Antibacterial Activity of Selected Beta-Lactam and Aminoglycoside Antibiotics Against Cephalothin-Resistant Enterobacteriaceae

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The in vitro antibacterial activity of four β-lactam antibiotics (cefatrizine [BL-S640], cefamandole, cefoxitin, and carbenicillin) and three aminoglycosides (amikacin, gentamicin, and tobramycin) was determined against 197 strains of cephalothin-resistant Enterobacteriaceae. Eighty strains were found to be gentamicin-sensitive, and 117 were found to be gentamicin-resistant. Carbenicillin was the most active β-lactam antibiotic against gentamicin-sensitive Serratia marcescens and Enterobacter spp. Cefoxitin was the most active β-lactam antibiotic against the remaining gentamicin-sensitive and -resistant Enterobacteriaceae, including Providencia stuartii and indole-positive Proteus spp. Cefatrizine exhibited little activity against the organisms studied. Cefamandole was less active than cefoxitin and carbenicillin. Amikacin was the most effective agent in vitro. With the exception of S. marcescens, cefoxitin appeared to be the next most promising agent in vitro against gentamicin- and cephalothin-resistant Enterobacteriaceae.

Many of the Enterobacteriaceae, in particular strains of Enterobacter spp., Providencia spp., Serratia spp., and indole-positive Proteus spp., are resistant to cephalothin. Strains of Pseudomonas aeruginosa are uniformly resistant. This resistance pattern was noted when the drug was introduced in 1962 (19). Subsequently, the widespread use of the cephalosporins has led to increasing resistance of some other clinical isolates to cephalothin (11).

Cefatrizine (BL-S640) (9, 10, 17, 21) and cefamandole (5, 16) are new semisynthetic cephalosporins that have been shown to be at least as active as cephalothin in vitro against gram-negative bacilli. Cefatrizine has been shown to be active in vitro against cephalosporin-resistant strains of Enterobacter spp., indole-positive Proteus spp., and P. stuartii (9) and to be efficacious in the treatment of experimental murine urinary tract and systemic infections caused by these same organisms (10). Cefamandole has also been shown to be active against cephalothin-resistant strains of Enterobacter spp., indole-positive Proteus spp., and Providencia spp., (5, 16), as well as some strains of S. marcescens (16); it is currently undergoing clinical investigation.

The cephamycins (18) comprise a new family of β-lactam antibiotics, isolated from species of Streptomyces. They are resistant to a variety of β-lactamases produced by different strains of cephalothin-resistant, gram-negative rods and are active in vitro against those organisms (3). Cefoxitin is the first semisynthetic cephamycin that has been made available for clinical investigation. It is structurally analogous to cephalothin, but perhaps in part, because of its resistance to β-lactamases (15), it has been reported to be more active than cephalothin against indole-positive Proteus spp., Providencia spp., and S. marcescens (2, 8, 14, 15), as well as some strains of Enterobacter (8, 15).

These reports of two new cephalosporins and a new family of compounds with a broader spectrum of activity than cephalothin against gram-negative rods, as well as the availability of newer aminoglycosides, provided the impetus for this study. We have determined and compared the activity of cefatrizine, cefamandole, cefoxitin, cephalothin, carbenicillin, gentamicin, amikacin, and tobramycin against cephalothin-resistant clinical isolates of Enterobacteriaceae at our hospital. We know of no other study that has compared the in vitro activity of these agents. Since cefatrizine, cefamandole, and cefoxitin have shown no activity against strains of P. aeruginosa, these organisms were not included in this study.
Gentamicin is usually the preferred agent in the treatment of many serious gram-negative infections, including those caused by cephalothin-resistant, gram-negative bacillary organisms. Many of our cephalothin-resistant Enterobacteriaceae are resistant to gentamicin as well. Amikacin (12; R. D. Meyer, R. P. Lewis, J. Halter, and M. White, Clin. Res., 24:114A, 1976) has been shown to be active against most gram-negative bacilli, including most gentamicin-resistant organisms. Tobramycin is likewise active against a wide spectrum of gram-negative bacillary organisms but has less activity than amikacin against gentamicin-resistant organisms (13, 23; Meyer et al., Clin. Res.). Cephalothin and tobramycin in combination have been shown to be synergistic against many Providencia spp. (7). Carbenicillin has been reported to be active against a large number of S. marcescens (6, 20, 22), Enterobacter spp., and indole-positive Proteus spp. (20). Since these other agents (carbenicillin, tobramycin, or amikacin) may be useful in various situations in treating infections caused by cephalothin-resistant, gram-negative bacilli, which may also be resistant to gentamicin, they were also included for evaluation with the newer β-lactam antibiotics.

**MATERIALS AND METHODS**

Different clinical isolates of Enterobacteriaceae resistant to cephalothin by standardized disk testing (1) in the Microbiology Laboratory of Wadsworth V.A. Hospital, a general hospital with 558 acute medical and surgical beds, were collected during a 7-month period in 1974 and 1975. Clinical isolates resistant to gentamicin were collected from August 1974 to October 1975. These organisms were identified by standard criteria in the Microbiology Laboratory and in the Anaerobic Bacteriology Research Laboratory at Wadsworth V.A. Hospital. S. marcescens was identified to species by the fermentation of arabinose. Organisms showing a zone size of ≤14 mm to a 30-µg cephalothin disk or ≤12 mm to a 10-µg gentamicin disk on repeated standardized testing (1) were then tested by the agar plate dilution method recommended by the International Collaborative Study of the World Health Organization (4). Approximately 100 organisms grown overnight at 37°C in Mueller-Hinton broth culture were inoculated with a replicating device onto media prepared from Mueller-Hinton broth solidified with 1.5% agar (Difco) and 5% defibrinated sheep blood prepared to contain no antibiotic (control), cefatrizine, cefamandole, cefoxitin, cephalothin, amikacin, gentamicin, or tobramycin in twofold increments from 1 to 128 µg/ml or carbenicillin in twofold increments from 32 to 512 µg/ml. Cefatrizine and amikacin sulfate were supplied by Edward Yevak of Bristol Laboratories. Tobramycin base, cefamandole, and cephalothin were gifts from R. S. Griffith of Eli Lilly & Co. Gentamicin sulfate was provided by George Hough of the Schering Corp. Cefoxitin was donated by C. Martin of Merck, Sharp and Dohme Research Laboratories. Carbenicillin was supplied by B. Horn-Borstel of Roerig Division of Pfizer Laboratories. The minimal inhibitory concentration (MIC) was recorded as the lowest concentration of antibiotic showing only a haze, one colony, or no growth after overnight incubation (4). Reference strains of Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 25922 were included in parallel tests.

Susceptibility to an antibiotic was based upon inhibition of strains at or below peak levels of antibiotics achieved in clinical use or in preclinical studies with volunteers. The following MICs in agar were considered as showing susceptibility: cefatrizine, ≤8 µg/ml; cefamandole, ≤32 µg/ml; cefoxitin, ≤32 µg/ml; cephalothin, ≤16 µg/ml; carbenicillin, ≤128 µg/ml; gentamicin, ≤8 µg/ml; tobramycin, ≤8 µg/ml; and amikacin, ≤16 µg/ml. All determinations were made in duplicate or triplicate, and the MICs were expressed as averages.

**RESULTS**

During the period of this study, 197 strains of Enterobacteriaceae with disk zone sizes of ≤14 mm against the 30-µg disk of cephalothin were confirmed to be cephalothin-resistant (MIC ≥ 32 µg/ml) by the ICS-WHO agar dilution method (4). Eighty strains proved to be gentamicin sensitive (MIC ≤ 8 µg/ml), and 117 were gentamicin resistant (MIC ≥ 16 µg/ml). During the initial phases of this study, we were interested primarily in collecting gentamicin-resistant organisms, so these figures do not reflect the true incidence of gentamicin resistance in our hospital (12; Meyer et al., Clin. Res.). There were appreciable differences in the activity of cefamandole, cefoxitin, carbenicillin, and tobramycin against gentamicin-sensitive as opposed to gentamicin-resistant strains. Table 1 and Fig. 1 through 3 summarize the data for the gentamicin-sensitive, cephalothin-resistant organisms. The data for the gentamicin- and cephalothin-resistant organisms are outlined in Table 2 and Fig. 4 through 7.

**Gentamicin-sensitive organisms.** Seventeen strains of cephalothin-resistant S. marcescens were sensitive to gentamicin. Of the β-lactam antibiotics studied, only carbenicillin was active against two-thirds or more of the strains. Cefatrizine had no activity at all, cefamandole had very little, and cefoxitin was active against only 35.2% of the gentamicin-sensitive S. marcescens (Table 1, Fig. 1).

Carbenicillin was the most active β-lactam antibiotic against our 36 strains of cephalothin-resistant, gentamicin-sensitive Enterobacter spp. Cefamandole was active against 58.3%, of which most were susceptible to carbenicillin.
Cefoxitin and cefatrizine exhibited no appreciable advantage over cephalothin (Table 1, Fig. 2).

Eight strains of Klebsiella spp., 15 of E. coli, and 4 of indole-positive Proteus spp. were sensitive to gentamicin. Since the susceptibility patterns to the antibiotics studied were similar, the data on these 27 strains were pooled. (Table 1, Fig. 3). Cefoxitin > cefamandole > carbenicillin were clearly superior to cephalothin, but again cefatrizine showed only minimal activity.

These 80 gentamicin-sensitive strains were almost uniformly susceptible to amikacin (78/80) and tobramycin (77/80).

Gentamicin-resistant organisms. Fifty strains of S. marcescens accounted for over 40% of the 117 gentamicin-resistant organisms. Cefoxitin was the only β-lactam antibiotic studied that showed any appreciable activity (12% susceptible at ≤32 µg/ml and 34% at ≤64 µg/ml). These organisms were almost uniformly resistant to cefatrizine, cefamandole, carbenicillin, and tobramycin. Amikacin, however, was active against 96% of the organisms (Table 2, Fig. 4).

Cefoxitin was the most active β-lactam antibiotic in vitro against 23 strains of Klebsiella spp. (91.3% susceptible at ≤32 µg/ml). Cefamandole was active against 91.1%, but cefatrizine, carbenicillin, and tobramycin exhibited no activity whatsoever. Amikacin was effective against 100% of these strains (Table 2, Fig. 5).

The data on six E. coli, five Enterobacter spp., seven indole-positive Proteus spp., and three P. mirabilis were pooled (Table 2, Fig. 6). These 21 strains had similar susceptibility patterns and were all gentamicin resistant. Cefoxitin was again the most active β-lactam antibiotic (96.2% susceptible at ≤32 µg/ml). Carbenicillin (23.8% susceptible) was more active than cefamandole (19.0% susceptible) and cefatrizine (4.8% susceptible). One-third of these strains were susceptible to tobramycin. All were inhibited by amikacin.

Twenty-three strains of P. stuartii were uniformly susceptible to amikacin (Table 2, Fig. 7). Cefoxitin (82.6% susceptible) and cefamandole (73.9% susceptible) also showed significant activity. Carbenicillin showed little activity, and

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<tbody>
<tr>
<td>Cefatrizine (MIC ≤ 2 µg/ml)</td>
<td>0</td>
<td>5.6</td>
<td>11.1</td>
</tr>
<tr>
<td>Cefamandole (MIC ≤ 32 µg/ml)</td>
<td>5.8</td>
<td>58.3</td>
<td>70.4</td>
</tr>
<tr>
<td>Cefoxitin (MIC ≤ 32 µg/ml)</td>
<td>35.2</td>
<td>5.6</td>
<td>85.2</td>
</tr>
<tr>
<td>Carbenicillin (MIC ≤ 128 µg/ml)</td>
<td>76.5</td>
<td>77.8</td>
<td>63.0</td>
</tr>
<tr>
<td>Amikacin (MIC ≤ 16 µg/ml)</td>
<td>94.1</td>
<td>100</td>
<td>96.3</td>
</tr>
<tr>
<td>Tobramycin (MIC ≤ 8 µg/ml)</td>
<td>94.1</td>
<td>97.2</td>
<td>96.3</td>
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**Fig. 1.** Antibiotic susceptibility patterns of 17 strains of gentamicin-sensitive, cephalothin-resistant S. marcescens.
Fig. 2. Antibiotic susceptibility patterns of 36 strains of gentamicin-sensitive, cephalothin-resistant Enterobacter.

Fig. 3. Antibiotic susceptibility patterns of 27 strains of gentamicin-sensitive, cephalothin-resistant Klebsiella (8), E. coli (15), and Proteus (4).

Table 2. Antibiotic susceptibility of gentamicin-resistant (MIC ≥ 16 μg/ml), cephalothin-resistant (MIC ≥ 32 μg/ml) organisms

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>S. marcescens (50 strains)</th>
<th>Klebsiella spp. (23 strains)</th>
<th>E. coli (6), Enterobacter spp. (5), Proteus spp. (10) (21 strains)</th>
<th>P. stuartii (23 strains)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftriaxone (MIC ≤ 8 μg/ml)</td>
<td>0</td>
<td>0</td>
<td>4.8</td>
<td>0</td>
</tr>
<tr>
<td>Cefamandole (MIC ≤ 32 μg/ml)</td>
<td>0</td>
<td>9.1</td>
<td>19.0</td>
<td>73.9</td>
</tr>
<tr>
<td>Cefoxitin (MIC ≤ 32 μg/ml)</td>
<td>12</td>
<td>91.3</td>
<td>95.2</td>
<td>82.6</td>
</tr>
<tr>
<td>Carbenicillin (MIC ≤ 128 μg/ml)</td>
<td>2</td>
<td>0</td>
<td>23.8</td>
<td>8.7</td>
</tr>
<tr>
<td>Amikacin (MIC ≤ 16 μg/ml)</td>
<td>96</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Tobramycin (MIC ≤ 8 μg/ml)</td>
<td>2</td>
<td>0</td>
<td>33.3</td>
<td>30.4</td>
</tr>
</tbody>
</table>
cefatrizine showed no activity. Tobramycin was active against only 30.4% of these strains.

**DISCUSSION**

Cefatrizine has a half-life in serum greater than that of cephalaxin and has been shown to be superior to other cephalosporins in the treatment of gram-negative bacillary experimental systemic or urinary tract infections in mice (10). On the basis of reasonable expectation of blood levels, our in vitro data suggest that cefatrizine cannot be expected to be very useful in treating cephalothin-resistant organisms.

Cefamandole was active against over 50% of our gentamicin-sensitive strains of *Enterobacter* spp., *Klebsiella* spp., *E. coli*, and indole-positive *Proteus*. These strains were, however, also usually sensitive to carbenicillin, as noted previously by others (16). Cefamandole showed appreciable (73.9% susceptible) activity against *P. stuartii*. These organisms are not usually susceptible to carbenicillin and were not in our study (Table 2, Fig. 7). Cefamandole appears to offer promise in the treatment of infections secondary to the organisms noted above.

Carbenicillin was the most active of the β-lactam antibiotics tested against gentamicin-sensitive *S. marcescens* and *Enterobacter* spp. Cefoxitin in turn was the most active β-lactam antibiotic tested against gentamicin-sensitive
and -resistant Klebsiella spp., E. coli, and indole-positive Proteus spp., as well as against gantamicin-resistant S. marcescens, Enterobacter spp., and P. stuartii. With the exception of S. marcescens, cefoxitin was almost as active (83 to 95% susceptible) as amikacin (100% susceptible) against gentamicin-resistant organisms.

At achievable serum levels, carbenicillin and cefoxitin had the greatest overall activity of the \(\beta\)-lactam antibiotics studied. The activity of cefoxitin against Enterobacteriaceae has previously been shown to be comparable to that of carbenicillin (D. Williams, M. Miller, S. Mann, and A. Folkens, Clin. Res. 23:55A, 1975). Our data indicate that cefoxitin is considerably more active than carbenicillin against our gentamicin-resistant strains. In contrast to the findings of others (D. Williams et al., Clin. Res. 23:55A, 1975), many of our S. marcescens are resistant to both cefoxitin and carbenicillin. The mechanism of broader gram-negative antibacterial activity of cefoxitin over that of cephalothin demonstrated here and in other studies (2, 8, 14) is unclear but probably relates in part to its relative resistance to \(\beta\)-lactamases (15).

These studies show that, of the new \(\beta\)-lactam antibiotics (ceftrizine, cefamandole, and cefoxitin) reported to have a broader gram-negative spectrum of activity than cephalothin, ce-
foxitin is most active in vitro. Cefamandole is less effective in vitro than cefoxitin against cephalothin-resistant organisms but has significant activity against some strains. With the exception of strains of *P. stuartii*, these are usually also sensitive to carbenicillin. Cefazolin had little activity against the organisms studied, and its usefulness may be limited by relatively low concentrations achieved after oral administration. Amikacin was the most active antibiotic tested against our multiple-drug-resistant *Enterobacteriaceae*.

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LITERATURE CITED