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Cefamandole: Antimicrobial Activity In Vitro of a New Cephalosporin

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Cefamandole, a new cephalosporin derivative, was found to have a broad spectrum of activity against a cross-section of both gram-positive and gram-negative bacteria isolated from clinical material. Gram-positive cocci, except for *Streptococcus faecalis*, were very susceptible. Penicillin G-resistant *Staphylococcus aureus* also was susceptible to cefamandole. Minimal bactericidal concentrations for gram-positive cocci approximated the minimal inhibitory concentrations. Strains of *Haemophilus influenzae* were very susceptible to the drug. Most strains of *Escherichia coli*, *Klebsiella* sp., and *Proteus* sp. were inhibited by low concentrations. Increasing resistance occurred with larger inocula. Strains of *Pseudomonas* sp. were resistant to cefamandole.

Cefamandole, 7-D-mandelamido-3-{[(1-methyl-1H-tetrazol-5-yl)-thio]methyl}-3-cephem-4 carboxylic acid (Fig. 1), is a new semisynthetic cephalosporin derivative with a broad spectrum of activity against a variety of gram-positive and gram-negative bacteria (3, 5, 7). Strains of *Haemophilus influenzae* are highly susceptible to this compound (3, 5). We report on the activity in vitro of this new antimicrobial agent against a representative cross-section of gram-negative and gram-positive bacterial isolates, including methicillin-resistant *Staphylococcus aureus*, from patients in a large general hospital.

MATERIALS AND METHODS

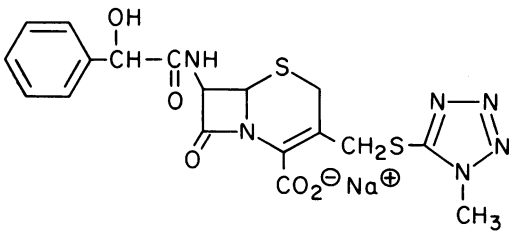
Strains of bacterial isolates from clinical specimens were obtained from the Diagnostic Microbiology Laboratory of The Mount Sinai Hospital. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of cefamandole for the isolates were determined by the standard two-fold tube dilution method. The inoculum was 0.5 ml of a 10^{-4} dilution of an overnight growth of the organisms. Trypticase soy broth (BBL) or heart infusion broth (Difco) was used as growth medium; Todd-Hewitt broth (Difco) was used for strains of *Streptococcus pneumoniae*. Strains of *H. influenzae* were grown in Levinthal broth (Difco); Kirby-Bauer studies (1) were performed with medium incorporated into Trypticase soy agar (Difco).

The MIC was defined as the lowest concentration of antibiotic in which no growth was observed after 18 h of incubation at 37 C. Methicillin-resistant strains of *S. aureus* were cultured at both 30 and 35 C (2), and the tubes were examined at 24 and 48 h. Subcultures of clear tubes then were made on Trypticase soy agar or heart infusion agar plates with the use of a wire loop and were incubated overnight at 37 C for determination of MBC. The MBC was defined as the lowest concentration of antibiotic at which wire loop subcultures onto agar plates showed no growth after 18 h. Disk susceptibility testing was performed by the Kirby-Bauer method (1) using Mueller-Hinton agar.

The effect of inoculum size on MICs of cefamandole for four strains of penicillin G- and methicillin-resistant stephylococci was determined by inoculating 100-fold dilutions of overnight growths of the organisms. The number of organisms was determined by colony counting using the standard pour-plate method. The effect of 50% human serum on antibacterial activity of cefamandole, compared to activity in standard broth media, was determined using four strains of penicillin G-resistant *S. aureus*.

RESULTS

Aerobic gram-positive bacteria, with the exception of strains of *Streptococcus faecalis*, were highly susceptible to cefamandole (Table 1); the



Cefamandole

FIG. 1. *Chemical structural formula of cefamandole.*

cumulative percentage of strains inhibited and killed at each concentration is shown in Fig. 2. *Streptococcus pyogenes* (group A) was exquisitely susceptible, with all strains inhibited at concentrations of 0.125 μg/ml or less. *S. pneumoniae* was also very susceptible, with 100% of strains inhibited by 0.18 μg/ml or less. Strains of *Streptococcus viridans* also were inhibited by low concentrations of cefamandole. For most gram-positive cocci the MICs and MBCs of cefamandole were equal. Enterococci were resistant to this compound (Fig. 2); the mean MIC was 39 μg/ml (Table 1). However, non-enterococcal group D streptococci such as *Streptococcus bovis* were highly susceptible, with MICs ranging from 0.32 μg/ml to 1.25 μg/ml (Table 1).

Cefamandole was highly active against both penicillin G-susceptible and penicillinase-producing strains of *S. aureus* (Table 1), the in vitro activity being equal against the two groups. The mean MICs were 0.50 and 0.44 μg/ml, respectively. The MBC was equal to the MIC or showed a one-tube difference in most cases.

Methicillin-resistant strains of *S. aureus* were susceptible to cefamandole when standard inocula of 10^{-4} dilutions of overnight growths were used (Table 1). Tube dilution and disk diffusion studies revealed these strains to be highly resistant to penicillin G and oxacillin. The average MIC of oxacillin was 26 μg/ml with a range from 12.5 to 50 μg/ml; the mean MIC of cefamandole was 2.5 μg/ml. The MBCs were two to four times the MICs. Agar dilution zone sizes averaged 31 mm with cefamandole, whereas none was seen with oxacillin. In a separate study, using inocula of 10^5 colony-forming units per ml, the activity of cefamandole at temperatures of 30 and 35 C was determined against methicillin-resistant *S. aureus* (Table 2). Although the MICs were generally higher in these studies, no differences between the MICs

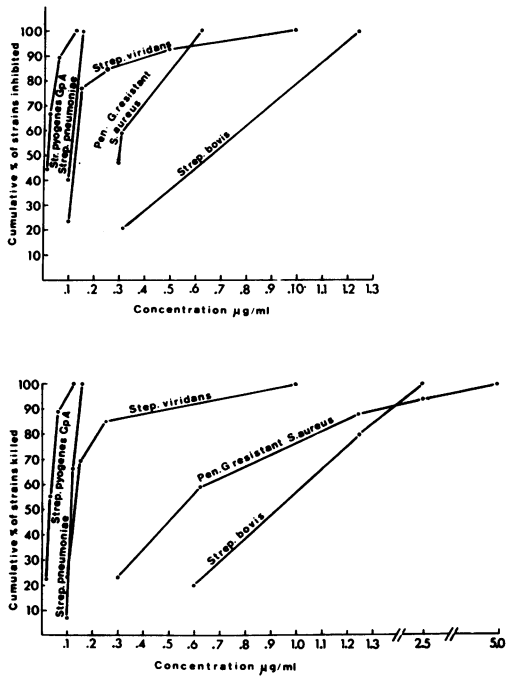


FIG. 2. *Cumulative percentage of strains of Streptococcus pyogenes (group A), Streptococcus pneumoniae, Streptococcus viridans, Streptococcus bovis, and penicillin G-resistant Staphylococcus aureus inhibited (top) and killed (bottom) by increasing concentrations of cefamandole.*

TABLE 1. *Antibacterial activity of cefamandole against gram-positive bacteria*

Bacteria	No. of strains	MIC (μg/ml)	MBC (μg/ml)	Kirby-Bauer (mm)
Streptococcus pneumoniae	15	0.179 ± 0.28[a] (0.0612–1.2)[b]	0.44 ± 1.2 (0.0612–4.8)	43 ± 4 (37–50)
S. pyogenes group A	9	0.04 ± 0.036 (0.015–0.125)	0.03 ± 0.02 (0.015–0.125)	41.2 ± 3.8 (36–45)
S. bovis	5	1.0 ± 0.41 (0.315–1.25)	1.37 ± 0.69 (0.6–1.25)	40.2 ± 3.8 (38–47)
S. viridans	13	0.21 ± 0.26 (0.06–1)	0.22 ± 0.26 (0.06–1)	39.3 ± 4.9 (26–45)
S. faecalis	11	38.9 ± 16.5 (3–50)	50 ± 0	16 ± 5.2 (13–22)
Penicillin G-susceptible *Staphylococcus aureus*	15	0.5 ± 0.27 (0.156–1.25)	2.47 ± 1.9 (0.312–5)	33.0 ± 3.6 (28–39)
Penicillin G-resistant *S. aureus*	17	0.43 ± 0.16 (0.3–0.625)	1.1 ± 1.1 (0.3–5)	30.7 ± 1.89 (23–34)
Methicillin-resistant *S. aureus*	12	2.4 ± 2.4 (1.25–10)	6.6 ± 3.19 (2.5–10)	24.2 ± 3.9 (19–31)

[a] Mean ± standard deviation.
[b] Range.

determined at either temperature were found at 24 h of incubation. The MICs at 48 h were approximately two to three times greater at both temperatures. The MBCs at 48 h were similar to the MICs.

Disk diffusion tests with cefamandole yielded large zone sizes with susceptible gram-positive bacteria (Table 1). The mean zone size was 44 mm for *S. pneumoniae* and 40 mm for susceptible streptococcal strains. Plates with *S. aureus* had smaller zone sizes, decreasing from an average of 31 mm for penicillin G-susceptible strains to 24 mm for methicillin-resistant strains. The resistant enterococcal strains yielded an average zone diameter of 16 mm.

TABLE 2. *Comparison of MICs of cefamandole for methicillin-resistant strains of Staphylococcus aureus at 35 and 30 C*

Strain	Cefamandole (μg/ml)		
	MIC		MBC (48 h)
	24 h	48 h	
24	6.8	6.8	13.5
	3.4	13.5	13.5
27	6.8	13.5	13.5
	6.8	13.5	13.5
13	3.4	13.5	13.5
	3.4	13.5	13.5
25	3.4	13.5	13.5
	3.4	13.5	13.5
26	3.4	13.5	27
	3.4	27	27
1	6.8	6.8	13.5
	3.4	13.5	13.5
9	6.8	6.8	6.8
	6.8	13.5	13.5
11	6.8	6.8	13.5
	3.4	13.5	13.5
P	3.4	13.5	13.5
	6.8	13.5	13.5

Cefamandole was active against a wide variety of gram-negative bacteria (Table 3). The cumulative percentage of strains inhibited and killed at each concentration is shown in Fig. 3 and 4. *H. influenzae* strains were the most susceptible organisms studied; the mean MIC of cefamandole was 1.43 μg/ml, with 14 of 20 strains tested inhibited by 1.25 μg/ml. The mean MIC for *Proteus mirabilis* was 2 μg/ml, and for indole-positive strains 3.36 μg/ml. Cefamandole was highly active against *Klebsiella* strains, with 95% of strains inhibited by a concentration of 6.75 μg/ml; the mean MIC was 2.44 μg/ml. Most *Escherichia coli* strains tested were susceptible to this compound; the mean MIC was 4.4 μg/ml. Three strains were inhibited by a concentration of 25 μg/ml. *Enterobacter* species varied in their susceptibility to cefamandole. Only 7 of 20 strains were inhibited by 3 μg/ml, whereas 10 strains required greater than 10 μg/ml for inhibition. *Serratia* species were more resistant to cefamandole, with 6 of 10 strains inhibited at concentrations of 14 to 28 μg/ml. *Pseudomonas aeruginosa* strains were highly resistant to this compound.

When the activity of cefamandole was studied with increasing inocula of penicillin G-resistant *S. aureus*, the MIC and the MBC increased at most eightfold in strains studied. Increasing inoculum size of methicillin-resistant *S. aureus* in some cases caused a greater than 10-fold increase in the MIC (Table 4). Some *E. coli* strains showed marked resistance

TABLE 3. *Antibacterial activity of cefamandole against gram-negative bacteria*

Bacteria	No. of strains	MIC (μg/ml)	MBC (μg/ml)	Kirby Bauer (mm)
Escherichia coli	27	4.43 ± 7.6[a] (0.35–25)[b]	8.5 ± 15.6 (0.75–50)	27.5 ± 3.7 (19–33)
Klebsiella sp.	22	2.4 ± 5.3 (0.35–25)	4.1 ± 10.5 (0.35–50)	27 ± 3.35 (17–31)
Haemophilus sp.	20	1.43 ± 1.45 (0.25–5)	1.97 ± 1.88 (0.25–5)	35.1 ± 4.32 (25–40)
Proteus mirabilis	16	2.09 ± 0.922 (0.75–3.0)	18.1 ± 17.0 (3–50)	27.8 ± 1.79 (23–31)
Proteus sp. (indole positive)	12	3.35 ± 4.1 (0.7–15)	7.7 ± 6.6 (0.7–15)	29.5 ± 2.4 (26–33)
Enterobacter sp.	20	19.5 ± 18.5 (0.9–55)	20.9 ± 17.7 (1.4–55)	26.5 ± 4.3 (17–32)
Serratia sp.	10	34.9 ± 13.6 (14–50)	45.9 ± 14.3 (14–55)	17.2 ± 5.67 (9–24)
Pseudomonas sp.	10	>50 (>50)	>50 (>50)	6.25

[a] Mean ± standard deviation.
[b] Range.

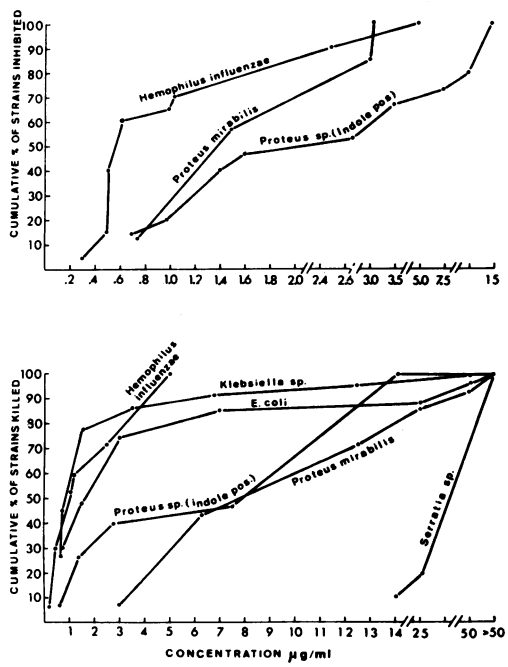


FIG. 3. *Cumulative percentage of strains of Haemophilus influenzae, Proteus mirabilis, and indole-positive Proteus sp. inhibited (top) and of H. influenzae, Klebsiella sp., Escherichia coli, P. mirabilis, indole-positive Proteus sp., and Serratia sp. killed (bottom) by increasing concentrations of cefamandole.*

at higher inocula (Table 4). MICs and MBCs were determined in 50% serum and compared to values obtained in Trypticase soy broth with four strains of penicillin G-resistant *S. aureus*. In two cases a twofold, and in two others a fourfold, increase in MIC was noted. In two cases the MBCs were significantly higher in serum.

DISCUSSION

Cefamandole was found to be very active against a wide variety of gram-positive and gram-negative bacteria. Eykyn et al. (3) found cefamandole to be slightly less active than either cephalothin or cephaloridine and more active than cephalexin, but the differences were slight when gram-positive bacteria were studied. Cefazolin is comparable to cephaloridine in activity (7) and thus would be slightly more active than cefamandole, though these differences probably would be of no clinical significance.

All our methicillin-resistant strains of *S. aureus* were inhibited at concentrations of 6.8 µg/ml or less of cefamandole at 35 C. Eykyn et al. (3) reported comparable results in that all their strains were inhibited at 12.5 µg/ml at 37 C. However, when our study was carried out to 48 h, the MICs increased two- to fourfold. Yet, for eight of the nine strains tested the MICs and MBCs were not greater than 13.5 µg/ml. Although greater resistance has been noted when methicillin-resistant strains of *S. aureus* are tested at an incubation temperature of 30 C (2), no real difference in susceptibility to cefamandole was noted at temperatures of 35 and 30 C. It should be cautioned that clinical studies will be required to determine whether cefamandole will have any efficacy in the treatment of infections caused by methicillin-resistant *S. aureus*.

H. influenzae strains were quite susceptible to cefamandole, with 90% of strains inhibited at 2.5 µg/ml; Eykyn et al. (3) found 100% inhibition at 3.12 µg/ml. These inhibitory concentrations are comparable to those observed with ampicillin (7). Indeed, cefamandole was much more active than either cephalothin, cephalexin, or cephaloridine against *H. influenzae* (3, 5–7). All *Pseudomonas* strains tested were highly resistant to cefamandole.

With gram-positive cocci the MIC of cefamandole was either equal to or twofold greater than

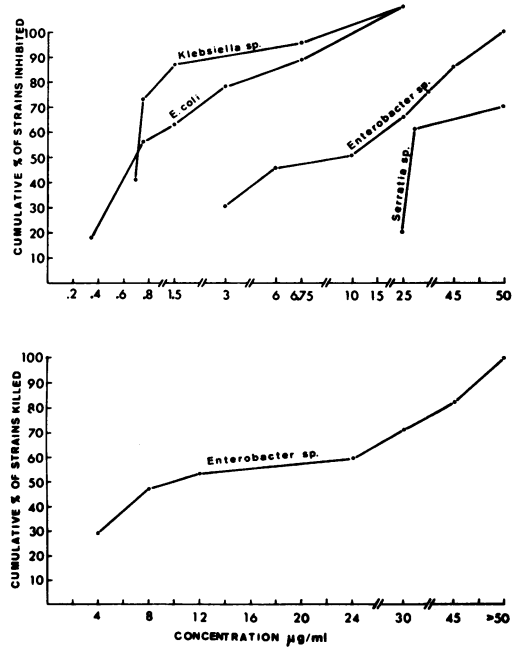


FIG. 4. *Cumulative percentage of strains of Klebsiella sp., Escherichia coli, Enterobacter sp., and Serratia sp. inhibited (top) and of Enterobacter sp. killed (bottom) by increasing concentrations of cefamandole.*

TABLE 4. *Effect of inoculum size on MIC and MBC of cefamandole for methicillin-resistant Staphylococcus aureus and Escherichia coli*

Bacteria	Strain	10^{-2} MIC	10^{-4} MIC	10^{-6} MIC	10^{-2} MBC	10^{-4} MBC	10^{-6} MBC
S. aureus	25	20	2.5	2.5	20	10	2.5
	26	10	2.5	2.5	20	10	2.5
	13	20	2.5	2.5	20	5	2.5
	9	20	2.5	1.25	20	10	1.25
	1	10	2.5	1.25	>20	5	2.5
	24	20	2.5	2.5	>20	2.5	5
E. coli	232	>50	3.12	1.5	50	3.12	1.5
	426	1.5	0.75	0.75	12.5	0.75	0.75
	411	>50	6.25	3.12	>50	6.25	3.12
	436	3.12	1.5	0.75	12.5	1.5	1.5
	087	1.5	0.75	0.38	1.5	1.5	0.75
	386	3.12	0.75	0.38	25	1.5	0.38

the MBC. Eykyn et al. (3) had reported the MBC for penicillinase-producing *S. aureus* to be approximately 10 times greater than the MIC. This was often true for gram-negative bacilli, although marked variation occurred. Increased resistance with larger inocula has been observed with other cephalosporin derivatives (4) and may reflect production of beta-lactamase. When the inoculum of penicillin G-resistant *S. aureus* was increased by 4 logs, the MIC increased fourfold with three of four stains tested. Eykyn et al. (3) found only a small change in the MIC of cefamandole with increasing inocula of penicillin G-resistant *S. aureus*, and they suggested that cefamandole may be relatively resistant to beta-lactamase of these organisms. Wick and Preston (7) reported that cefamandole was more resistant to penicillinases derived from *S. aureus* and *Bacillus cereus* than either cephaloridine, cephanone, or cefazolin. Resistance of gram-negative bacteria to cephalosporins also may be mediated by beta-lactamases, although high-level resistance can occur in the absence of these enzymes (5). Cefamandole has been found to be more resistant to hydrolysis by some of these enyzmes than either cephalothin and cephaloridine. The drug seems to merit further study for the treatment of infections in vivo.

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