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In Vitro Studies of Cefamandole

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Cefamandole is a new cephalosporin antibiotic that was tested in vitro against 540 clinical isolates of gram-positive cocci and gram-negative bacilli. A concentration of 0.39 μg/ml inhibited 95% of the isolates of *Staphylococcus aureus*. A concentration of 6.25 μg/ml inhibited over 90% of the isolates of *Proteus mirabilis* and *Escherichia coli*, 69% of the isolates of *Klebsiella pneumoniae*, and 31% of the isolates of indole-positive *Proteus* spp. and *Enterobacter* spp. It was active against most cephalothin-resistant isolates of *E. coli* but not of *K. pneumoniae*.

Gram-negative bacillary infections continue to be a major cause of morbidity and mortality among hospitalized patients. The introduction of newer antibiotics, such as gentamicin and carbenicillin, has altered the spectrum of these infections (H. Y. Chang, G. Narboni, V. Rodriguez, G. P. Bodey, and E. J. Freireich, Medicine, in press). An increasing number of infections is caused by *Klebsiella pneumoniae* and *Serratia marcescens* (3). A substantial number of these organisms is resistant to most of the currently available antibiotics. Hence, the search for new antibiotics and new congeners of existing antibiotics continues to be important.

Cefamandole is one of the new cephalosporin derivatives that is highly active against many gram-positive cocci and gram-negative bacilli. The sodium salt of the formyl ester of this antibiotic (cefamandole nafate) has been formulated for clinical use. This ester rapidly hydrolyzes in vivo to the parent compound. Since cefamandole has been reported to be more active than other cephalosporins in vitro, we have evaluated its activity against clinical isolates at this institution where a substantial proportion of isolates of *Escherichia coli* and *K. pneumoniae* is resistant to other cephalosporins.

MATERIALS AND METHODS

Susceptibility tests were conducted on 408 clinical isolates of gram-negative bacilli and 132 clinical isolates of gram-positive cocci, using the serial dilution technique with an automatic microtiter system (Canalco; Autotiter IV instruction manual). The organisms were inoculated into Mueller-Hinton broth (Difco) and incubated at 37 C for 18 h. A 0.05-ml sample of 10^{-3} dilution of the broth cultures of gram-negative bacilli (approximately 10^5 colony-forming units/ml) was used as the inoculum for the susceptibility tests. A 0.05-ml sample of a 10^{-2} dilution of the broth cultures of gram-positive cocci (approximately 10^6 colony-forming units/ml) was used as the inoculum.

All gram-negative bacilli used in this study were cultured from blood specimens collected from cancer patients at this institution between 1970 and 1975. A total of 100 isolates each of *E. coli, K. pneumoniae,* and *Proteus mirabilis,* 50 isolates of *S. marcescens,* and 29 isolates each of indole-positive *Proteus* spp. and *Enterobacter* spp. were tested. All gram-positive cocci were cultured from specimens collected from hospitalized patients, most of whom did not have cancer. A total of 100 isolates of *Staphylococcus aureus,* 25 isolates of *Streptococcus pyogenes,* and 7 isolates of *S. pneumoniae* were tested. The susceptibility of isolates of *S. aureus* to penicillin G was determined by the broth dilution method. Isolates inhibited by less than 0.10 μg/ml were selected as penicillin G susceptible, and isolates resistant to more than 25 μg/ml were selected as penicillin G resistant.

Cefamandole, cephalothin, and cephalexin used in this study were supplied by Eli Lilly & Co., Indianapolis, Ind. Cefoxitin was supplied by Merck, Sharp and Dohme Research Laboratories, Rahway, N.J. Twofold serial dilutions of the antibiotics were prepared with Mueller-Hinton broth, and the minimum inhibitory concentration (MIC) was determined after incubation at 37 C for 18 h. All wells containing trace growth or no discernible growth were subcultured on sheep blood agar. A calibrated pipette was used to transfer 0.01 ml of the inoculum. The minimum bactericidal concentration (MBC) was determined after incubation at 37 C for 18 h. The MBC was defined as the lowest concentration of drug that yielded less than 25 colonies on subculture (95% lysis of the initial inoculum).

RESULTS

The in vitro activity of cefamandole against gram-positive cocci and gram-negative bacilli is shown in Fig. 1. All of the penicillin G-sensitive isolates and 90% of the penicillin G-resistant isolates of *S. aureus* were inhibited by 0.39 μg

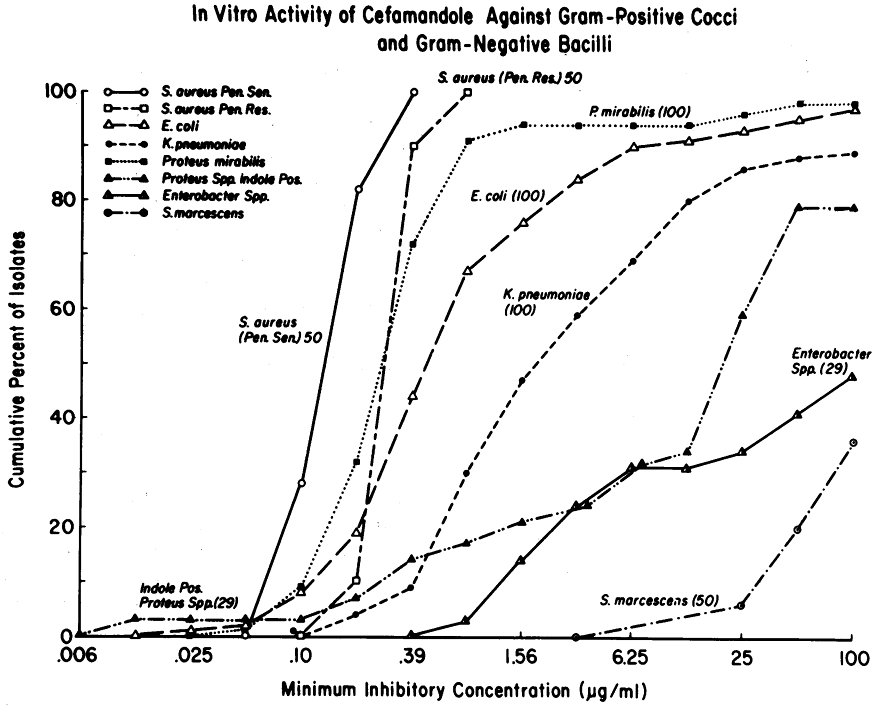

FIG. 1. *In vitro activity of cefamandole against gram-positive cocci and gram-negative bacilli. The numbers in parentheses indicate the number of isolates studied.*

of cefamandole per ml. All of the 25 isolates of *S. pyogenes* and the 7 isolates of *S. pneumoniae* were inhibited by 0.025 µg of cefamandole per ml. At a concentration of 6.25 µg/ml, cefamandole inhibited 94% of the isolates of *P. mirabilis*, 90% of the isolates of *E. coli*, and 69% of the isolates of *K. pneumoniae* but only 31% of the isolates of indole-positive *Proteus* spp. and *Enterobacter* spp. Cefamandole inhibited only 6% of the isolates of *S. marcescens* at a concentration of 25 µg/ml. The MIC was the MBC for all isolates of *S. pyogenes* and *S. pneumoniae*. For the majority of isolates of other organisms, the MIC was the MBC also (Table 1).

The in vitro activity of cefamandole was compared to that of cefoxitin, cephalothin, and cephalexin (Fig. 2 through 6). Cephalothin was the most active cephalosporin against both penicillin G-sensitive and -resistant *S. aureus*, but cefamandole was only slightly less active. Ce-

TABLE 1. *Comparison of MIC and MBC of cefamandole against microorganisms*[a]

Concn of cefamandole (µg/ml)	*S. aureus*		*E. coli*		*K. pneumoniae*		*Proteus* spp.[b]		*S. marcescens*		*Enterobacter* spp.	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
0.10	14	11	8	5			8	7				
0.20	46	43	19	13	4	4	26	17				
0.39	95	93	44	37	9	8	59	43				
0.78	100	100	67	59	30	30	74	70			3	3
1.56			76	73	47	47	78	76			14	10
3.12			84	81	59	59	78	77			24	24
6.25			90	87	69	68	80	78	2	2	31	31
12.5			91	90	80	77	81	78	4	2	31	31
25			93	91	86	84	88	85	6	6	34	34
50			95	94	88	87	94	93	20	16	41	41
100			97	96	89	88	94	94	46	40	48	48

[a] Expressed as cumulative percentage of isolates.

[b] Since results were similar for indole-negative and indole-positive *Proteus* spp., they were combined.

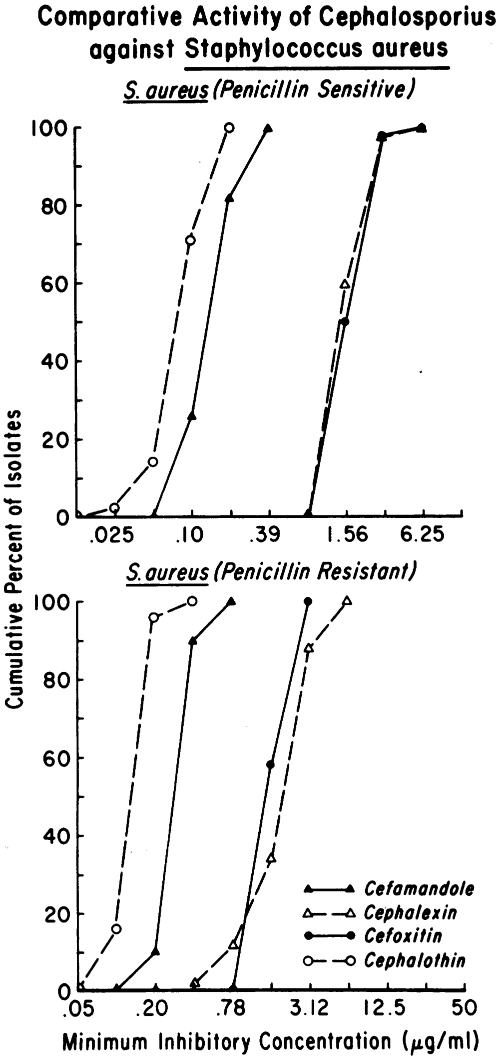

FIG. 2. *Comparative activity of cephalosporins against S. aureus. Fifty isolates of penicillin G-sensitive and 50 isolates of penicillin G-resistant S. aureus were studied.*

foxitin and cephalexin were considerably less active than the other two cephalosporins. Cefamandole was the most active cephalosporin against *E. coli*. At a concentration of 1.56 μg/ml, it inhibited 76% of the isolates, whereas at the same concentration cefoxitin inhibited 28%, and cephalothin and cephalexin inhibited only 5% of isolates. All four cephalosporins had similar activity against *K. pneumoniae*. However, only 4% of these isolates was resistant to 25 μg of cephalexin per ml, whereas 14% was resistant to the same concentration of cefamandole. Cefamandole was the only cephalosporin that had substantial activity against isolates of *Enterobacter* spp. Cefoxitin was the most active cephalosporin against indole-positive *Proteus* spp., whereas cefamandole was the most active against *P. mirabilis*. At a concentration of 12.5 μg/ml, cefoxitin inhibited 97% of the former isolates and cefamandole inhibited 34%, but cephalothin and cephalexin inhibited less than 10% of the isolates. Although cefamandole was active against some isolates of *S. marcescens*, cefoxitin was more active. At a concentration of 50 μg/ml, cefamandole inhibited 20% of the isolates, whereas cefoxitin inhibited 52%. Cephalexin and cephalothin were inactive against *S. marcescens*.

The susceptibility to cefamandole of cephalothin-resistant isolates of *E. coli* and *K. pneumoniae* is shown in Table 2. Thirteen of these 22 isolates of *E. coli* were susceptible to 12.5 μg or less of cefamandole per ml. Only 2 of the 18 isolates of *K. pneumoniae* were susceptible to cefamandole. All eight isolates that were resistant to 100 μg of cephalothin per ml were also resistant to 100 μg of cefamandole per ml.

DISCUSSION

Cefamandole was effective in vitro against gram-positive cocci, including penicillin G-resistant isolates of *S. aureus*. Our results are similar to those of Neu who found that isolates of *S. aureus* and *S. pyogenes* were quite sensitive to cefamandole (2). Eykyn et al. compared cefamandole to cephalothin and cephalexin against isolates of *S. aureus* and found results similar to the present study (1).

The activity of cefamandole against gram-negative bacilli was impressive since it included some isolates of *Enterobacter* spp. and *S. marcescens*, which usually are resistant to cephalosporins. It was also active against isolates of *E. coli*, which were resistant to cephalothin. Cefamandole was superior to cephalothin and cephalexin against *E. coli* and *P. mirabilis*, which is in agreement with the results of Neu et al. (2) and Eykyn et al. (1). In our study these three cephalosporins were equally active against *K. pneumoniae*, whereas these other investigators found cefamandole to be more active. Although cefamandole was active against most cephalothin-resistant isolates of *E. coli*, it was not active against most cephalothin-resistant isolates of *K. pneumoniae*. Cefamandole is more resistant to degradation by β-lactamases produced by some gram-negative bacilli, which may explain its activity against cephalothin-resistant *E. coli* (2). Other mechanisms of resistance exist since strains of gram-negative bacilli that fail to hydrolyze cefamandole also may be resistant (2). Although cefamandole

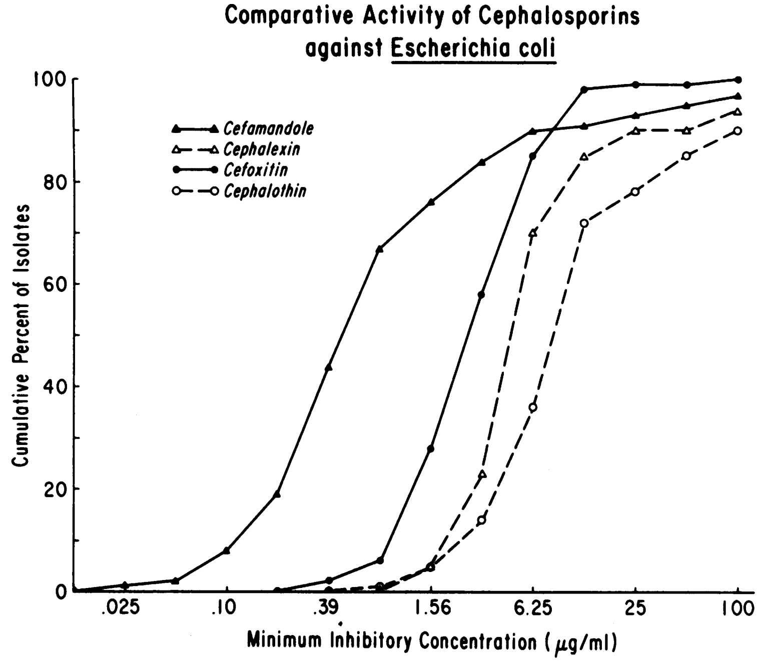

FIG. 3. *Comparative activity of cephalosporins against 100 isolates of E. coli.*

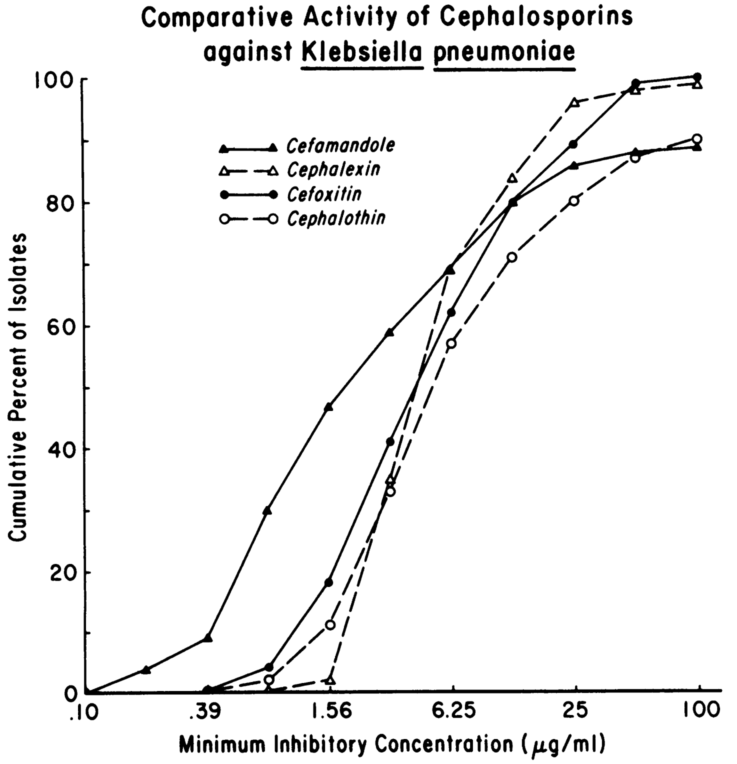

FIG. 4. *Comparative activity of cephalosporins against 100 isolates of K. pneumoniae.*

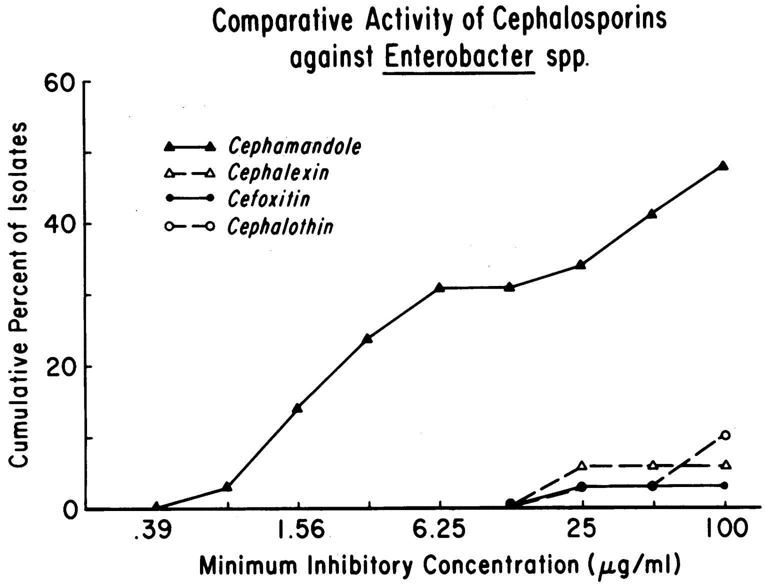

FIG. 5. *Comparative activity of cephalosporins against 29 isolates of Enterobacter spp.*

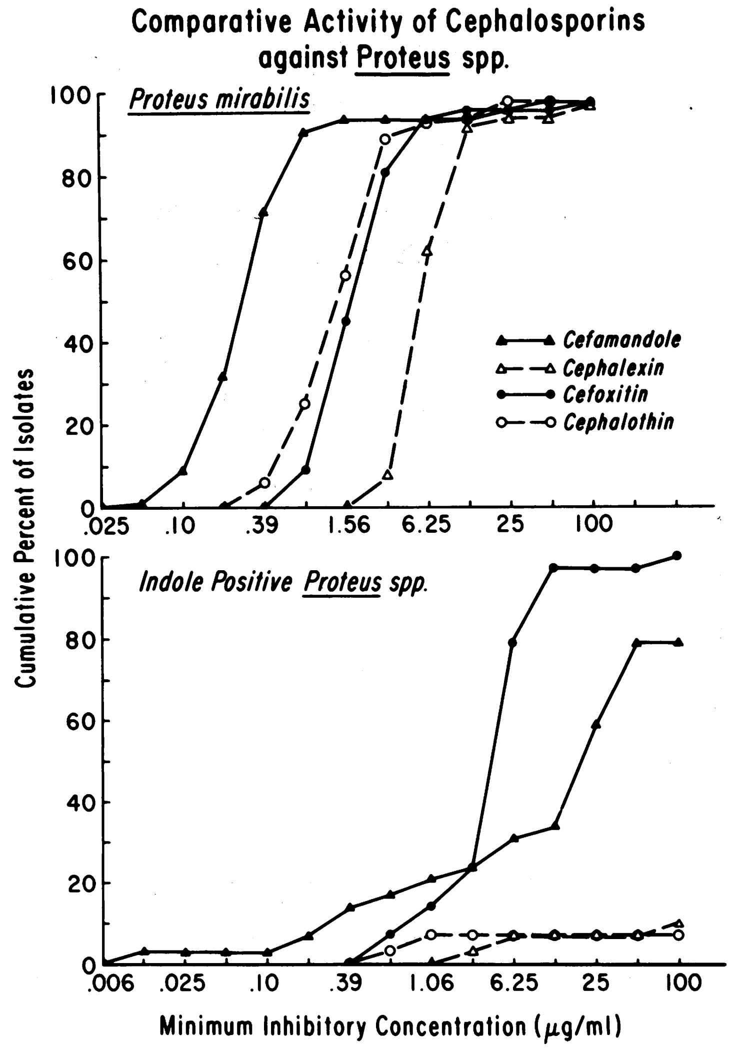

FIG. 6. *Comparative activity of cephalosporins against Proteus spp. One hundred isolates of P. mirabilis and 29 isolates of indole-positive Proteus spp. were studied.*

TABLE 2. *Susceptibility to cefamandole of cephalothin-resistant isolates*

MIC of cephalothin (μg/ml)	No. of isolates at an MIC of cefamandole (μg/ml)											Total no.
	0.20	0.39	0.78	1.56	3.12	6.25	12.5	25	50	100	>100	
E. coli												
50	0	0	1	2	0	0	0	0	1	2	0	6
100	1	0	1	0	1	0	0	0	1	0	2	6
>100	0	1	0	2	1	2	1	2	0	0	1	10
K. pneumoniae												
50	0	0	0	0	0	1	1	3	1	1	0	7
100	0	0	0	0	0	0	0	1	1	0	1	3
>100	0	0	0	0	0	0	0	0	0	0	8	8

was active against a few isolates of *S. marcescens*, it is unlikely to be of clinical importance for the treatment of *Serratia* infections. In our study cefamandole was bactericidal against most isolates at the MIC. Eykyn et al. found the MBC of many isolates to be considerably higher than the MIC, which is difficult to reconcile with our results (1).

ACKNOWLEDGMENT

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