

## Piperacillin: In Vitro Evaluation

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The in vitro activity of a new semisynthetic penicillin, piperacillin, was determined against 577 clinical isolates of gram-positive cocci and gram-negative bacilli. A concentration of 12.5  $\mu\text{g/ml}$  inhibited 92% of isolates of *Pseudomonas aeruginosa*, 82% of *Serratia marcescens*, 73% of *Escherichia coli*, 61% of *Klebsiella* spp., and 42% of *Enterobacter* spp. Most *Proteus* spp. were extremely susceptible; over 85% were inhibited by 0.10  $\mu\text{g/ml}$ . Piperacillin failed to inhibit the growth of gram-negative bacilli when large inocula were used. The type of media and pH had variable effects on the activity of piperacillin, depending upon the organism. Piperacillin was generally less active than PC-904 against gram-negative bacilli, but was consistently more active than carbenicillin and ticarcillin.

Infection continues to be a major cause of morbidity and mortality among hospitalized patients. Many of these infections are caused by gram-negative bacilli. *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* are responsible for the majority of gram-negative bacillary infections in most hospitals, although epidemics caused by other organisms including *Serratia marcescens* and *Proteus* spp. have been recognized in recent years (3, 6). No antibiotic has been entirely satisfactory for treatment of these infections. The aminoglycosides provide broad-spectrum activity but are associated with nephrotoxicity and appear to be somewhat less effective in compromised hosts (2, 8). Penicillins are less toxic, but most derivatives have a limited spectrum of activity. Consequently, new analogs are being synthesized and evaluated. Piperacillin [sodium 6-(D(-)- $\alpha$ -(4 ethyl-2,3-dioxo-1-piperazinylcarbonylamino)- $\alpha$  phenylacetamido)penicillanate] is a new semisynthetic penicillin with broad-spectrum activity. This report presents an in vitro evaluation of piperacillin and compares its activity with other semisynthetic penicillins.

### MATERIALS AND METHODS

Susceptibility tests were conducted on 471 clinical isolates of gram-negative bacilli and 106 clinical isolates of gram-positive cocci, using a dilution technique with an automatic microtiter system (Canalco Inc., Rockville, Md., autotiter instruction manual). All gram-negative bacilli and *Staphylococcus aureus* isolates tested were incubated in Mueller-Hinton broth (pH 7.4) for 18 h at 37°C. *Streptococcus pyogenes* and *Streptococcus pneumoniae* were incubated in tryptose phosphate broth. Approximately  $10^8$  colony-forming units (CFU)/ml were used in inoculum for gram-neg-

ative bacilli and *S. aureus*. For the remaining gram-positive cocci, an inoculum of  $10^8$  CFU/ml was used for the in vitro susceptibility tests.

All gram-negative bacilli used in this study were cultured from blood specimens of patients who were hospitalized at this institution and had underlying malignant diseases. A total of 100 isolates each of *P. aeruginosa*, *Klebsiella* spp., and *E. coli*, 56 isolates of *Proteus* spp., 36 isolates of *Serratia* spp., and 79 isolates of *Enterobacter* spp. were used. All gram-positive cocci used in this study were cultured from specimens obtained from hospitalized patients, most of whom did not have cancer. A total of 50 isolates of *S. pyogenes*, 7 isolates of *S. pneumoniae*, and 49 isolates of *S. aureus* was used. Isolates of *S. aureus* were divided according to their susceptibility to penicillin G. Those isolates which were inhibited by less than 0.10  $\mu\text{g/ml}$  were selected as penicillin G susceptible, and those isolates resistant to more than 25  $\mu\text{g/ml}$  were selected as penicillin G resistant.

Organisms used for studies of the effect of inoculum size on the activity of piperacillin were incubated in Mueller-Hinton broth for 18 h at 37°C. It was assumed that approximately  $10^8$  CFU/ml were present after incubation, which was subsequently confirmed by subculturing 0.1-ml aliquots on sheep blood agar and performing colony counts after 24 h of incubation at 37°C. Serial 10-fold dilutions of the broth culture were made, using Mueller-Hinton broth, so that  $10^7$  and  $10^6$  CFU/ml were used as inocula. An inoculum of  $10^6$  CFU/ml was used in all other studies of gram-negative bacilli. Studies of the effect of pH on the activity of piperacillin were conducted in Mueller-Hinton broth, and the pH was adjusted to 6.4, 7.2, and 8.2 with phosphate buffer. Studies comparing the activity of piperacillin with carbenicillin, ticarcillin, amoxicillin, mezlocillin, azlocillin, and PC-904 were conducted in Mueller-Hinton broth. Fifty isolates each of *E. coli*, *Klebsiella* spp., *P. aeruginosa*, *Enterobacter* spp., and *Proteus mirabilis*, 32 isolates of *S. marcescens*, and 7

isolates of indole-positive *Proteus* spp. were used. Isolates were selected so that organisms with differing susceptibilities to piperacillin were included.

Piperacillin was supplied as a white powder by Lederle Laboratories, Pearl River, N.Y. Azlocillin and mezlocillin were supplied as powders by Delbay Pharmaceuticals Inc., Bloomfield, N.J. Carbenicillin and ticarcillin were supplied by Beecham Pharmaceuticals, Bristol, Tenn. PC-904 was supplied by Sumitomo Chemical Co. Ltd., Hyogo, Japan. All antibiotics were diluted serially in Mueller-Hinton broth or tryptose phosphate broth. No visible growth after 18 h of incubation at 37°C was accepted to be the minimal inhibitory concentration (MIC). The minimum bactericidal concentration (MBC) was defined as the lowest concentration of drug which yielded less than five colonies on subculture to sheep blood agar (99% kill). A 0.01-ml calibrated pipette was utilized to transfer the inoculum. Comparative studies were performed simultaneously in triplicate.

## RESULTS

Figure 1 summarizes the in vitro activity of piperacillin against gram-positive cocci and gram-negative bacilli. All gram-positive cocci, except penicillin G-resistant *S. aureus*, were inhibited by 0.78 µg/ml. The majority of the latter

organisms were resistant to 25 µg of piperacillin per ml. Most *Proteus* spp. were quite susceptible to piperacillin; a concentration of 0.10 µg/ml inhibited 85% indole-positive *Proteus* spp. and 92% of *P. mirabilis*. A majority of most species of gram-negative bacilli were inhibited by 12.5 µg of this antibiotic per ml. At this concentration, 92% of *P. aeruginosa*, 82% of *S. marcescens*, 73% of *E. coli*, 61% of *Klebsiella* spp., and 42% of *Enterobacter* spp. were inhibited. The MBC was the same as the MIC for most organisms. For the exceptions, the MBC was twofold higher than the MIC.

The effect of inoculum size on the MIC for 10 isolates each of *E. coli*, *K. pneumoniae*, and *P. aeruginosa* were determined. All of the isolates of *E. coli*, nine of the isolates of *P. aeruginosa*, and eight of the isolates of *K. pneumoniae* were inhibited by 12.5 µg of piperacillin per ml when an inoculum of 10<sup>5</sup> CFU/ml was used. However, when an inoculum of 10<sup>7</sup> CFU/ml was used, none of the isolates was inhibited by 400 µg/ml.

The effect of pH and media variation was ascertained for 10 isolates of *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. Piperacillin was more active against *E. coli* and *K. pneumoniae*

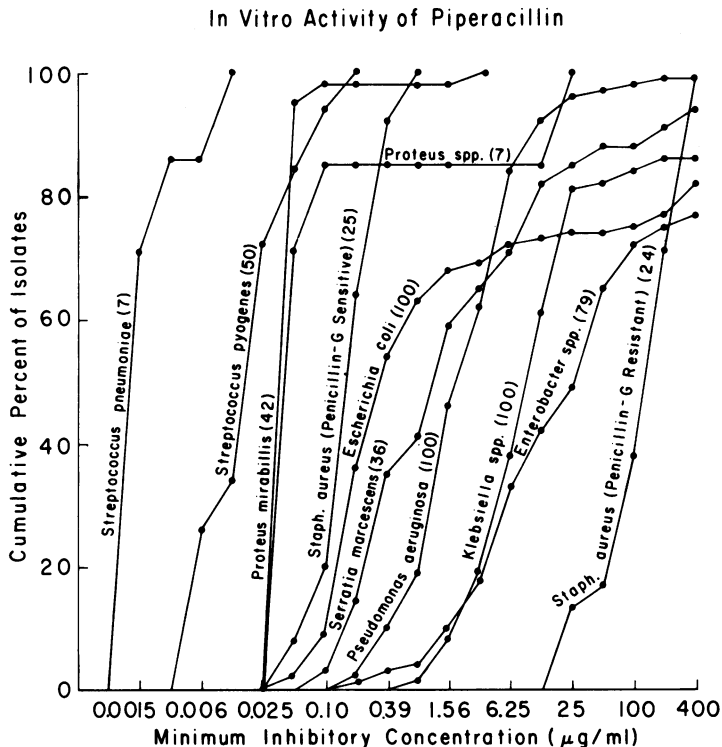


FIG. 1. In vitro activity of piperacillin against gram-positive cocci and gram-negative bacilli. The numbers in parentheses indicate the number of isolates tested.

at an alkaline pH (Fig. 2). This effect was most pronounced for *K. pneumoniae*. The pH had only a minimal effect on the activity of piperacillin against *P. aeruginosa*. Piperacillin was

most active against *E. coli* and *K. pneumoniae* in brain heart infusion broth, but least active against *P. aeruginosa* in this medium (Fig. 3). In general, differences in activity of piperacillin

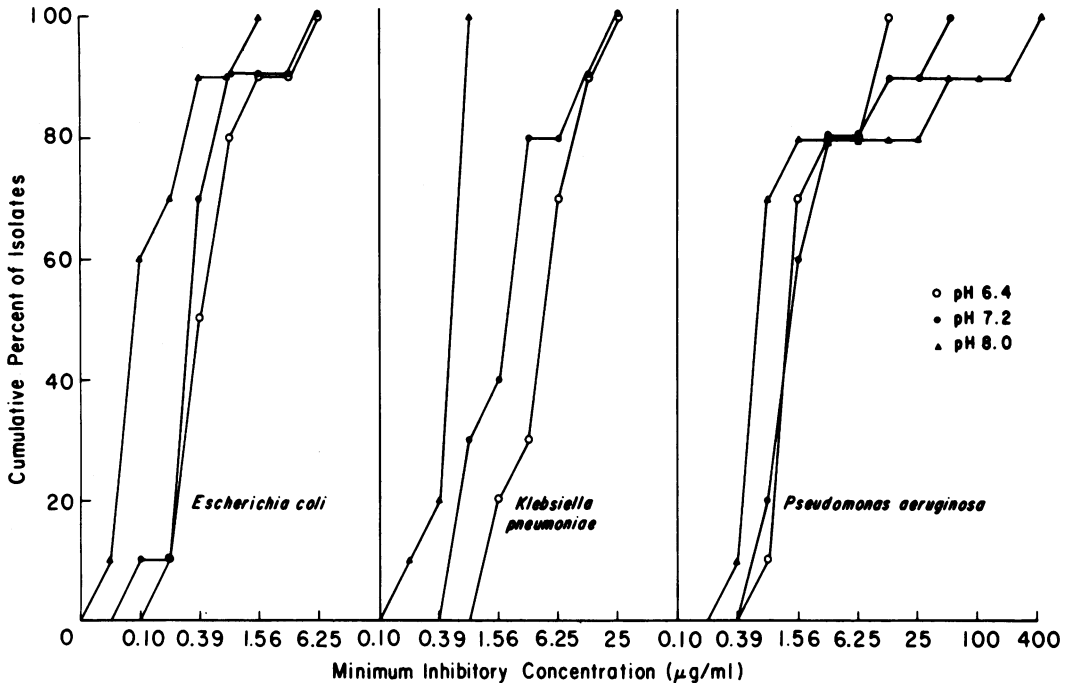


FIG. 2. Effect of pH on in vitro activity of piperacillin against 10 isolates each of *E. coli*, *K. pneumoniae*, and *P. aeruginosa*.

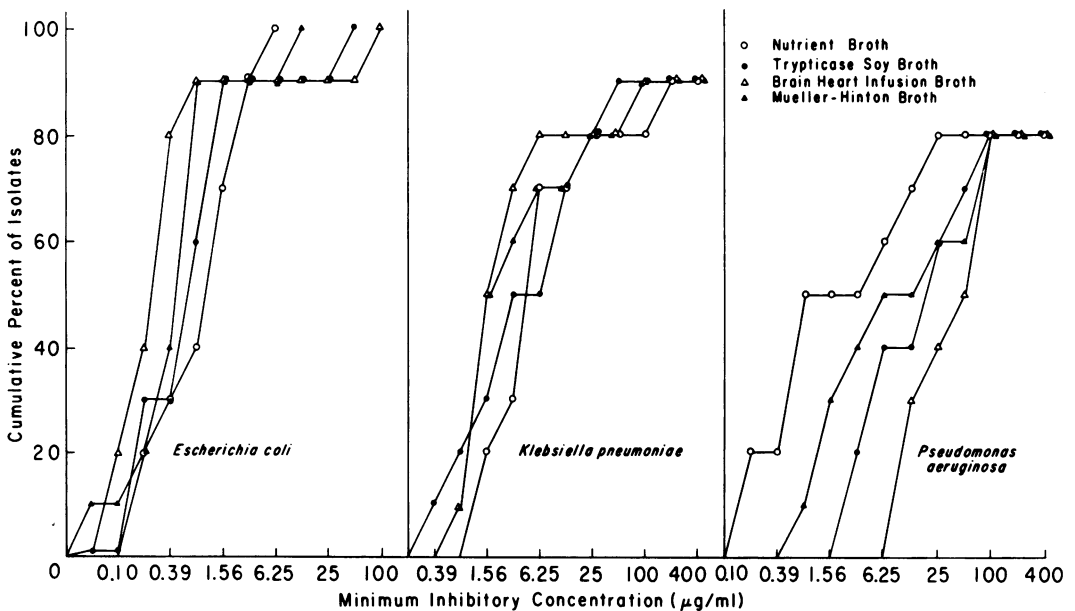


FIG. 3. Effect of media on in vitro activity of piperacillin against 10 isolates each of *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. Some of the isolates tested were different from those in Fig. 2.

against *E. coli* and *K. pneumoniae* in various media were minimal. However, the difference between the activities of piperacillin against *P. aeruginosa* in nutrient broth and brain heart infusion broth were substantial.

The susceptibility of isolates of *Enterobacteriaceae* to piperacillin, carbenicillin, ticarcillin, amoxicillin, PC-904, mezlocillin, and azlocillin was determined. PC-904 was the most active penicillin against isolates of *E. coli* (Fig. 4). However, mezlocillin and piperacillin were only slightly less active, and the greater activity of PC-904 was only demonstrable against isolates inhibited by  $\leq 0.78 \mu\text{g/ml}$ . PC-904 also was the most active penicillin against isolates of *Klebsiella* spp. (Fig. 5). Against most isolates, there

was only a twofold difference in activity between PC-904 and piperacillin. The activity of mezlocillin was nearly identical to that of piperacillin.

Piperacillin was the most active penicillin against isolates of *S. marcescens* (Fig. 6). Mezlocillin and PC-904 also had considerable activity; nearly 90% of isolates were inhibited by  $100 \mu\text{g}$  of these three penicillins per ml. Over 40% of these isolates were resistant to  $400 \mu\text{g}$  of amoxicillin, carbenicillin, and ticarcillin per ml. Mezlocillin was the most active penicillin against *Enterobacter* spp., but piperacillin and PC-904 demonstrated only slightly less activity (Fig. 7). However, 20 to 30% of these isolates were resistant to  $400 \mu\text{g}$  of these three penicillins per ml. Piperacillin was the most active penicillin

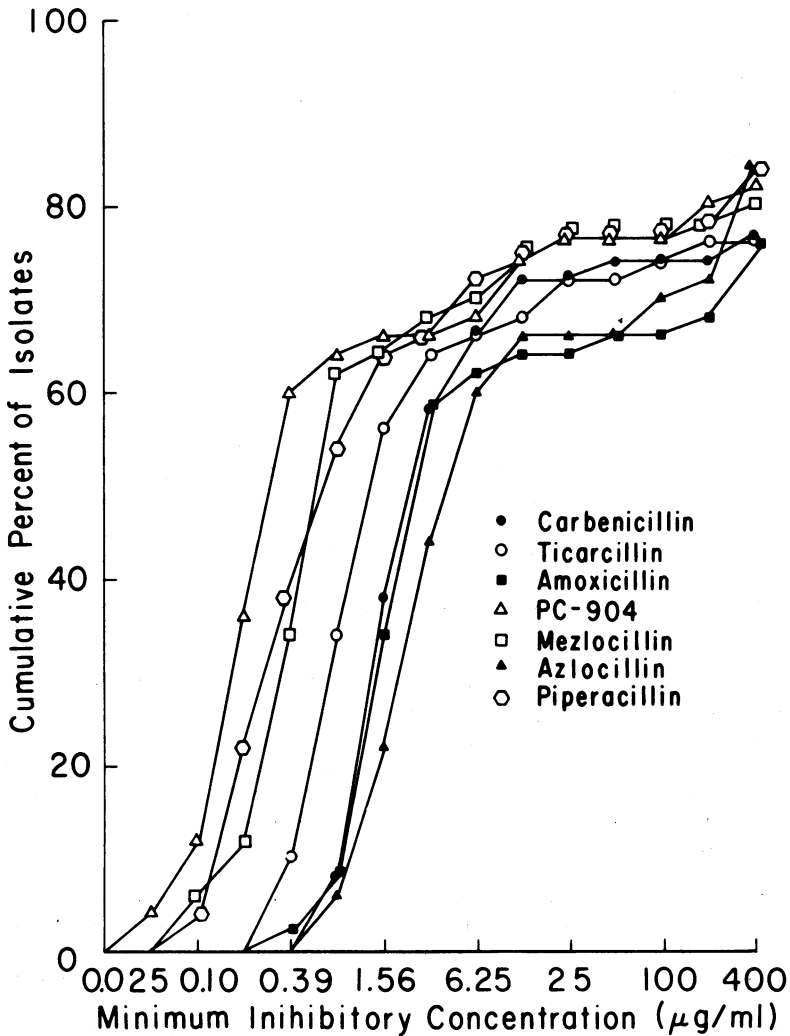


FIG. 4. Comparative activity of penicillins against 50 isolates of *E. coli*.

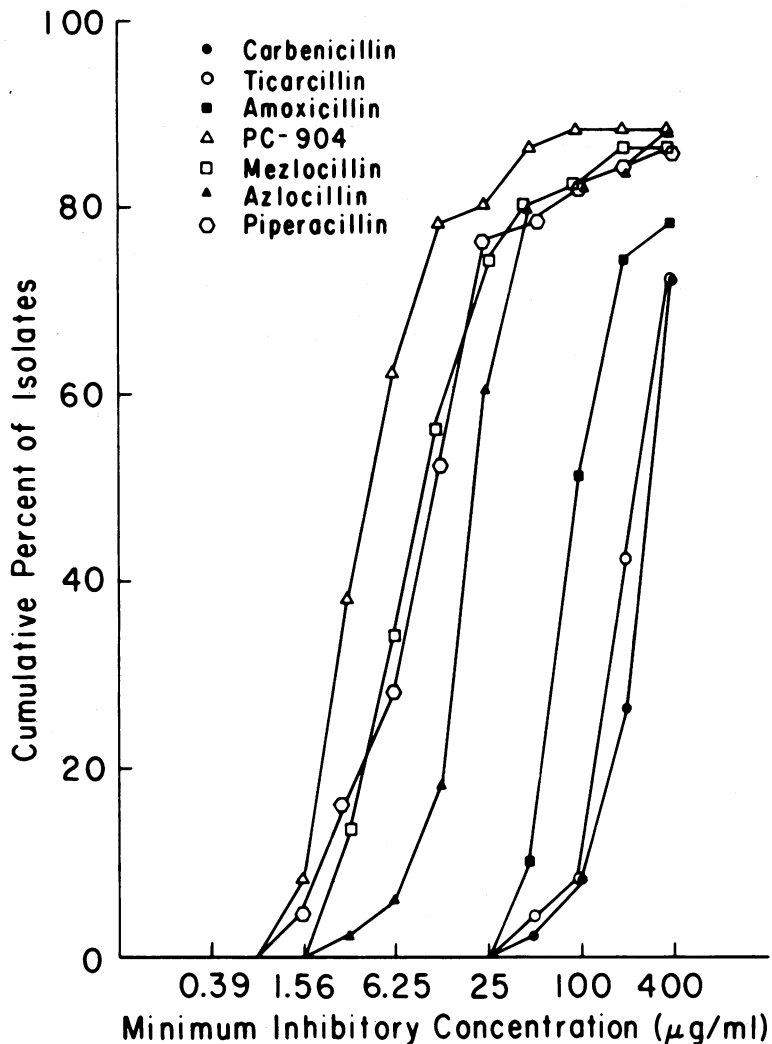


FIG. 5. Comparative activity of penicillins against 50 isolates of *Klebsiella* spp.

against both *P. mirabilis* and indole-positive *Proteus* spp. (Fig. 8). Against indole-positive isolates, it was eight times more active than carbenicillin, which was the second most active penicillin. It was also two to eight times more active than other penicillins against *P. mirabilis*.

Figure 9 illustrates the activity of the 7 penicillins against 50 isolates of *P. aeruginosa*. PC-904 was the most active penicillin, eight times more active than piperacillin against most isolates. Piperacillin was 2 times as active as azlocillin, 4 to 8 times more active than ticarcillin, and 16 times more active than carbenicillin against most isolates.

## DISCUSSION

Substitution of different side chains at the  $\alpha$  position on the penicillin molecule has resulted in derivatives with different antibacterial spectra. Ampicillin was the first such derivative introduced with activity against gram-negative bacilli. The synthesis of carbenicillin resulted in a penicillin with antipseudomonal activity (1). Subsequently, a variety of new derivatives have been synthesized with greater activity than carbenicillin against *P. aeruginosa* and a broader spectrum of activity against other gram-negative bacilli. Piperacillin is one of these semisynthetic

penicillins which has broad-spectrum activity against *E. coli*, *K. pneumoniae*, *S. marcescens*, *Proteus* spp., and *P. aeruginosa*.

The results of this study are in general agreement with those of other investigators who have found piperacillin to be more active against gram-negative bacilli than ampicillin, carbenicillin, and ticarcillin (R. Jones, P. Fuchs, and A. Barry, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 17th, New York, N.Y., Abstr. no. 318, 1977; W. J. Martin, D. J. Winston, D. Wang, L. S. Young, and W. L. Hewitt, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 17th, New York, N.Y., Abstr. no. 319, 1977; and R. T. Ives and L. O. Gentry, Program Abstr. Intersci. Conf. Antimicrob.

Agents Chemother. 17th, New York, N.Y., Abstr. no. 320, 1977). Ives et al. found *E. coli* to be less susceptible to piperacillin than other gram-negative bacilli, whereas piperacillin was quite active against most of our isolates of *E. coli*. Although they used different media, Ueo et al. obtained results which were similar to the results of our studies (7). Both the type of culture medium and pH had little effect on the in vitro activity of piperacillin in both studies. They also found that the MBC was the same as the MIC for most organisms. However, for penicillinase-producing strains of *Klebsiella* spp., *Proteus* spp., and *P. aeruginosa*, the MBC was four to eight times higher than the MIC.

Inoculum size had a major effect on the activ-

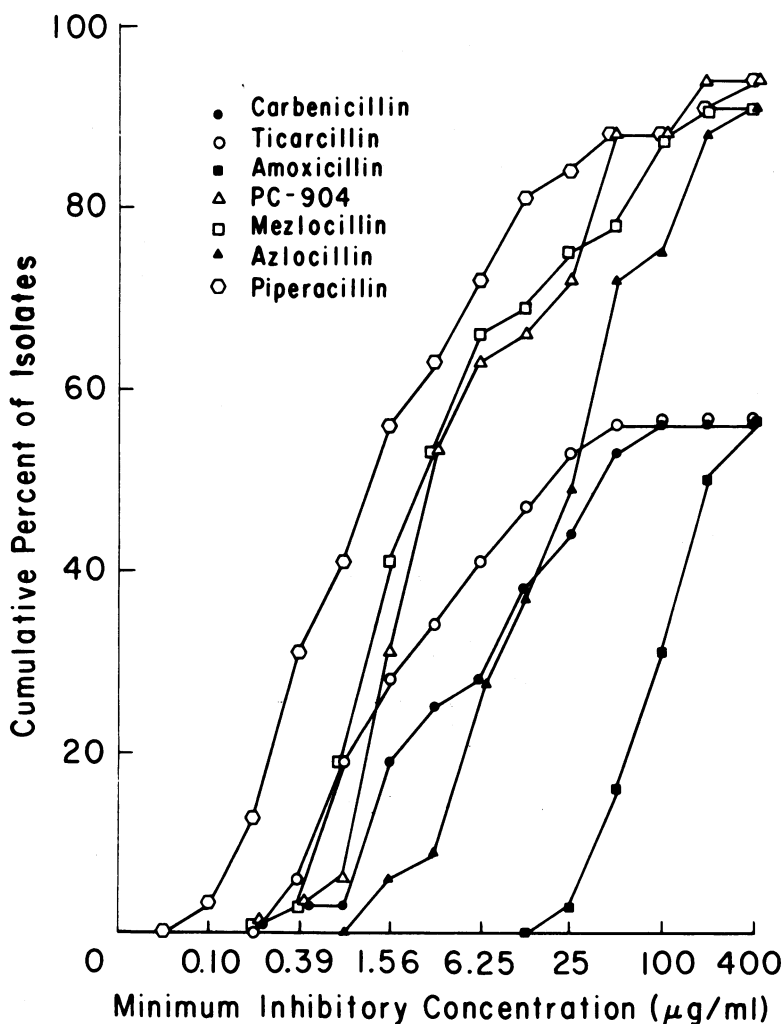


FIG. 6. Comparative activity of penicillins against 36 isolates of *S. marcescens*.

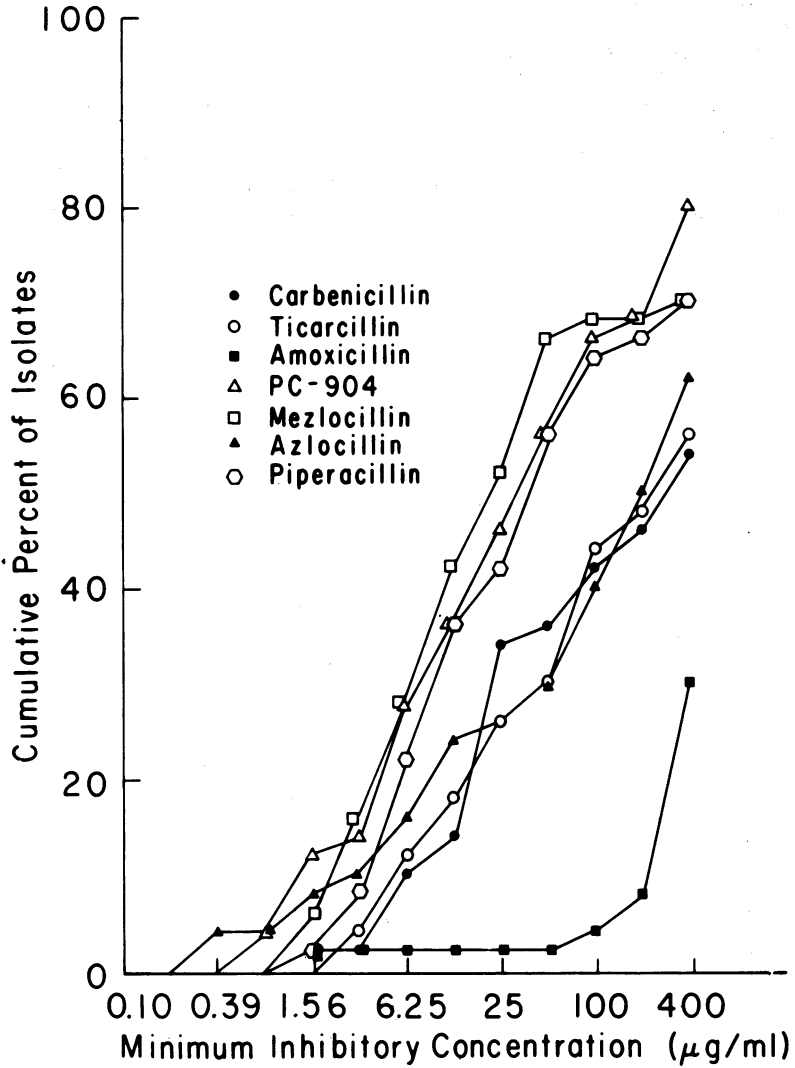


FIG. 7. Comparative activity of penicillins against 50 isolates of *Enterobacter* spp.

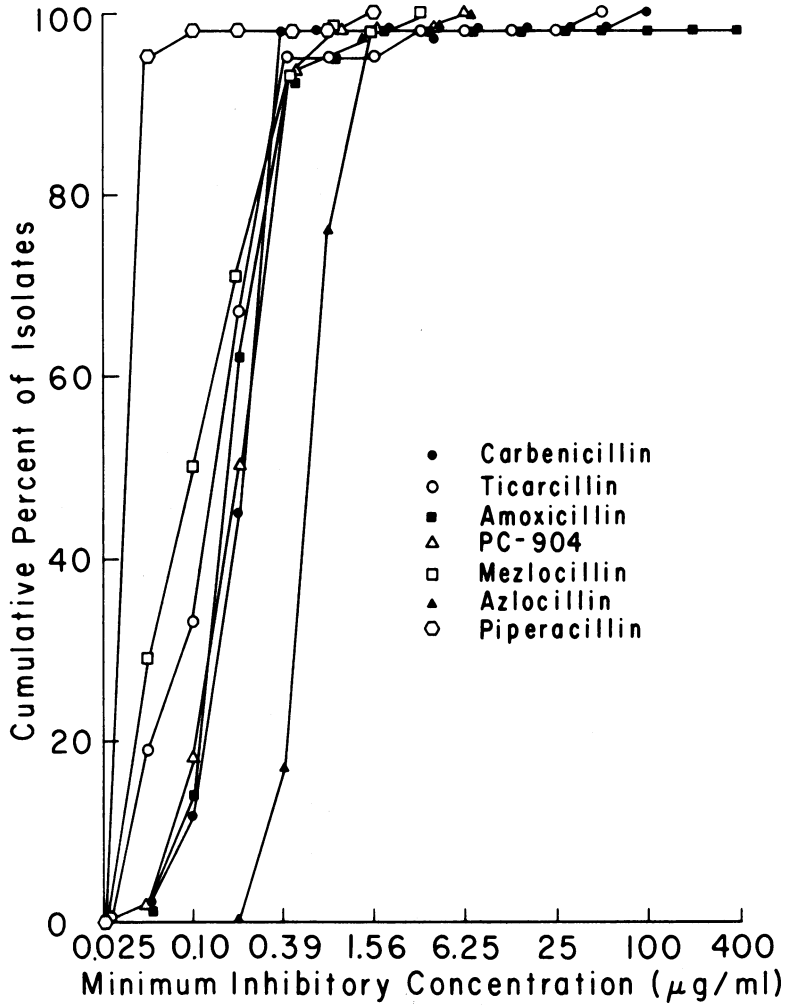


FIG. 8. Comparative activity of penicillins against 42 isolates of *P. mirabilis*.



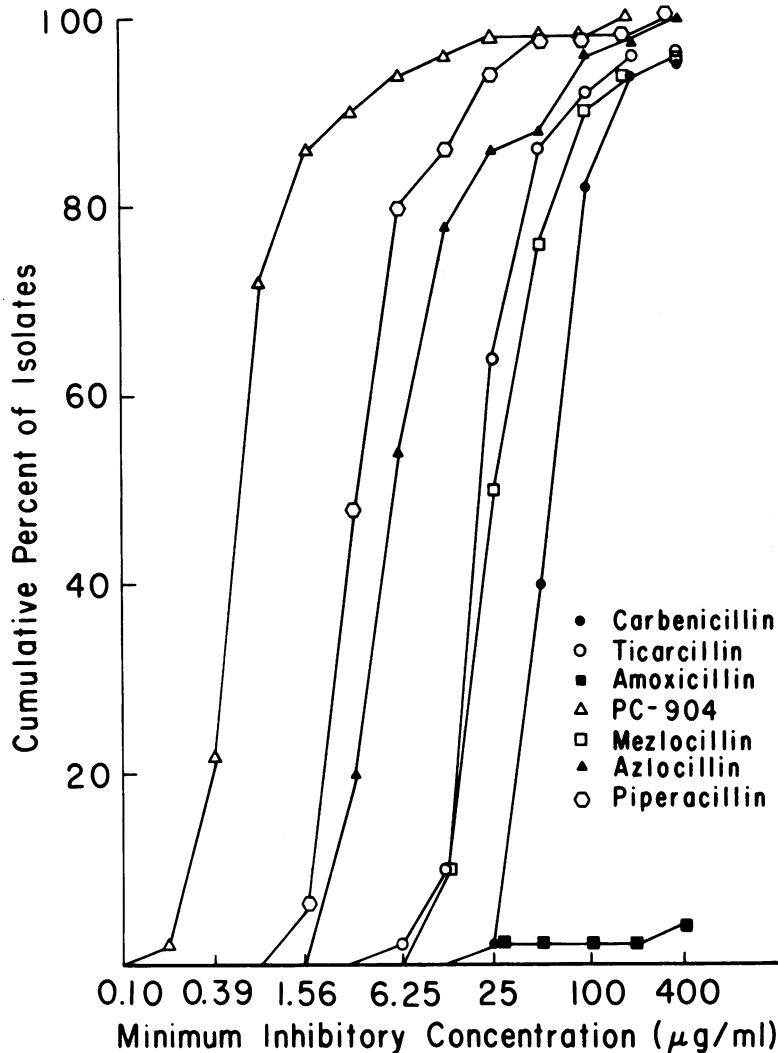


FIG. 9. Comparative activity of penicillins against 50 isolates of *P. aeruginosa*.

ity of piperacillin. Like azlocillin and PC-904, piperacillin was inactive against inocula of *E. coli*, *P. aeruginosa*, and *K. pneumoniae* containing  $10^7$  CFU. Several investigators have reported this adverse effect of large inocula on the activity of other semisynthetic penicillins (4, 5). The clinical importance of this observation is not clear, but it has been suggested that this is due to the presence of a greater number of resistant cells in the larger inocula.

Piperacillin is an interesting new semisynthetic penicillin of potential importance. Not only is it more active in vitro than most other penicillins against gram-negative bacilli, but it encompasses a broader spectrum of activity. It is

active against the *Klebsiella-Enterobacter-Serratia* group and has substantially greater activity against *P. aeruginosa* than carbenicillin and ticarcillin.

#### ACKNOWLEDGMENTS

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