

## In Vitro Activity of *N*-Formimidoyl Thienamycin (MK0787) Against Resistant Strains of *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Serratia marcescens*, and *Enterococcus* spp.

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The in vitro activities of *N*-formimidoyl thienamycin (MK0787) and nine other antibiotics were tested against 129 clinical isolates, including 71 of enterococci, 34 of *Staphylococcus epidermidis*, 17 of *Pseudomonas aeruginosa*, and 7 of *Serratia marcescens*. These isolates exhibited a variety of resistant patterns: 97% of the enterococci were resistant to moxalactam; 71 and 81% of the *S. epidermidis* isolates were resistant to methicillin and penicillin, respectively; 47, 53, and 53% of the *P. aeruginosa* isolates were resistant to carbenicillin, cefotaxime, and moxalactam, respectively; and all *S. marcescens* isolates were resistant to amikacin, gentamicin, and tobramycin. With respect to concentrations required to inhibit growth of 90% of the isolates, *N*-formimidoyl thienamycin was more active than any compound tested. Determination of the concentration required to inhibit growth of 50% of the isolates showed *N*-formimidoyl thienamycin to be more active than any other agent against *S. epidermidis*, *S. marcescens*, and enterococci, but against *Pseudomonas* isolates it was less active than amikacin, gentamicin, and tobramycin. This preparation is potentially useful for patients with serious infection caused by resistant bacteria; enterococcal and *S. epidermidis* endocarditis infections may be special situations which merit clinical trials.

*N*-formimidoyl thienamycin (MK0787), a new beta-lactam derivative, has broad activity against gram-positive and gram-negative bacteria, including enterococci and strains of *Staphylococcus epidermidis* resistant to cephalosporins and other penicillinase-resistant penicillins (3, 4). This observation led to the study of the comparative activities of *N*-formimidoyl thienamycin and 13 other antimicrobial agents in vitro against clinical isolates of the above microorganisms, *Pseudomonas aeruginosa*, and *Serratia marcescens* with the results recorded in this report.

### MATERIALS AND METHODS

**Microorganisms.** The 129 isolates examined in this report were obtained from as many different patients at University Hospital, Birmingham, Alabama. These included 34 strains of *S. epidermidis*, 71 strains of enterococci, 7 strains of *S. marcescens*, and 17 strains of *P. aeruginosa*. All of the enterococci grew in 6.5% NaCl and were bile esculin positive. The seven *S. marcescens* isolates represented strains which were found in a previous study to be most resistant to aminoglycosides among 50 strains examined (2). Table 1 shows the percentages of strains used in this study which exhibited minimal inhibitory concentrations (MICs) greater than levels usually achievable in serum (5).

All isolates were stored in frozen skim milk at  $-60^{\circ}\text{C}$  after isolation. During testing they were maintained on nutrient agar slants (BBL Microbiology Systems, Cockeysville, Md.) at room temperature.

**Antimicrobial agents.** Sterile standardized powders were kindly supplied by their respective manufacturers: *N*-formimidoyl thienamycin (MK0787) by Merck, Sharp and Dohme Research Laboratories; moxalactam (LY127935), tobramycin, vancomycin, and cephalothin by Eli Lilly & Co.; carbenicillin and ticarcillin by Beecham Laboratories; gentamicin by Schering Corp.; clindamycin by The Upjohn Co., amikacin and methicillin by Bristol Laboratories; cefotaxime (HR756) by Hoechst-Roussel Pharmaceuticals, Inc.; and cefoperazone by Pfizer Pharmaceuticals.

**Determination of MICs.** MICs were determined by a microdilution technique described by Barry (1). Antibiotic dilutions were prepared in Mueller-Hinton broth and distributed in 50- $\mu\text{l}$  volumes to the wells of a U-shaped microtiter plate (Dynatech, Inc., Alexandria, Va.).

Colonies of isolates to be tested were picked from nutrient agar slants, suspended in 1 ml of Mueller-Hinton broth, and incubated overnight at  $37^{\circ}\text{C}$ . Fifty microliters of a 1:1,000 dilution of this culture in Mueller-Hinton broth was added to each well of the microtiter plate, resulting in final inocula (determined by representative colony counts) of from  $4.5 \times 10^4$  to  $1.0 \times 10^5$  colony-forming units per ml. The concentrations of antimicrobial agents in these wells ranged from 0.12 to 100  $\mu\text{g/ml}$ . The MIC was defined as the lowest

TABLE 1. Percentage of bacterial isolates with MIC greater than a concentration indicated

| Agent         | MIC ( $\mu\text{g/ml}$ ) | <i>P. aeruginosa</i><br>(17 strains) | <i>S. epidermidis</i><br>(34 strains) | <i>S. marcescens</i><br>(7 strains) | <i>Enterococcus</i> spp.<br>(71 strains) |
|---------------|--------------------------|--------------------------------------|---------------------------------------|-------------------------------------|--|
| Amikacin      | 32                       | 18                                   | NT <sup>a</sup>                       | 100                                 | NT                                       |
| Carbenicillin | 125                      | 47                                   | NT                                    | NT                                  | NT                                       |
| Cefoperazone  | 32                       | 12                                   | NT                                    | NT                                  | 21                                       |
| Cefotaxime    | 32                       | 53                                   | NT                                    | NT                                  | NT                                       |
| Cephalothin   | 32                       | NT                                   | 0                                     | NT                                  | NT                                       |
| Clindamycin   | 2                        | NT                                   | 0                                     | NT                                  | NT                                       |
| Gentamicin    | 8                        | 12                                   | NT                                    | 100                                 | NT                                       |
| Methicillin   | 3.12                     | NT                                   | 71                                    | NT                                  | NT                                       |
| Moxalactam    | 32                       | 53                                   | NT                                    | 0                                   | 97                                       |
| Penicillin G  | 0.1                      | NT                                   | 81                                    | NT                                  | NT                                       |
| Ticarcillin   | 125                      | 12                                   | NT                                    | NT                                  | NT                                       |
| Tobramycin    | 8                        | 12                                   | NT                                    | 100                                 | NT                                       |
| Vancomycin    | 5                        | NT                                   | 6                                     | NT                                  | NT                                       |

<sup>a</sup> NT, Not tested.

concentration of antibiotic that inhibited visible growth after incubation of the plate for 18 h at 37°C.

**Determination of MBCs.** The minimal bactericidal concentrations (MBCs) of the isolates were determined by transferring 2  $\mu\text{l}$  of the broth from each well exhibiting no visible growth onto a culture plate of Mueller-Hinton agar by using a replicator-type inoculator. The MBC was defined as the lowest concentration of antibiotic yielding two or fewer colonies after overnight incubation at 37°C (the concentration at which at least 99.8% of the bacteria were rendered nonculturable). Since MBCs for all isolates tested were either equal to or no more than twofold greater than the MICs, detailed MBC data are not included.

## RESULTS

The activities of *N*-formimidoyl thienamycin against 17 strains of *P. aeruginosa* were compared with those of tobramycin, gentamicin, amikacin, cefoperazone, cefotaxime, moxalactam, ticarcillin, and carbenicillin in vitro (Table 2). Against this selected group of microorganisms, *N*-formimidoyl thienamycin was the most active beta-lactam agent. Fifty percent of the *P. aeruginosa* isolates were inhibited by 3.13  $\mu\text{g/ml}$ , and 90% were inhibited by 6.25  $\mu\text{g/ml}$ . In comparison, the next most active beta-lactam agent, cefoperazone, achieved 50 and 90% inhibition at concentrations of 12.5 and 64  $\mu\text{g/ml}$ , respectively. Gentamicin and tobramycin were the most active of the aminoglycoside agents tested, with 50% inhibition at 0.5  $\mu\text{g/ml}$  each.

The activities of clindamycin, cephalothin, vancomycin, penicillin G, and methicillin were compared with those of *N*-formimidoyl thienamycin against 34 strains of *S. epidermidis*. *N*-Formimidoyl thienamycin was the most active agent, with 50 and 90% of the strains being inhibited by 0.10 and 0.20  $\mu\text{g/ml}$ , respectively. The next most active agent was clindamycin, with 90% inhibition by 1.56  $\mu\text{g/ml}$ .

The activities of *N*-formimidoyl thienamycin against seven selected strains of *S. marcescens*

were compared with those of moxalactam, amikacin, gentamicin, and tobramycin. *N*-Formimidoyl thienamycin demonstrated the most activity, with 90% inhibition at a concentration of 1.56  $\mu\text{g/ml}$ . Moxalactam was the next most active agent, with 90% inhibition at a concentration of 12.5  $\mu\text{g/ml}$ . Tobramycin was inactive at 100  $\mu\text{g/ml}$  against all *S. marcescens* isolates.

The activities of *N*-formimidoyl thienamycin against 71 strains of *Enterococcus* sp. were compared with those of moxalactam and cefoperazone. *N*-Formimidoyl thienamycin was clearly the most active of the beta-lactam antibiotics tested. All strains were susceptible to 6.25  $\mu\text{g/ml}$ . Cefoperazone achieved 50 and 90% inhibition at 25 and 50  $\mu\text{g/ml}$ , respectively.

## DISCUSSION

Earlier studies demonstrated that thienamycin is a potent antibiotic with a broad spectrum of activity (6, 7). *N*-Formimidoyl thienamycin, a derivative of thienamycin, has been shown to be more stable, more active in vitro, and more effective for treatment of infections in mice (H. Kropp, J. G. Sundelof, J. S. Kahan, F. M. Kahan, and J. Birnbaum, Program Abstr. Int. Congr. Chemother. 11th and Intersci. Conf. Antimicrob. Agents Chemother. 19th, Boston, Mass., abstr. no. 231).

In the present study, with respect to the MIC required to inhibit growth of 90% of the isolates (MIC-90), *N*-formimidoyl thienamycin was more active than any antimicrobial agent tested. Comparison of the MIC-50 of each agent showed *N*-formimidoyl thienamycin to be more active than any other agent against *S. epidermidis*, *S. marcescens*, and enterococci, but against *P. aeruginosa* isolates it was less active than were amikacin, gentamicin, and tobramycin.

Infectious disorders caused by the bacteria examined in the present study can provide dif-

TABLE 2. Comparative *in vitro* activities of *N*-formimidoyl thienamycin and other antibiotics against clinical bacterial isolates

| Species (n)                   | Antibiotic                        | Range ( $\mu\text{g/ml}$ ) | MIC for: |      |
|-------------------------------|-----------------------------------|----------------------------|----------|------|
|                               |                                   |                            | 50%      | 90%  |
| <i>P. aeruginosa</i> (17)     | <i>N</i> -Formimidoyl thienamycin | 1.56–12.5                  | 3.13     | 6.25 |
|                               | Cefoperazone                      | 3.13–64                    | 12.5     | 64   |
|                               | Cefotaxime                        | 8–64                       | 32       | 64   |
|                               | Moxalactam                        | 16–64                      | 32       | 64   |
|                               | Carbenicillin                     | 16–64                      | 64       | 64   |
|                               | Ticarcillin                       | 16–64                      | 32       | 64   |
|                               | Amikacin                          | 0.5–64                     | 1.0      | 64   |
|                               | Gentamicin                        | 0.25–64                    | 0.5      | 8    |
|                               | Tobramycin                        | 0.25–16                    | 0.5      | 16   |
| <i>S. epidermidis</i> (34)    | <i>N</i> -Formimidoyl thienamycin | 0.05–25                    | 0.10     | 0.20 |
|                               | Cephalothin                       | 0.2–6.25                   | 0.4      | 1.67 |
|                               | Clindamycin                       | 0.2–1.56                   | 0.8      | 1.56 |
|                               | Methicillin                       | 0.78–25                    | 6.25     | 25   |
|                               | Penicillin G                      | 0.1–25                     | 1.6      | 25   |
|                               | Vancomycin                        | 0.05–12.5                  | 6.25     | 12.5 |
| <i>S. marcescens</i> (7)      | <i>N</i> -Formimidoyl thienamycin | 0.39–1.56                  | 0.78     | 1.56 |
|                               | Amikacin                          | 25–50                      | 50       | 50   |
|                               | Gentamicin                        | 50–100                     | 100      | 100  |
|                               | Tobramycin                        | 100                        | 100      | 100  |
|                               | Moxalactam                        | 3.12–12.5                  | 6.25     | 12.5 |
| <i>Enterococcus</i> spp. (71) | <i>N</i> -Formimidoyl thienamycin | 0.20–6.25                  | 0.78     | 3.13 |
|                               | Cefoperazone                      | 6.25–100                   | 25       | 50   |
|                               | Moxalactam                        | 25–100                     | 100      | 100  |

ficult therapeutic challenges to the clinician. Our *in vitro* results demonstrate that *N*-formimidoyl thienamycin has excellent antibacterial activity against enterococci, *S. epidermidis*, *P. aeruginosa*, and resistant strains of *S. marcescens*, and its activity against these isolates exceeds that of the other beta-lactam antibacterial agents tested. This agent is potentially very useful for patients with serious infectious disorders caused by resistant bacteria. Enterococcal and *S. epidermidis* endocarditis infections may be special situations which merit therapeutic trials with *N*-formimidoyl thienamycin.

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