strain were tested (0.5× MIC, 1.0× MIC, and 5.0× MIC), as well as a control (no antibiotic added). Strains were incubated in a shaking (150 rpm) incubator at 37°C with 1-ml samples removed after 0, 4, 10, and 24 h of incubation and inoculated after appropriate 10-fold serial dilution in PBS, in duplicate, on sBHI agar plates; bacterial density was then determined. The minimal accurately detectable number of CFU per milliliter was 10. The antibiotics were not inactivated, but drug carry-over effects were eliminated by serial 10-fold dilutions of initial suspensions in PBS before plating.

Detection of PBPs. The amount of unlabeled antibiotic needed to decrease the [^{3}H]penicillin binding by 50% (IC_{50}) was determined for ceftibuten, cefixime, cefpodoxime, and cefotaxime with the ampicillin-susceptible strain MAP as described previously (13). Cell membranes were prepared from cells grown to the logarithmic phase (A_{600} of 0.6; 5 × 10^8 CFU/ml) as described previously (13): the membranes were incubated with twofold increasing concentrations of unlabeled antibiotic from 0.002 to 4 μg/ml for 10 min before the addition of [^{3}H]penicillin (5.42 Ci; 0.1 μg/100-μl volume) and incubated at 37°C for 30 min. Electrophoresis in sodium dodecyl sulfate-polyacrylamide gel, fixation, enhancement, and autofluorography were performed as described previously (13, 19).

TABLE 1. MICs for *H. influenzae* isolates

Phenotype (no. of isolates)[a]	MIC for individual isolates (μg/ml)[b]	
	Amoxicillin	Amoxicillin-clavulanate
Amp^s (18)	0.125_2, 0.25_8, 0.5_8	0.125_1, 0.25_2, 0.5_{15}
Amp^{r}BLP (13)	8_2, 16_2, $>16_9$	1_5, 2_8
Amp^{r}NBLP (7)	1_2, 2_1, 4_2, 16_2	1_1, 2_1, 4_3, 8_1, 16_1

[a] All Amp^r NBLP strains had ampicillin MICs of ≥1.5 μg/ml at an inoculum of 10^3 CFU, with the agar dilution technique when tested with SBHI media (15).

[b] HTM (8) and an inoculum of 10^5 CFU were used with the agar dilution technique. The subscript indicates the number of isolates with the MIC indicated.

RESULTS

MIC and MBC. Table 1 shows the amoxicillin and amoxicillin-clavulanate MICs for the three phenotypic groups tested. Although no overlap was observed between Amp^s and Amp^{r}NBLP strains, MICs for two of the latter strains were 1 μg/ml with HTM media, whereas previously MICs for all strains were ≥1.5 μg/ml at the lower inoculum of 10^3 CFU when tested on sBHI agar (15).

Table 2 shows the MIC range and MICs for 50 and 90% (MIC_{50} and MIC_{90}) of 38 strains of *H. influenzae* by phenotype with the 10 antibiotics tested at an inoculum of 10^5 CFU. Except for amoxicillin and cefaclor, the Amp^s and Amp^{r}BLP strains showed nearly identical MIC_{50}s and MIC_{90}s of all other antimicrobial agents tested. All three cephems had excellent activity against these strains, with MIC_{50}s and MIC_{90}s of 0.04 to 0.08 μg/ml; these values were two- to fourfold higher than those for cefotaxime and 8- to 16-fold higher than those for ceftriaxone. In contrast to the Amp^s and Amp^{r}BLP strains, the Amp^{r}NBLP strains were less susceptible to the three cephems: MIC_{50}s and MIC_{90}s were 3- to 96- and 12- to 192-fold higher, respectively. Similarly, there was a 2- to 24-fold increase in MIC_{50} and MIC_{90} for the Amp^{r}NBLP strains compared with Amp^s and Amp^{r}BLP strains with the other antibiotics tested (amoxicillin-clavulanate, cefaclor, cefuroxime, ceftriaxone, and cefotaxime).

Comparison of geometric mean titers revealed that there was no significant effect on the MIC by increasing inoculum from 10^3 to 10^5 CFU for any of the three groups of strains with the 10 antibiotics. In contrast, at the 10^6 inoculum, only the Amp^{r}NBLP isolates revealed a striking inoculum effect with the three cephems and ceftriaxone (Table 3).

Table 4 shows the broth MICs and $MBC_{99.9}$ for two strains of each phenotype with seven antibiotics. The broth MICs for the Amp^{r}NBLP isolates with the cephems were 16- to >375-fold higher than those for the Amp^s and Amp^{r}BLP strains. Similarly, but less so, the ceftriaxone MICs were 8- to 32-fold higher for the Amp^{r}NBLP isolates. For the Amp^s

TABLE 2. Agar dilution MICs of three cephems and comparative agents[a]

Antibiotic	MIC (μg/ml)								
	Amp^s (n = 18)			Amp^{r}BLP (n = 13)			Amp^{r}NBLP (n = 7)		
	Range	50%	90%	Range	50%	90%	Range	50%	90%
Ceftibuten	0.01–0.08	0.04	0.08	0.04–0.08	0.08	0.08	0.48–15.36	3.84	7.68
Cefpodoxime	0.02–0.12	0.04	0.08	0.04–0.12	0.08	0.08	0.48–15.36	0.48	1.92
Cefixime	0.005–0.08	0.04	0.04	0.02–0.04	0.04	0.04	0.12–0.96	0.24	0.96
Cefmetazole	0.5–4	2	2	2–4	2	4	0.125–>16	8	>16
Amoxicillin	0.125–0.05	0.25	0.5	8–>16	>16	>16	1.0–16	4	16
Amoxicillin-clavulanate	0.125–0.5	0.5	0.5	1–2	2	2	1.0–16	4	8
Cefaclor	0.125–8	4	8	4–>16	8	>16	4–>64	32	64
Cefuroxime	0.125–2	0.5	1	0.5–2	1	1	2–16	4	8
Ceftriaxone	0.005–0.04	0.005	0.005	0.005–0.24	0.005	0.005	0.04–0.12	0.08	0.08
Cefotaxime	0.005–0.08	0.01	0.02	0.01–0.02	0.02	0.02	0.12–0.48	0.24	0.48

[a] HTM (8) and an inoculum of 10^5 CFU were used.

and Amp^{r}BLP strains with all six cephalosporins, the broth MICs were nearly identical to the agar MIC_{50}s and MIC_{90}s. In contrast, for certain Amp^{r}NBLP strains, the broth MICs of the cephems were higher than the comparable agar MICs. Specifically, cefpodoxime revealed a 5-fold increase in MIC for one strain and equivalence for the other, and ceftibuten revealed equivalence for one strain and a >4-fold increase in MIC for the other strain, comparing solid and liquid media. Of interest, ceftriaxone revealed no MIC difference for either isolate on either medium.

One of two Amp^s strains appeared to be tolerant of cefixime and ceftibuten (MBC 32-fold greater than the MIC) but not tolerant of cefpodoxime. No Amp^{r}BLP strains were tolerant of the cephems, however; the $MBC_{99.9}$ for one was 16-fold the MIC of ceftriaxone. With the two Amp^{r}NBLP isolates, no bactericidal concentration of cefaclor or cefpodoxime was determined.

Killing kinetics. Figure 1 shows the killing kinetics of cefaclor, cefuroxime, and ceftibuten for a single strain of each phenotype (Amp^s, Amp^{r}BLP, and Amp^{r}NBLP) at 0.5×, 1.0×, and 5.0× MIC of the antibiotic tested. However, because a cefaclor MIC was not achieved for the Amp^{r}NBLP strain (MIC, >30 and >140 μg/ml), no data for cefaclor killing kinetics could be determined for that strain. At 1.0× and 5.0× MIC for cefuroxime and ceftibuten for all three strains, the kill kinetics appeared similar, whereas at 0.5× MIC cefuroxime did not appear to kill the Amp^s and Amp^{r}BLP strains and ceftibuten did not appear to kill the Amp^{r}NBLP strain.

PBPs. Table 5 shows the IC_{50}s of cefotaxime, ceftibuten, cefpodoxime, and cefixime for the PBPs of the ampicillin-susceptible strain MAP. PBPs 2, 4, and 5 appear to have high affinity (lowest IC_{50}) for all three cephems and for cefotaxime. The IC_{50}s of PBPs 1, 3, 7, and 8 exceed the cephem MICs for this strain (data not shown), making them less likely targets.

TABLE 3. Geometric mean titers of MICs for *H. influenzae* at high and low inocula[a]

Antibiotic	Geometric mean titer					
	Amp^s (n = 5)		Amp^{r}BLP (n = 4)		Amp^{r}NBLP (n = 7)	
	10^3	10^6	10^3	10^6	10^3	10^6
Cefpodoxime	0.04	0.36	0.06	1.92	0.29	12.60*
Ceftibuten	0.04	0.21	0.05	3.23*	1.58	22.82*
Cefixime	0.03	0.14	0.03	1.14	0.26	11.41*
Cefotaxime	0.01	0.06	0.02	0.20	0.18	1.58
Ceftriaxone	0.01	0.09	0.01	0.10	0.04	4.24*
Cefuroxime	0.57	0.84	1.00	1.68	4.42	14.49

[a] Titers were calculated from agar dilution MICs, using HTM (8). *, Groups demonstrating an inoculum effect, $P < 0.05$.

DISCUSSION

Reflecting their excellent β-lactamase stability, the three cephems have potent and comparable activities against Amp^s and Amp^{r}BLP strains of *H. influenzae*. Our cefixime MIC_{90}s of 0.04 μg/ml for both Amp^s and Amp^{r}BLP strains are consistent with certain studies (10–12), but 3- to 6.25-fold lower than those observed by others (1, 4, 5, 9, 18). Similarly, our cefpodoxime and ceftibuten MIC_{90}s of 0.08 μg/ml for these strains are close to those reported previously: ≤0.06 and ≤0.12 μg/ml for cefpodoxime (4, 5, 10) and 0.06 μg/ml for ceftibuten (5, 6).

In contrast to the data for Amp^s and Amp^{r}BLP strains, the Amp^{r}NBLP isolates are markedly less susceptible to the cephems. Although uncommon, Amp^{r}NBLP strains of *H. influenzae* have been isolated worldwide (2, 16), and their primary mechanism of resistance is reduced affinity of certain PBPs for ampicillin (17, 19). It follows that strains resistant by the above mechanism would also be resistant to other β-lactams. Our cefixime MIC_{90}s of 0.96 μg/ml were 2- and 8- to 16-fold higher than those reported previously (references 18 and 1, respectively) but were within the range reported by others (7, 10). Similarly, our cefpodoxime MIC_{90} of 1.92 μg/ml was consistent with that reported by Knapp et al. (10). Ceftibuten MIC_{90}s have not been reported previously with Amp^{r}NBLP strains, and our MIC_{90} was four- to eightfold higher than those of the other two cephems. However, it should be noted that the peak concentrations in serum reported for ceftibuten (11.6 μg/ml after a 200-mg dose) are three- to fourfold higher than cefixime (4 μg/ml after a 400-mg dose) and cefpodoxime (3 μg/ml after a 200-mg dose) (5, 6, 12).

Fuchs et al. (3), studying cefixime, defined organisms with a MIC lower than 1 μg/ml as being susceptible and MICs of ≥1 and ≤4 μg/ml as being of intermediate resistance. By these criteria, three of seven of the Amp^{r}NBLP strains appear to have intermediate resistance to cefixime on solid media with an inoculum of 10^5 CFU, whereas one was resistant and three were susceptible. At the higher inoculum of 10^6 CFU, six of seven were resistant and one was intermediate. Similarly, in broth, one isolate was resistant and the other was intermediate. Because the peak serum

TABLE 4. Broth MIC and $MBC_{99.9}$ for the cephems and comparative antibiotics[a]

Antibiotic	Broth MIC (μg/ml)			$MBC_{99.9}$ (μg/ml)		
	Amp^s	Amp^{r}BLP	Amp^{r}NBLP	Amp^s	Amp^{r}BLP	Amp^{r}NBLP
Amoxicillin	≤0.47	30, >30	3.76, 7.5	≤0.47, 1.88	>30	15
Cefaclor	1.88, 3.76	3.76, 7.5	>30, >140	7.5	15, >30	>30, >140
Cefuroxime	0.94	1.88	7.5, >30	0.94, 1.88	1.88, 15	15, >30
Ceftriaxone	0.004, 0.008	0.004, 0.008	0.063, 0.125	0.008, 0.06	0.008, 0.13	0.13, 0.5
Cefixime	0.04, 0.08	0.04, 0.08	1.25, 5.0	0.08, 2.5	0.08	2.5, 7.5
Cefpodoxime	0.08	0.08	>30, >30	0.16	0.16, 0.31	>30, >30
Ceftibuten	0.08	0.08	7.5, >30	0.08, 7.5	0.08, 0.16	30, >30

[a] An inoculum of 10^5 CFU was used. Two strains of each phenotype were tested; a single value is listed when both strains had identical MIC and MBC.

levels of the cephems appear to be only 1.5- to 4-fold higher than the MIC_{90} for the Amp^{r}NBLP strains even at the lower inoculum of 10^5 CFU, their therapeutic efficacy appears to be limited with these strains. In contrast, achievable levels in serum are 38- to 145-fold higher than the cephem MIC_{90}s for Amp^s and Amp^{r}BLP strains.

The basis for the potent activity of the cephems for gram-negative bacteria has only been reported for cefixime (20). It has been shown in *Escherichia coli* that outer membrane permeability does not play a role (20), but that PBPs 3, 1a, and 1b of *E. coli* have very high and equal affinities for cefixime. Similarly, three PBPs in *H. influenzae*, PBPs 2, 4, and 5, appeared to have high affinities for the cephems at concentrations below the MIC for the susceptible strain tested. PBPs 4 and 5 of *H. influenzae* are known to be involved in cell wall synthesis and appear to have transpeptidase activity which, coupled with the affinity data, implicate them as important targets for the cephems (14). No role for PBP 2 of *H. influenzae* has been defined; however, it may not be essential, as a wild-type strain lacking this PBP has been observed (16).

Consistent with our data revealing no significant inoculum effect comparing 10^3 and 10^5 CFU, no inoculum effect was found previously with ceftibuten at inocula of 10^4 and 10^5

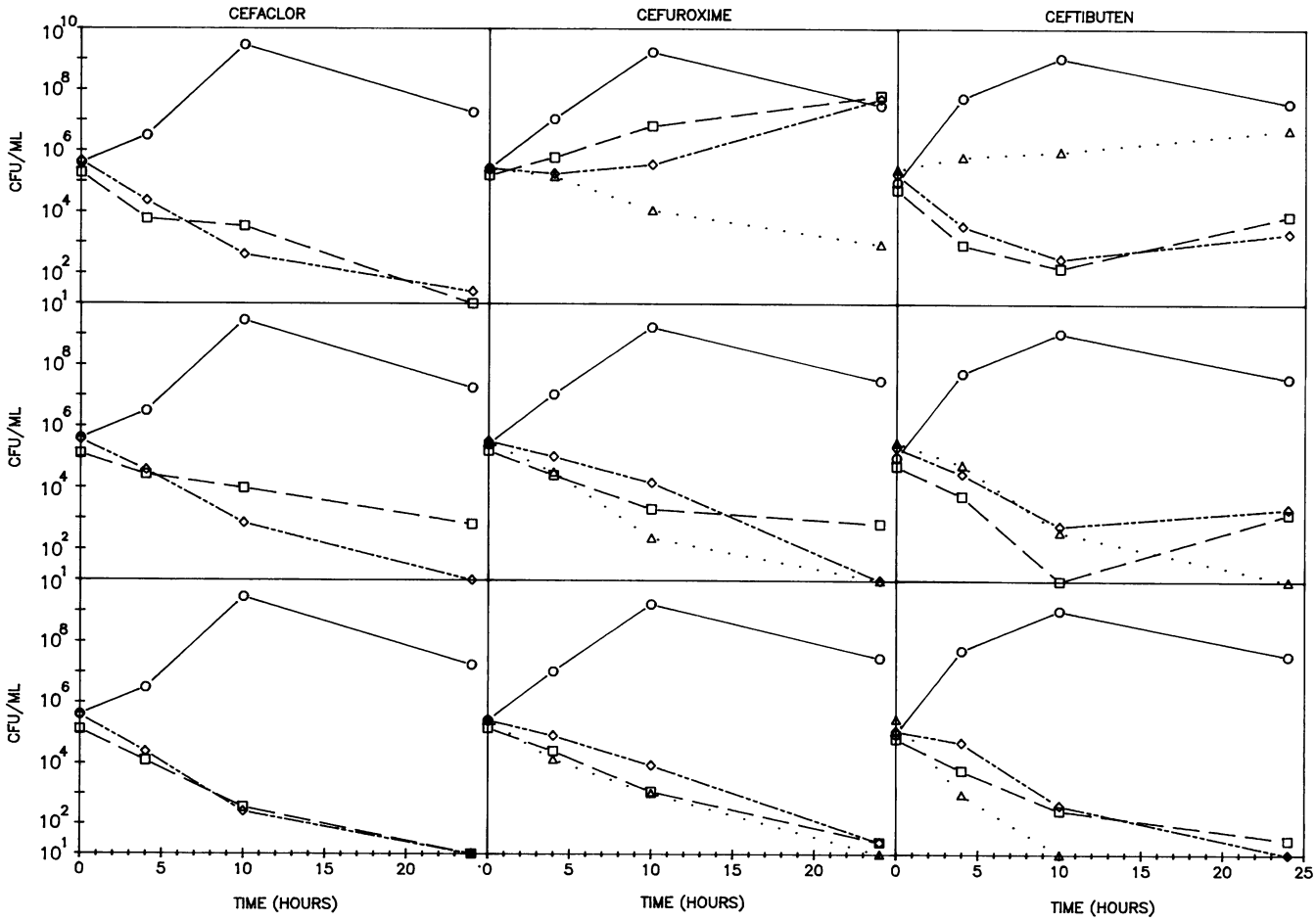

FIG. 1. Killing curves of three strains of *H. influenzae*: Amp^s, ◇; Amp^{r}BLP, ■; Amp^{r}NBLP, △. The control (○) represents the average of the three strains with no antibiotic added. The top panels represent 0.5× MIC; the middle panels, 1× MIC; and the lower panels, 5× MIC. MIC (micrograms per milliliter) are as follows: cefaclor—Amp^s = 3.75; Amp^{r}BLP = 7.5, and Amp^{r}NBLP = >140 (thus could not be tested); cefuroxime—Amp^s = 0.94, Amp^{r}BLP = 1.88, and Amp^{r}NBLP = 7.5; ceftibuten—Amp^s = 0.08, Amp^{r}BLP = 0.08, and Amp^{r}NBLP = 7.5.

TABLE 5. Affinity of the PBPs[a] of *H. influenzae* MAP for four cephalosporins expressed in IC_{50}s[b]

PBP	IC_{50} (μg/ml)			
	Cefotaxime	Ceftibuten	Cefpodoxime	Cefixime
1	0.2 (0.05)	2.37 (0.54)	0.3 (0.05)	0.4 (0.1)
2	0.037 (0.015)	0.016 (0.003)	0.013 (0.001)	0.0089 (0.0023)
3	0.28 (0.44)	ND[c]	0.2 (0.07)	0.48 (0.25)
4	0.00076 (0.00002)	0.038 (0.021)	0.042 (0.006)	0.0093 (0.0033)
5	0.002 (0.0009)	0.023 (0.003)	0.12 (0.02)	0.017 (0.003)
6	ND	ND	ND	ND
7	>8.0	>4.0	>4.0	>4.0
8	0.02 (0.02)	0.47 (0.11)	0.18 (0.07)	ND

[a] PBPs were detected with cell membranes of the ampillin-susceptible strain MAP as indicated in the text.
[b] IC_{50} is the concentration of unlabeled antibiotic required to decrease the binding to [^{3}H]penicillin G by 50‰. The number in parentheses is the standard deviation.
[c] ND, Not detected.

CFU (6). With a 10^6 CFU inoculum with the Amp^{r}NBLP strains, we observed an inoculum-dependent increase in MIC for all three cephems. Others have reported, with Amp^{r}BLP isolates, a 2-fold increase in ceftibuten MIC, a 2- to 8-fold increase in cefpodoxime MIC and a 6- to >256-fold increase in cefixime MIC with an inoculum of 10^7 CFU (6, 10). These data suggest that, at an inoculum higher than we tested, there may be an inoculum-dependent increase in MIC of the cephems with strains of this phenotype.

The bactericidal activities we observed for cefixime and ceftibuten with the Amp^{r}BLP strains are comparable to those reported by Bergeron et al. (1) and Jones and Barry (6). In addition, we observed tolerance to cefixime for a single Amp^s isolate, which is in agreement with the report of Bergeron et al. (1) and in contrast to that of Kumar and Kelly (12). However, not previously known (6), we also observed tolerance to ceftibuten for one of two Amp^s isolates tested. For the Amp^{r}NBLP strains, cefixime was the most bactericidal, with clear endpoints. Of note, our cefixime $MBC_{99.9}$s were at least an order of magnitude higher (0.24 versus 2.5 μg/ml) than those reported by Bergeron et al. (1) for strains with this phenotype.

We conclude that the three cephems have excellent in vitro activities against Amp^s and Amp^{r}BLP isolates; however, their activities against Amp^{r}NBLP strains appear to be limited.

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