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Susceptibility of clinical isolates of bacteria to cefamandole, cefoxitin and cephalothin

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The in vitro susceptibility was determined of 274 isolates to cephalothin and two new antibiotics, cefamandole and cefoxitin. Cefamandole was comparable to cephalothin in preventing growth of cultures of the gram positive organisms except for penicillin-resistant Staphylococcus aureus *which was more sensitive to cephalothin. Cefamandole was more active than cephalothin against all the gram negative bacteria including* Haemophilus influenzae *and in addition it was active against many strains of* Enterobacter sp. *Cefoxitin was less active than cephalothin against the gram positive organisms but it was more active against most of the gram negative bacteria. In addition, it was active against* Serratia *and indole positive* Proteus *which are uniformly resistant to cephalothin.*

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THE cephalosporin antibiotics have a wide spectrum of activity against gram positive and gram negative bacteria. However, certain *Enterobacteriaceae* such as *Serratia, Enterobacter* and indole positive *Proteus* are resistant to the commercially available cephalosporins. This resistance is related, at least in part, to the susceptibility of the antibiotics to hydrolysis by the β-lactamases produced by these gram negative organisms.[1,2] In the case of *Pseudomonas aeruginosa* the resistance to β-lactam antibiotics seems to be primarily due to an intrinsic resistance rather than to β-lactamase.[3]

Two investigational antibiotics: cefamandole, a new cephalosporin and cefoxitin, a cephamycin derivative, have been shown to have a wider spectrum of activity against gram negative organisms than the currently available cephalosporins.[4-7] In addition, cefoxitin is also active against *Bacteroides fragilis* which is usually resistant to the cephalosporin antibiotics.[8] Cefoxitin has an increased resistance to inactivation by the β-lactamase of certain gram negative bacteria probably related to the presence of an alpha methoxy group in position C7 of its lactam ring[9] (Figure 1).

The purpose of this study was to compare the in vitro activity of cefamandole and cefoxitin with that of cephalothin against recent isolates of bacteria from Henry Ford Hospital.

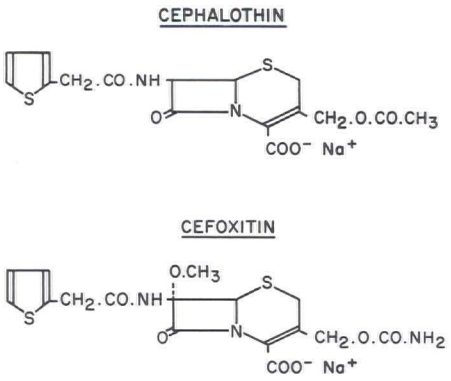

Figure 1
Chemical structure of cephalothin and cefoxitin.

Material and methods

The activity of cefamandole, cefoxitin and cephalothin against 274 isolates was determined by the agar dilution method[10] utilizing Mueller-Hinton agar (BBL), except in the case of *Haemophilus influenzae* where GC Medium Base (BBL) was used. Inoculation of the agar plates, containing two fold dilutions of the antibiotics, was performed using the Steers replicator.[11] Approximately 10^5 organisms were delivered to each plate for each representative organism. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of antibiotic which prevented visible growth after 18 hours of incubation (24 hours for *H. influenzae*) at 37°C. The susceptibility of the following bacteria was determined: 39 strains of *Escherichia coli* (including 19 cephalothin-resistant strains), 24 strains each of penicillin resistant *Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella* sp., and *Streptococcus faecalis*, 23 strains of *P. aeruginosa*, 22 strains of group A beta hemolytic streptococcus, 20 strains of penicillin sensitive *Staphylococcus aureus*, 18 strains of alpha streptococcus, 15 strains each of indole positive *Proteus* and *H. influenzae*, 14 strains of *Enterobacter* sp., and 12 of *Serratia* sp.

Disc susceptibility testing was done according to the standardized disc technique recommended by the Food and Drug Administration.[12,13] Thirty microgram discs were used for the three antibiotics. The zone diameters were then plotted against the MIC values obtained with the agar dilution method, and a regression line was calculated by the method of least squares.

Results and discussion

Table 1 compares the MIC's and the zones of inhibition of the three antibiotics against the gram positive organisms tested. It can be seen that cefamandole was as active as cephalothin against all of them except penicillin resistant *S. aureus* which was more sensitive to cephalothin. Cefoxitin was less active than cephalothin against all the gram positive organisms tested. All three antibiotics were inactive against *S. faecalis*.

Table 2 compares the MIC's and the zones of inhibition of the three antibiotics against the gram negative organisms. Cefamandole was more active than cephalothin against all the gram negative bacteria including *H. influenzae*, and in addition it was active against many strains of *Enterobacter* sp. Cefamandole was more active than cefoxitin against all the gram negative bacteria except *Serratia* sp. and indole positive *Proteus*. Cefoxitin compared favorably with cephalthin and in addition it was active against *Serratia* sp. and indole positive *Proteus*. All three antibiotics were inactive against *P. aeruginosa*.

Figures 2 to 8 show the activity of the three antibiotics against some of the organisms tested, expressed as cumulative percent of strains inhibited at increasing MIC's. One hundred percent of strains of group A beta hemolytic streptococcus were inhibited by 0.048 μg/ml of cefamandole, 0.39 μg/ml of cephalothin and 0.78 μg/ml of cefoxitin (Figure 2). All strains of penicillin resistant *S. aureus* were inhibited by 0.39 μg/ml of cephalothin, whereas 3.1 μg/ml of cefa-

Table 1.
In Vitro Activity of Cefamandole, Cefoxitin and Cephalothin Against Gram Positive Organisms

Organisms and Number of Strains	Cefamandole		Cefoxitin		Cephalothin	
	MIC[a] (μg / ml)	Zone of Inhibition[b] (mm)	MIC (μg / ml)	Zone of Inhibition (mm)	MIC (μg / ml)	Zone of Inhibition (mm)
Group A hemolytic streptococcus (22)	<0.033	41.8	0.625	34.0	<0.075	34.9
S. aureus						
Penicillin res. (24)	0.984	27.6	3.51	29.2	0.329	31.3
Penicillin sen. (20)	0.209	40.2	2.61	30.1	0.193	39.5
Alpha streptococcus (18)	0.143	43.9	1.82	32.7	0.389	37.7
S. faecalis (24)	34.3	12.9	50.0	6	25.0	14.9

[a] Geometric mean
[b] Arithmetic mean

Table 2.
In Vitro Activity of Cefamandole, Cefoxitin and Cephalothin Against Gram Negative Organisms

Organisms and Number of Strains	Cefamandole		Cefoxitin		Cephalothin	
	MIC[a] (μg / ml)	Zone of Inhibition[b] (mm)	MIC (μg / ml)	Zone of Inhibition (mm)	MIC (μg / ml)	Zone of Inhibition (mm)
E. coli (20	0.984	26.5	3.71	26.1	8.47	17.6
Klebsiella sp. (24)	1.43	25.5	4.95	22.8	4.60	21.4
Enterobacter sp (14)	7.99	23.4	>33.6	10.6	>50	6.0
P. mirabilis (24)	0.989	28.2	2.77	23.9	5.14	24.5
Indole positive Proteus (15)	>17.3	16.5	8.63	19.3	>50	6.0
Serratia sp. (12)	>26.5	14.9	18.7	17.6	>50	6.0
P. aeruginosa (23)	>50	6.0	>50	6.0	>50	6.0
H. influenzae (15)	0.389	26.2	6.84	21.1	1.70	23.1

[a] Geometric mean
[b] Arithmetic mean

mandole and 6.2 μg/ml of cefoxitin were needed to inhibit all strains (Figure 3).

Against *E. coli*, a concentration of 1.56 μg/ml of cefamandole inhibited 90% of strains, whereas at the same concentration, only about 20% of strains were inhibited by cephalothin and cefoxitin (Figure 4). We also tested 19 strains of cephalothin resistant *E. coli* (not shown in the graph), and found that both cefamandole and cefoxitin inhibited about 50% of them at a concentration of 12.5 μg/ml. This concentration can be readily achieved with doses of cefamandole and cefoxitin recommended in current clinical trials. Previous studies have shown that the

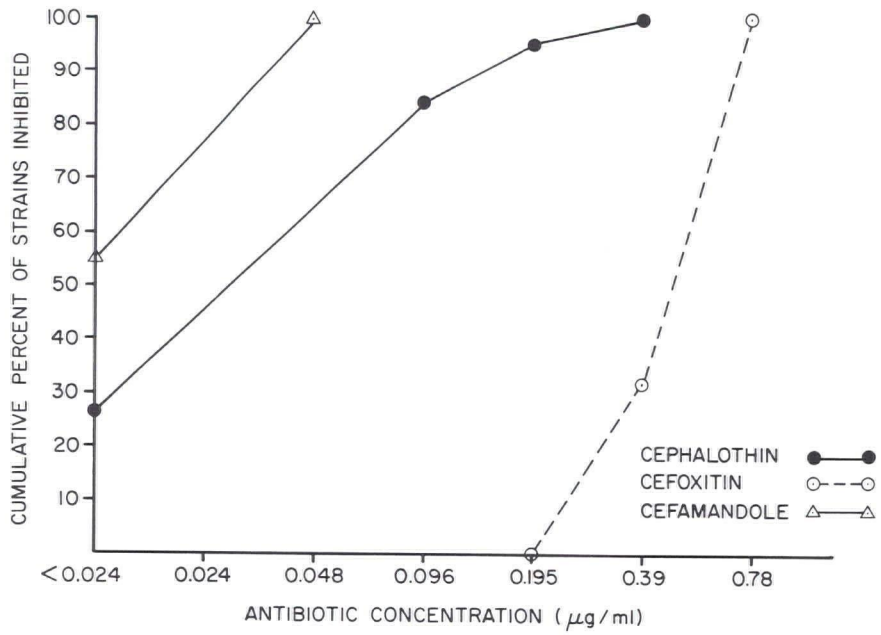

Figure 2
Cumulative percentage of group A hemolytic streptococcus inhibited by increasing concentrations of cefamandole, cefoxitin and cephalothin.

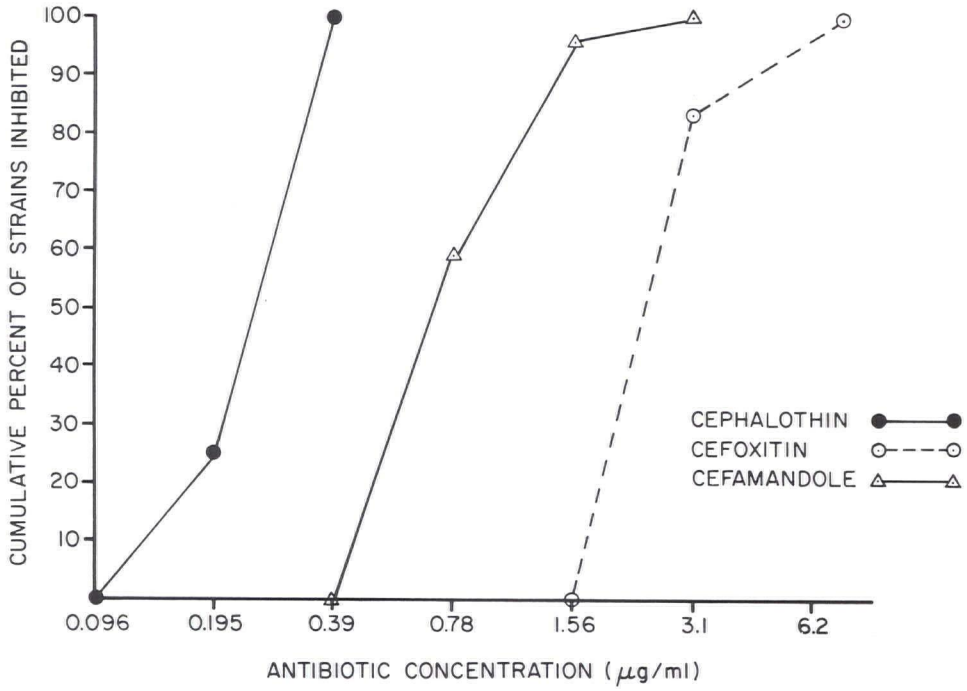

Figure 3
Cumulative percentage of penicillin resistant *S. aureus* inhibited by increasing concentrations of cefamandole, cefoxitin and cephalothin.

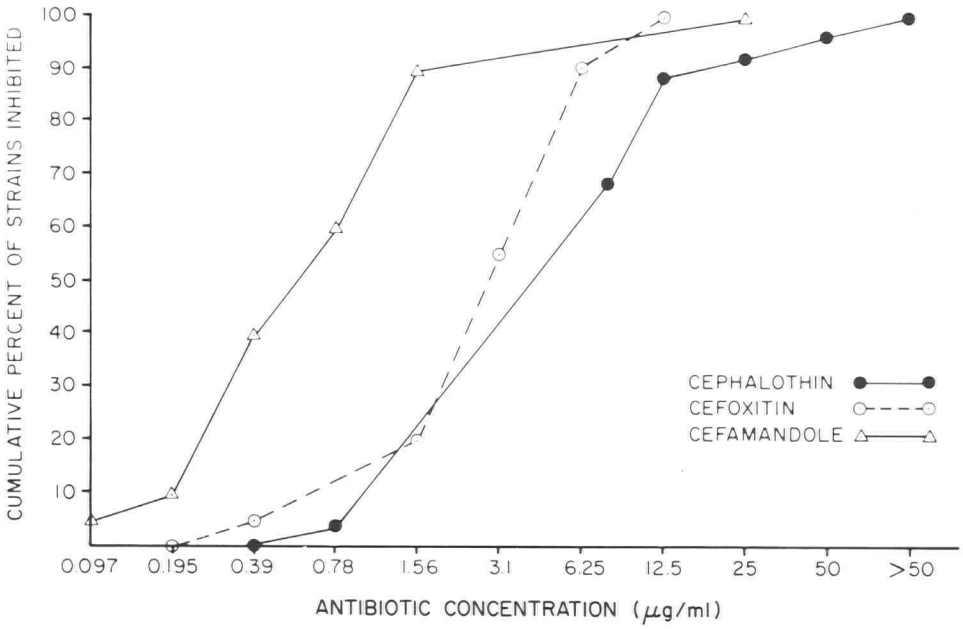

Figure 4
Cumulative percentage of *E. coli* inhibited by increasing concentrations of cefamandole, cefoxitin and cephalothin.

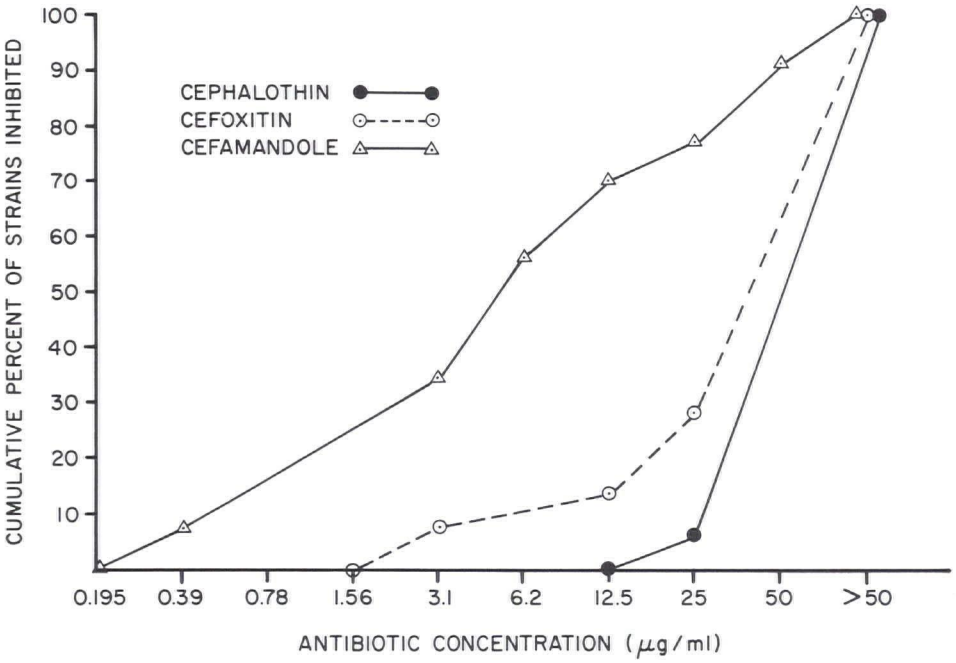

Figure 5
Cumulative percentage of *Enterobacter sp.* inhibited by increasing concentrations of cefamandole, cefoxitin and cephalothin.

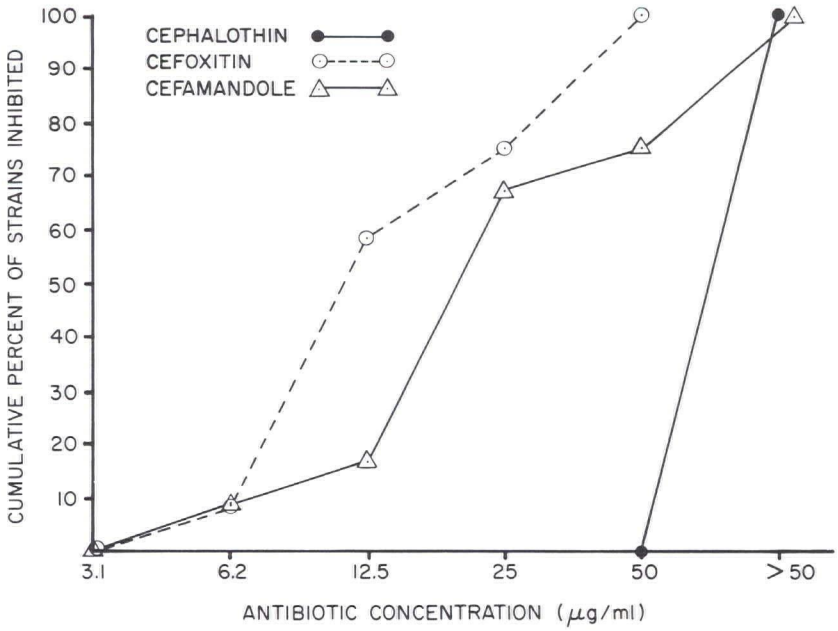

Figure 6
Cumulative percentage of *Serratia sp.* inhibited by increasing concentrations of cefamandole, cefoxitin and cephalothin.

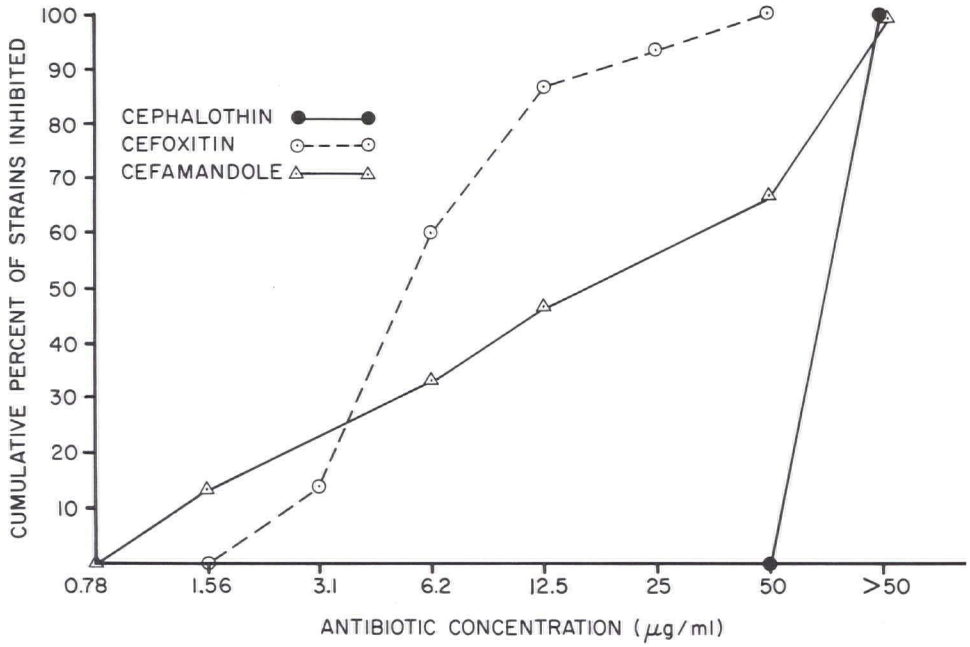

Figure 7
Cumulative percentage of indole positive *Proteus* inhibited by increasing concentrations of cefamandole, cefoxitin and cephalothin.

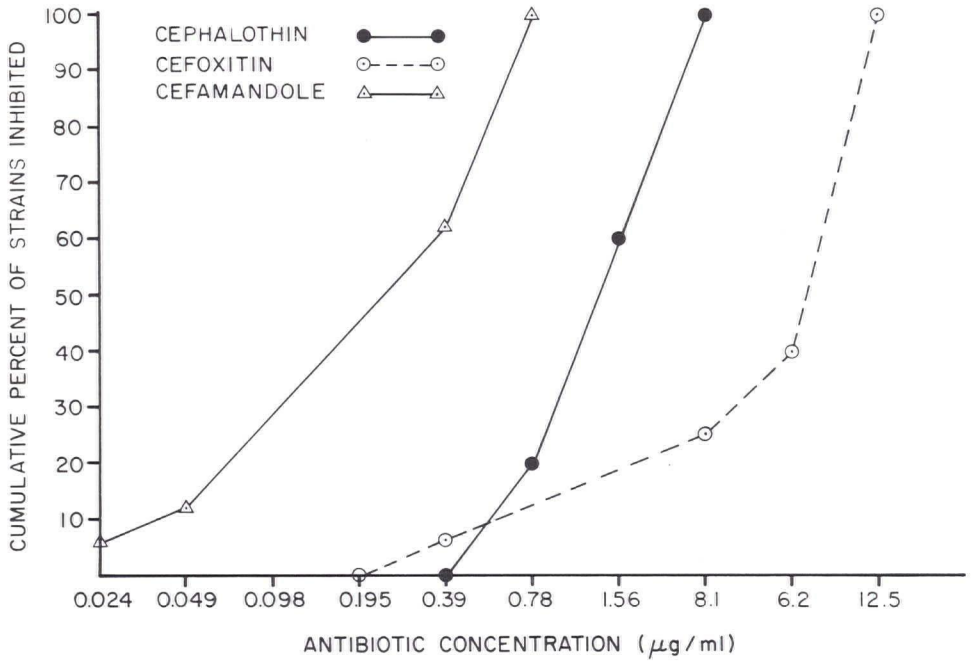

Figure 8
Cumulative percentage of *H. influenzae* inhibited by increasing concentrations of cefamandole, cefoxitin and cephalothin.

peak serum levels of cefamandole and cefoxitin are comparable to those of cephalothin and their serum half lives are more prolonged.[7,14,15]

Against *Enterobacter* sp., cefamandole at a concentration of 12.5 μg/ml inhibited 70% of strains, whereas cefoxitin inhibited 15% and cephalothin inhibited none of them (Figure 5). Against *Serratia* sp., cefoxitin was the most active antibiotic, and at a concentration of 12.5 μg/ml, it inhibited 60% of strains while cefamandole inhibited only 15% and cephalothin was uniformly inactive (Figure 6). Cefoxitin was also the most active against indole positive *Proteus*; at a concentration of 12.5μg/ml it inhibited 85% of strains, while cefamandole inhibited only half of the strains and cephalothin none of them (Figure 7). All strains of *H. influenzae* were inhibited by 12.5 μg/ml or less of the three antibiotics. However, cefamandole was much more active, and at a concentration of less than 1 μg/ml, it inhibited all strains (Figure 8).

Disc susceptibility tests: Using the established criteria for all cephalosporin antibiotics (i.e., that a zone of inhibition of 18 mm or more, with a 30 μg antibiotic disc, indicates susceptibility) we found that all the gram positive organisms tested (except *S. faecalis*) were susceptible to the three antibiotics. With the gram negative organisms, however, we found that 72% of the isolates were sensitive to cefoxitin, 68% to cefamandole

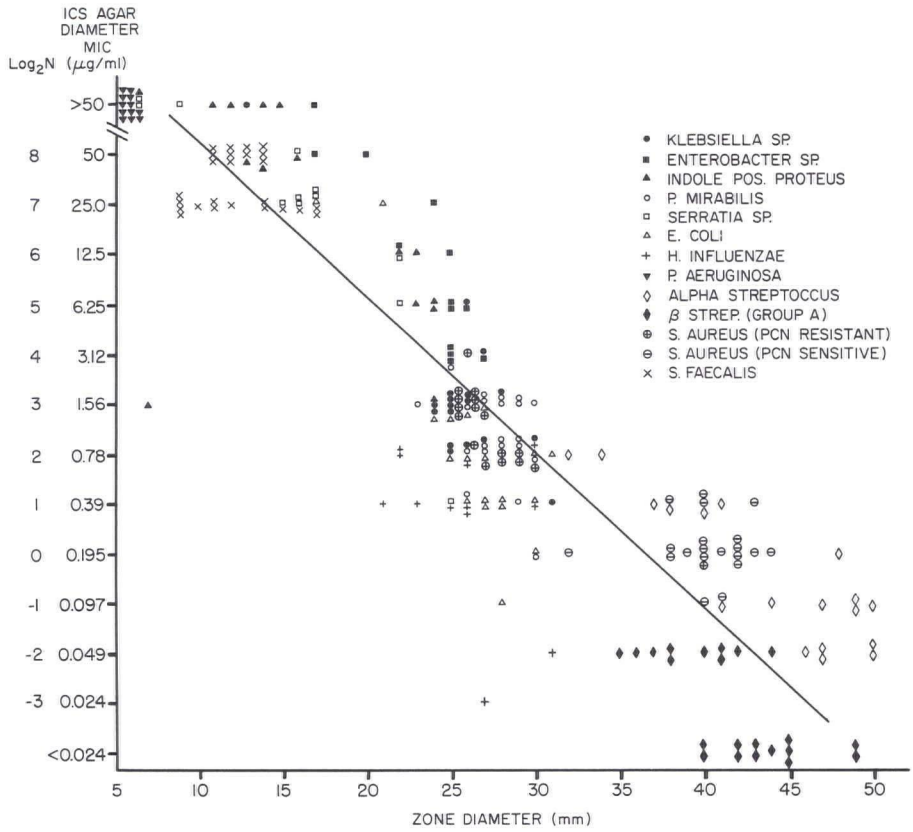

Figure 9
Regression line correlating disc zone sizes with MIC's of cefamandole.

and only 47% were sensitive to cephalothin. The greater number of susceptible organisms to cefoxitin and cefamandole was related mainly to the increased susceptibility of *Serratia* sp. indole positive *Proteus*, *Enterobacter* sp. and *E. coli*.

The correlation of activity of cefamandole and cefoxitin as determined by the standardized disc technique and the agar dilution method is shown in Figures 9 and 10. The regression curve for cefamandole shows that the accepted cutoff point for susceptibility of the cephalosporins (18 mm), corresponds to an MIC value of 10 μg/ml (Figure 9). For cefoxitin an 18 mm inhibition zone corresponds to an MIC of 12.5 μg/ml. It should be

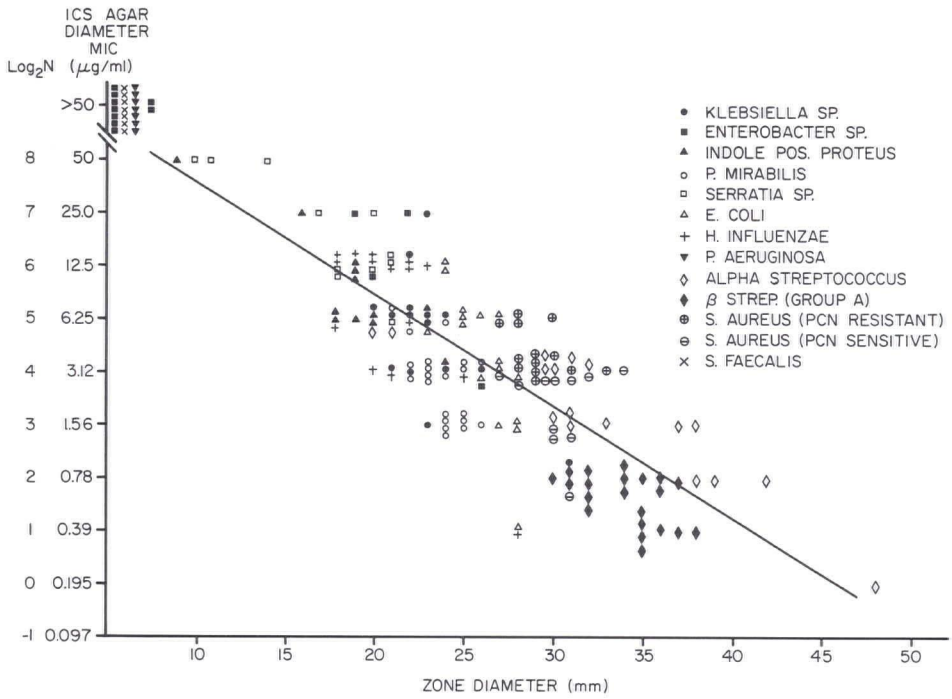

Figure 10
Regression line correlating disc zone sizes with MIC's of cefoxitin.

kept in mind, however, that before establishing a zone diameter and MIC value to define susceptibility of an organism to any antibiotic, we must await the results of clinical trials with the antibiotic.

In conclusion, this study demonstrates that cefamandole and cefoxitin have an increased in vitro activity as compared to cephalothin, especially against the gram negative bacteria. This data indicates that clinical trials with these two new antibiotics are warranted.

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