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In Vitro Comparison of Cefoxitin, Cefamandole, Cephalexin, and Cephalothin

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The in vitro effect of cefoxitin, cefamandole, cephalexin, and cephalothin was tested against 645 strains of bacteria recently isolated from clinical sources. Against gram-positive organisms cephalothin and cefamandole were the most effective, generally being three- to fourfold more active than cephalexin or cefoxitin. Enterococci were not inhibited by less than 25 μg of any of the antibiotics per ml. Against *Enterobacteriaceae*, cefoxitin and cefamandole were the most active. An exception was the *Enterobacter* strains, against which cefoxitin was the least effective. None of the *Pseudomonas aeruginosa* strains were susceptible to 100 μg of any of the cephalosporins per ml. Cefamandole was the most active agent against *Neisseria meningitidis* and *Neisseria gonorrhoeae*. It was also the most effective agent against *Haemophilus influenzae*, even when taking into account a threefold inoculum effect.

Two promising antibiotics have recently been developed in the search for a cephalosporin that would combine good in vitro activity with relative resistance to hydrolysis by beta-lactamase. Cefamandole is a new cephalosporin with the formula 7-D-mandelamido-3-[[(1-methyl-1H-tetrazol-5-yl)-thio]methyl]-3-cephem-4-carboxylic acid, formate (ester) sodium salt. Cefoxitin is a semisynthetic cephalosporin-like antibiotic with the formula 3-carbamoyloxymethyl-7-α-methoxy-7[2-(2-thienyl)acetamido]-3-cephem-4-carboxylic acid. Figure 1 illustrates the comparative structural formulas of the antibiotic tested. The purpose of this study is to compare the in vitro antibacterial activity of cefamandole, cefoxitin, cephalothin, and cephalexin against a wide spectrum of bacteria from clinical sources.

MATERIALS AND METHODS

Bacteria. A total of 645 strains of bacteria were tested. These were distributed as follows: 75 *Staphylococcus epidermidis*, 75 *Staphylococcus aureus*, 75 *Escherichia coli*, 50 *Pseudomonas aeruginosa*, 50 *Klebsiella*, 35 *Enterobacter*, 16 *Serratia*, 43 *Proteus mirabilis*, 47 *Proteus rettgeri*, 26 *Salmonella*, 10 *Shigella*, 25 group D streptococci, 21 alpha-hemolytic streptococci (non-enterococcal), 23 group A beta-hemolytic streptococci, 12 *Streptococcus pneumoniae*, 27 *Neisseria gonorrhoeae*, 20 *Neisseria meningitidis*, and 15 *Haemophilus influenzae*. Most of these organisms were recently isolated from clinical sources and identified in the Clinical Microbiology Laboratory of Colorado General Hospital under the direction of L. Barth Reller. The strains of *N. gonorrhoeae* were supplied by the Colorado General Hospital Veneral Disease Clinic Laboratory under the direction of Peter E. Dans. Most of the strains of *Salmonella* and *Shigella* were obtained from the Colorado State Public Health Laboratory. The *P. rettgeri* isolates were provided by F. Marc LaForce, of the Denver Veterans Administration Hospital.

Cephalosporins. Standard reference powders of cefamandole lithium, cephalexin monohydrate, and sodium cephalothin were kindly provided by Eli Lilly and Co. Sodium cefoxitin was kindly provided by Merck, Sharp and Dohme.

Susceptibility testing methods: (i) Broth MIC. The minimal inhibitory concentrations (MIC) of *S. aureus*, *S. epidermidis*, *E. coli*, *P. aeruginosa*, *Klebsiella*, *Enterobacter*, *Serratia*, *Proteus*, *Salmonella*, *Shigella*, and group D streptococci were determined by a microtiter broth dilution technique. Serial twofold dilutions of freshly prepared antibiotics were made in Mueller-Hinton broth, with the exception of the group D streptococci which were tested in Trypticase soy broth. Overnight broth cultures of the organisms were diluted 10^{-5}, and 0.05 ml was added to 0.05 ml of the diluted antibiotic in microtiter plates (Cooke Engineering Co.). Plates were incubated overnight in ambient air at 35 C. The MIC was defined as the lowest concentration of antibiotic in which there was no visible growth. The minimal bactericidal concentration was determined by using an adaptation of the Steers, Foltz, and Graves replicator (11). Each well of all microtiter plates was subcultured on Mueller-Hinton agar. The group D streptococci were subcultured on Mueller-Hinton agar supplemented with 4% defibrinated sheep blood. All subcultures were incubated in ambient air at 35 C for 24 h.

(ii) Agar dilution method. The antibiotic dilution technique, using the inocula-replicating method of Steers et al. (11), was used to test the strains of *S. pneumoniae*, alpha-hemolytic streptococci (non-enterococcal), group A beta-hemolytic streptococci, *N. meningitidis*, *N. gonorrhoeae*, and *H. influenzae*.

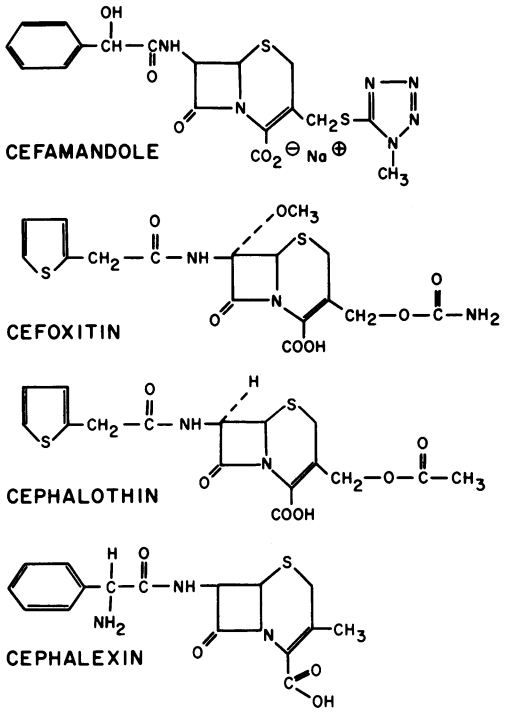

FIG. 1. *Chemical structures of cefamandole, cefoxitin, cephalothin, and cephalexin.*

The *S. pneumoniae*, alpha-hemolytic streptococci (non-enterococcal), and group A beta-hemolytic streptococci were tested on Mueller-Hinton agar supplemented with 4% defibrinated sheep blood. The *N. meningitidis*, *N. gonorrhoeae*, and *H. influenzae* strains were tested for susceptibility on GC medium base (Difco) supplemented with 2% hemoglobin (BBL) and 1% IsoVitaleX (BBL).

As inoculum, a volume of approximately 0.003 ml of an overnight undiluted broth culture was applied to the surface of the antibiotic-containing agar plates with the Steers replicator. The group A beta-hemolytic streptococci, the *S. pneumoniae*, and the alpha-hemolytic streptococci (non-enterococcal) were grown in Trypticase soy broth with 4% defibrinated sheep blood added. The group A beta-hemolytic streptococci and the *S. pneumoniae* inocula contained approximately 10^5 colony-forming units/ml. The alpha-hemolytic streptococci (non-enterococcal) contained 10^6 colony-forming units/ml. The *N. meningitidis* and the *N. gonorrhoeae* were grown overnight in Mueller-Hinton broth supplemented with 2% hemoglobin and 1% IsoVitaleX. Each of these inovula contained approximately 10^6 colony-forming units/ml. The *H. influenzae* were also grown overnight in the hemoglobin- and IsoVitaleX-supplemented Mueller-Hinton broth, but diluted 10^{-3}. This gave an inoculum of approximately 7×10^4 colony-forming units/ml. The plates were incubated for 24 to 36 h at 35 C in a 5% CO_2 atmosphere.

Inoculum effect. The effect of inoculum size was evaluated by using two inocula sizes, a 10^{-2} and a 10^{-5} dilution of an overnight broth culture for five strains each of *S. aureus*, *E. coli*, *Klebsiella*, and *P. mirabilis*. All 15 strains of *H. influenzae* were tested by using both a 10^{-3} dilution and an undiluted overnight broth culture.

RESULTS

The six panels of Fig. 2 show the cumulative percentages of the common gram-positive organisms susceptible to increasing concentrations of each antibiotic. Cefoxitin and cephalexin were much less effective than cephalothin and cefamandole against most gram-positive organisms. Group D streptococci were relatively unsusceptible to all of the cephalosporins tested. Cephalothin and cefamandole were again the most effective antibiotics tested, with 88% of the strains inhibited at 100 μg/ml.

A different pattern was found with gram-negative organisms (Fig. 3). Generally, cefamandole and cefoxitin appeared to be the most effective agents, with cephalexin third and cephalothin the least effective. Cefamandole inhibited 77% of the *E. coli* tested at 1.6 μg/ml, whereas cefoxitin inhibited only 24%. Similar results are shown for *Klebsiella*. Cefamandole was quite effective against *Shigella*, with 0.4 μg/ml inhibiting 9 out of 10 strains tested. All 10 strains were inhibited by 6.2 μg of cefamandole or cefoxitin per ml.

The *Salmonella* tested seemed to have a bimodal distribution when tested against cefamandole, with 62% of the strains inhibited by 1.6 μg/ml. Cefoxitin inhibited 96% of the strains at 6.2 μg/ml. *P. mirabilis* was quite susceptible to cefamandole, with 100% of the strains inhibited by 3.2 μg/ml. Cefoxitin was the next most active drug, with 98% of strains inhibited by 6.2 μg/ml. Forty-seven strains of *P. rettgeri* were tested and, again, cefamandole was the most active, with 64% of the strains susceptible to 0.8 μg/ml, whereas only 6% were susceptible to cefoxitin at that level. This activity of cefamandole is of particular interest, since disk susceptibility testing had previously demonstrated the multiple antibiotic resistance of these strains. Fifty-five percent were resistant to cephalothin, 73% to kanamycin, 100% to tetracycline, 100% to furadantin, 54% to ampicillin, 47% to gentamicin, and 43% to carbenicillin. *Serratia*, although intrinsically resistant to cephalosporin antibiotics, showed susceptibility to cefamandole, with 14 out of 16 strains inhibited by 25 μg/ml or less. A similar result was seen with cefoxitin, 15 out of 16 strains being inhibited by 25 μg/ml or less. Cefamandole was markedly more effective against *Enterobacter* than any of the other three antibiotics. *P. aeruginosa* maintained its characteristic

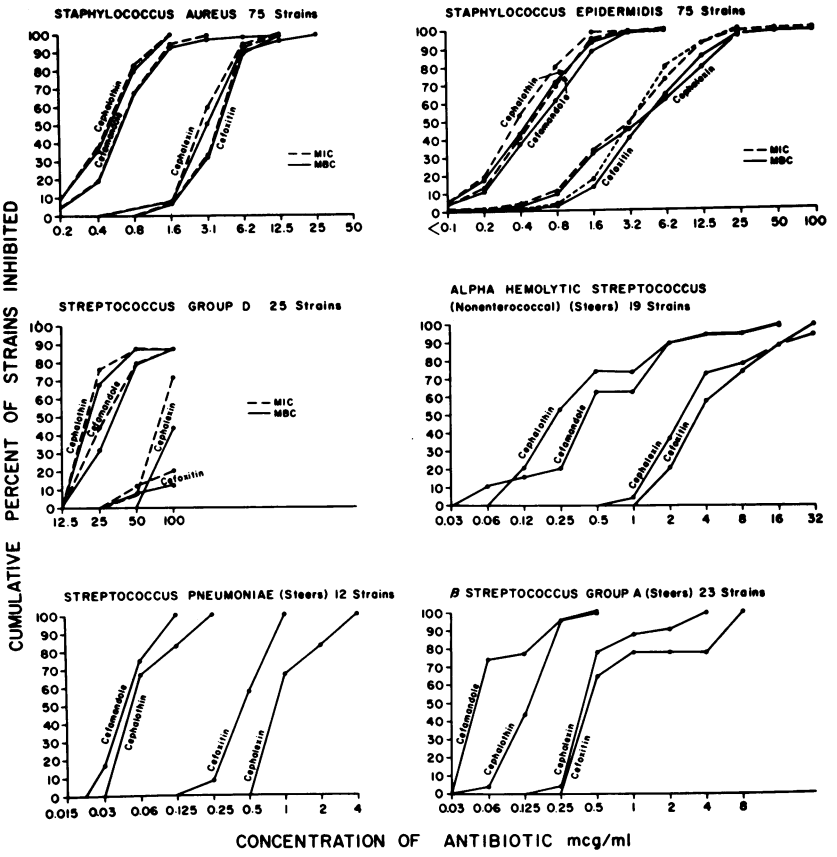

FIG. 2. *Susceptibility of gram-positive cocci to cephalothin, cefamandole, cephalexin, and cefoxitin.*

resistance to cephalosporins. Fifty strains were tested, and none of them were susceptible to any of the four antibiotics at a concentration of 100 μg/ml.

N. meningitidis, *N. gonorrhoeae*, and *H. influenzae* were also examined (Fig. 4). Cefamandole again appeared to be the most effective drug, especially with *N. meningitidis*.

Inoculum effect. To evaluate the inoculum effect on *H. influenzae*, all 15 strains (including six organisms that had MICs to ampicillin ranging from 6.2 to 100 μg/ml) were tested against two inocula, an overnight broth culture (7×10^7 organisms/ml) and a 10^{-3} dilution of the culture (7×10^4 organisms/ml). Cefamandole and cephalothin both demonstrated a significant inoculum effect (Table 1). The ampicillin-susceptible strains showed no or, at the most, a onefold increase in their MICs to cefamandole, whereas the ampicillin-resistant strains showed a three- to fourfold increase. With cephalothin the effect was strain variable, with some strains showing a fourfold or more increase. There was no correlation between ampicillin resistance and inoculum effect.

The MIC of *S. aureus* was not significantly altered by the size of the inoculum (Table 2). The minimal bactericidal concentration, however, was usually raised approximately fivefold by the heavier inoculum. This was true for all four of the antibiotics tested.

When the *Enterobacteriaceae* were tested against cefoxitin both the MIC and the minimal bactericidal concentration were unchanged by the inoculum size (Table 3). With the other three cephalosporins there was strain variation with no consistent pattern.

DISCUSSION

Our study comparing two new cephalosporins, cefamandole and cefoxitin, with two older ones, cephalexin and cephalothin, indicates that both new drugs have specific advantages. As found in other studies, cefamandole has a striking in vitro antibacterial spectrum (2, 7).

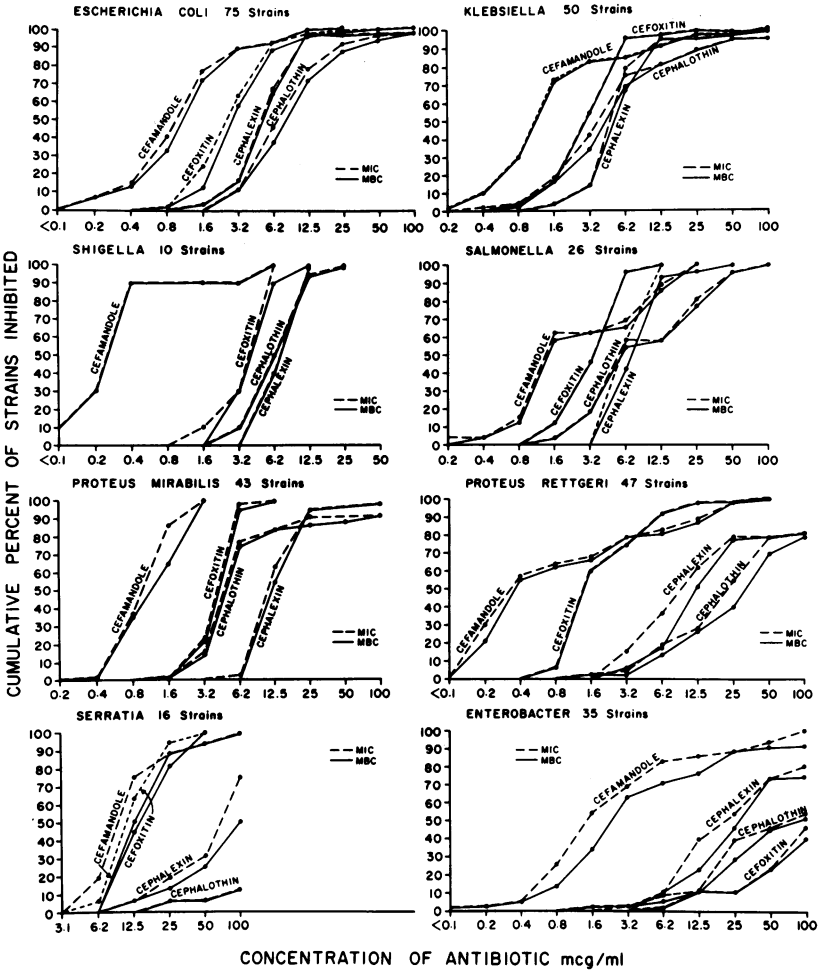

FIG. 3. *Susceptibility of Enterobacteriaceae to cephalothin, cefamandole, cephalexin, and cefoxitin.*

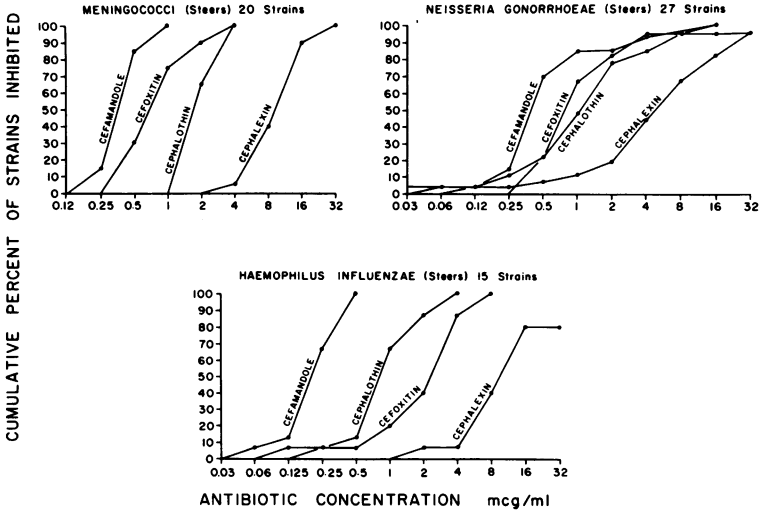

FIG. 4. *Susceptibility of N. meningitidis, N. gonorrhoeae, and H. influenzae to cephalothin, cefamandole, cephalexin, and cefoxitin.*

Its activity against gram-positive organisms is comparable to cephalothin and clearly superior to cefoxitin and cephalexin. Its activity against the *Enterobacteriaceae* is generally comparable to that of cefoxitin. Like cefoxitin, it inhibits a number of *Serratia* and indole-positive *Proteus*

TABLE 1. *Inoculum effect against H. influenzae*

Strain	MIC (μg/ml)								
	Ampicillin (10^{-4})	Cefoxitin		Cefamandole		Cephalexin		Cephalothin	
		Undiluted	10^{-3}	Undiluted	10^{-3}	Undiluted	10^{-3}	Undiluted	10^{-3}
1	0.2	4	2	0.5	0.25	16	8	1	1
2	<0.1	4	1	0.5	0.25	32	16	2	2
3	<0.1	4	2	0.5	0.25	>32	32	>32	4
4	<0.1	4	4	0.5	0.5	>32	32	>32	2
5	0.4	8	8	1	1	>32	32	>32	4
6	0.2	4	4	0.5	0.5	>32	16	>32	2
7	0.2	4	2	2	0.5	16	16	2	1
8	0.4	1	0.125	0.125	0.06	4	2	0.5	0.25
9	0.2	4	4	1	0.5	16	8	2	1
10	3.1	4	1	4	0.25	16	8	4	0.5
11	>100	4	4	4	0.25	16	16	2	1
12	25	4	4	2	0.5	16	8	2	1
13	12.5	4	4	4	0.25	16	16	2	1
14	100	4	4	2	0.25	16	16	4	1
15	6.2	4	4	2	0.25	16	8	4	1

TABLE 2. *Inoculum effect against five strains of S. aureus*

Strain	Cefoxitin				Cefamandole				Cephalexin				Cephalothin			
	10^{-2}		10^{-5}		10^{-2}		10^{-5}		10^{-2}		10^{-5}		10^{-2}		10^{-5}	
	MIC[a]	MBC[a]	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
1	6.2	6.2	6.2	6.2	3.1	100	0.4	0.4	6.2	>100	6.2	6.2	1.6	>100	0.4	0.4
2	3.1	>100	3.1	3.1	1.6	>100	0.8	0.8	6.2	>100	6.2	6.2	0.4	>100	1.6	1.6
3	3.1	>100	3.1	3.1	0.4	>100	0.8	0.8	3.1	>100	3.1	3.1	1.6	>100	0.8	0.8
4	3.1	>100	3.1	3.1	6.2	>100	1.6	1.6	12.5	>100	6.2	6.2	1.6	>100	1.6	1.6
5	3.1	>100	3.1	3.1	0.8	>100	0.4	0.4	12.5	>100	12.5	12.5	0.4	>100	1.6	1.6

[a] MIC and minimal bactericidal concentrations (MBC) are given in micrograms per milliliter

TABLE 3. *Inoculum effect against Enterobacteriaceae*

Organism	Cefoxitin				Cefamandole				Cephalexin				Cephalothin			
	10^{-2}		10^{-5}		10^{-2}		10^{-5}		10^{-2}		10^{-5}		10^{-2}		10^{-5}	
	MIC[a]	MBC[a]	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. coli	6.2	6.2	6.2	6.2	>100	>100	6.2	6.2	>100	>100	6.2	6.2	>100	>100	25	25
	6.2	6.2	6.2	6.2	3.1	3.1	1.6	1.6	12.5	12.5	12.5	12.5	6.2	6.2	6.2	6.2
	3.1	3.1	6.2	6.2	3.1	3.1	1.6	1.6	25	50	12.5	12.5	50	50	12.5	12.5
	6.2	6.2	12.5	12.5	3.1	3.1	1.6	3.1	50	100	12.5	12.5	50	50	25	25
	6.2	6.2	3.1	3.1	3.1	3.1	0.8	1.6	25	25	6.2	6.2	50	50	6.2	6.2
Klebsiella	25	25	25	25	25	25	6.2	6.2	25	25	12.5	12.5	50	50	25	25
	>100	>100	>100	>100	>100	>100	3.1	3.1	>100	>100	>100	>100	>100	>100	>100	>100
	6.2	6.2	6.2	6.2	6.2	6.2	0.4	0.4	6.2	6.2	3.1	3.1	12.5	12.5	3.1	3.1
	6.2	6.2	6.2	6.2	12.5	12.5	1.6	1.6	6.2	6.2	6.2	6.2	12.5	12.5	6.2	6.2
	3.1	3.1	3.1	3.1	12.5	12.5	1.6	1.6	6.2	6.2	6.2	6.2	12.5	12.5	1.6	1.6
P. mirabilis	25	25	6.2	6.2	12.5	12.5	3.1	3.1	50	50	12.5	12.5	12.5	12.5	6.2	6.2
	25	25	6.2	6.2	12.5	12.5	3.1	3.1	50	50	12.5	12.5	6.2	6.2	6.2	6.2
	25	25	3.1	6.2	0.8	1.6	1.6	1.6	50	50	12.5	12.5	6.2	6.2	3.1	3.1
	6.2	6.2	3.1	3.1	3.1	3.1	0.8	0.8	25	25	12.5	12.5	6.2	6.2	1.6	1.6
	6.2	6.2	3.1	3.1	3.1	3.1	0.8	0.8	25	25	12.5	12.5	6.2	6.2	3.1	3.1

[a] MIC and minimal bactericidal concentrations (MBC) are given in micrograms per milliliter.

sp. It was significantly more active than cefoxitin when tested against *P. mirabilis, P. rettgeri,* and *Enterobacter*. As reported by other investigators, the low MIC range for cefamandole when tested against *H. influenzae* is impressive (2, 5), but account should be taken of the inoculum effect when testing beta-lactamase-producing, ampicillin-resistant strains.

Cefoxitin has been shown to be less active against gram-positive cocci than cephalothin (1, 3, 6, 8). This study also shows it to be less active than cefamandole and comparable to cephalexin in its activity. One of the properties of cefoxitin is its resistance to hydrolysis by gram-negative-produced beta-lactamase (EC 3.5.2.6, penicillin [cephalosporin] amido-beta-lactam hydrolase) (9). As a consequence it has been shown that there is not a significant inoculum effect when cefoxitin is tested against *Enterobacteriaceae*. With four out of five strains of *S. aureus* the MIC was not affected by increased inoculum, but the minimal bactericidal concentration was elevated. This may suggest that cefoxitin is less resistant to staphylococcal penicillinase. It has been shown that staphylococcal penicillinase can hydrolyze cephaloridine and cephalothin (4, 10).

Against the *Enterobacteriaceae* other than *Enterobacter,* cefoxitin was markedly superior to cephalexin and cephalothin. This is in agreement with other recent studies (1, 3, 6, 8). Cefamandole is slightly more effective against *E. coli* than cefoxitin and significantly more effective against *Proteus* and *Enterobacter*. However, cefoxitin seems to be more effective than cefamandole against some strains of *Salmonella* and *Serratia*. Cefoxitin was somewhat less active against *H. influenzae* than cefamandole and cephalothin but, unlike them, did not show an inoculum effect.

This in vitro study has shown that cefamandole, with its increased spectrum and high intrinsic activity, and cefoxitin, with its resistance to hydrolysis by beta-lactamase, both offer distinct advantages over cephalexin and cephalothin. This data would support further in vitro and in vivo investigation of these two drugs.

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