

## Comparative Study of the In Vitro Antibacterial Activity of Cefoxitin, Cefuroxime, and Cephaloridine

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The in vitro antibacterial effects of cefoxitin, a semisynthetic cephamycin, cefuroxime, a new cephalosporin antibiotic, and cephaloridine were compared. With gram-positive bacteria, marked differences were found only in the effects against *Streptococcus faecalis*, where cephaloridine and cefuroxime were superior to cefoxitin. With gram-negative aerobic bacteria, cefoxitin, which is known to be more resistant to beta-lactamases from gram-negative bacteria than any cephalosporin, was found to be more effective than cefuroxime and cephaloridine against ampicillin-resistant strains of *Escherichia coli* and indole-positive strains of *Proteus*. *Haemophilus influenzae* was found to be more susceptible to cefuroxime than to cefoxitin and cephaloridine. When ampicillin-resistant strains of *H. influenzae* were tested, markedly higher minimal inhibitory concentration values were obtained with cephaloridine in comparison to those obtained with ampicillin-susceptible strains. These increases in the minimal inhibitory concentration values were not observed with cefoxitin and cefuroxime, probably due to the resistance of these two compounds to beta-lactamases. Strains of *Bacteroides fragilis* were found to be much more susceptible to cefoxitin than to cefuroxime, which in turn was superior to cephaloridine. The results obtained indicate that cefoxitin and cefuroxime both are superior in their antibacterial spectra to the cephalosporins that are now in clinical use.

Since the introduction of cephalothin and cephaloridine as the first clinically available cephalosporins, a large number of new cephalosporins have been synthesized and are now in routine use. Most of these new compounds have only minor advantages over cephalothin and cephaloridine as regards antibacterial activity. It is therefore of considerable interest that cefoxitin and cefuroxime, two new compounds presently undergoing clinical trials, seem to have markedly broader antibacterial spectra than any of the cephalosporins in clinical use (1, 6, 6a, 7a, 10, 12). It should be noted that, although cefuroxime [-6R, 7R-3-carbamoyloxymethyl-7-(2Z)-2-methoximino(fur-2-yl)-acetamidoceph-3-em-4-carboxylate] is a cephalosporin, synthesized from cephalosporin C, cefoxitin is a cephamycin, derived from cephamycin C (Fig. 1). Cephamycins differ from cephalosporins especially by a 7-alpha-methoxy group.

In this study the antibacterial activities of cefoxitin and cefuroxime were compared to each other and to that of cephaloridine.

### MATERIALS AND METHODS

**Bacterial strains.** All strains tested were recent clinical isolates. Thirty-two strains of *Haemophilus*

*influenzae* from patients with upper-respiratory tract diseases were used. Six of the strains were ampicillin resistant. Thirty-five indole-positive and 32 indole-negative strains of *Proteus* spp., all isolated from patients with urinary tract infections, were employed. Sixty-one strains of *Escherichia coli*, 36 from blood cultures and 25 from patients with urinary tract infections, were also included in the study. The latter strains were all characterized by transferable multiple antibiotic resistance and were considered as R factor bearing (R<sup>+</sup>). Of these strains 12 were completely resistant to ampicillin as determined by a disk test (3). Two of the strains from the blood cultures were found to be ampicillin resistant. Other strains employed were: 27 strains of *Bacteroides fragilis*, obtained from patients with various forms of deep-seated infections, 20 strains of type A and 16 strains of type B *Neisseria meningitidis*, all isolated from patients with clinically manifest meningococcal infections; 36 strains of *Streptococcus faecalis*, isolated from patients with urinary tract infections; 32 strains of *Streptococcus pneumoniae*, from patients with upper and lower respiratory tract infections; and 36 beta-lactamase-producing strains of *Staphylococcus aureus*, obtained from patients with various forms of staphylococcal infection.

**Media.** The antibiotic susceptibility studies were in most cases performed on the ASM medium described by Wretling et al. (13). Rabbit blood (8%) and IsoVitaleX (2%, BBL) were added to the ASM me-

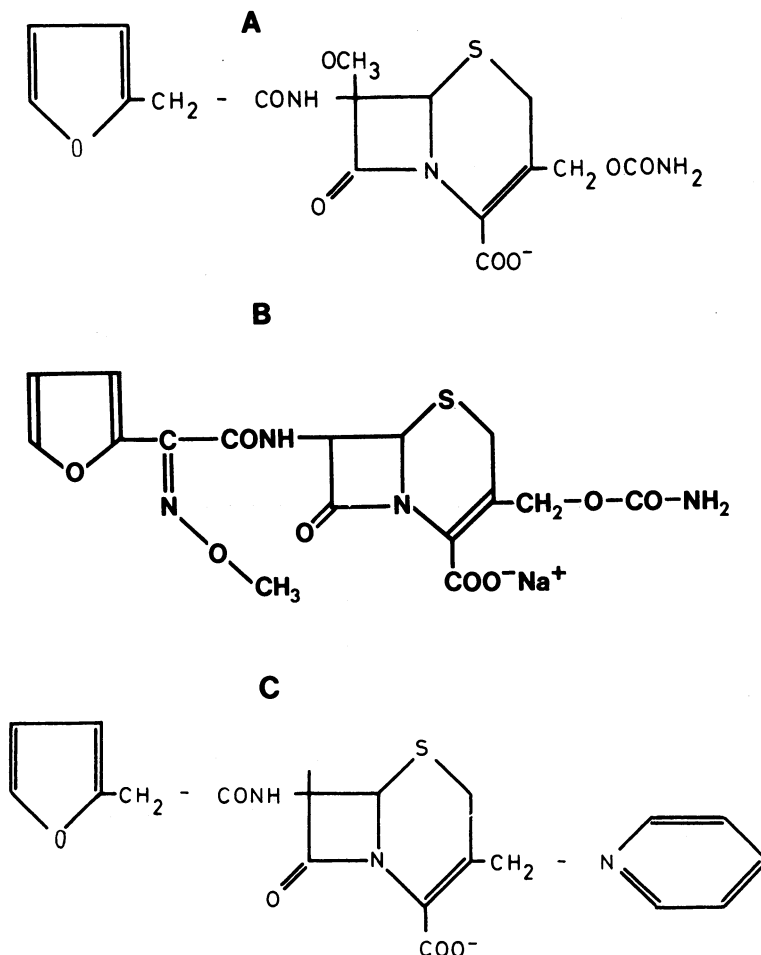


FIG. 1. Chemical structures of (A) cefoxitin, (B) cefuroxime, and (C) cephaloridine.

dium when strains of meningococci, pneumococci, *Haemophilus*, and *Bacteroides* were tested. The medium was chocolateized before inoculation with *H. influenzae*, pneumococci, and meningococci. When the *Proteus* strains were tested, a cysteine-lactose-electrolyte-deficient agar (Oxoid) was used.

**Antibiotics.** Cefoxitin (Mefoxin, Merck, Sharpe & Dome Research Laboratories), cefuroxime (Glaxo Research Ltd.) and cephaloridine (Ceporan, Glaxo) were all used as freshly prepared solutions of dry substance in saline.

**Determinations of MIC.** The agar dilution technique described by Ericsson and Sherris (3) was used for the determination of minimal inhibitory concentrations (MIC). The bacterial inocula consisted of 4-h nutrient broth cultures diluted in saline to give a concentration of  $5 \times 10^4$  bacteria/ml (in some experiments,  $5 \times 10^5$ /ml). Aliquots of 0.001 ml were inoculated on the agar plates using a slight modification of the device described by Steers et al. (8). After incubation at 37 C for 18 h the plates were read, and the concentrations of antibiotics giving 100% or 50 to 90% inhibition of bacterial growth

were determined. When the *B. fragilis* strains were tested, incubation in 95% H<sub>2</sub>-5% CO<sub>2</sub> was used.

## RESULTS

The accumulated percentages of bacterial strains completely inhibited by the various antibiotics tested are presented in Fig. 2.

**Effect on gram-positive bacteria.** The MIC of cefuroxime and cephaloridine against gram-positive bacteria tended to be lower than the MICs of cefoxitin. This was most pronounced against strains of *S. faecalis*. Of the 37 strains tested, only 3 were inhibited by 128  $\mu$ g of cefoxitin per ml, whereas all strains were inhibited by MICs of less than 16  $\mu$ g of cephaloridine or cefuroxime per ml. On penicillinase-producing staphylococci, cephaloridine was found to be more active than either cefuroxime or cefoxitin.

**Effect on gram-negative bacteria.** Against *E. coli* no differences between the compounds were noted when the MICs for all strains were

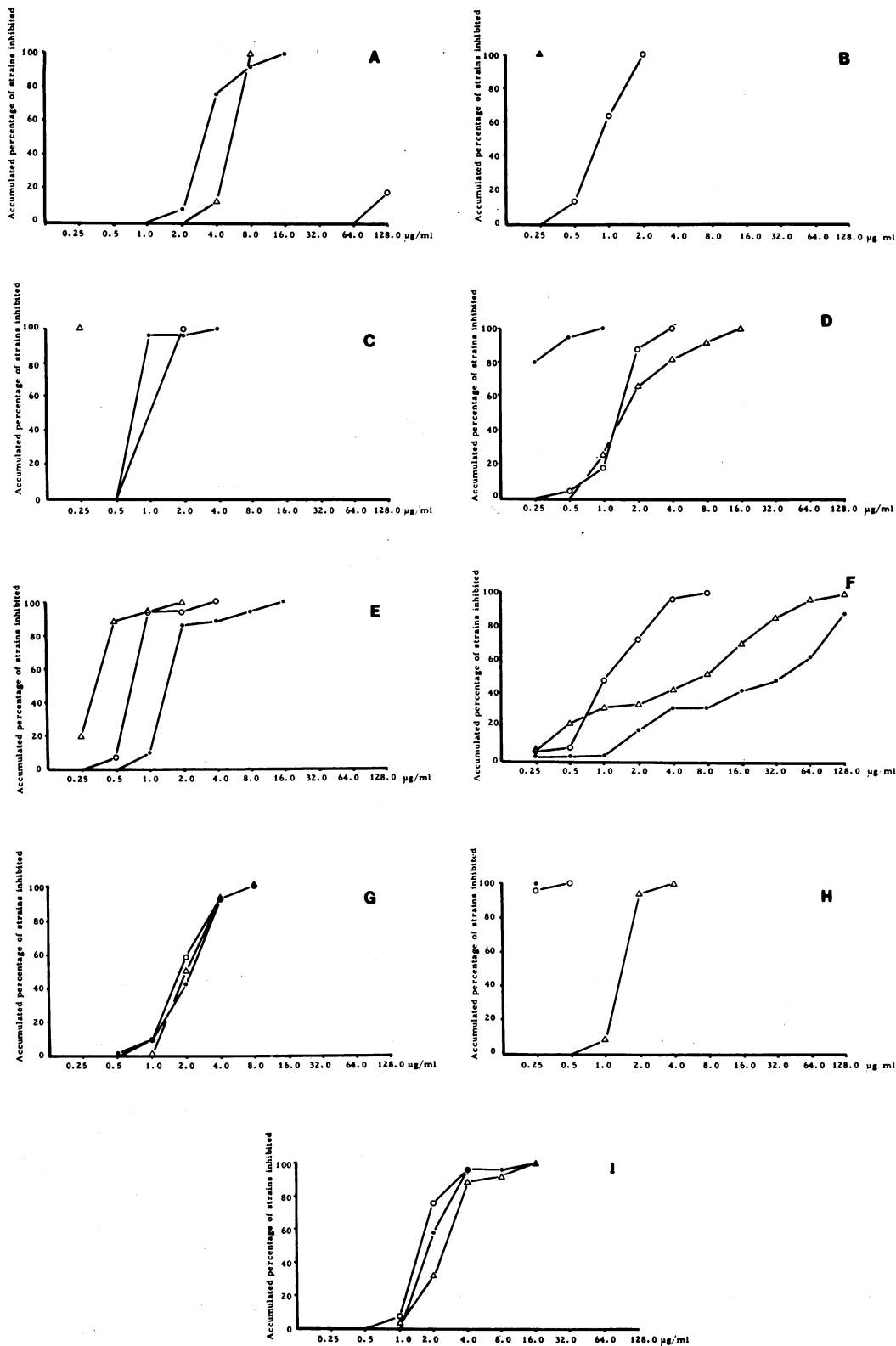


FIG. 2. Cumulated percentages of 100% inhibition of bacterial growth obtained with various concentrations of cefoxitin (○), cefuroxime (●), and cephaloridine (△). (A) *S. faecalis*, n = 36; (B) pneumococci, n = 32; (C) *S. aureus*, n = 36; (D) *H. influenzae*, n = 38; (E) indole-negative *Proteus* spp., n = 35; (F) indole-positive *Proteus* spp., n = 35; (G) *E. coli*, n = 61; (H) meningococci, n = 36; (I) *B. fragilis*, n = 32.

considered together. Table 1 demonstrates, however, that there seems to be a better antibacterial effect produced by cefoxitin on ampicillin-resistant strains of *E. coli* than by cefuroxime, which in turn was superior to cephaloridine. The same tendency was observed with indole-positive *Proteus* strains, which were all inactivated by 8  $\mu\text{g}$  of cefoxitin per ml, whereas the MICs were above 16  $\mu\text{g}/\text{ml}$  for 28% of the strains when cephaloridine was tested and for 54% of the strains when cefuroxime was tested. Against indole-negative strains of *Proteus*, cephaloridine seemed more effective than either cefoxitin or cefuroxime.

*H. influenzae* was found to be more susceptible to cefuroxime than to cephaloridine or cefoxitin. Six of the *Haemophilus* strains tested were ampicillin resistant, and these strains were most susceptible to cefuroxime, whereas cephaloridine was found to be inferior to cefoxitin.

Against meningococci both cephaloridine and cefuroxime inactivated all strains at concentrations below 0.5  $\mu\text{g}/\text{ml}$ , whereas a concentration of 2 to 4  $\mu\text{g}$  of cefoxitin per ml was required to inactivate all strains.

Cefoxitin was found to be effective against the strains of *B. fragilis* tested. Cefuroxime and cephaloridine were both less active against *Bacteroides*. To study the importance of the size of the inoculum, it was increased 10-fold with 11 of the *B. fragilis* strains. This did not affect the MIC values obtained with cefoxitin, whereas considerable increases were noted with cefuroxime and even more so with cephaloridine (Table 2).

## DISCUSSION

The antibacterial activity of the clinically used cephalosporins is characterized by low

TABLE 1. MIC for inhibition of ampicillin-resistant strains of *E. coli*.

Strain	MIC ( $\mu\text{g}/\text{ml}$ ) obtained with:		
	Cefoxitin	Cefuroxime	Cephaloridine
C 1	8	8	4
C 5	4	4	4
R 7	1	1	1
R 8	1	1	1
R 14	1	2	4
R 17	1	2	2
R 18	1	1	2
R 19	1	1	2
R 20	1	1	2
R 22	1	2	2
R 23	1	2	2
R 25	4	8	8
R 26	1	2	8
R 27	1	1	2

MIC values against most gram-positive bacteria and most aerobic gram-negative bacteria that do not produce beta-lactamases. Enterococci, *Pseudomonas*, *Serratia*, and *Providentia* have all been reported to be less susceptible to cephalosporins, as have *Bacteroides* strains. Cefoxitin, which is not a cephalosporin but a cephamycin, has been found to be much more resistant to gram-negative beta-lactamases than cephalosporins and also to have a remarkably good effect on *B. fragilis* (1, 6, 7, 10, 12). Cefuroxime, which was synthesized later than cefoxitin and which is a cephalosporin, has been found to be more beta-lactamase resistant than other cephalosporins and, in addition, to have a better antibacterial effect on some gram-negative bacteria, such as *H. influenzae* and meningococci (2, 9).

The present study presents evidence of the superior antibacterial effect of cefoxitin on indole-positive *Proteus* and *B. fragilis* compared to cefuroxime and cephaloridine. These differ-

TABLE 2. MIC required for 100% inhibition of bacterial growth with various inocula of *B. fragilis*

Strain	Inoculum size (no. of bacteria per ml)	MIC ( $\mu\text{g}/\text{ml}$ ) obtained with:		
		Cefoxitin	Cefuroxime	Cephaloridine
B 1	5 $\times$ 10 <sup>4</sup>	2	4	8
	5 $\times$ 10 <sup>6</sup>	2	4	8
B 2	5 $\times$ 10 <sup>4</sup>	4	2	2
	5 $\times$ 10 <sup>6</sup>	8	2	8
B 8	5 $\times$ 10 <sup>4</sup>	2	64	64
	5 $\times$ 10 <sup>6</sup>	2	128	128
B 9	5 $\times$ 10 <sup>4</sup>	1	8	2
	5 $\times$ 10 <sup>6</sup>	1	8	8
B 14	5 $\times$ 10 <sup>4</sup>	8	8	4
	5 $\times$ 10 <sup>6</sup>	8	8	4
B 15	5 $\times$ 10 <sup>4</sup>	2	4	8
	5 $\times$ 10 <sup>6</sup>	2	8	16
B 18	5 $\times$ 10 <sup>4</sup>	2	2	4
	5 $\times$ 10 <sup>6</sup>	2	4	8
B 22	5 $\times$ 10 <sup>4</sup>	8	16	16
	5 $\times$ 10 <sup>6</sup>	8	16	16
B 24	5 $\times$ 10 <sup>4</sup>	8	32	32
	5 $\times$ 10 <sup>6</sup>	8	32	64
B 26	5 $\times$ 10 <sup>4</sup>	4	8	8
	5 $\times$ 10 <sup>6</sup>	4	16	8
B 31	5 $\times$ 10 <sup>4</sup>	1	2	8
	5 $\times$ 10 <sup>6</sup>	1	2	8

ences seem to be of clinical significance, as the MIC values obtained with cefuroxime and cephaloridine in many cases were so high that antibacterial concentrations in serum were not likely to be obtained even after administration of high doses. Cefoxitin also seemed more effective against ampicillin-resistant strains of *E. coli* than cefuroxime and cephaloridine, but the differences observed were less pronounced than had been expected. Cefoxitin was found to be less effective than cefuroxime against pneumococci, *S. faecalis*, and *H. influenzae*. Only 8% of the strains of *S. faecalis* were inhibited by a concentration of 128  $\mu\text{g}$  of cefuroxime/ml, whereas the highest MIC value obtained for cefuroxime and cefoxitin was 16  $\mu\text{g}$ /ml. The differences in antibacterial effect on pneumococci and *H. influenzae* were less pronounced but must be considered important, as these bacteria are commonly etiological agents in cases of bacterial meningitis, and the cephalosporins penetrate the blood brain barrier only to a low degree and thus give low drug concentrations in the central nervous system. Cefuroxime therefore seems to be an antibiotic which might be useful for the treatment of bacterial meningitis, as not only *Haemophilus* and pneumococci but also meningococci were found to be extremely susceptible to this compound. Studies on the capacity of cefuroxime to enter into the central nervous system are, however, lacking and need to be performed before any firm conclusions can be drawn on this point. An interesting finding is that the differences in the antibacterial effects on *H. influenzae* between cefuroxime and cefoxitin were not restricted to the ampicillin-susceptible strains but were also observed when ampicillin-resistant, beta-lactamase-producing strains were tested. This finding indicates that cefuroxime is equally or more resistant to the beta-lactamases produced by *H. influenzae* than is cefoxitin.

Cephaloridine was found to have a better antibacterial effect than cefoxitin against gram-positive bacteria, and it was also superior to cefuroxime in its effect on penicillinase-producing *S. aureus*. On gram-negative bacteria cephaloridine seemed to be less effective than the other two compounds, with the exception of indole-negative *Proteus* spp. The results obtained with cephaloridine were in agreement with earlier studies (4, 9, 11).

Pending the availability of further clinical and pharmacokinetic data on cefoxitin and cefuroxime, it is not possible to predict the clinical usefulness of these two compounds compared to the commercially available cephalo-

sporins. The antibacterial spectra of these two new compounds do, however, indicate that they might become the two most important members of the cephalosporin group.

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