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# Comparison of Cefoperazone, Cefotaxime, and Moxalactam (LY127935) Against Aerobic Gram-Negative Bacilli

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This study compares the minimum inhibitory concentrations of cefoperazone, cefotaxime, and moxalactam (LY127935) for 446 aerobic gram-negative bacillary isolates and further compares the minimum inhibitory concentrations of LY127935 and these third-generation cephalosporins with those of thienamycin for *Pseudomonas aeruginosa*. Each antibiotic at low concentrations inhibited nearly all *Enterobacteriaceae* tested. Minimum inhibitory concentrations for *P. aeruginosa* were higher, but for a majority of strains they fell below achievable serum levels. Thienamycin and cefoperazone showed significantly greater anti-pseudomonal activity than did cefotaxime or LY127935. Cefoxitin-inducible resistance to LY127935 and the two cephalosporins was demonstrated among *Enterobacter* species but did not occur with thienamycin.

Refinements in the chemistry of the cephalosporins have provided a third generation in this family of antimicrobial agents. Although second-generation cephalosporins—cefoxitin, cefamandole, and cefuroxime-inhibit the majority of *Enterobacteriaceae*, they have little or no activity against *Pseudomonas aeruginosa*. Third-generation compounds now being tested include cefoperazone (T1551) (5) and cefotaxime (HR756) (1,2,3,8). Moxalactam (LY127935) (4) and another new beta-lactam antimicrobial agent, thienamycin (11), though not cephalosporins, resemble them in that spectrum of activity and are currently under study. The purpose of the present study was to compare and contrast the in vitro efficacy of these four antibiotics against aerobic gram-negative bacilli isolated in the clinical laboratory of a large teaching hospital.

## MATERIALS AND METHODS

We determined minimum inhibitory concentrations (MICs) by agar dilution by the procedure of Washington and Barry (9).

**Preparation of inoculum.** Aerobic, gram-negative bacilli isolated by the Duke Clinical Microbiology Laboratory were streaked on nutrient agar (Difco Laboratories) to provide single colonies. After incubation and inspection for purity, plates were held at 4°C until used. Approximately five colonies were touched with a bacteriological loop and inoculated into tubes containing 2 to 5 ml of tryptic soy broth, which were incubated at 35°C in air for 2 to 5 h. Turbidity was adjusted to that of a 0.5 MacFarlane standard by addition of further tryptic soy broth, the suspension was blended in a Vortex mixer, and a final dilution of 1:20 was made in tryptic soy broth. Plates were inoculated with a Steers replicator within 30 min of standardizing the inoculum. This method delivered approximately $10^4$ colony-forming units CFU of each organism to the plates.

**Preparation of agar plates.** LY127935 assay powder was supplied by Lilly Research Laboratories, cefoperazone was supplied by Pfizer, cefotaxime was supplied by Hoechst-Roussel, and thienamycin was supplied by Merck Sharp & Dohme. Cefoperazone and cefotaxime were dissolved in sterile, deionized water, LY127935 was dissolved in 0.1 M phosphate-buffered saline (PBS; pH 7.0), and thienamycin was dissolved in 0.01 M PBS. To make plates containing twofold dilutions, antibiotics were prepared in 50-ml, screw-topped tubes so that each held a final volume of 2.5 ml that contained 10 times the concentration of antibiotic required in each agar plate. A 22.5-ml amount of Mueller-Hinton agar from a single batch (control no. 656889) at a concentration of 1.5% at 50 to 55°C was added to each tube to give a final volume of 25 ml. Antibiotic and agar were mixed by gentle inversion of the tube, which was then emptied into a 100-mm petri dish. Final concentrations of antibiotics in agar ranged from 64 μg to 0.03 μg of thienamycin. To test swarming *Proteus*, agar was prepared at a 3% concentration. All plates were inoculated within 4 h of beginning antibiotic dilutions.

**Controls.** Each plate was inoculated with the following control organisms: *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, and *Staphylococcus aureus* ATCC 25923.

**Incubation and interpretation.** Plates were incubated for 18 h at 35°C in air. Control plates without antibiotic were included at the beginning and end of each series of inoculations. An adequate inoculum was defined by confluent or near-confluent growth on control plates. The MIC of antibiotic was taken as the lowest concentration that prevented growth. A single colony or faint haze was defined as "no growth."

## RESULTS

**Antibacterial activity.** Table 1 shows the comparative in vitro activity of LY127935 and

TABLE 1. *In vitro susceptibility of 446 aerobic gram-negative bacilli to LY127935, cefoperazone (T1551), and cefotaxime (HR756) and of 43 P. aeruginosa strains to thienamycin*

| Organism | No. of isolates | MIC (μg/ml) inhibiting the following % of strains: | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | LY127935 | | | Cefoperazone | | | Cefotaxime | | | Thienamycin | | |
| | | 50 | 80 | 90 | 50 | 80 | 90 | 50 | 80 | 90 | 50 | 80 | 90 |
| *P. aeruginosa* | 148 | 16 | 64 | 64 | 4 | 16 | 32 | 16 | 64 | 64 | | | |
| | 43 | | | | | | | | | | 8 | 8 | 8 |
| *E. coli* | 110 | 0.06 | 0.12 | 0.12 | 0.12 | 0.50 | 1 | ≤0.03 | 0.06 | 0.06 | | | |
| *Klebsiella* species[a] | 69 | 0.12 | 0.25 | 0.25 | 0.25 | 0.50 | 1 | ≤0.03 | 0.06 | 0.12 | | | |
| *Enterobacter* species[b] | 25 | 0.06 | 0.12 | 0.25 | 0.12 | 0.25 | 0.50 | 0.12 | 0.50 | 0.50 | | | |
| *Proteus mirabilis* | 28 | 0.06 | 0.12 | 0.12 | 0.50 | 1 | 1 | ≤0.03 | ≤0.03 | ≤0.03 | | | |
| *Proteus* (indole +),[c] *Providencia* and *Morganella* species | 25 | 0.12 | 0.12 | 1 | 1 | 2 | 4 | ≤0.03 | 0.06 | 0.25 | | | |
| *Citrobacter* species[d] | 12 | 0.06 | 0.12 | 0.12 | 0.12 | 0.25 | 0.50 | 0.06 | 0.12 | 0.25 | | | |
| *Serratia marcescens* | 9 | 0.25 | 1 | 2 | 1 | 2 | >32 | 0.25 | 4 | 8 | | | |
| *Acinetobacter* species[e] | 12 | 64 | 64 | >64 | 32 | 64 | 64 | 8 | 16 | 32 | | | |
| Other *Pseudomonas* species[f] | 4 | 8 | 32 | 32 | 8 | 32 | 32 | 1 | 16 | 16 | | | |
| Miscellaneous[g] | 4 | 0.12 | 64 | 64 | 1 | 16 | 16 | 0.12 | ≤16 | ≤16 | | | |

[a] 54 *K. pneumoniae*, 13 *K. oxytoca*, and 2 *K. ozaenae*.
[b] 17 *E. cloacae*, 6 *E. aerogenes*, and 2 *E. agglomerans*.
[c] 11 *M. morganii*, 7 *P. stuartii*, 4 *P. vulgaris*, and 3 *P. rettgeri*.
[d] Nine *C. freundii* and three *C. diversus*.
[e] 10 *A. calcoaceticus* subsp. *anitratus* and 2 *A. calcoaceticus* subsp. *lwoffi*.
[f] *P. putrifaciens*, *P. stutzeri*, *P. maltophilia*, and *P. fluorescens*.
[g] *Hafnia alvei*, *Aeromonas hydrophila*, *Alkaligenes faecalis*, and *Flavobacterium meningosepticum*.

two third-generation cephalosporins, cefoperazine and cefotaxime, against 446 aerobic gram-negative bacilli. It also shows the MICs of thienamycin for 43 strains of *P. aeruginosa*. Of the four antimicrobial agents, thienamycin proved to be most active by weight against *P. aeruginosa*, inhibiting more than 95% of strains at 16 μg/ml. This concentration of each of the other three agents inhibited more than 50% of *P. aeruginosa* isolates, whereas 64 μg/ml inhibited over 90%. Cefoperazone was slightly more active than cefotaxime and LY127935 against the strains tested.

Most aerobic, gram-negative species other than *P. aeruginosa* were inhibited by LY127935 and both cephalosporins at low concentrations: for example, over 90% of 204 strains of *E. coli*, *Klebsiella* and *Enterobacter* were inhibited by each of these three antimicrobial agents at ≤1 μg/ml (Table 1). Cefoperazone was somewhat less active, however, than cefotaxime or LY127935 against the other species tested.

Only five (1.7%) non-*P. aeruginosa* strains were resistant to LY127935 or the two cephalosporins at 64 μg/ml. These were two strains of *Acinetobacter calcoaceticus* subsp. *antitratus*, two *Providencia stuartii*, and one *Klebsiella oxytoca*. Overall, strains of *Acinetobacter calcoaceticus* var. *anitratus* and *Providencia stuartii* were more resistant than other species.

Isolates relatively resistant to LY127935 or either of the newer cephalosporins were usually relatively resistant to all. We noted some exceptions: for example, a strain of *Klebsiella oxytoca* was resistant to 64 μg of cefoperazone per ml but susceptible to 0.125 μg of cefotaxime and LY127935 per ml. This disparity was confirmed by three separate determinations. We could not evaluate thienamycin against most of the non-*P. aeruginosa* isolates because insufficient compound was available. However, MICs for 13 strains of *Enterobacteriaceae* were determined; nine *E. coli*, one *K. oxytoca*, and one *Enterobacter cloacae* were susceptible to ≤0.5 μg of thienamycin per ml; a single strain of *Providencia rettgeri* was inhibited by thienamycin at 8 μg/ml.

**Resistant isolates.** Disk susceptibilities of all our isolates had been determined by the clinical microbiology laboratory, using the Bauer-Kirby method. Table 2 lists the percentage of *P. aeruginosa* strains, non-*P. aeruginosa* strains, and all strains that were resistant to cephalothin, cefamandole, carbenicillin, and three aminoglycosides, compared with the percent resistant to the two new cephalosporins, to LY127935, and to thienamycin by MICs. We also analyzed the in vitro efficacy of these four β-lactam antibiotics, using an arbitrary breakpoint of 32 μg/ml, against *P. aeruginosa* that were resistant to carbenicillin and those resistant to gentamicin or tobramycin or both (Fig. 1). Strains resistant to these established antipseudomonal agents tended to be resistant to LY127935 and cefotaxime, but most were inhibited by cefoperazone, and all 10 strains tested were inhibited by thienamycin.

**Inoculum effect.** We studied the effect of increasing inoculum by determining MICs for 47 isolates, including 12 *P. aeruginosa* isolates using inocula of both $10^4$ and $10^6$ colony-forming units (CFU)/ml. Figure 2 illustrates the magnitude of the inoculum effect by showing the cumulative percentage of strains at each inoculum that were inhibited by drug concentrations ranging from 0.03 to 64 μg/ml. Usually the higher inoculum caused only modest increases in MICs. A four-fold or greater increase in MIC was ob-

TABLE 2. *Isolates resistant to established antibiotics by disk testing, with those resistant to LY127935 and the third-generation cephalosporins and thienamycin by agar dilution*[a]

| Resistant to:[b] | *P. aeruginosa* (*n* = 148) | non-*Pseudomonas* (*n* = 298) | All isolates (*n* = 446) |
|---|---|---|---|
| By disk | | | |
| Cepalothin | 100 | 31 | 48 |
| Cefamandole | 100 | 12 | 41 |
| Carbenicillin | 29 | 35 | 33 |
| Gentamicin/tobramycin | 22 | 3 | 9 |
| Amikacin | 6 | 1 | 3 |
| By MIC | | | |
| LY127935 | 24 | 3 | 10 |
| Cefotaxime | 22 | 1 | 8 |
| Cefoperazone | 3 | 3 | 3 |
| Thienamycin | 2[c] | ND | ND |

[a] Values represent percent. ND, Not done.
[b] Using >32 μg/ml to indicate resistance, except for thienamycin, where >16 μg/ml was used.
[c] 43 strains.

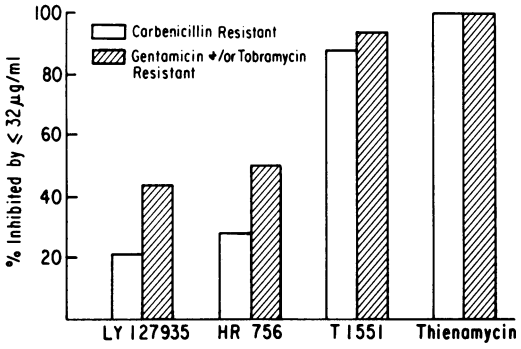


FIG. 1. *Percent P. aeruginosa resistant to carbenicillin, and percent resistant to gentamicin or tobramycin or both, inhibited by LY 127935, cefoperazone, cefotaxime, and thienamycin at 32 µg/ml. (43 carbenicillin-resistant strains (10 versus thienamycin) and 32 aminoglycoside-resistant strains (10 versus thienamycin).*

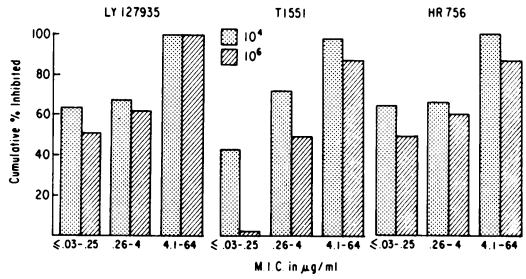


FIG. 2. *Effect of inoculum on MICs of LY127935, cefoperazone, and cefotaxime for 47 strains of aerobic, gram-negative bacilli: 12 P. aeruginosa, 12 E. coli, 7 K. pneumoniae, 4 E. cloacae, 2 E. aerogenes, 2 S. marcescens, 2 Citrobacter freundii, 2 Acinetobacter calcoaceticus var. anitratus, 2 Providencia spp., 1 Morganella morganii, and 1 P. maltophilia.*

tained by using an inoculum of $10^6$ organisms for 34% of isolates tested for susceptibility to cefoperazone, 19% in the case of cefotaxime, and 13% with LY127935. For a few isolates among *E. coli*, *S. marcescens*, *E. cloacae*, and *Citrobacter freundii* species, the inoculum effect was striking for one or more of the cephalosporins. For example, three strains of *E. coli* that were inhibited by cefoperazone at 2 to 16 µg/ml with an inoculum of $10^4$ were resistant to cefoperazone at 64 µg/ml at an inoculum of $10^6$ CFU. These same three organisms showed either no change or only a twofold increase in resistance to cefotaxime and LY127935 at the higher inoculum. Conversely, a few isolates showed a greater inoculum effect with LY127935 or cefotaxime than with cefoperazone; the MIC of cefoperazone for one strain of *Citrobacter freundii* showed a fourfold increase, using $10^6$ CFU, whereas MICs of LY127935 and cefotaxime increased 64- and 128-fold, respectively. We could discern no specific relationship between the MIC determined by using $10^4$ organisms and the magnitude of the inoculum effect.

**Cefoxitin-inducible resistance.** We tested for cefoxitin-inducible resistance in three strains of *E. cloacae*, one *E. aerogenes*, two *Serratia marcescens*, and one strain each of *K. oxytoca*, *Providencia stuartii*, *Providencia rettgeri*, and *Morganella morganii*, using the screening method devised by Waterworth and Emmerson (10). Cefoxitin-induced resistance was found in all four strains of *Enterobacter* for cefotaxime, cefoperazone, and LY 127935, but not in the other organisms. In the case of thienamycin, however, no resistance was induced among the four *Enterobacter* strains by cefoxitin.

**Effect of serum on MICs.** To estimate the importance of protein binding, we determined the MICs of the three cephalosporins for three control organisms (*S. aeureus* ATCC 25923, *E. coli* ATCC 25922, and *P. aeruginosa* ATCC 27853) in both Mueller-Hinton broth and in the same medium plus 50% inactivated human serum, using a standard microdilution procedure (7). The inoculum used was $10^5$ CFU/ml. MICs of these antibiotics for *S. aureus* increased two- to eightfold in 50% serum. On two separate determinations, we found four- and eightfold increases in the MIC of cefoperazone against this organism, compared with two- and fourfold increases in the case of LY127935 and fourfold increases in the case of cefotaxime. Against *E. coli*, MICs were either unchanged or increased only twofold. MICs for this strain of *P. aeruginosa* showed either no change or two- to fourfold reduction in 50% serum.

**Bactericidal activity.** To determine the minimum bactericidal concentrations (MBCs) of the cephalosporins for control organisms, we used the microdilution procedure and subsequently plated 0.01 ml onto agar from those wells that did not show turbidity after overnight incubation in air at 35°C. After further overnight incubation, the MBC was read as the least concentration of antibiotic permitting growth of five or fewer colonies. For *E. coli* and *S. aureus*, the MBC of each antibiotic was the same as its MIC. For *P. aeruginosa*, the MBC of LY127935 (32 µg/ml) was 4 times its MIC, that of cefotaxime (64 µg/ml) was 8 times its MIC, and that of cefoperazone (>64 µg/ml) was more than 16 times greater than its MIC.

## DISCUSSION

The third generation of cephalosporins, now being evaluated, includes cefoperazone and ce-

fotaxime. These agents, and LY127935 and thienamycin, other new β-lactam antibiotics, are all potentially useful for treatment of severe bacterial infections, because each has a remarkably wide spectrum of antibacterial activity and each is likely to prove as free from toxicity as well-tried predecessors in the β-lactam series. Recent reports have documented the in vitro activities of these new agents and compared them with other antimicrobial agents (1,2,3,4,5,8,11). Here we compare all four in a single study of 446 aerobic gram-negative isolates from the clinical microbiology laboratory of a teaching hospital.

Our findings confirm that the third-generation cephalosporins, cefoperazone and cefotaxime, as well as the β-lactam LY127935, are highly active against the majority of *Enterobacteriaceae*, inhibiting at 2 μg/ml, over 90% of strains. This represents a significant increase in both spectrum and potency over previous generations of cephalosporins. Although *P. aeruginosa* isolates were relatively resistant compared with most *Enterobacteriaceae*, the high serum levels achievable with these new antimicrobial agents exceed MICs for the majority of this species.

Thienamycin, a noncephalosporin β-lactam antibiotic, was the most effective of the four agents studied against *P. aeruginosa*. For this species, cefoperazone also was somewhat more active than either LY127935 or cefotaxime. On the other hand, LY127935 and cefotaxime held an advantage over cefoperazone against most *Enterobacteriaceae*, but since this difference became inapparent at concentrations well below serum levels achievable with LY127935 or either of the two cephalosporins, its significance is uncertain.

Because *Pseudomonas* strains comprise an important proportion of resistant isolates, we compared MICs of the four new antibiotics for *P. aeruginosa* isolates that were resistant to carbenicillin, or were resistant to gentamicin or tobramycin or both by disk testing. At an arbitrary break point of 32 μg/ml, thienamycin inhibited all resistant strains, whereas cefoperazone inhibited 88 and 94% of carbenicillin and aminoglycoside-resistant strains, respectively. On the other hand, cefotaxime and LY127935 inhibited half or fewer of these resistant isolates at 32 μg/ml. In this series of clinical isolates, the proportion of gram-negative bacilli inhibited by these new agents was comparable to that sensitive to gentamicin or tobramycin. However, for *P. aeruginosa*, amikacin inhibited significantly more strains than any other tested except thienamycin.

Although the aminoglycosides were equivalent to or, in the case of amikacin, superior to LY127935 and the third-generation cephalosporins with respect to the percentage of aerobic gram-negative bacilli inhibited, most aminoglycoside-resistant isolates, including those resistant to amikacin, were nevertheless susceptible to one or more of the cephalosporins or LY127935. Moreover, the disparities between MICs of LY127935, cefoperazone, and cefotaxime for particular isolates indicates that no one of these agents has an overall advantage. Clinical microbiology laboratories will find it necessary to determine the susceptibilities of isolates to each rather than adopt a generic disk.

Cefoxitin can induce resistance in vitro to cefuroxime and cefotaxime (10), and to cefamandole (6), among some strains of *Enterobacteriaceae*. We found that cefoxitin similarly induced resistance to LY127935 and cefoperazone in four strains of *Enterobacter*. In contrast, cefoxitin did not induce resistance to thienamycin in these same strains. In this respect, thienamycin may possess an advantage over LY127935 and the cephalosporins.

Cefoxitin-inducible resistance is especially prevalent among *Enterobacter* species. It may prove to be of clinical significance because those organisms exhibiting this phenomenon also contain a subpopulation of mutants that are naturally resistant to all cephalosporins tested (10). In our study, some but not all strains showing cefoxitin-inducible resistance appeared considerably more resistant to LY127935 and the third-generation cephalosporins when $10^6$ rather than $10^4$ CFU were used as the inoculum. However, not all isolates for which MICs increased markedly at the higher inoculum showed enhanced resistance in the presence of cefoxitin.

We found little change in the MICs of LY127935 and the two cephalosporins for most of 47 isolates when tested at an inoculum of $10^6$ in addition to $10^4$ CFU. The MICs of cefoperazone were most affected, and those of LY127935 least often showed significant change. Individual organisms showed a marked inoculum effect which generally applied to one or two, but not all, of the cephalosporins and LY127935. No single agent emerged as superior for all organisms tested.

The addition of 50% heat-inactivated serum to broth lowered the MICs of LY127935 and the two cephalosporins for *P. aeruginosa* ATCC 27853, but raised those for *S. aureus* ATCC 25923 two- to eightfold, an extent in keeping with protein binding reported by the manufacturers of these agents: 50% for LY127935, 36 to 50% for cefotaxime, and 85 to 90% for cefoperazone.

LY127935 and the two cephalosporins were bactericidal for *S. aureus* ATCC 25923 and *E.*

*coli* ATCC 25922, but concentrations significantly above their MICs were required to kill *P. aeruginosa* ATCC 27853. The MBC of cefoperazone for this organism exceeded 64 μg/ml, even though the MIC was only 4 μg/ml. However, other investigators have found that MBCs for most clinical isolates are only two- to fourfold greater than corresponding MICs [R. Auckenthaler and F. A. Waldvogel, 1st Int. Symp. Cefoperazone Sodium (T1551), abstr. no. 11, 1979].

These new antimicrobial agents cannot be regarded merely as redundant congeners of earlier cephalosporins, offering only marginal improvements over existing antibiotics. Each has significant advantages in its in vitro spectrum, especially for *Pseudomonas* spp., over previously licensed β-lactam drugs.

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