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# In Vitro Antagonism of Beta-Lactam Antibiotics by Cefoxitin

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We assessed the extent and mechanisms of antagonism of beta-lactam antibiotics by cefoxitin. In tests with 41 gram-negative isolates, cefoxitin antagonized cephalothin, cefamandole, cefsulodin, cefotaxime, moxalactam, ampicillin, carbenicillin, piperacillin, mezlocillin, and azlocillin, but not cephalexin, mecillinam, or *N*-formimidoyl thienamycin. The extent of antagonism varied with the beta-lactam and genus studied. However, antagonism occurred most often with strains possessing inducible cephalosporinases. Antagonism of cephalothin and cefamandole correlated closely with the induction of beta-lactamases capable of inactivating these drugs. Although antagonism of the remaining drugs occurred more often with strains possessing inducible beta-lactamases, these enzymes did not inactivate the drugs. Morphological studies revealed that cefoxitin inhibited filamentation and lysis produced by various beta-lactam drugs. Results of this investigation suggest that cefoxitin antagonizes beta-lactams via (i) induction of drug-inactivating beta-lactamases, and (ii) the induction of beta-lactamases that cannot inactivate the drug but serve as barriers against access to target proteins. This barrier appears most efficient for drugs that bind to penicillin-binding proteins 1 and 3.

Cefoxitin is a relatively new semisynthetic cephamycin that is highly stable to a variety of different beta-lactamases (1, 17). Studies in this and other laboratories have shown that cefoxitin antagonizes the in vitro activity of cefamandole, cefuroxime, cefotaxime, carbenicillin, piperacillin, azlocillin, and ticarcillin (2, 5, 8, 9, 11, 22, 27). Proposed mechanisms responsible for these antagonisms include induction of beta-lactamases by cefoxitin (8, 9, 22, 27) and competition for binding sites within the cell (8). Since each of the previous studies included only a few beta-lactams, the purpose of this study was to evaluate the ability of cefoxitin to antagonize a broad range of beta-lactam antibiotics and determine (i) the relative frequency with which antagonism occurs with each drug, (ii) the relative frequency with which antagonism occurs with specific genera of gram-negative bacilli, and (iii) possible mechanisms responsible for the antagonism.

## MATERIALS AND METHODS

**Antibiotics.** Laboratory standard powders of each antibiotic were obtained from the following manufacturers: cephalothin, cefamandole, cephalexin, and moxalactam (Eli Lilly & Co., Indianapolis, Ind.); cefotaxime (Hoechst-Roussel Pharmaceuticals, Somerville, N.J.); cefsulodin (Abbott Laboratories, North Chicago, Ill.); cefoxitin and *N*-formimidoyl thienamycin (Merck, Sharp & Dohme, Rahway, N.J.); mecillinam (Hoffmann-LaRoche Inc., Nutley, N.J.); carbenicillin (Beecham Laboratories, Bristol, Tenn.); piperacillin (Lederle Laboratories, Pearl River, N.Y.); azlocillin and mezlocillin (Miles Pharmaceuticals, West Haven, Conn.); and penicillin G (Bristol Laboratories, Syracuse, N.Y.). Solutions of each drug were prepared (weight corrected for potency) the day of use. Commercially prepared disks were either purchased from BBL microbiology associates, Cockeysville, Md. or kindly supplied by the drug manufacturer. These disks included ampicillin (10 μg), carbenicillin (100 μg), piperacillin (100 μg), azlocillin (75 μg), mezlocillin (75 μg), mecillinam (16 μg), cephalothin (30 μg), cefamandole (30 μg), cefotaxime (30 μg), moxalactam (30 μg), cefoxitin (30 μg), and cefsulodin (30 μg).

**Bacterial strains.** The 41 strains tested were randomly selected clinical isolates representing those species of nonfastidious gram-negative bacilli commonly recovered from infected sites of hospitalized patients. They included 4 *Escherichia coli*, 3 *Klebsiella pneumoniae*, 3 *Proteus mirabilis*, 3 *Citrobacter* spp., 4 *Serratia* spp., 5 *Enterobacter* spp., 5 indole-positive *Proteus* spp., 4 *Providencia* spp., and 10 *Pseudomonas aeruginosa*. *Enterobacter cloacae* P99, a fully derepressed producer of type Ia cephalosporinase (6), and its beta-lactamase-negative mutant, $P99^-$ (7), were kindly provided by L. Koupal of Merck, Sharp & Dohme.

**Susceptibility tests.** Minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) were determined by twofold dilution tests in 3 ml of Mueller-Hinton broth (MHB; Difco Laboratories) with an inoculum of $10^5$ colony-forming units per ml. Tests were incubated for 18 h at 35°C in air. After incubation, 0.01-ml samples from each clear tube were subcultured to blood agar plates. The MBC was defined as the lowest concentration with no growth on subculture (99.9% kill).

**Disk approximation tests for antagonism.** The ability of cefoxitin to antagonize the activity of other beta-

TABLE 1. Results of tests with cefoxitin on 41 clinical isolates

| Strain | MIC[a] (μg/ml) | Cefoxitin concn used for antagonism tests (μg/ml)[b] |
|---|---|---|
| *Escherichia coli* 10 | 2 | 7.5 |
| *Escherichia coli* 14 | 1 | 10 |
| *Escherichia coli* L39 | 2 | 10 |
| *Escherichia coli* L57 | 8 | 30 |
| *Klebsiella pneumoniae* 1 | 4 | 10 |
| *Klebsiella pneumoniae* L69 | 16 | 15 |
| *Klebsiella pneumoniae* L37 | 4 | 15 |
| *Proteus mirabilis* 20 | 2 | 10 |
| *Proteus mirabilis* L46 | 2 | 15 |
| *Proteus mirabilis* 32 | 2 | 15 |
| *Citrobacter amalonaticus* 19 | 4 | 10 |
| *Citrobacter diversus* L54 | 8 | 15 |
| *Citrobacter freundii* L68 | 8 | 30 |
| *Serratia liquefaciens* 36 | >64[c] | C |
| *Serratia liquefaciens* L29 | 32 | 30 |
| *Serratia marcescens* L6 | 32 | 30 |
| *Serratia marcescens* L40 | >64[c] | C |
| *Enterobacter cloacae* 7 | >64[c] | C |
| *Enterobacter cloacae* 20 | >64[c] | C |
| *Enterobacter aerogenes* 13 | >64[c] | C |
| *Enterobacter sakozaki* 6 | >64[c] | C |
| *Enterobacter agglomerans* 26 | >64[c] | C |
| *Proteus vulgaris* 125 | 2 | 15 |
| *Morganella morganii* L18 | 16[c] | 30 |
| *Morganella morganii* L67 | 32[c] | 30 |
| *Morganella morganii* 14 | 2 | 10 |
| *Morganella morganii* 132 | 4 | 30 |
| *Providencia stuartii* 122 | 1 | 10 |
| *Providencia stuartii* 43 | 1 | 10 |
| *Providencia stuartii* 133 | 4 | 7.5 |
| *Providencia stuartii* L2 | 16 | 15 |
| *Pseudomonas aeruginosa* L3 | >64[c] | C |
| *Pseudomonas aeruginosa* L21 | >64[c] | C |
| *Pseudomonas aeruginosa* L28 | >64[c] | C |
| *Pseudomonas aeruginosa* L47 | >64[c] | C |
| *Pseudomonas aeruginosa* L49 | >64[c] | C |
| *Pseudomonas aeruginosa* L58 | >64[c] | C |
| *Pseudomonas aeruginosa* L13 | >64[c] | C |
| *Pseudomonas aeruginosa* L11 | >64[c] | C |
| *Pseudomonas aeruginosa* L19 | >64[c] | C |
| *Pseudomonas aeruginosa* L32 | >64[c] | C |
| *Enterobacter cloacae* P99 | >64[c] | C |
| *Enterobacter cloacae* P99⁻ | 16 | 30 |

[a] MIC, Minimal inhibitory concentration.

[b] Commercially prepared disks are indicated by the letter C.

[c] Resistant to cefoxitin in disk diffusion assays.

lactam antibiotics was determined in disk approximation tests, as described in detail previously (22). Briefly, a cefoxitin disk producing no zone of inhibition was placed on a seeded plate at a distance from the test disk equivalent to the radius of the zone produced by the beta-lactam when tested alone. After overnight incubation at 35°C in air, the radii of the zone (i) between the cefoxitin and other beta-lactam disks and (ii) on the far side of the beta-lactam disk were measured. If the radius of (i) was smaller than that of (ii) by 4 mm or more, then antagonism was considered to have occurred. For the marketed drugs, tests were performed only on those strains susceptible to the drugs in standard disk diffusion assays (16). For the investigative drugs, tests were performed only on those strains giving zones of ≥15 mm in disk diffusion assays. Commercially prepared disks were used for all drugs except cephalexin and *N*-formimidoyl thienamycin. For these two drugs, disks were prepared the day of use from drug solutions. Disk approximation tests were performed with cefoxitin disks that gave no zone of inhibition for the test strain. For some strains, these were commercially prepared 30-μg disks; for others, disks of lower potency were prepared from drug solutions (Table 1).

**Beta-lactamase assay.** Enzyme inactivation of cephalosporins, moxalactam, and *N*-formimidoyl thienamycin was determined with whole cells, using a spectrophotometric assay described in detail previously (22). Briefly, cells were suspended in phosphate buffer containing 100 μM of each drug. After incubation for 4 h at 37°C in air, the cells were removed by filtration. The amount of residual drug remaining after incubation was determined by measuring the absorbance at the wavelength corresponding to the beta-lactam ring. Inactivation of the penicillins was determined in a cylinder-cup bioassay, using *Sarcina lutea* (for penicillin G) or *Bacillus subtilis* ATCC 6633 (for other penicillins) as the assay organism. Beta-lactamases were induced by growing strains on MH agar (Difco) containing cefoxitin at the highest concentration that was not inhibitory to the test strain (22). Enzymes were considered inducible if levels obtained in cells grown on the cefoxitin agar exceeded levels obtained in cells grown on drug-free agar by at least two standard deviations for the assay employed.

**Morphological studies.** Strains were inoculated into MHB, MHB with 30 μg of cefoxitin per ml, MHB with other beta-lactams at a concentration equivalent to their MBC, and MHB with other beta-lactams plus cefoxitin. After incubation for 4 at 35°C in air, samples of each broth were removed and placed on cleaned glass slides. Cellular morphology was examined immediately by phase-contrast microscopy in simple wet mounts of each sample. Additionally, samples of each aliquot were heat-fixed and stained with methylene blue. These were examined under the high dry objective (50×), and photographs were taken with a microscope-mounted Zeiss 35-mm camera that provided an additional 12.5× magnification. Cellular morphology in wet mounts and stained preparations was the same.

## RESULTS

**Disk approximation tests for antagonism.** Cefoxitin's ability to antagonize the activity of other beta-lactam antibiotics correlated closely with the test strain's susceptibility to cefoxitin, as determined in disk diffusion tests (Tables 1 and 2). For most beta-lactams, antagonism was more likely to occur in tests with cefoxitin-resistant strains than with susceptible strains. For the "penicillin-group" beta-lactams (Table

TABLE 2. Antagonism of beta-lactam antibiotics by cefoxitin in disk approximation tests

| Beta-lactam antibiotic[a] | Antagonism of *Enterobacteriaceae*[b] | | Antagonism of *P. aeruginosa*[b] |
|---|---|---|---|
| | CX-Susceptible[c] | CX-Resistant[c] | |
| AM | 1/6 (17) | | |
| CB | 1/10 (10) | 6/8 (75) | 2/7 (29) |
| PIP | 3/21 (14) | 7/9 (78) | 10/10 (100) |
| MZ | 3/20 (15) | 5/8 (63) | 7/8 (88) |
| AZ | 4/16 (25) | 7/7 (100) | 10/10 (100) |
| MC | 0/14 | 0/4 | |
| TH | 0/22 | 0/9 | 0/10 |
| CF | 2/12 (17) | | |
| CL | 0/12 | | |
| CM | 5/18 (28) | 7/7 (100) | |
| CT | 4/22 (18) | 8/9 (89) | 7/7 (100) |
| MX | 0/22 | 4/9 (44) | 6/6 (100) |
| CS | | | 4/10 (40) |

[a] Drug abbreviations: ampicillin (AM), carbenicillin (CB), piperacillin (PIP), mezlocillin (MZ), azlocillin (AZ), mecillinam (MC), *N*-formimidoyl thienamycin (TH), cephalothin (CF), cephalexin (CL), cefamandole (CM), cefotaxime (CT), moxalactam (MX), cefsulodin (CS).

[b] No. of strains showing antagonism/no. of strains tested. The percentage is given in parentheses.

[c] Cefoxitin (CX) susceptibility was based upon results in disk diffusion assays.

2), azlocillin was most frequently antagonized, followed by piperacillin, mezlocillin, and carbenicillin. Cefoxitin did not antagonize mecillinam or *N*-formimidoyl thienamycin. For the "cephalosporin-group" beta-lactams (Table 2), cefotaxime was most frequently antagonized, followed by cefamandole and moxalactam. The activity of cefsulodin against *Pseudomonas* sp. was antagonized in several tests. No antagonism of cephalexin was observed.

**Enzyme studies.** Tests were performed on a number of the 41 isolates to determine the presence of cefoxitin-inducible beta-lactamases. For cephalothin and cefamandole, there was an association between the presence of inducible cephalosporinases active against these drugs and a positive disk test for antagonism (Table 3). The low prevalence of inducible enzymes capable of inactivating cephalexin in strains with negative tests for antagonism was similar to that observed for strains with negative antagonism tests for cefamandole and cephalothin. Two strains of *Citrobacter* spp. possessed cefoxitin-inducible cephalosporinases that inactivated cephalothin and cefamandole but not cephalexin. Cefoxitin antagonized the activity of the former two drugs but not cephalexin against these strains (Table 2). Thus, the stability of cephalexin to these enzymes in part may have been responsible for the lack of antagonism of this drug by cefoxitin.

In contrast to cephalothin, cefamandole, and cephalexin, there was little correlation between the results of antagonism tests and the presence of cefoxitin-inducible beta-lactamases capable of inactivating ampicillin, the anti-*Pseudomonas* penicillins, cefotaxime, moxalactam, or cefsulodin. For example, only one *Pseudomonas* showed enhanced inactivation of mezlocillin, azlocillin, and piperacillin, but not carbenicillin, after induction with cefoxitin. However, cefoxitin antagonized all four drugs in disk approximation tests with this strain. In general, very few strains showed enhanced inactivation of any of these substrates after cefoxitin induction. However, when penicillin G and cefamandole were used as substrates, a large percentage of strains were found to possess enhanced activity after induction with cefoxitin. The relationship between the presence of this inducible enzyme activity and the results of antagonism tests is shown in Table 4. For cefoxitin-susceptible *Enterobacteriaceae*, strains possessing inducible cephalosporinase activity (measured with cefamandole as a substrate) were more likely to show antagonism than those strains possessing only inducible penicillinase activity (measured with penicillin G as a substrate) or no inducible enzyme activity. All cefoxitin-resistant *Enterobacteriaceae* and *P. aeruginosa* possessed inducible penicillinase and cephalosporinase activity. Antagonism between cefoxitin and other beta-lactam antibiotics was most frequent among these strains. The only beta-lactams that did not show an association between the presence of these enzymes and antagonism were mecillinam and *N*-formimidoyl thienamycin. Regardless of the presence or absence of cefoxitin-inducible beta-lactamases, no antagonism was observed with these drugs. The occurrence of antagonism in tests with each species grouped by its susceptibility to cefoxitin and the presence

TABLE 3. Presence of cefoxitin-inducible cephalosporinase activity in *Enterobacteriaceae* and its relationship to results in disk approximation tests for antagonism

| Substrate | Disk antagonism test | No. of strains tested | No. (%) with inducible beta-lactamases[a] |
|---|---|---|---|
| Cephalothin | Negative | 4 | 1 (25) |
| | Positive | 2 | 2 (100) |
| Cephalexin | Negative | 7 | 1 (14) |
| Cefamandole | Negative | 7 | 2 (29) |
| | Positive | 12 | 12 (100) |

[a] Detected utilizing drug indicated as substrate.

TABLE 4. Presence of cefoxitin-inducible penicillinase/cephalosporinase[a] activity and its relationship to results in disk approximation tests for antagonism

| Bacterium | Presence of cefoxitin-inducible activity | | Cefoxitin antagonism in disk approximation tests[b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Penicillinase | Cephalosporinase | AM | CB | PIP | MZ | AZ | CT | MX | CS |
| Cefoxitin-susceptible | – | – | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | |
| *Enterobacteriaceae* | + | – | | | 0/2 | 0/2 | 0/1 | 0/2 | 0/2 | |
| | – | + | | | 1/1 | 1/1 | 1/1 | 0/2 | 0/2 | |
| | + | + | 1/1 | 0/2 | 1/4 | 1/4 | 2/2 | 2/4 | 0/4 | |
| Cefoxitin-resistant *Enterobacteriaceae* | + | + | | 5/7 | 6/7 | 4/6 | 6/6 | 6/7 | 3/7 | |
| *P. aeruginosa* | + | + | | 2/4 | 6/6 | 4/5 | 6/6 | 4/4 | 4/4 | 2/6 |
| All bacteria | +/– | + | 1/1 | 7/13 | 14/18 | 10/16 | 15/15 | 12/17 | 7/17 | 2/6 |
| All bacteria | +/– | – | 0/2 | 0/2 | 0/4 | 0/4 | 0/3 | 0/4 | 0/4 | |

[a] Penicillinase detected with penicillin G as the substrate; cephalosporinase detected with cefamandole as the substrate. See Table 2, footnote [a] for drug abbreviations.
[b] No. of strains showing antagonism/no. of strains tested.

of inducible cephalosporinase activity is summarized in Table 5. Clearly, the presence of an inducible cephalosporinase was more predictive of antagonism than the susceptibility of the species to cefoxitin.

To further assess the role of inducible beta-lactamases in the antagonisms observed, studies were performed with *E. cloacae* P99 and its beta-lactamase-negative mutant, P99$^-$. The P99 strain was found to be resistant to all beta-lactam antibiotics evaluated in this except mecillinam and *N*-formimidoyl thienamycin. Disk antagonism tests with these latter two drugs were negative. Resistance precluded disk antagonism tests with the other drugs. The P99$^-$ strain was susceptible to all antibiotics, including cefoxitin. Disk antagonism tests with this strain were negative for all drugs. Enzyme assays revealed no beta-lactamase activity in P99$^-$ and noninducible penicillinase and cephalosporinase activity in P99. Among the substrates tested, the P99 strain inactivated penicillin G, cephalothin, cephalexin, cefamandole, and cefotaxime, but not moxalactam or *N*-formimidoyl thienamycin.

TABLE 5. Relationship between inducible cephalosporinases and cefoxitin susceptibility to the occurrence of antagonism in tests with specific organisms

| Species | Antagonism[a] in group that was: | | |
|---|---|---|---|
| | CX-S ENZ – | CX-S ENZ + | CX-R ENZ + |
| *E. coli* | 0/4 | | |
| *K. pneumoniae* | 0/3 | | |
| *P. mirabilis* | 0/3 | | |
| *Citrobacter* spp. | 0/1 | 2/2 | |
| *Enterobacter* spp. | | | 5/5 |
| *Serratia* spp. | 1/1 | 1/1 | 2/2 |
| Indole-positive *Proteus* spp. | 0/3 | | 2/2 |
| *Providencia* spp. | 0/1 | 3/3 | |
| *P. aeruginosa* | | | 10/10 |
| All species | 1/16 | 6/6 | 19/19 |

[a] No. of strains showing antagonism in tests with any beta-lactam antibiotic/no. of strains tested. Cefoxitin susceptible (CX-S); cefoxitin resistant (CX-R) inducible cephalosporinase absent (ENZ –); inducible cephalosporinase present (ENZ +), using cefamandole as substrate.

**Morphological studies.** Microscopic analyses were performed to determine the influence of cefoxitin on the morphological effects produced by beta-lactam antibiotics. For these studies, one strain each of *E. cloacae* and *P. aeruginosa* (both resistant to cefoxitin) was incubated in broth containing a variety of beta-lactams at their MBC, with and without 30 μg of cefoxitin per ml. After 4 h, cells were stained with methylene blue and examined for morphological effects produced by the beta-lactams. For the *E. cloacae*, cefoxitin inhibited cell lysis and disintegration (Fig. 1E and F) and decreased filamentation (Fig. 1C and D) induced by carbenicillin, mezlocillin, cefotaxime, moxalactam, and cefamandole. For the *P. aeruginosa*, cefoxitin (i) did not affect morphological changes induced by carbenicillin (Fig. 2C and D) or mezlocillin, (ii) decreased lysis and filamentation produced by cefotaxime (Fig. 2E and F) and moxalactam, and (iii) enhanced filamentation produced by cefsulodin. Results of disk approximation tests with the two strains correlated with these morphological effects observed in broth, i.e, cefoxitin antagonized each beta-lactam against the *E. cloacae*; did not antagonize carbenicillin, mezlocillin, or cefsulodin against the *P. aeruginosa*; but did antagonize cefotaxime and moxalactam. Although cefoxitin alone did not alter the growth rate or viability of the two strains, slight mor-

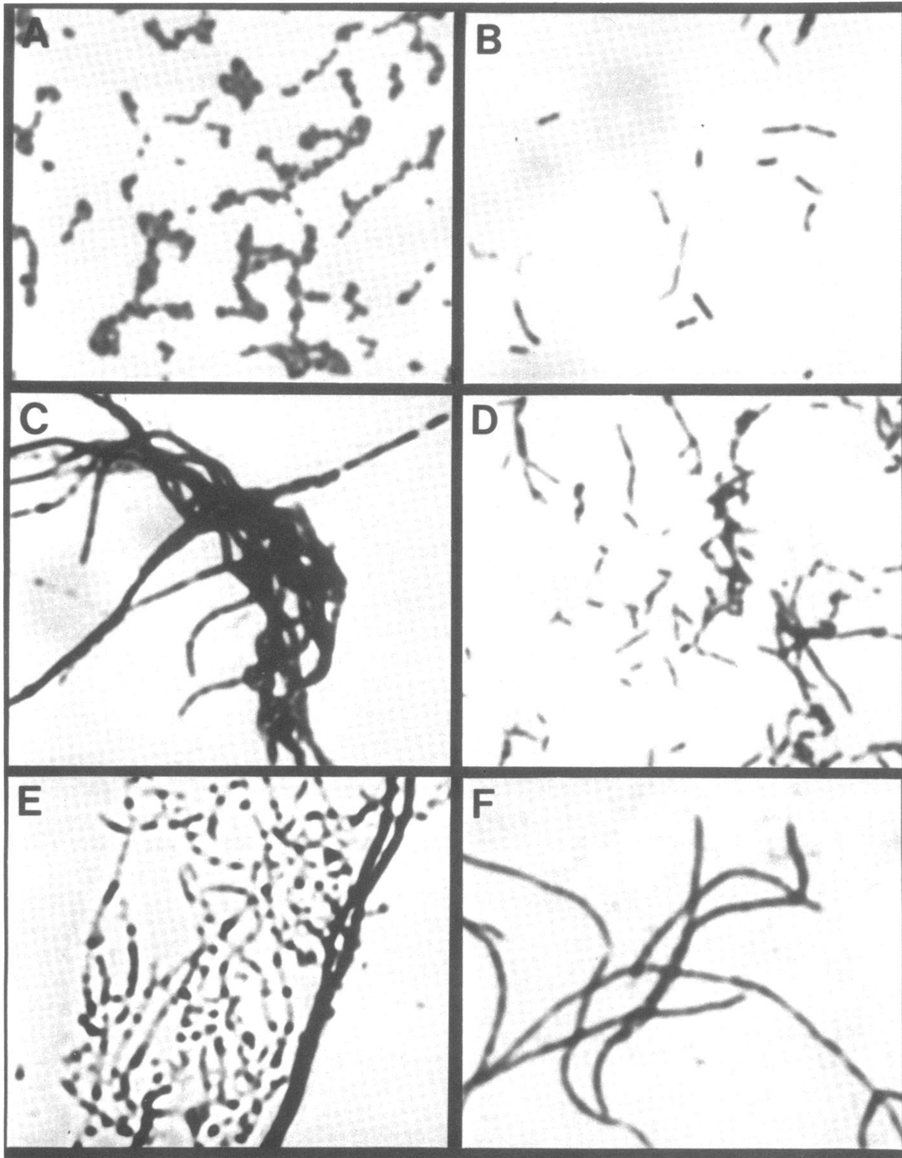


FIG. 1. Alteration of the morphological effects of beta-lactam antibiotics on *E. cloacae* by 30 μg of cefoxitin per ml (625×). Cells were exposed for 4 h to each drug in broth at its MBC, with and without cefoxitin. (A) Drug-free control. (B) Cefoxitin. (C) Carbenicillin (16 μg/ml). (D) Carbenicillin plus cefoxitin. (E) Moxalactam (2.0 μg/ml). (F) Moxalactam plus cefoxitin.

phological alterations were produced (Fig. 1B and 2B).

## DISCUSSION

This study clearly indicated the ability of cefoxitin to antagonize a variety of beta-lactam antibiotics in vitro. These included many anti-*Pseudomonas* penicillins and "third-generation" cephalosporins. The frequency with which antagonism occurred was lowest in tests with strains lacking inducible cephalosporinases and highest with strains possessing such enzymes. Several possible mechanisms appeared to be responsible for the antagonisms observed; both involved beta-lactamases.

The first mechanism involved the induction of drug-inactivating enzymes by cefoxitin. This mechanism appeared to be responsible for the

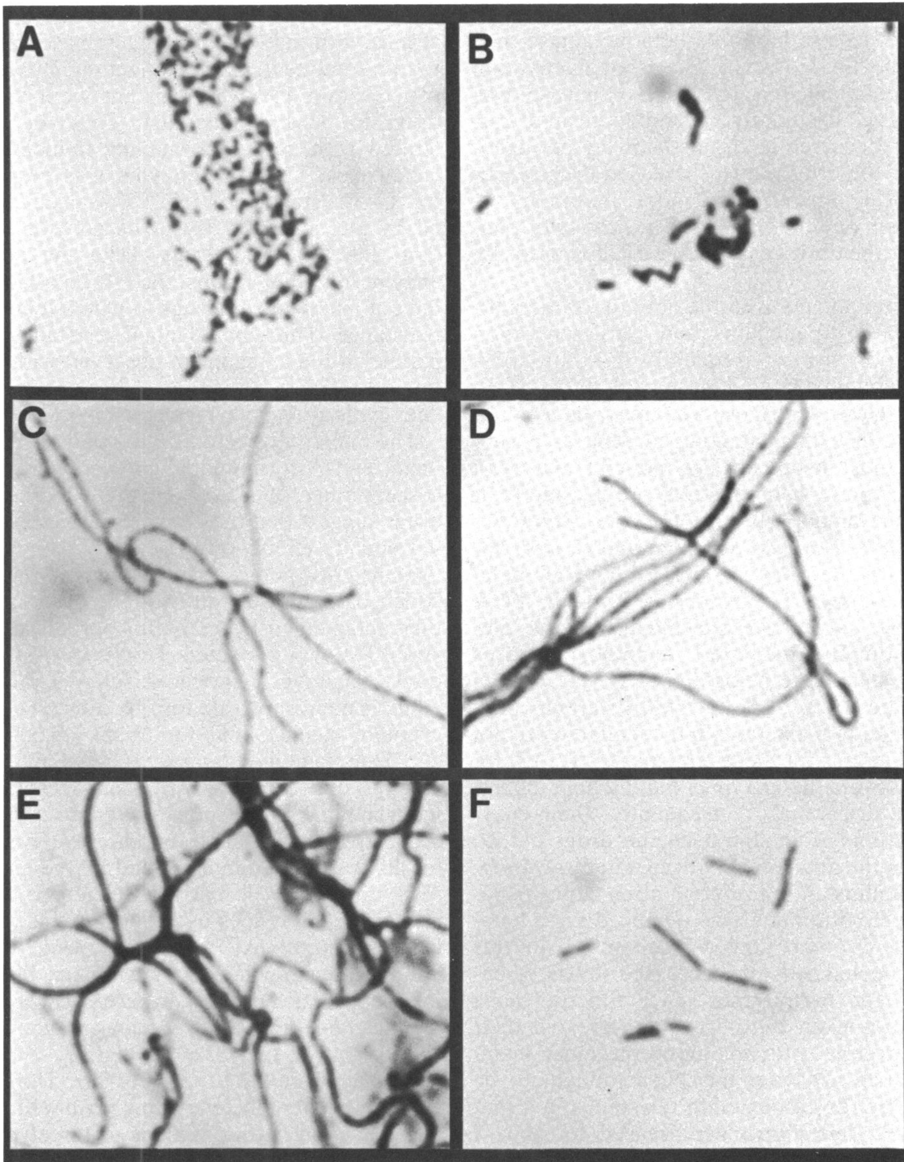


FIG. 2. Alteration of the morphological effects of beta-lactam antibiotics on *P. aeruginosa* by 30 μg of cefoxitin per ml (625×). Cells were exposed for 4 h to each drug in broth at its MBC, with and without cefoxitin. (A) Drug-free control. (B) Cefoxitin. (C) Carbenicillin (100 μg/ml). (D) Carbenicillin plus cefoxitin. (E) Cefotaxime (100 μg/ml). (F) Cefotaxime plus cefoxitin.

antagonism of both cephalothin and cefamandole. The low prevalence of inducible cephalosporinases in cephalothin-susceptible *Enterobacteriaceae* was probably responsible for the low frequency of antagonism of both cephalothin and cefamandole observed with these strains. Also, the stability of cephalexin to the inducible enzymes in these strains may explain the inability of cefoxitin to antagonize this drug. The higher prevalence of inducible cephalosporinases in cephalothin-resistant *Enterobacteriaceae*, especially those also resistant to cefoxitin, was probably responsible for the more frequent antagonism of cefamandole by cefoxitin with these strains. Induction of drug-inactivating enzymes may also be in part responsible for the more frequent antagonism by cefoxitin of the ureidopenicillins in comparison to carbenicillin

in tests with *Pseudomonas* sp., as the former are somewhat less stable to its beta-lactamase than is carbenicillin. However, increased inactivation of the ureidopenicillins by *Pseudomonas* sp. was not observed frequently, and antagonism of carbenicillin occurred in strains showing no inactivation of the drug. Thus, increased drug inactivation did not explain most instances of antagonism of either the anti-*Pseudomonas* penicillins or the third-generation cephalosporins by cefoxitin.

Cefoxitin antagonized the activity of the anti-*Pseudomonas* penicillins and third-generation cephalosporins most frequently in strains possessing inducible penicillinase-cephalosporinase activity. However, these enzymes did not appear to utilize the antagonized drug as a substrate in most instances. The inducible nature of these enzymes, their substrate profile, and their prevalence among strains of *Enterobacter*, indole-positive *Proteus, Serratia*, and *P. aeruginosa* suggest that they are very likely Richmond and Sykes type I enzymes (24). Such beta-lactamases are characteristically inducible, chromosomally mediated cephalosporinases that also inactivate penicillin G, but at a much slower rate (10, 21, 24). Cefoxitin, carbenicillin, cefotaxime, moxalactam, and cefsulodin are not good substrates for these enzymes (4, 12, 17, 19, 24). However, the enzymes have a high affinity for these drugs and, consequently, their enzymatic activity is inhibited by the drugs (4, 19, 24). Thus the antagonism of the anti-*Pseudomonas* penicillins and third-generation cephalosporins by cefoxitin may have occurred via a beta-lactamase-derived barrier mechanism. In this second mechanism, the increased levels of enzyme in the periplasmic space function as a barrier to nonsubstrate drugs and prevent their access to target proteins along the inner membrane. Such a barrier has been thought to be responsible for carbenicillin resistance in a mutant of *P. aeruginosa* derepressed for type I beta-lactamase production (24), cefoxitin resistance in *E. coli* and *E. cloacae* producing high levels of cephalosporinase (17, 25), and resistance to carbenicillin and sulbenicillin in *E. coli* and *Salmonella typhimurium* carrying plasmids for type IIIa and Va beta-lactamases (28). Our results are the first indication that such a barrier mechanism may be responsible for antagonism between two drugs.

The data obtained with *E. cloacae* P99 and its mutant lend further support to this barrier mechanism. *E. cloacae* P99 produced the prototype Ia enzyme (24), which is similar to those found in other strains of *Enterobacter* spp. except its production in P99 is stably derepressed (6, 10, 24). Thus, this strain consistently possesses high levels of beta-lactamase in its periplasmic space and in this respect is phenotypically similar to strains with inducible beta-lactamases after cefoxitin treatment. The beta-lactam antibiotics to which strain P99 was resistant were the same drugs for which antagonism could be demonstrated with strains possessing inducible beta-lactamases. That the enzyme was responsible for this resistance in strain P99 was demonstrated by the susceptibility of its enzymeless mutant, P99$^-$, to the drugs. Like the inducible enzymes in other strains, the P99 beta-lactamase did not inactivate all drugs to which it conferred resistance. Thus, beta-lactam resistance in P99 paralleled the antagonism observed with cefoxitin, and both appeared to be due either to enzymatic or barrier mechanisms.

The inability of cefoxitin to antagonize mecillinam and *N*-formimidoyl thienamycin and the susceptibility of *E. cloacae* P99 to these two drugs suggest that the enzyme-derived barrier is not equally effective for all drugs. Since mecillinam is not a good substrate for type I enzymes which have little affinity for the drug (18), neither antagonism by cefoxitin nor resistance in strain P99 was expected. However, *N*-formimidoyl thienamycin, like moxalactam and carbenicillin, is not a substrate for type I enzymes but is a potent suicide inhibitor of the enzymes (19, 20). Thus, one might have expected antagonism and resistance to this drug, as occurred with moxalactam. Since none was observed, this suggests that the barrier is not equally effective for all drugs that can be bound by the enzymes. This difference in barrier efficiency may be related to differences in target proteins (penicillin-finding proteins, PBPs) in the cell. *N*-formimidoyl thienamycin, like mecillinam, binds primarily to PBP 2 (3, 26), whereas carbenicillin, moxalactam, and other drugs antagonized by cefoxitin bind to PBPs 1A, 1B, 3, and many nonlethal PBPs (3, 13, 23, 26, 29). Thus differences in barrier efficiency may result either from differences in (i) the number of target proteins that must be bound by a drug for its antibacterial effect or (ii) the location of the beta-lactamase barrier relative to the lethal PBPs. In the first situation, under conditions of limited access to target proteins, drugs like carbenicillin and moxalactam that bind to many PBPs are greatly affected since the limited drug molecules passing the barrier bind both lethal and nonlethal PBPs. In contrast, drugs like *N*-formimidoyl thienamycin that bind only to a limited number of PBPs are less affected since each molecule passing the barrier is likely to bind a lethal PBP. In the second situation, the beta-lactamase barrier may be located in greater proximity to PBPs 1A, 1B, or 3. Thus, drugs with these PBPs as primary lethal targets would be more greatly affected than those binding primarily to PBP 2. Clearly,

more extensive studies are required to delineate which of these two possibilities is responsible for the differences in the barrier efficiency observed in this study. However, there is a good association between low barrier efficiency and PBP 2 as a primary lethal target.

Although competition for binding proteins has been proposed as a mechanism responsible for cefoxitin's antagonism of some beta-lactams, this mechanism was not addressed in our study. However, the close correlation between the antagonism profile observed with strains with cefoxitin-inducible beta-lactamases and the resistance profile observed with a strain stably derepressed for enzyme production (in the absence of cefoxitin) makes this mechanism unlikely to be of great importance in the antagonisms reported here.

The clinical significance of these antagonisms is unknown. However, recently Kuck et al. have shown that cefoxitin antagonizes the in vivo efficacy of piperacillin or mezlocillin in an animal infection model (14). Since cefoxitin does appear to be antagonistic to a variety of beta-lactam antibiotics in vitro, we designed an animal infection model to further assess cefoxitin's antagonistic potential in vivo. That study is the subject of the succeeding paper.

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