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Cefoxitin, a Semisynthetic Cephamycin Antibiotic: Susceptibility Studies

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Cefoxitin, 3-carbamoyloxymethyl-7-α-methoxy-7-[2-(2-thienyl)acetamido]-3-cephem-4-carboxylic acid, is a new semisynthetic cephamycin with broad antibacterial activity. It is highly active against gram-negative microorganisms including indole-positive *Proteus* and *Serratia* strains, which are ordinarily resistant to the cephalosporins. Cefoxitin is also highly active against many strains of *Escherichia coli* and *Proteus mirabilis* which are resistant to the cephalosporins. Furthermore, *E. coli* and *Klebsiella* strains which are susceptible to the cephalosporins are generally more susceptible to the cephamycin analog. The susceptibility of the gram-positive bacteria falls well within the effective range of the antibiotic for gram-negative organisms, but cefoxitin is less active than cephalothin or cephaloridine. As is the case with the cephalosporins, strains of *Pseudomonas* and group D streptococci are resistant to cefoxitin. Changes in pH, inoculum density, and growth medium have no significant effect on the activity of the antibiotic.

Recently, the discovery of a new family of antibiotics, the cephamycins, has been reported (1, 3, 6-9, 11). These 7-methoxylated cephalosporins possess marked resistance to the action of β-lactamases from gram-positive and gram-negative organisms (10). One of these, cephamycin C, has broad gram-negative activity but lacks significant activity against gram-positive organisms. Cefoxitin, 3-carbamoyloxymethyl-7-α-methoxy-7-[2-(2-thienyl)acetamido]-3-cephem-4-carboxylic acid (Fig. 1), is the result of chemical effort directed at improving the antibacterial spectrum of the cephamycins, particularly their gram-positive activity, without detracting from their gram-negative activity and their stability to β-lactamases. This communication presents data obtained from an in vitro evaluation of cefoxitin and two clinically used cephalosporins, cephalothin and cephaloridine, by using a wide variety of clinical isolates.

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MATERIALS AND METHODS

Antibiotics. Cefoxitin was prepared in the Merck Sharp and Dohme Research Laboratories as the sodium salt. The sodium salt of cephalothin and the cephaloridine used were the commericial products (Keflin and Loridine; Eli Lilly & Co.).

Bacterial cultures. Fresh clinical isolates representing several genera of gram-positive and gram-negative bacteria were obtained from hospitals at several different geographical areas. These cultures were identified by the respective hospital microbiological laboratories, and some of the identifications were verified in our laboratories. A total of 1,483 random clinical isolates were obtained for evaluation.

A second group of 174 gram-negative microorganisms which were selected for their resistance to cephalothin were also tested.

Disk susceptibility testing. The zone of inhibition obtained with each bacterial culture with the respective antibiotics was determined by means of the Kirby-Bauer disk susceptibility testing method (2). Mueller-Hinton (MH) agar (BBL) medium was used for these studies. When isolates of *Streptococcus* and *Diplococcus pneumoniae* were tested, the medium was supplemented with 5% defibrinated sheep blood and the tubes or plates were incubated in the presence of 10% CO_2.

All disks (0.25-inch [about 0.07 cm] diameter) contained 30 μg of the respective antibiotics. The cephalothin disks were purchased from either Baltimore Biological Laboratories or Difco Laboratories. The cefoxitin and cephaloridine disks were prepared in our laboratories.

Minimal inhibitory concentration. The minimal inhibitory concentration (MIC) was determined by the broth dilution or agar dilution procedures. The inoculum was grown in Trypticase soy broth (BBL) overnight at 37 C for all cultures except streptococci and *D. pneumoniae;* these cultures were grown in brain heart infusion broth (Difco) supplemented with 5% defibrinated sheep blood, and the incubation was

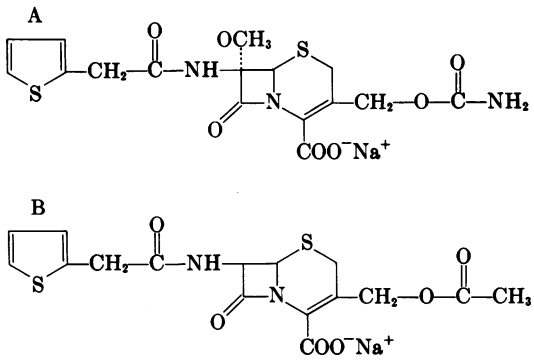

FIG. 1. *Chemical structures of cefoxitin (A) and cephalosporin (B).*

carried out in 10% CO_2. The overnight culture broths for the broth dilution tests were diluted 10^{-4} for all cultures except the streptococci and pneumococci, which were diluted 10^{-2} in MH broth prepared in twice the normal medium concentration. A total of 0.5 ml of the diluted culture in MH broth (2×) was added to tubes containing 0.5 ml of antibiotic diluted in twofold increments in distilled water. Thus, the final cell concentration per tube was approximately 10^5 colony-forming units (CFU). The MIC was defined as the lowest concentration of antibiotic which prevented visible growth after overnight incubation at 37 C.

In the agar dilution tests, the antibiotics were added aseptically in twofold increments to autoclaved MH medium cooled to 50 C, and plates were poured. The inoculum was diluted 10^{-2}, and the agar plates were inoculated with a modified Steers replicator (12) so that the number of organisms deposited on the plate was approximately 5×10^4 CFU.

Minimal bactericidal concentration. The minimal bactericidal concentration (MBC) was determined by streaking a loopful of broth from tubes selected from the MIC tests on Trypticase soy agar medium. The MBC was defined as the lowest concentration of antibiotic from which no colonies were detected after overnight incubation at 37 C.

Correlation of MIC values with disk test results. The MIC values obtained by the broth dilution test were plotted against zone diameters obtained with the disks containing 30 μg of antibiotic. From these experimental points, a regression curve calculated by the method of least squares could be drawn.

Effect of pH. The effect of pH upon the activity of the respective antibiotics was determined by the agar dilution method. The pH of the medium was adjusted with either NaOH or HCl to 6, 7, and 8 after sterilization and cooling to 50 C.

Effect of inoculum size. The effect of inoculum on MIC was assessed also by the agar dilution method. Appropriate dilutions of overnight broth were made so that the final numbers of CFU deposited on the agar surface were approximately 10^6, 10^5, 10^4, and 10^3.

RESULTS AND DISCUSSION

Broth dilution tests. The antibacterial activities of cefoxitin and cephalothin were compared by using the broth dilution method. The MIC values obtained for these two antibiotics with 407 clinical isolates comprising 78 gram-positive and 329 gram-negative organisms are presented in Table 1 and Figure 2 as the cumulative percentage of isolates inhibited by increasing concentrations of antibiotic. Concurrent with the broth dilution test, the isolates were also tested by the Kirby-Bauer sensitivity test in order to allow an evaluation of the relationship between MIC and the size of the zone of inhibition. This will be discussed in a later section.

It is apparent from the MIC data that, for the gram-negative microorganisms generally susceptible to cephalothin, i.e., *E. coli*, *Proteus mirabilis*, and *Klebsiella*, cefoxitin was significantly more active than cephalothin. Extrapolation from the graphs in Fig. 2 indicates that for these microorganisms it takes at least four times more cephalothin than cefoxitin to inhibit 90% of the isolates. Although isolates of *Enterobacter* were less susceptible than the other gram-negative organisms, they were more susceptible to cefoxitin than to cephalothin. Of even greater interest is the susceptibility to cefoxitin noted among the isolates of indole-positive *Proteus* species and *Providencia*, two groups of gram-negative organisms ordinarily resistant to cephalothin (4, 13, 15). At least 90% of the 59 isolates comprising these groups had MIC values of 12.5 μg/ml or less with cefoxitin. In addition, about one-half of the *Serratia* isolates had MIC values of 25 μg or less, and 96% of them were inhibited by 100 μg of cefoxitin per ml, whereas all were insusceptible to 100 μg of cephalothin per ml.

The gram-positive microorganisms, on the other hand, were less susceptible to cefoxitin than to cephalothin. All 53 isolates of *Staphyloccus aureus* were inhibited by 6.25 μg or less of cefoxitin per ml, whereas cephalothin was effective against all of the cultures at levels below 0.78 μg/ml, the lowest level tested. Similarly, all five pneumococcal isolates were inhibited by 3.1 μg of cefoxitin per ml, a level appreciably higher than the <0.78 μg of cephalothin per ml required to inhibit the same isolates. The streptococci apparently are more susceptible to cefoxitin than are the staphylococci, their MICs being 0.63 μg/ml or less.

Since the MBCs for cefoxitin and cephalothin were generally equal to or within one to two dilution tubes of the MIC readings, the data are not presented. There was no significant shift upward in the MBC values relative to the MIC determinations.

Based on these in vitro MIC data, it can be concluded that, against gram-negative microor-

TABLE 1. *Cumulative percent of isolates susceptible to cefoxitin and cephalothin*[a]

Organism	Antibiotic	No. of Isolates	MIC (μg/ml)									
			<0.78	0.78	1.56	3.12	6.25	12.5	25	50	100	>100
Escherichia coli	Cefoxitin	60		2	13	60	92	98	100			
	Cephalothin			2	3	7	35	68	88	97	98	100
Proteus mirabilis	Cefoxitin	64			5	78	94	98				100
	Cephalothin				11	41	75	83	88	89	91	100
P. vulgaris	Cefoxitin	21			5	29	67	100				
	Cephalothin					5	10			14		100
P. morganii	Cefoxitin	15					40	93	100			100
	Cephalothin					7						100
P. rettgeri	Cefoxitin	11			9	36	64	90	100			
	Cephalothin										9	100
Providencia	Cefoxitin	12			25	67	92					100
	Cephalothin									8		100
Klebsiella	Cefoxitin	70		4	6	34	70	83	94	96		100
	Cephalothin			1	1	24	39	59	63	69	71	100
Enterobacter	Cefoxitin	36			6	17	25	42	48	53	61	100
	Cephalothin					6	17	31	33	39	47	100
Serratia	Cefoxitin	23						22	48	65	96	100
	Cephalothin											100
Paracolon	Cefoxitin	17				12	29	41		59	77	100
	Cephalothin					6	18	24		29		100
Staphylococcus aureus	Cefoxitin	53			2	93	100					
	Cephalothin		100									
Diplococcus pneumoniae	Cefoxitin	5				100						
	Cephalothin		100									
Streptococcus	Cefoxitin	20	100									
	Cephalothin		100									

[a] Percentage figures are to the nearest whole number.

ganisms, cefoxitin is superior to cephalothin. The antibiotic was more effective against strains susceptible to cephalothin and was also effective against groups of organisms which are generally resistant to cephalothin. Against gram-positive microorganisms, cefoxitin was less active than cephalothin. Nonetheless, the MIC values for the gram-positive organisms tested fell between 0.6 and 6 μg, well within the range of the MICs for the gram-negative bacteria.

Disk susceptibility tests. In order to obtain a more extensive comparison of cefoxitin with cephalothin, a large number of clinical isolates were evaluated by using the Kirby-Bauer disk susceptibility test procedure. Since in laboratory practice the demarcation point for susceptibility and resistance for the cephalosporins has been established by using cephalothin as the standard in the disk test, we used the same criterion, namely, that the development of a zone of inhibition of 18-mm diameter or greater with a 30-μg antibiotic disk indicates susceptibility of the organism to the antibiotic.

The susceptibility data obtained with cefoxitin, cephalothin, and cephaloridine disks against gram-positive organisms and isolates of *Haemophilus* and *Neisseria meningitidis* are presented in Table 2, and those obtained with gram-negative organisms are presented in Table 3. The data in Table 2 indicate that all of the organisms tested, with the exception of the methicillin-resistant staphylococci, enterococci (including *S. faecalis*), and 4 of 92 streptococci, were susceptible to cefoxitin and cephalothin. Enterococci other than *S. faecalis* were all resistant to cefoxitin.

Data obtained with 1,085 fresh clinical gram-negative isolates are shown in Table 3. Of these, 933 (86%) were sensitive to cefoxitin compared with 784 (72%) and 769 (71%) susceptible to cephalothin and cephaloridine, respectively. The increased numbers of organisms susceptible to cefoxitin can be accounted for, in part, by the indole-positive *Proteus* and *Serratia* isolates, two groups known to be resistant to the other cephalosporins. It will also be noted that a greater number of isolates of *E. coli*, *Proteus mirabilis*, and *Klebsiella* were sensitive to cefoxitin. Isolates of *Pseudomonas*, *Herella*, and *Mima* were resistant to cefoxitin and the other cephalosporins.

Because of the greater susceptibility of several gram-negative species to cefoxitin observed in the above evaluation, we deliberately selected organisms designated resistant to cepha-

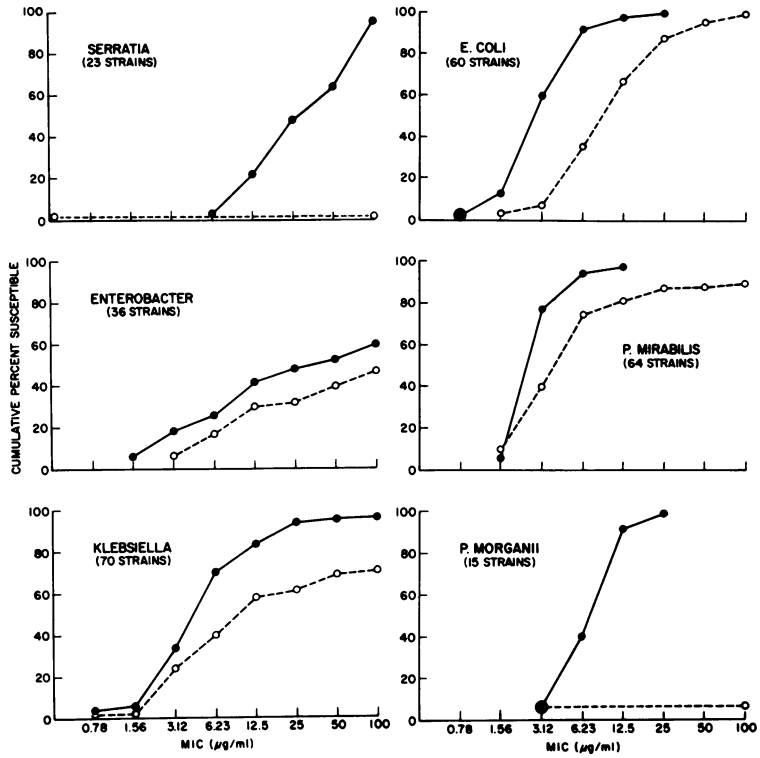

FIG. 2. *Cumulative percentage of isolates of Enterobacteriaceae inhibited by increasing concentrations of cefoxitin and cephalothin.* ——, *Cefoxitin;* ----- *cephalothin.*

lothin by several hospital laboratories for testing against cefoxitin by the disk procedure. The results obtained with 174 such isolates are shown in Table 4. They indicate that 66% of these organisms were susceptible to cefoxitin compared with 25 and 19% to cephalothin and cephaloridine, respectively. A greater number of isolates in each group, with the exception of *Citrobacter* (which was resistant to the three antibiotics), was susceptible to cefoxitin. Again, as in the study with random isolates, the enhanced activity of cefoxitin was due in part to the indole-positive *Proteus*, *Providencia*, and *Serratia* isolates against which cephalothin and cephaloridine are generally resistant. It is also significant that a respectable number of cephalothin-resistant *E. coli*, *Proteus mirabilis*, and *Klebsiella* were susceptible to cefoxitin. This demonstrated activity of cefoxitin against these cephalothin-resistant strains may have been due, at least in part, to the greater stability of the antibiotic to the action of β-lactamases (10). It is of interest that approximately 25% of the cultures in this group were found to be susceptible to cephalothin by our laboratory procedure, although they were selected on the basis of resistance to cephalothin. This difference was probably due to differences among laboratories in the methods used for susceptibility testing and the marginal sensitivity of some of the cultures.

Effect of pH. The effect of initial pH of the medium on antibiotic activity was evaluated by using the agar dilution method with four gram-negative genera and *S. aureus*. The antibiotics were added and pH was adjusted after the medium was cooled to 50 C. The data in Table 5 indicate that there is no appreciable effect of initial pH of medium on the activity of either antibiotic. The variation in average MIC with change in initial pH was no greater than two dilution tubes, and in most instances it was no greater than one dilution tube.

Effect of inoculum size. The effect of inoculum density on antibiotic activity also was examined by using the agar dilution method with isolates of *E. coli*, *Klebsiella*, *Enterobacter*, and *P. mirabilis*. An overnight broth culture was diluted so that the number of CFU inoculated on the surface of each plate varied from approximately 10^3 to 10^6 (Table 6). As a means of evaluating the effect of inoculum size on antibiotic activity, the ratio of the average MIC values obtained with inocula of 10^3 and 10^6 CFU

TABLE 2. *Susceptibility of gram-positive isolates to cefoxitin, cephalothin, and cephaloridine*[a]

Organism	No.	No. of cultures susceptible[b]		
		Cefoxitin	Cephalothin	Cephaloridine
Staphylococcus aureus	152	152	152	152
Staphylococcus aureus (Meth. Res.)	40	2	4	18
Staphylococcus epidermidis	13	13	13	13
Diplococcus pneumoniae	14	14	14	14
Streptococcus (α and β)	92	88	88	89
Streptococcus faecalis	20	8	8	8
Other enterococci	59	0	18	53
Micrococcus tetragenes	3	3	2	3
Haemophilus	3	3	2	3
Neisseria meningitidis	2	2	2	2

[a] Cultures were selected at random and included *Haemophilus* and *Neisseria meningitidis*.

[b] The Kirby-Bauer method for susceptibility testing was used. A zone diameter of 18 mm or greater was the criterion for susceptibility. All disks contained 30 μg of the respective antibiotic.

was calculated for each antibiotic for each genus tested. It can be seen that inoculum size had no marked effect on the activity of any of the antibiotics studied except cephalothin. In the case of cephalothin, a significant inoculum effect was noted with isolates of *E. coli*, a phenomenon ascribed to the ability of strains of *E. coli* to deacetylate the antibiotic to its less active desacetyl analogue (14).

Medium studies. Four growth media were tested for expression of antibiotic activity against one strain each of *E. coli* (L-112) and *Klebsiella* (L-127). The tests were conducted by the broth dilution method. The average MIC values for all three antibiotics generally fell within the range of the experimental error of the procedure (Table 7). In the case of cephaloridine, the average MIC value for *E. coli* in nutrient broth was eightfold lower than noted with two of the other media. This was not observed with the strain of *Klebsiella*, and its significance is not clear.

Correlation of MIC with inhibition zones. To establish a basis for determining susceptibility of clinical isolates to an antibiotic, a comparison usually is made of the MIC values and size of the zones of inhibition obtained by the disk susceptibility test. With few exceptions, an inverse linear relationship is observed between the log MIC and the size of the zone of inhibition with a large number of microorganisms. Such relationships obtained with six representative groups of gram-negative microorganisms and *S. aureus* are shown in the family of graphs in Fig. 3. There is, in general, good agreement between MIC values and inhibition zone sizes. The spread of points in the graphs is typical of

TABLE 3. *Susceptibility of gram-negative clinical isolates to cefoxitin, cephalothin, and cephaloridine*[a]

Organism	No.	No. of cultures susceptible[b]		
		Cefoxitin	Cephalothin	Cephaloridine
Escherichia coli	461	455	419	417
Klebsiella	140	133	128	121
Proteus mirabilis	174	173	164	160
Proteus (indole positive)	51	50	4	3
Enterobacter	91	37	30	27
Serratia	29	24	0	0
Salmonella	13	12	12	13
Shigella	8	8	6	6
Herella	5	0	0	0
Mima	1	0	0	0
Alkaligenes faecalis	13	3	2	2
Pseudomonas	58	0	0	0
Paracolon and unidentified gram-negative rods	41	38	19	20
Total	1,085	933	784	769

[a] Cultures were selected at random.

[b] The Kirby-Bauer method for susceptibility testing was used. A zone diameter of 18 mm or greater was the criterion for susceptibility. All disks contained 30 μg of the respective antibiotic.

TABLE 4. *Susceptibility of Enterobacteriaceae to cefoxitin, cephalothin, and cephaloridine*[a]

Organism	No.	No. of Cultures susceptible[b]		
		Cefoxitin	Cephalothin	Cephaloridine
Escherichia coli	21	17	7	10
Klebsiella	41	20	5	2
Enterobacter	22	2	1	0
Citrobacter	3	0	0	0
Serratia	9	6	0	0
Herella	1	1	0	0
Proteus mirabilis	44	42	28	20
Proteus vulgaris	21	19	2	1
Proteus morganii	2	1	0	0
Providencia	10	7	0	0
Total	174	115	43	33
Percent susceptible		66.1	24.7	19.0

[a] Cultures were selected on the basis of resistance to cephalothin.

[b] The Kirby-Bauer method for susceptibility testing was used. A culture was considered susceptible if a zone diameter of 18 mm or greater was obtained. All disks contained 30 μg of the respective antibiotic.

what is observed with most antibiotics except possibly in the case of the *Serratia* isolates. A composite regression curve constructed with the data obtained for the 407 isolates shown in Fig. 3 is given in Fig. 4. The curve was plotted by using the method of least squares. Because of the large number of cultures involved in the construction of the curve, it was not practical to designate each culture on the curve. In defining susceptibility of an organism in terms of diameter of inhibition zone, consideration must be given to the MIC values obtained and the achievable blood levels. Therefore, the zone diameter used to define susceptibility of an organism to cefoxitin must await pharmacological studies in man and clinical experience with the antibiotic. It is clear from the cefoxitin regression curve (Fig. 4) that an 18-mm inhibition zone, which is the present cut-off point for susceptibility to the cephalosporin antibiotics, corresponds to an MIC value of 14 μg/ml.

Since the data presented indicate that the spectrum of cefoxitin is broader than that of the presently used cephalosporins, it is proposed that, in susceptibility testing, a cefoxitin disk be used in addition to the cephalothin disk, which is used to represent the clinically used cephalosporins in the Food and Drug Administration standardized procedure.

It is clear from the data presented in this paper that cefoxitin has distinct advantages in

TABLE 5. *Effect of pH upon the activity of cefoxitin and cephalothin*[a]

Organism	Average MIC (μg/ml)					
	Cefoxitin			Cephalothin		
	pH 6	pH 7	pH 8	pH 6	pH 7	pH 8
Escherichia coli (16)[b]	5.3	2.7	1.7	6.6	6.4	13.6
Enterobacter (9)	11.2	5.6	2.5	14.3	7.5	8.1
Klebsiella (12)	6.8	3.3	3.1	5.5	3.0	7.3
Proteus mirabilis (7)	3.0	2.7	5.4	10.2	4.2	9.6
Staphylococcus aureus (4)	0.9	2.6	2.6	0.2	0.2	0.2

[a] Tests were conducted in Mueller-Hinton agar by the agar dilution method. Approximately 10^5 cells of each culture were placed on the surface of the plates.

[b] Numbers in parentheses indicate the number of isolates averaged.

TABLE 6. *Effect of inoculum density upon the activity of cefoxitin, cephalothin, and cephaloridine*[a]

Inoculum CFU[b]	Average MIC (μg/ml)		
	Cefoxitin	Cephalothin	Cephaloridine
Escherichia coli (31)[c]			
10^3	2.8	7.9	3.3
10^4	3.8	11.5	6.0
10^5	6.8	38.5	16.8
10^6	10.7	>100	28.2
Ratio, $10^6:10^3$	3.8	>12.7	8.5
Proteus mirabilis (16)			
10^3	3.8	6.7	7.1
10^4	4.1	7.4	9.9
10^5	6.0	8.0	8.3
10^6	21.5	28.6	17.4
Ratio, $10^6:10^3$	5.7	4.3	2.5
Klebsiella (18)			
10^3	3.7	7.7	6.8
10^4	4.0	6.7	6.5
10^5	12.0	17.4	13.6
10^6	24.8	67.5	46.4
Ratio, $10^6:10^3$	6.7	8.7	6.8
Enterobacter (10)			
10^3	7.0	11.0	10.1
10^4	11.4	15.7	12.6
10^5	20.3	39.2	32.3
10^6	21.0	95.0	62.8
Ratio, $10^6:10^3$	3.0	8.6	6.2

[a] Tests were conducted in Mueller-Hinton agar by the agar dilution method.

[b] Approximate number of colony-forming units deposited on the agar surface.

[c] Numbers in parentheses indicate the number of isolates averaged.

TABLE 7. *Effect of medium upon the activity of cefoxitin, cephalothin, and cephaloridine*[a]

Medium	MIC (μg/ml)					
	Escherichia coli L-122			*Klebsiella* L-127		
	Cefoxitin	Cephalothin	Cephaloridine	Cefoxitin	Cephalothin	Cephaloridine
Mueller-Hinton	6.25	25	25	6.25	25	6.25
Trypticase soy broth	12.5	50	50	6.25	12.5	25
Nutrient broth	3.12	50	6.25	3.12	12.5	12.5
Brain heart infusion	12.5	50	50	6.25	25	12.5

[a] The broth dilution procedure was used, and each tube was inoculated with approximately 10^5 CFU.

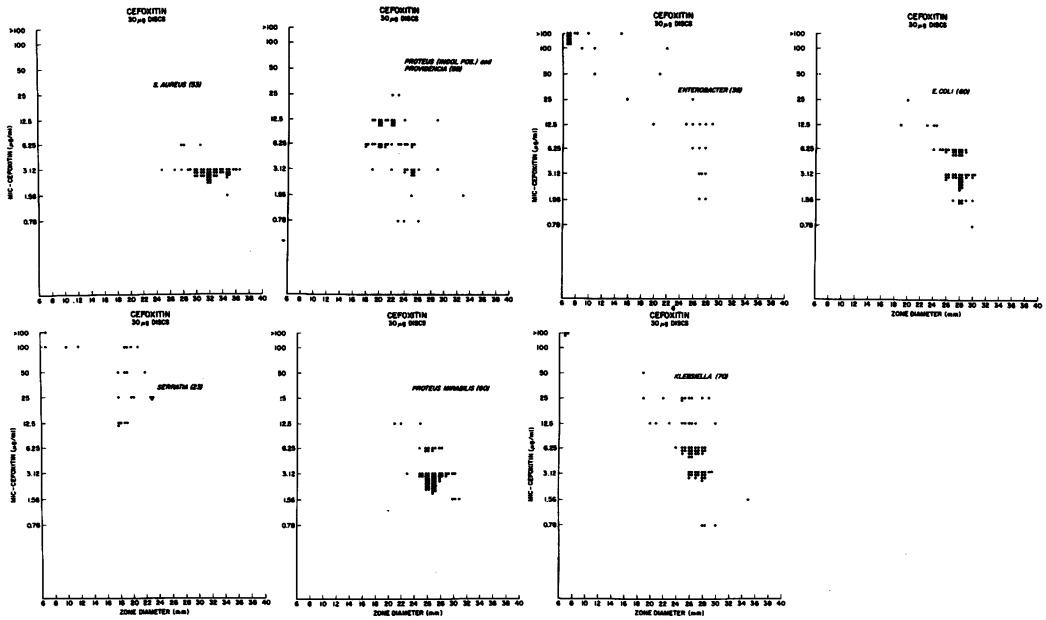

FIG. 3. *Relationship of zone diameters (30-μg disks) by the Kirby-Bauer disk susceptibility procedure to broth dilution MIC values for Staphylococcus aureus and six genera of gram-negative organisms.*

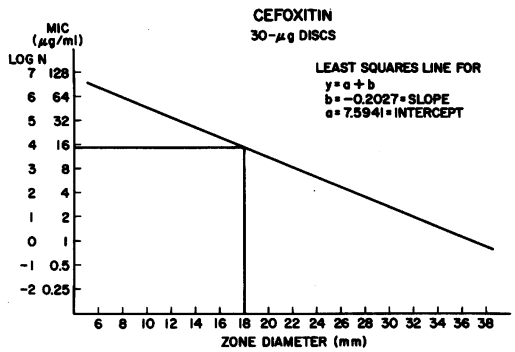

FIG. 4. *Relationship of zone diameters (30-μg disks) by the Kirby-Bauer disk susceptibility procedure to broth dilution MIC values for 407 clinical isolates (gram-positive and -negative).*

antibacterial activity over the commercially available cephalosporin-type antibiotics. Cefoxitin is effective against a number of gram-negative organisms against which cephalothin and cephaloridine are ineffective. Because of these interesting in vitro data, preliminary pharmacological and extensive efficacy studies against systemic infections have been carried out in mice (5).

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