

# In Vitro Studies of Semisynthetic $\alpha$ - (Substituted-Ureido) Penicillins

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The activity of three  $\alpha$ -(substituted-ureido) penicillins was evaluated in vitro against 599 clinical isolates of gram-negative bacilli, by use of the broth-dilution technique. At a concentration of 12.5  $\mu$ g or less/ml, BL-P1597 inhibited 90% of isolates of *Pseudomonas* sp., 56% of *Enterobacter* sp., 67% of indole-positive *Proteus* spp., 72% of *Escherichia coli*, and 85% of *Proteus mirabilis*. BL-P1654 had similar activity, whereas BL-P1532 was much less active. At a concentration of 25  $\mu$ g or less/ml, BL-P1597 also inhibited nearly 60% of isolates of *Klebsiella* sp. and nearly 40% of *Serratia* sp. BL-P1597 and BL-P1654 were as active as ampicillin and carbenicillin against *E. coli* and *P. mirabilis*. They were less active than carbenicillin against indole-positive *Proteus* spp. Both drugs were substantially more active than carbenicillin against *Pseudomonas* sp. A strain of *Pseudomonas* sp. which developed resistance to carbenicillin also developed resistance to the  $\alpha$ -(substituted-ureido) penicillins simultaneously.

Chemical modifications of benzylpenicillin have yielded several very useful antibiotics. The introduction of semisynthetic penicillins has broadened the spectrum of activity of penicillin to include penicillinase-producing *Staphylococcus aureus* and some *Enterobacteriaceae*. The recent synthesis of carbenicillin has provided a penicillin with activity against *Pseudomonas* sp. and indole-positive *Proteus* spp. (1, 4). The introduction of carbenicillin has been of special benefit to patients with impaired host defense mechanisms, who have a high frequency of *Pseudomonas* bacteremia (7). Nearly 80% of these patients have responded to carbenicillin therapy, whereas only 24% responded to therapy with a polymyxin antibiotic (2, 12).

Although carbenicillin is very effective against *Pseudomonas* infections, large doses of drug are required in the treatment of systemic infections. Further research has led to the synthesis of other penicillins which have activity against *Pseudomonas* sp. greater than that of carbenicillin. Among these semisynthetic penicillins are three  $\alpha$ -(substituted-ureido) penicillins which have been made available for laboratory investigation (10). The studies reported herein indicate that two of these penicillins have substantial activity against *Pseudomonas* sp., *Proteus* spp., *Escherichia coli*, and *Enterobacter* spp.

## MATERIALS AND METHODS

Susceptibility tests were conducted on 599 clinical isolates of gram-negative bacilli, by use of the broth-dilution technique (6). The organisms were inoculated into Mueller-Hinton Broth (Difco) and incubated at 37 C for 18 hr. A 0.1-ml sample of a  $10^{-4}$  dilution of this broth (approximately  $10^8$  colony-forming units) was used as the inoculum for susceptibility testing. The effect of type of media on the activity of these penicillins was determined in Mueller-Hinton Broth (Difco) and Nutrient Broth (BBL), by use of a micro-autodilutor (9). A 50- $\mu$ liter sample of inoculum (prepared as above) was added to 50  $\mu$ liters of antibiotic solution and incubated at 37 C for 18 hr. These studies were performed in triplicate.

The three semisynthetic penicillins studied were 6-[D- $\alpha$ -(3-guanylureido)-phenylacetamido]-penicillanic acid (BL-P1654), sodium 6-[D- $\alpha$ -(3-carbamoylureido)-phenylacetamido]-penicillanate (BL-P1532), and potassium 6-[D- $\alpha$ -(3-(2-furoyl)ureido)-phenylacetamido]-penicillanate (BL-P1597; Fig. 1). The drugs were supplied by Bristol Laboratories, Syracuse, N.Y. Solutions were prepared by dissolving the antibiotics in Mueller-Hinton Broth to a final concentration of 400  $\mu$ g/ml. BL-P1532 dissolved readily, and BL-P1597 dissolved after agitation on a Vortex mixer for 5 min. BL-P1654 was relatively insoluble, and dissolved only after being agitated on a Vortex mixer for 45 min. Twofold serial dilutions of the antibiotics were made with Mueller-Hinton Broth, and the minimal inhibitory concentration (MIC) was determined after incubation at 37 C for 18 hr. All tubes containing trace growth or no discernible growth were subcultured on sheep blood-agar. A wire loop with an inside

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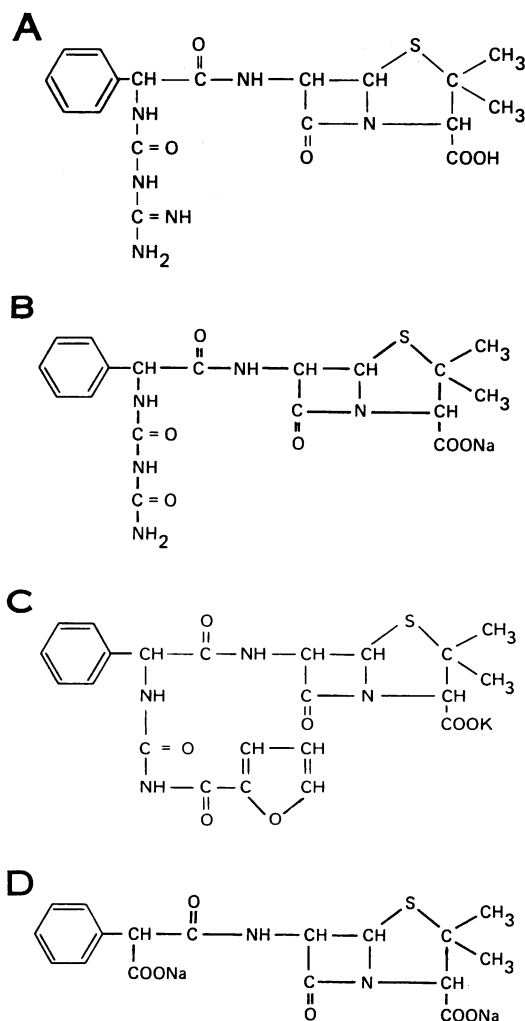


FIG. 1. Chemical structures of  $\alpha$ -(substituted-ureido) penicillins. (A) 6-[D- $\alpha$ -(3 guanylyureido)-phenylacetamido]-penicillanic acid (BL-P1654); (B) sodium 6-[D- $\alpha$ -(3 carbamoylureido)-phenylacetamido]-penicillinate (BL-P1532); (C) potassium 6-[D- $\alpha$ -(3-(2-furoyl)ureido)-phenylacetamido]-penicillanate (BL-P1597); (D) Carbenicillin (disodium  $\alpha$ -carboxyl benzyl penicillin) is included for comparison.

diameter of 5 mm was used to transfer the inoculum. The drugs were considered to be bactericidal against those isolates which yielded less than 10 colonies on subculture of the tube containing the MIC.

All organisms used in this study were cultured from blood specimens obtained from patients between December 1966 and May 1970. The majority of these patients were hospitalized at this institution and had underlying malignant disease. A total of 150 isolates of *Pseudomonas* sp., 150 isolates of *E. coli*, 87 isolates of *Proteus* spp., 46 isolates of *Enterobacter* sp., 66 isolates of *Serratia* sp., and 100 isolates of *Klebsiella* sp. were studied.

## RESULTS

The activity of the three penicillins against gram-negative bacilli is shown in Fig. 2 to 4. BL-P1597 was most active against *Pseudomonas* sp., inhibiting 90% of these isolates at a concentration of 12.5  $\mu$ g or less/ml. In addition, 56% of isolates of *Enterobacter* sp., 67% of indole-positive *Proteus* spp., 72% of *E. coli*, and 85% of *P. mirabilis* also were inhibited by 12.5  $\mu$ g or less/ml. At a concentration of 25  $\mu$ g or less/ml, BL-P1597 inhibited nearly 60% of isolates of *Klebsiella* sp. and nearly 40% of isolates of *Serratia* sp. BL-P1654 was most active against *P. mirabilis*, inhibiting 79% of these isolates at a concentration of 1.56  $\mu$ g or less/ml. A concentration of 12.5  $\mu$ g or less/ml inhibited 41% of isolates of *Enterobacter* sp., 75% of *E. coli*, and 69% of *Pseudomonas* sp. BL-P1654 inhibited over 60% of isolates of *Klebsiella* sp., 35% of *Serratia* sp., and only 30%

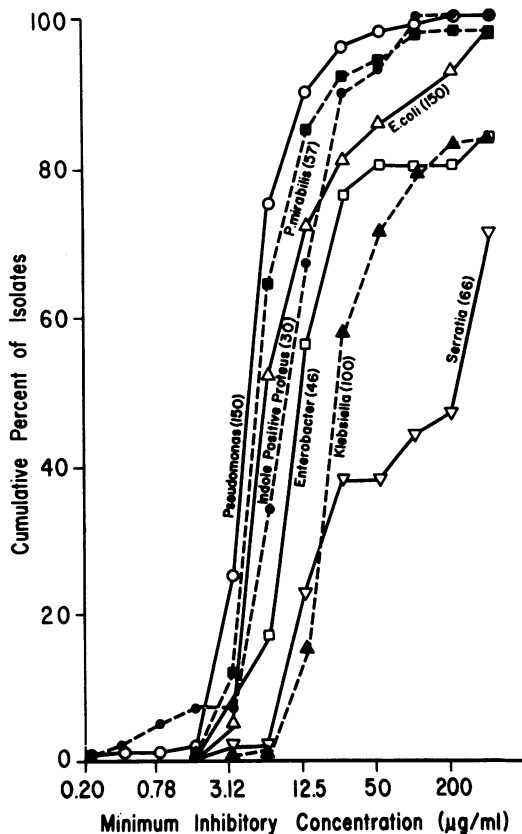


FIG. 2. *In vitro* activity of BL-P1597 against gram-negative bacilli, determined by the broth-dilution technique. The MIC is plotted on a log<sub>2</sub> scale, and the cumulative percentage of susceptible strains is shown. The numbers in parentheses indicate the number of isolates tested.

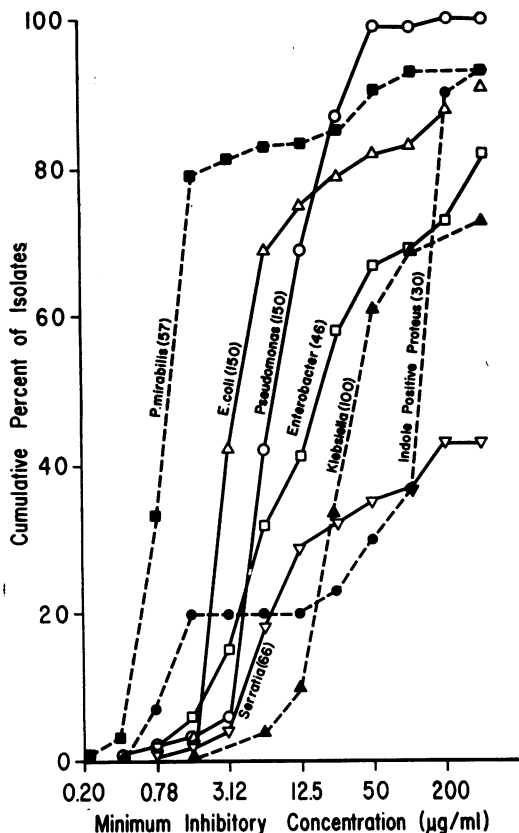


FIG. 3. In vitro activity of BL-P1654 against gram-negative bacilli.

of indole-positive *Proteus* spp. at a concentration of 50 µg or less/ml. In general, BL-P1532 was the least active antibiotic. A concentration of 25 µg or less/ml inhibited 80% of isolates of *P. mirabilis*, 57% of *Pseudomonas* sp., and 54% of *E. coli*. BL-P1532 was much less active against indole-positive *Proteus* spp., *Enterobacter* sp., *Klebsiella* sp., and *Serratia* sp.

BL-P1597 and BL-P1532 had greater bactericidal activity than BL-P1654 (Table 1). Bactericidal activity was greatest against *E. coli*, *Proteus* spp., *Enterobacter* sp., and *Klebsiella* sp. None of the three drugs was bactericidal against *Pseudomonas* sp.

The effect of inoculum size on the MIC of each drug was determined against 10 isolates each of *Pseudomonas* sp., *P. mirabilis*, *E. coli*, and *Serratia* sp. (Fig. 5 to 7). The inocula used were  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ , and  $10^{-6}$  dilutions of an 18-hr broth culture of the test organisms (containing approximately  $10^8$  colony-forming units/ml). In general, the antibiotics were less active against the largest inoculum. This was especially true for *Pseudomonas* sp. There was no substantial dif-

ference in activity of the drugs when  $10^{-4}$  and  $10^{-5}$  dilutions of the organisms were used.

BL-P1654 was more active when tested against organisms in Nutrient Broth than in Mueller-Hinton Broth (Fig. 8 and 9). This effect was more prominent for *Pseudomonas* sp. than for *E. coli* or *Proteus* spp. The type of medium had little

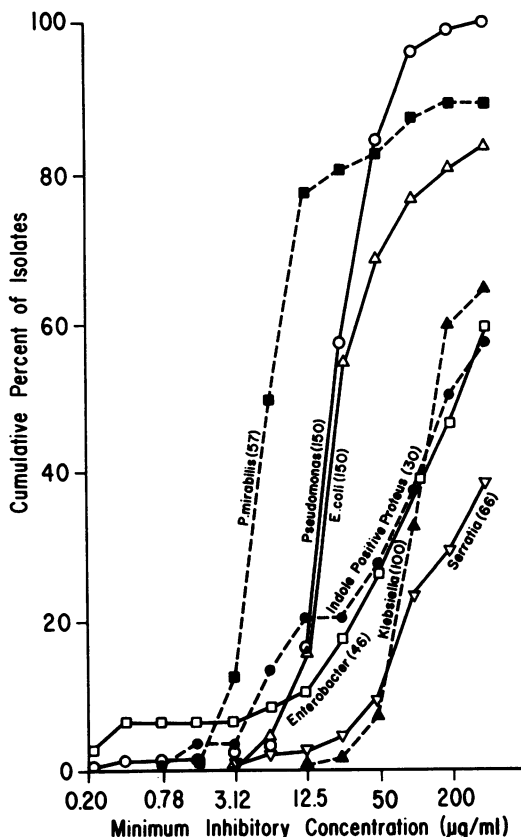


FIG. 4. In vitro activity of BL-P1532 against gram-negative bacilli.

TABLE 1. Bactericidal activity of  $\alpha$ -(substituted-ureido) penicillins

Organism	Bactericidal activity <sup>a</sup>		
	BL-P1597	BL-P1654	BL-P1532
<i>Escherichia coli</i> . . . . .	51	33	65
<i>Proteus</i> spp. . . . .	34	13	38
<i>Enterobacter</i> sp. . . . .	52	20	26
<i>Klebsiella</i> sp. . . . .	31	26	20
<i>Serratia</i> sp. . . . .	27	7	5
<i>Pseudomonas</i> sp. . . . .	3	2	3

<sup>a</sup> Defined as the percentage of isolates which yielded less than 10 colonies on subculture of the tube containing the MIC.

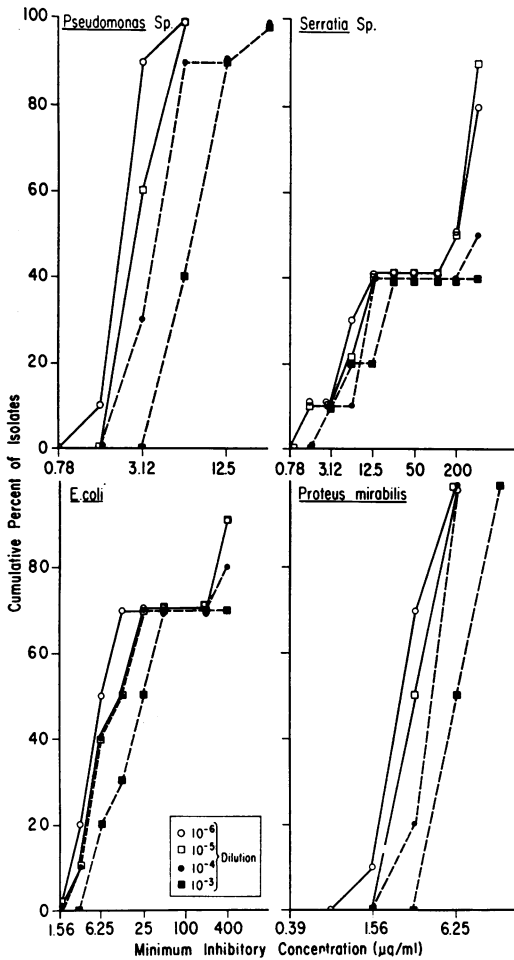


FIG. 5. Effect of inoculum size on the activity of BL-P1597 against gram-negative bacilli. These studies were conducted with 10 clinical isolates each of *Pseudomonas sp.*, *Serratia sp.*, *E. coli*, and *P. mirabilis*.

effect on the activity of BL-P1597, BL-P1532, and carbenicillin against *Pseudomonas sp.*

The activity of the  $\alpha$ -(substituted-ureido) penicillins and ampicillin was compared simultaneously against 50 isolates of *E. coli*, 16 isolates of indole-positive *Proteus spp.*, and 34 isolates of *P. mirabilis*. The  $\alpha$ -(substituted-ureido) penicillins also were compared simultaneously against 50 isolates of *Pseudomonas sp.* Results of previous studies with carbenicillin and BL-P1462 are included for comparison. The activities of ampicillin, BL-P1654, carbenicillin, and BL-P1597 were similar against *E. coli* (Fig. 10). These antibiotics inhibited more than 75% of the isolates of *E. coli* at a concentration of 12.5 µg or less/ml, whereas only 32% were inhibited by BL-P1532 and 10% by BL-P1462 at the same concentration.

BL-P1597 was the most active antibiotic against *Pseudomonas sp.* (Fig. 11). Over 90% of the isolates were inhibited by 12.5 µg or less/ml. BL-P1654 was also quite active, inhibiting 76% of isolates at the same concentration. BL-P1532 was less effective, inhibiting only 30% of isolates at 25 µg or less/ml. Carbenicillin and BL-P1462 inhibited only 10% of isolates at this concentration.

BL-P1654 and carbenicillin were most active against *P. mirabilis* (Fig. 12). Both drugs inhibited 70% of the isolates at a concentration of 1.56 µg or less/ml. BL-P1462 inhibited 50% of the isolates, and the other three penicillins were virtually inactive, at this concentration. At a concentration of 50 µg/ml, BL-P1597 was the most active antibiotic. Carbenicillin was the most active penicillin against indole-positive *Proteus spp.*, and was more active against these organisms than against *P. mirabilis*. BL-P1597 was equally active against indole-negative and indole-positive *Proteus spp.*, but was less active than carbenicillin. The other penicillins were much less active against indole-positive *Proteus spp.*

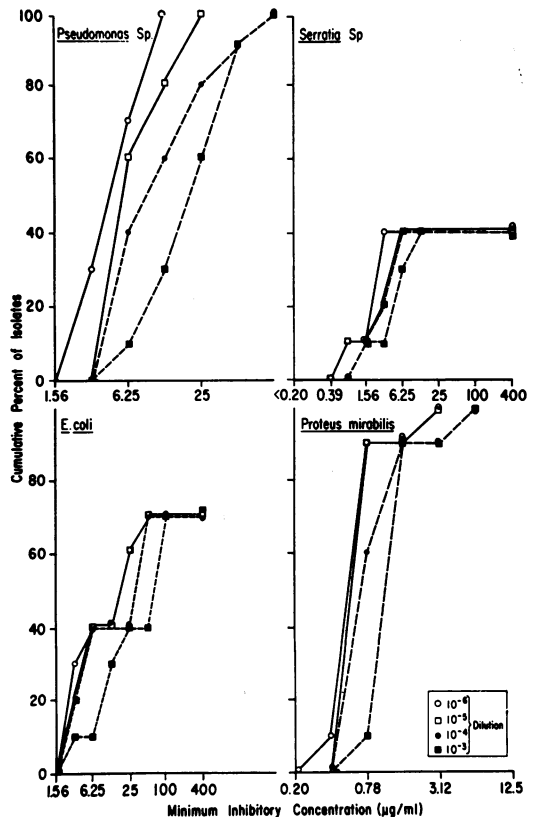


FIG. 6. Effect of inoculum size on the activity of BL-P1654 against gram-negative bacilli.

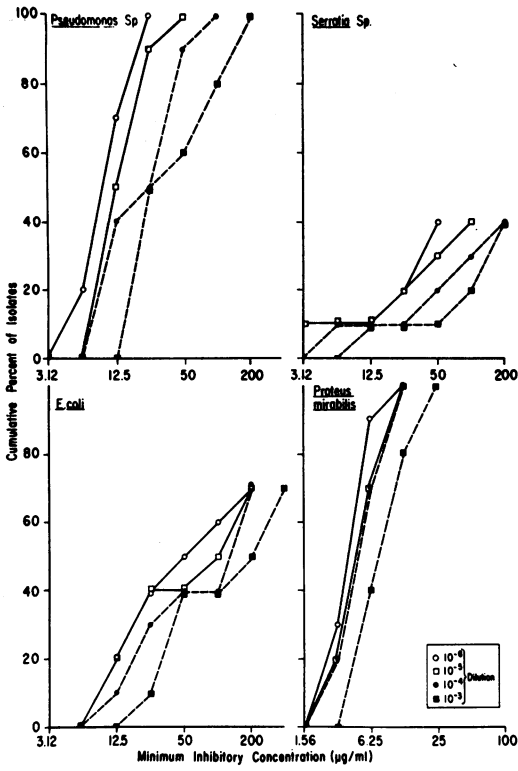


FIG. 7. Effect of inoculum size on the activity of BL-P1532 against gram-negative bacilli.

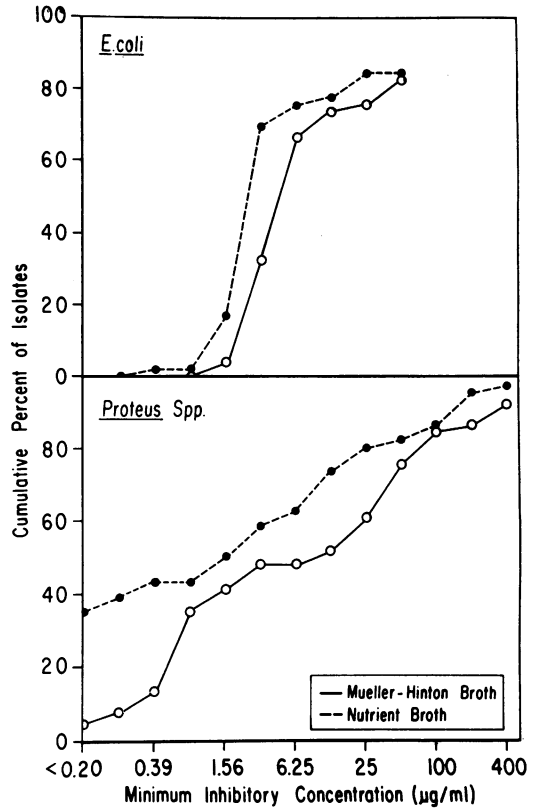


FIG. 9. Effect of media on the activity of BL-P1654 against *E. coli* and *Proteus* spp.

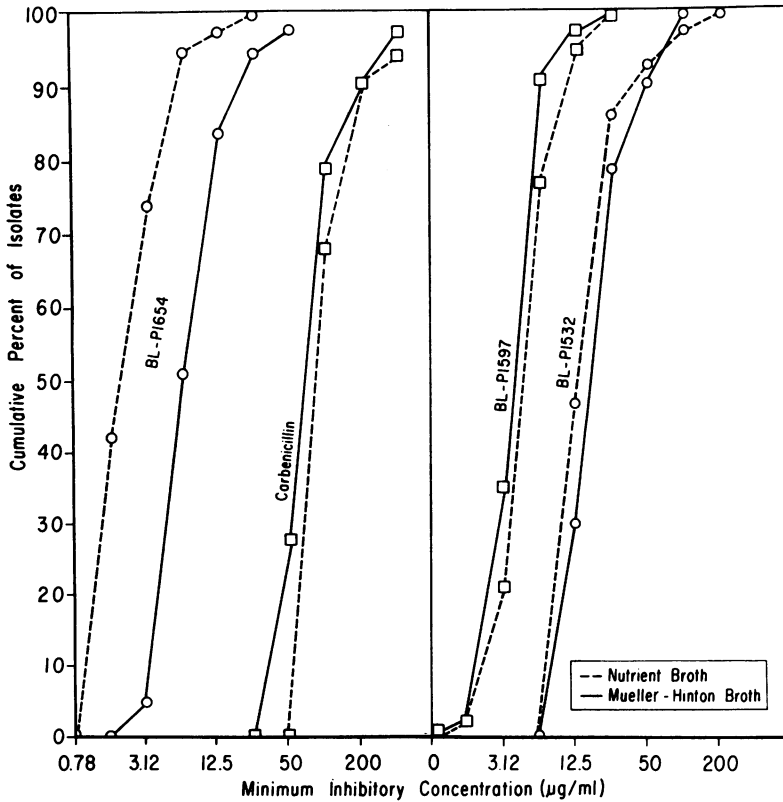


FIG. 8. Effect of media on the activity of semisynthetic penicillins against *Pseudomonas* sp.

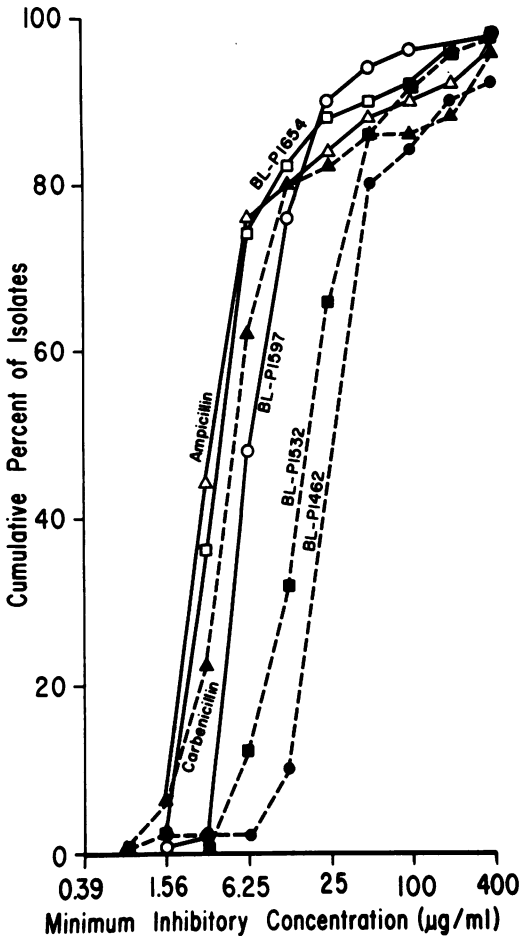


FIG. 10. Activity of semisynthetic penicillins against 50 clinical isolates of *E. coli*.

The activity of the  $\alpha$ -(substituted-ureido) penicillins and BL-P1462 was determined against a strain of *Pseudomonas* sp. which had developed resistance to carbenicillin (Table 2). *Pseudomonas* sp. was cultured from the blood of a patient who was treated with 30 g of carbenicillin for 12 days. The patient failed to respond, and *Pseudomonas* sp. of the same pyocine type was cultured from his blood on several occasions between days 7 and 12. This strain of *Pseudomonas* sp. also developed resistance in vitro to all of the other semisynthetic penicillins tested.

DISCUSSION

The  $\alpha$ -(substituted-ureido) penicillins are an interesting group of semisynthetic penicillins which have broad-spectrum activity against gram-negative bacilli. They are especially active against *Pseudomonas* sp. and *Proteus* spp., but also are active against *E. coli* and *Enterobacter*

sp. BL-P1597 and BL-P1654 have very similar activity, whereas BL-P1532 is a much less active compound.

The size of inoculum and type of medium affect the results obtained with the  $\alpha$ -(substituted-ureido) penicillins. Like other semisynthetic penicillins, these three drugs are less active against larger inocula (1, 3). The type of media had the greatest effect on the activity of BL-P1654 against *Pseudomonas* sp. The increased activity of BL-P1654 in nutrient agar was first demonstrated by K. E. Price (*unpublished data*). The activity of other semisynthetic penicillins varies only slightly in different media.

BL-P1597 and BL-P1654 compare favorably with other semisynthetic penicillins which are active against gram-negative bacilli. Both of these penicillins are as active as ampicillin and carbenicillin against *E. coli*. BL-P1654 is as active as carbenicillin, and more active than ampicillin, against *P. mirabilis*. However, BL-P1597 is the most effective antibiotic against the more resistant isolates of *P. mirabilis*. None of the  $\alpha$ -(substituted-ureido) penicillins is as active as carbenicillin against indole-positive *Proteus* spp. The  $\alpha$ -

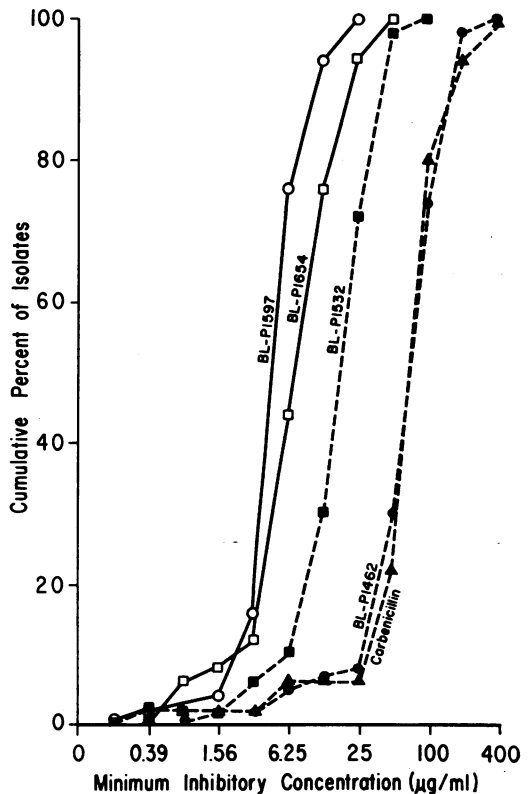


FIG. 11. Activity of semisynthetic penicillins against 50 clinical isolates of *Pseudomonas* sp.

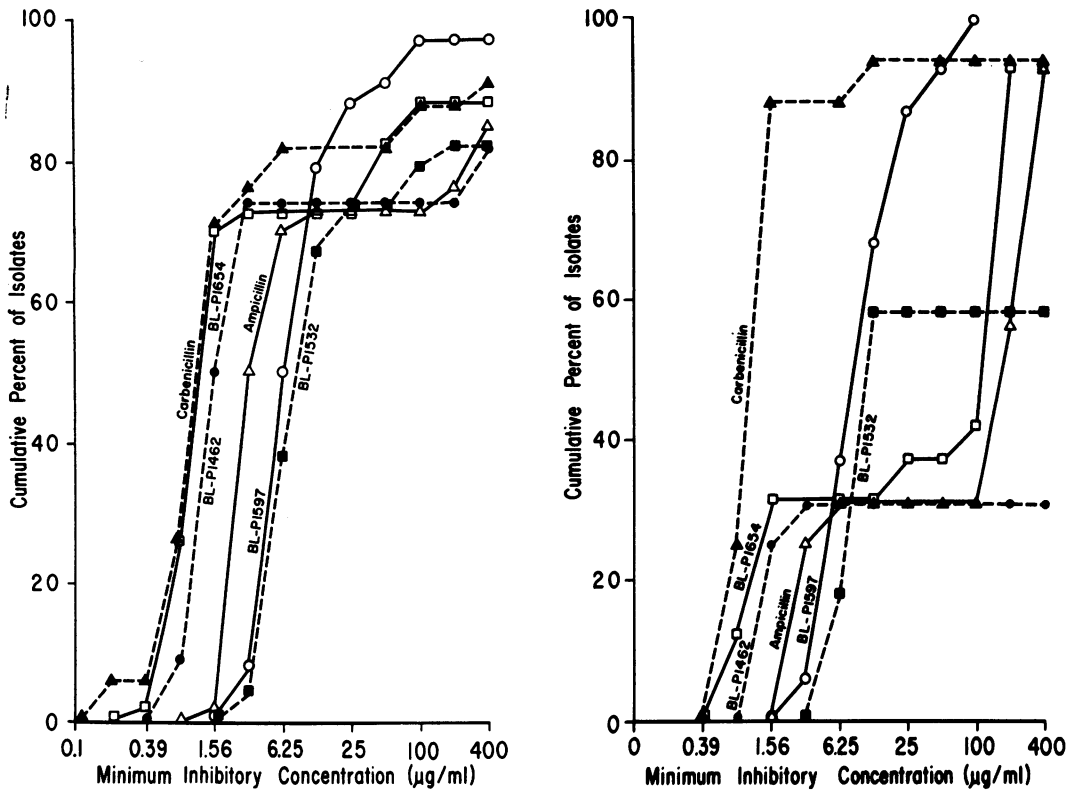


FIG. 12. Activity of semisynthetic penicillins against 34 clinical isolates of *P. mirabilis* (left) and 16 clinical isolates of indole-positive *Proteus* spp. (right).

TABLE 2. Susceptibility to semisynthetic penicillins of a strain of *Pseudomonas* sp. which developed resistance to carbenicillin

Day of carbenicillin therapy	Minimal inhibitory concn (µg/ml)				
	Carbenicillin	BL-P1462	BL-P1532	BL-P1597	BL-P1654
1	50	200	50	6.25	12.5
7	100	200	50	6.25	25
8	200	>400	200	—	100
9	200	>400	200	50	100
12	>400	>400	>400	400	>400

(substituted-ureido) penicillins are substantially more active against *Pseudomonas* sp. than is carbenicillin or BL-P1462. BL-P1597 inhibits over 90% of isolates at a concentration of 12.5 µg or less/ml. However, the  $\alpha$ -(substituted-ureido) penicillins are not bactericidal against *Pseudomonas* sp., whereas carbenicillin and BL-P1462 are bactericidal against approximately 30% of isolates (3, 4). Other investigators have found similar results with BL-P1654 against gram-negative bacilli (10, 11).

Most isolates of *Klebsiella* sp. and *Serratia* sp. are resistant to all of the semisynthetic penicillins, and superinfections caused by these organisms have been a problem in patients receiving carbenicillin (2). Nearly 60% of isolates of *Klebsiella* sp. and nearly 40% of isolates of *Serratia* sp. are inhibited by 25 µg or less of BL-P1597/ml. All three  $\alpha$ -(substituted-ureido) penicillins are nearly as active against *Klebsiella* sp. as they are against *Enterobacter* sp., which is not true for carbenicillin and BL-P1462 (3, 4).

At some institutions, a substantial number of isolates of *Pseudomonas* sp. have developed resistance to carbenicillin (5, 8). Carbenicillin-resistant isolates have been found infrequently at our institution. *Pseudomonas* sp. was cultured repeatedly from the blood of one patient who failed to respond to carbenicillin therapy. This organism developed resistance in vitro to carbenicillin and to all of the other antipseudomonal penicillins. It has been suggested that resistance to carbenicillin is due to production of a specific carbenicillinase (8). This is unlikely in our case, since the organism simultaneously developed resistance to these other penicillins which have different chemical structures.

Carbenicillin has been extremely effective in the treatment of *Pseudomonas* infections (2). However, large doses of drug are required for effective therapy. These in vitro studies suggest that BL-P1597 and BL-P1654 should be effective at much lower doses. However, a major limitation in the development of BL-P1654 for clinical trials is its minimal solubility in aqueous solutions. BL-P1597 does not present this problem. The  $\alpha$ -(substituted-ureido) penicillins appear most promising and deserve further evaluation as potential antibiotics for clinical use.

#### ACKNOWLEDGMENTS

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