

In Vitro Evaluation of Cefoxitin and Cefamandole

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Cefoxitin and cefamandole were evaluated in vitro against 263 organisms. Studies were performed in Mueller-Hinton and nutrient broth and agar employing inoculum sizes of 10^6 and 10^8 organisms per ml. At obtainable serum levels both antibiotics were bactericidal for nearly all strains of *Escherichia coli*, *Klebsiella*, *Proteus mirabilis*, and *Staphylococcus aureus* but were inactive against *Pseudomonas aeruginosa* and enterococcus. In agar, cefamandole appeared to be active against most strains of *Enterobacter* and indole-positive *Proteus*, whereas cefoxitin was active against indole-positive *Proteus* but not *Enterobacter*. Moreover, in broth medium most strains of *Enterobacter* were not readily inhibited by either antibiotic and only 40 and 73% of indole-positive *Proteus* were inhibited by 10 μ g of cefamandole per ml in Mueller-Hinton and nutrient broth, respectively. However, in both broth media, 10 μ g of cefoxitin per ml continued to be inhibitory and bactericidal for most isolates of indole-positive *Proteus*. Cefoxitin also was bactericidal against four cephalothin-resistant strains of *E. coli*. These data suggest that cefoxitin broadens the spectrum of existing cephalosporins by enhancing the activity against indole-positive *Proteus* species as well as some other *Enterobacteriaceae*. On the other hand, with the exception of strains of *Enterobacter aerogenes*, the apparent increased in vitro activity of cefamandole was demonstrated in agar and not in broth.

Several cephalosporin antibiotics currently are available for the therapy of infection. Two more recently studied compounds, cefoxitin and cefamandole, by virtue of their apparent resistance to degradation by cephalosporinase enzymes, are reported to extend the antibacterial spectrum of the cephalosporin antibiotics (2-6). Cefoxitin, although resembling cephalothin structurally, is actually a member of the cephamycin group of antibiotics (11). The present studies were performed to assess the in vitro antibacterial activity of cefoxitin and cefamandole.

MATERIALS AND METHODS

Antibacterial activity was determined by antibiotic dilution methods performed in nutrient (Difco) broth (NB) and agar and Mueller-Hinton (Difco) broth (MHB) and agar. Studies in agar were performed by use of a Steers replicator device (8). In broth both minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) were determined by methods described previously from this laboratory (7, 10). The MBC was defined as the lowest concentration of antibiotic in which fewer than three viable colonies were recovered when about 0.005 ml of broth from each clear tube was subcultured onto agar without antibiotic. A total of 173 isolates of *Enterobacteriaceae* and 30 isolates each of *Pseudomonas aeruginosa* and enterococcus

were tested. Also studied were four cephalothin-resistant strains of *Escherichia coli* that had demonstrated no zone of inhibition when tested with 30 μ g-content cephalothin discs (1). A laboratory reference strain of *E. coli* inhibited by 2.5 μ g of cefoxitin and 0.5 μ g of cefamandole/ml was used as a control organism in all experiments.

RESULTS

Cefoxitin. The cumulative percentage of 173 isolates of *Enterobacteriaceae* susceptible to increasing concentrations of cefoxitin is shown in Fig. 1, which also summarizes the effects of medium and inoculum size on the antibacterial activity of this compound in agar. At 20 μ g of antibiotic/ml, a level attainable in serum after a 1-g parenteral dose, cefoxitin inhibited all isolates of *E. coli*, *Proteus mirabilis*, *Klebsiella* and indole-positive *Proteus* species when tested against both inoculum sizes of bacterial cells. However, less than 20% of *Enterobacter* isolates were inhibited in agar by 20 μ g of cefoxitin/ml. Moreover, the percentage of *Enterobacter* isolates susceptible to cefoxitin did not increase until the concentration of antibiotic was at least 50 μ g/ml.

Figures 2 and 3 summarize the inhibitory (MIC) and bactericidal (MBC) activities of cefoxitin against these same isolates of *Entero-*

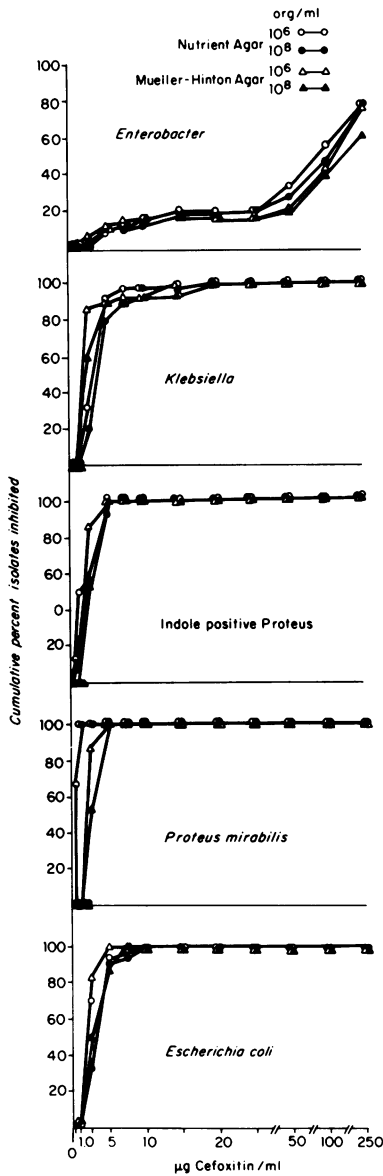


FIG. 1. Cumulative percentage of 30 isolates each of *E. coli*, *Klebsiella*, *P. mirabilis*, and indole-positive *Proteus* and 53 isolates of *Enterobacter* inhibited by increasing concentrations of cefoxitin tested in agar medium with bacterial inocula of two different sizes.

bacteriaceae tested in broth medium with two inoculum sizes of bacterial cells. In MHB the MICs obtained when the lower inoculum size was employed were nearly comparable to those obtained in agar. In addition, when studied in MHB, 20 µg of cefoxitin/ml was bactericidal against all isolates of *E. coli* and killed 28 of 30 strains of *P. mirabilis* even when tested

against the higher inoculum of microorganisms. Although more than 90% of *Klebsiella* isolates were both inhibited and killed by 20 µg of cefoxitin or less/ml when tested against 10⁶ organisms/ml, 50 µg/ml was required to inhibit and kill a comparable number of strains when tested against 10⁸ organisms/ml. Similarly, al-

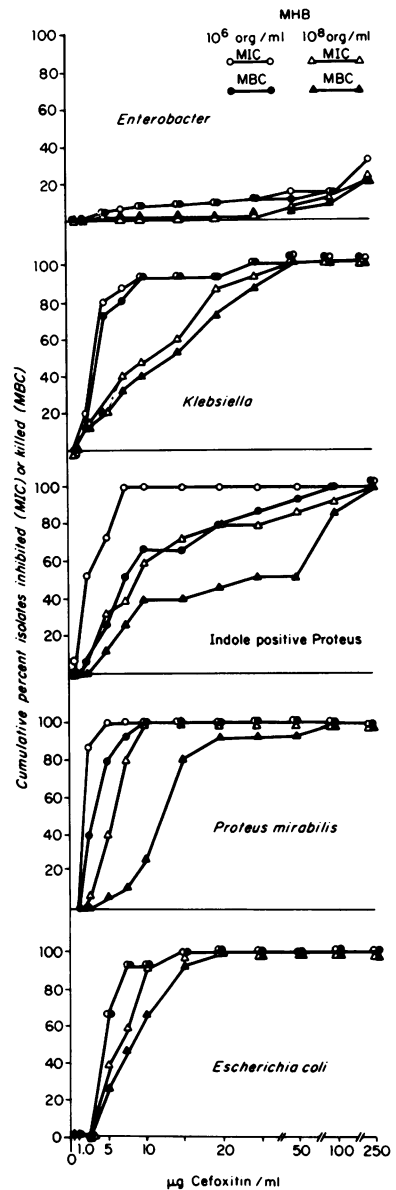


FIG. 2. Cumulative percentage of 30 isolates each of *E. coli*, *Klebsiella*, *P. mirabilis*, and indole-positive *Proteus* and 53 isolates of *Enterobacter* inhibited (MIC) or killed (MBC) by increasing concentrations of cefoxitin tested in MHB medium with bacterial inocula of two different sizes.

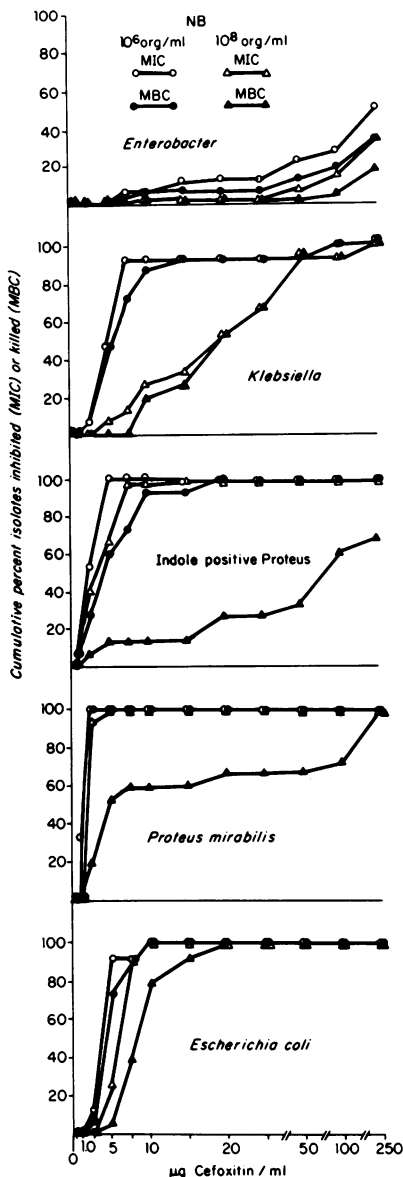


FIG. 3. Cumulative percentage of 30 isolates each of *E. coli*, *Klebsiella*, *P. mirabilis*, and indole-positive *Proteus* and 53 isolates of *Enterobacter* inhibited (MIC) or killed (MBC) by increasing concentrations of cefoxitin tested in NB medium with bacterial inocula of two different sizes.

though 20 µg of cefoxitin/ml was bactericidal against 80% of isolates of indole-positive *Proteus* strains when the lower inoculum size was employed, only 47% of strains were killed at the higher inoculum. Cefoxitin showed negligible activity in MHB against isolates of *Enterobacter*, and most isolates grew in concentrations of 250 µg of antibiotic/ml. Results obtained using

NB were similar to those obtained with MHB, except that cefoxitin appeared to exert less bactericidal activity against some genera in NB than in MHB.

Cefoxitin at 20 µg or less/ml inhibited and killed all 30 isolates of *S. aureus* in both broth media when tested against the lower inoculum of bacterial cells (data not shown). However, in MHB 14% of these isolates resisted killing when the higher inoculum size was tested. No isolate of *P. aeruginosa* was inhibited by cefoxitin even at a concentration of 250 µg/ml. Cefoxitin exerted some activity against the enterococcus, but the majority of isolates grew in concentrations of 100 µg/ml.

In agar, all four of the cephalothin-resistant isolates of *E. coli* were susceptible to 20 µg or less of cefoxitin/ml, and three of the four isolates were killed in broth at 20 µg of cefoxitin/ml, even when tested against the higher inoculum of bacterial cells.

Fifty-three isolates of *Enterobacter* were speciated into five subgroups, e.g., *E. hafnia* (four isolates), *E. liquefaciens* (five isolates), *E. agglomerans* (four isolates), *E. cloacae* (twenty-seven isolates), and *E. aerogenes* (thirteen isolates). In general, isolates of *E. hafnia* were more sensitive to cefoxitin than the other four species. In broth, 10 µg of cefoxitin/ml killed all four isolates of *E. hafnia*, whereas none of the other species was inhibited or killed until concentrations of at least 50 µg/ml were achieved.

Cefamandole. The activity of cefamandole against 173 isolates of *Enterobacteriaceae* tested in agar medium is summarized in Fig. 4. At 20 µg of cefamandole/ml all 30 isolates each of *E. coli*, *P. mirabilis*, and *Klebsiella* were inhibited in both agar media regardless of the size of the inoculum. In addition, approximately 75% of 30 isolates of indole-positive *Proteus* strains and 60% of 53 isolates of *Enterobacter* also were susceptible to 20 µg or less of cefamandole/ml in agar.

The results of testing these same isolates with cefamandole in broth are summarized in Fig. 5 and 6, which also depict the effects of medium and inoculum size on the MIC and MBC. In MHB medium cefamandole showed much less activity against isolates of *Enterobacter* and indole-positive *Proteus* species than was demonstrated in agar. This discrepancy was most apparent when the MBC was determined against a high inoculum of bacterial cells. Although the results obtained with cefamandole were similar in both broth media, there was less bactericidal activity against *Klebsiella* and *P. mirabilis* in NB than in MHB.

Cefamandole at 10 µg or less/ml was bacteri-

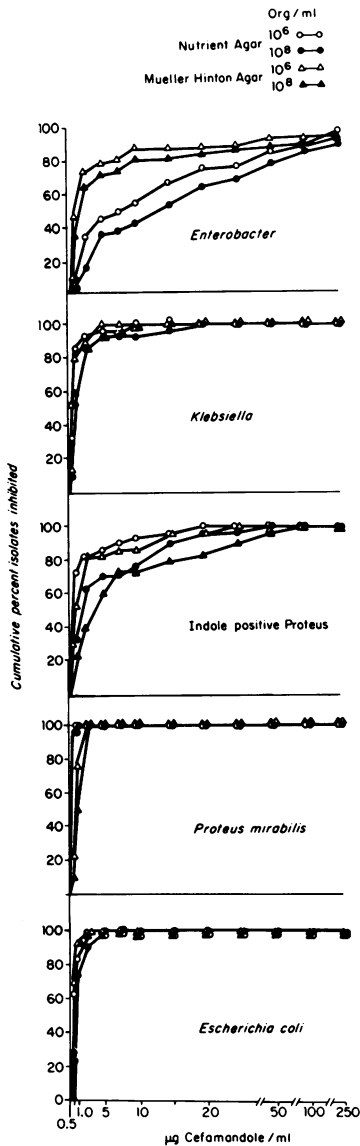


FIG. 4. Cumulative percentage of 30 isolates each of *E. coli*, *Klebsiella*, *P. mirabilis*, and indole-positive *Proteus* and 53 isolates of *Enterobacter* inhibited by increasing concentrations of cefamandole tested in agar medium with bacterial inocula of two different sizes.

cidal against all 30 isolates of *S. aureus* in NB at both inoculum sizes of bacterial cells (data not shown). When tested against the high inoculum size in MHB, 28 of the 30 strains were killed by 10 µg of cefamandole or less/ml. Similar to cefoxitin, cefamandole was completely inactive against *P. aeruginosa*. However, cefamandole was somewhat more active against the enterococcus. For example, when the lower inoculum size of bacterial cells was tested in

broth, 50 µg of cefamandole or less/ml killed 80% of the isolates. On the other hand, 20 µg/ml was rarely bactericidal.

The cephalothin-resistant isolates of *E. coli* showed variable susceptibility to cefamandole. For example, in broth medium, 20 µg of cefamandole/ml inhibited only one of the four cultures.

Isolates of *E. aerogenes* were more sensitive to cefamandole than were the other species of

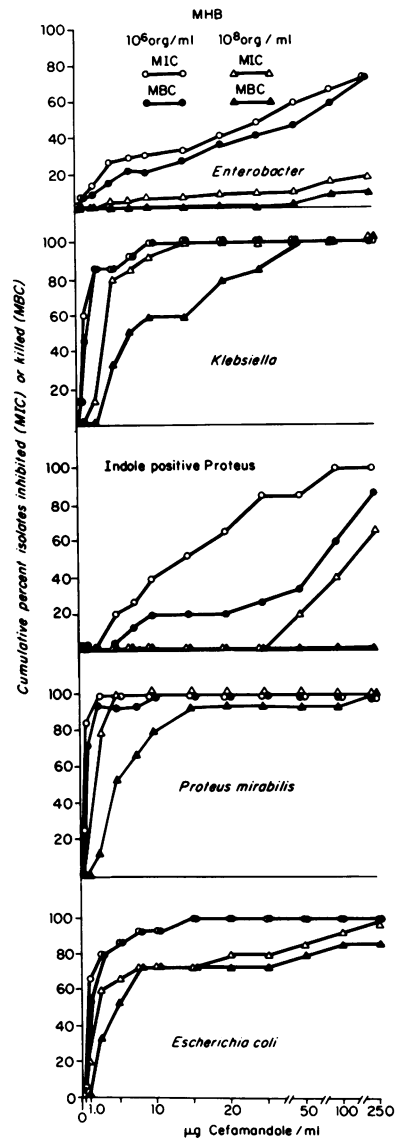


FIG. 5. Cumulative percentage of 30 isolates each of *E. coli*, *Klebsiella*, *P. mirabilis*, and indole-positive *Proteus* and 53 isolates of *Enterobacter* inhibited (MIC) or killed (MBC) by increasing concentrations of cefamandole tested in MHB medium with bacterial inocula of two different sizes.

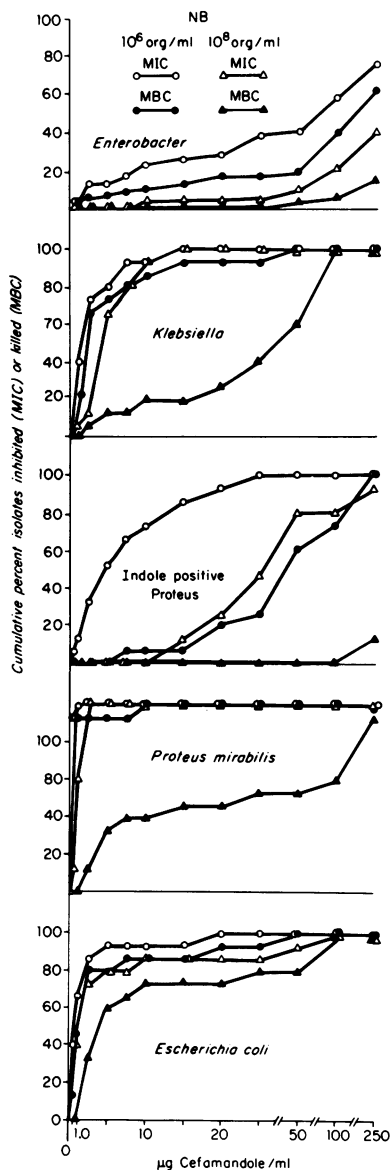


FIG. 6. Cumulative percentage of 30 isolates each of *E. coli*, *Klebsiella*, *P. mirabilis*, and indole-positive *Proteus* and 53 isolates of *Enterobacter* inhibited (MIC) or killed (MBC) by increasing concentrations of cefamandole tested in NB medium with bacterial inocula of two different sizes.

Enterobacter, and all 13 strains were inhibited and killed by 20 μg of cefamandole or less/ml. On the other hand, only 25% of the other species of *Enterobacter* were susceptible to that concentration of cefamandole in broth.

DISCUSSION

It appears that at attainable serum levels (20 $\mu\text{g}/\text{ml}$) both cefoxitin and cefamandole are ac-

tive against the majority of *E. coli*, *P. mirabilis*, *Klebsiella*, and *Staphylococcus aureus* strains. Although cefoxitin and, to a lesser degree, cefamandole are less active in vitro against *S. aureus* than other currently available cephalosporins, this is unlikely to be a clinical problem. In addition, similar to other cephalosporins, cefoxitin and cefamandole were inactive against all isolates of *P. aeruginosa*. Cefamandole appeared somewhat more active against enterococci than other clinically available cephalosporins.

Unlike the cephalosporins, cefoxitin demonstrated appreciable activity in both agar and broth medium against indole-positive *Proteus* species. Moreover, cefoxitin was active against four clinical isolates of cephalothin-resistant *E. coli*. This enhanced activity of cefoxitin may be related to its apparent resistance to degradation by beta-lactamase, a characteristic of the cephamycins (6). Cefoxitin also has been reported to be active in vitro against strains of *Bacteroides fragilis* and *Serratia* (4, 9).

In contrast to cefoxitin, the primary in vitro advantage of cefamandole appeared to be its activity against some strains of *Enterobacter*. However, against most of these strains, the activity of cefamandole was demonstrated in agar but not in broth. Cefamandole also has been reported to be more active than other cephalosporins against *Haemophilus influenzae* (3). This enhanced activity also may be related to better stability of cefamandole against certain cephalosporinase-degrading enzymes. The reasons for the differences observed in the susceptibility of some strains of organisms to cefamandole when tested in broth are unclear. Both cefoxitin and cefamandole appear stable in MHB and NB, and simple degradation of the antibiotics is unlikely to account for the differences. Since in our studies we did not measure residual antibiotic after addition of microorganisms, we are unable to comment on the possibility of enzymatic inactivation of cefamandole when tested against a high inoculum size of bacterial cells in broth.

In conclusion, cefoxitin and cefamandole possess some in vitro characteristics that may provide some advantages over the currently available cephalosporin compounds. The results of ongoing clinical trials hopefully will define these specific attributes.

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