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Azlocillin: In Vitro Studies of a New Semisynthetic Penicillin

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The activity of azlocillin, a new semisynthetic penicillin, was determined against 582 clinical isolates of gram-negative bacilli and gram-positive cocci. Over 75% of the isolates of *Pseudomonas aeruginosa* were inhibited at a concentration of 12.5 μg or less per ml. Azlocillin is also active against indole-negative and -positive *Proteus* spp., inhibiting 98 and 71%, respectively, at a concentration of 12.5 μg or less per ml. Isolates of *Klebsiella* spp. and *Enterobacter* spp. showed less susceptibility than isolates of *Escherichia coli* and *Serratia* spp. Gram-positive cocci except penicillin G-resistant *Staphylococcus aureus* were susceptible to azlocillin. Azlocillin failed to inhibit the growth of gram-negative bacilli when large inocula were used. It was more active in alkaline pH, but the type of medium used had little effect on its activity. Azlocillin was more active than mezlocillin, ticarcillin, and carbenicillin and as active as BLP-1654 against isolates of *P. aeruginosa*. It was not as active as mezlocillin against the majority of the other gram-negative bacilli.

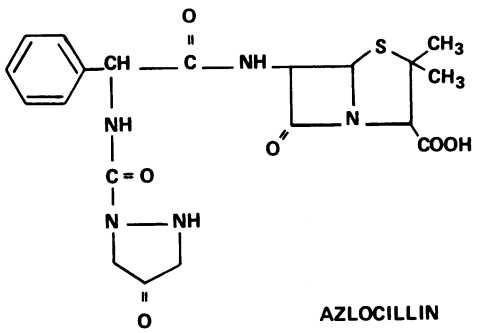
Gram-negative bacilli, especially *Klebsiella* spp. and *Pseudomonas aeruginosa*, continue to be a major cause of infection among patients with malignant diseases and patients receiving immunosuppressive therapy (6, 7). Some of these patients fail to improve even when an appropriate antibiotic is administered on the basis of in vitro susceptibility testing (2). In general, penicillins have been more effective than other antibiotics against susceptible organisms in the compromised host (1). Since penicillins have been more effective than other classes of antimicrobial agents for the treatment of such infections, new penicillin derivatives with broad-spectrum activity, particularly against gram-negative bacilli, are of special interest. Azlocillin, a new semisynthetic penicillin, is of interest because of its activity against these organisms and especially *P. aeruginosa*. Figure 1 shows the chemical structure of azlocillin (6[D-2-(2-oxoimidazolidine-1-carboxamido)-2-phenylacetamido]-penicillanic acid, sodium salt). This report presents an in vitro evaluation of azlocillin and compares its activity with other semisynthetic penicillins, including another new derivative, mezlocillin.

MATERIALS AND METHODS

Susceptibility tests were conducted on 479 clinical isolates of gram-negative bacilli and 99 clinical isolates of gram-positive cocci, using a dilution technique with an automatic microtiter system (Canalco, *Autotiter Instruction Manual*). All gram-negative bacilli and *Staphylococcus aureus* isolates to be tested were incubated in Mueller-Hinton broth (pH 7.4) for 18 h at 37°C. *Streptococcus pyogenes* and *S. pneumoniae* were incubated in tryptose phosphate broth. Approximately 10^5 colony-forming units (CFU) per ml was used as the inoculum for gram-negative bacilli and *S. aureus*. For the remaining gram-positive cocci, an inoculum of 10^6 CFU/ml was used for the in vitro susceptibility testing.

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 *Escherichia coli*, 79 isolates of *Proteus* spp., 50 isolates each of *Serratia* spp., and *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 40 isolates of *S. pyogenes*, 9 isolates of *S. pneumoniae*, and 50 isolates of *S. aureus* were used. Isolates of *S. aureus* were divided according to their susceptibility to penicillin G. Those isolates that were inhibited by less than 0.10 μg/ml were selected as penicillin G susceptible and those isolates resistant to more than 25 μg/ml were selected as penicillin G resistant.

Organisms used for studies of the effect of inoculum size on the activity of azlocillin 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 portions on sheep blood agar and performing colony counts after 14 h of incubation at 37°C. Serial 10-fold dilutions of the broth culture were made, with Mueller-Hinton broth, so that 10^7 and 10^5 CFU/ml were used as inocula. An inoculum of 10^5 CFU/ml was used in all other studies of gram-negative bacilli. Studies of the



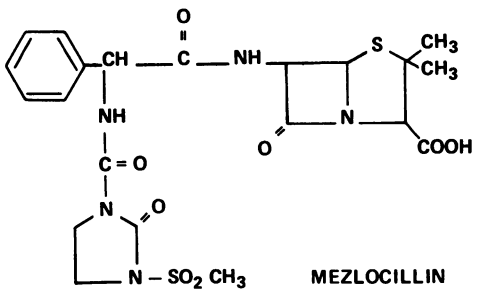


FIG. 1. *Chemical structure of azlocillin and mezlocillin.*

effect of pH on the activity of azlocillin 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 azlocillin with mezlocillin, BLP-1654, carbenicillin, and ticarcillin were conducted in Mueller-Hinton broth. Fifty isolates each of *E. coli*, *Klebsiella* spp., *P. aeruginosa*, *Serratia* spp., *Enterobacter* spp., and *P. mirabilis* and 14 isolates of indole-positive *Proteus* spp. were used. Isolates were selected so that organisms with differing susceptibilities to azlocillin were included.

Azlocillin and mezlocillin (Bay 6905 and 1353) were supplied as powders by Delbay Pharmaceuticals Inc., Bloomfield, N.J. Carbenicillin and ticarcillin were supplied by Beecham Pharmaceuticals, Bristol, Tenn. BLP-1654, 6 [D-α(3-guanylureido)-phenylacetamido]-penicillanic acid, was supplied by Bristol Laboratories, Syracuse, N.Y. All antibiotics were diluted serially in Mueller-Hinton broth or tryptose phosphate broth. The minimum inhibitory concentration (MIC) was determined as no visible growth after 18 h of incubation at 37°C. The minimum bactericidal concentration (MBC) was defined as the lowest concentration of drug that yielded less than 5 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

The in vitro susceptibility of azlocillin against gram-positive cocci and gram-negative bacilli is shown in Fig. 2. At a concentration of 12.5 μg/ml, over 75% of the isolates of *P. aeruginosa* were inhibited. Both indole-negative and -positive *Proteus* spp. were susceptible to azlocillin, with 75% of *P. mirabilis* inhibited at 1.56 μg/ml and 71% of indole-positive *Proteus* spp. inhibited at 12.5 μg/ml. Azlocillin exhibited moderate activity against isolates of *E. coli* and *Serratia* spp., inhibiting 63 and 46%, respectively, at a concentration of 12.5 μg/ml. *Klebsiella* spp. and *Enterobacter* spp. showed less activity with only 21 and 26%, respectively, inhibited at the same concentration of 12.5 μg/ml. All isolates of *S. pyogenes* were inhibited by a concentration of 0.10 μg or less per ml. However, only four of nine isolates of *S. pneumoniae* were inhibited at 0.10 μg/ml. The majority of the penicillin G-susceptible *S. aureus* were inhibited by 0.20 μg or less of azlocillin per ml, whereas the penicillin G-resistant *S. aureus* was also resistant to azlocillin. The MBC generally was the same as, or one concentration higher than, the MIC.

The effect of inoculum size on the MIC and MBC was determined for 10 isolates each of *K. pneumoniae*, *E. coli*, and *P. aeruginosa*. All of the isolates of *E. coli* were completely inhibited at 6.25 μg/ml when an inoculum of 10^5 CFU/ml was used. At a concentration of 50 μg/ml or less, 8 of the 10 isolates of *K. pneumoniae* and 8 of the 10 isolates of *P. aeruginosa* were inhibited when an inoculum of 10^5 CFU/ml was used. With the exception of one isolate of *P. aeruginosa*, all the isolates had similar MICs and MBCs. None of the isolates was inhibited at a concentration of 400 μg/ml when an inoculum of 10^7 CFU/ml was used.

The effect of pH on susceptibility of 10 isolates each of *E. coli*, *K. pneumoniae*, and *P. aeruginosa* to azlocillin is shown in Fig. 3. The activity of azlocillin increased against all organisms as the pH became more alkaline. As shown in Fig. 4, media variation had little effect on the activity of azlocillin. However, isolates of *P. aeruginosa* were slightly more susceptible in nutrient broth, whereas isolates of *E. coli* and *K. pneumoniae* were not.

The susceptibility of isolates of Enterobacteriaceae was determined for azlocillin, carbenicillin, mezlocillin, ticarcillin, and BLP-1654 (Fig. 5). Mezlocillin was consistently more active than azlocillin. Mezlocillin was the most active penicillin against *Klebsiella* spp. Azlocillin was as active as BLP-1654 and inhibited 54% of the isolates at a concentration of 25 μg/ml. Azlocillin was the least active penicillin against *Enterobacter* spp.: 70% of the isolates were resistant to 25 μg/ml. Mezlocillin and carbenicillin were the most active penicillins against *Enterobacter* spp., although about half

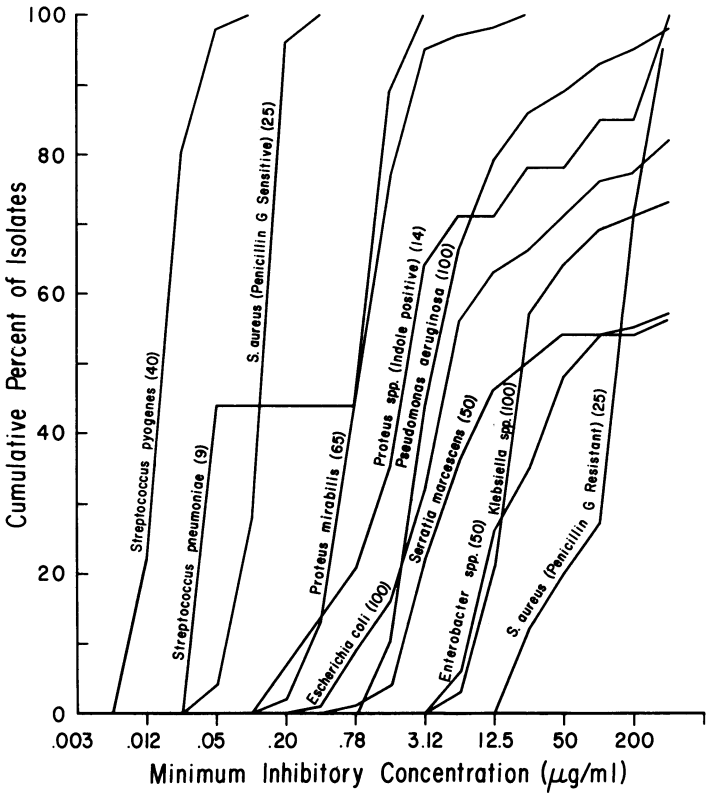


FIG. 2. *In vitro activity of azlocillin.*

of the isolates were resistant to 25 μg of these penicillins per ml. Azlocillin inhibited fewer isolates of *S. marcescens* at a concentration of 12.5 μg/ml than the other penicillins. However, some of the isolates resistant to carbenicillin and ticarcillin were inhibited by azlocillin. Mezlocillin was the most active penicillin against *Serratia* spp., *E. coli*, and *P. mirabilis*. Azlocillin, carbenicillin, BLP1654, and ticarcillin had similar activity against isolates of *E. coli*. All of these antibiotics were active against most of the isolates of *Proteus mirabilis* at a concentration of 3.12 μg/ml. At lower concentrations, azlocillin was the least active penicillin. Azlocillin also was less active than mezlocillin against indole-positive *Proteus* spp., but more active than BLP-1654. It inhibited 64% of these isolates at a concentration of 3.12 μg/ml.

The activity of the five penicillins against isolates of *P. aeruginosa* is shown in Fig. 6. Azlocillin and BLP-1654 were the most active penicillins and were substantially more active than carbenicillin. Mezlocillin and ticarcillin were intermediate in their activity. Eleven isolates of *P. aeruginosa* that were resistant to carbenicillin (MIC, ≥400 μg/ml) were tested for their susceptibility to the other penicillins (Table 1). Five isolates of *E. coli*, five isolates of *S. marcescens*, and four isolates of *Enterobacter* spp. were also tested. Those isolates of *P. aeruginosa*, which were inhibited by 400 μg of carbenicillin per ml, were generally susceptible to the other penicillins, and azlocillin was the most active. Most of the isolates of *P. aeruginosa* resistant to 400 μg of carbenicillin per ml were also resistant to the other penicillins. All of the isolates of other gram-negative bacilli were not inhibited by 400 μg of any of these penicillins per ml.

DISCUSSION

Azlocillin is a new ureido-substituted penicillin with activity against gram-negative bacilli. In general, its spectrum of activity is similar to that of BLP-1654 (5). The only major advantage of azlocillin is its antipseudomonal activity, which is substantially greater than carbenicillin. Against most isolates of *P. aeruginosa*, it is four times more active than ticarcillin. Another new penicillin, mezlocillin, has a broader spectrum of activity than azlocillin, but is not as active against *P. aeruginosa*. A major disadvantage of BLP-1654 was renal damage, which was observed in toxicology studies. Azlocillin

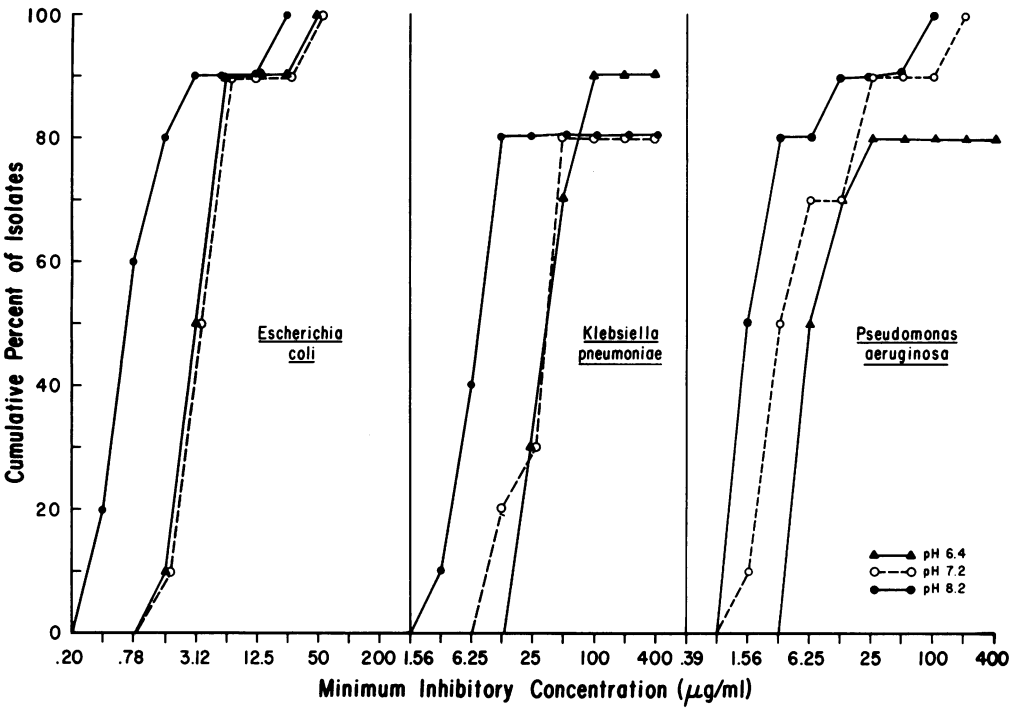


FIG. 3. *Effect of pH on activity of azlocillin.*

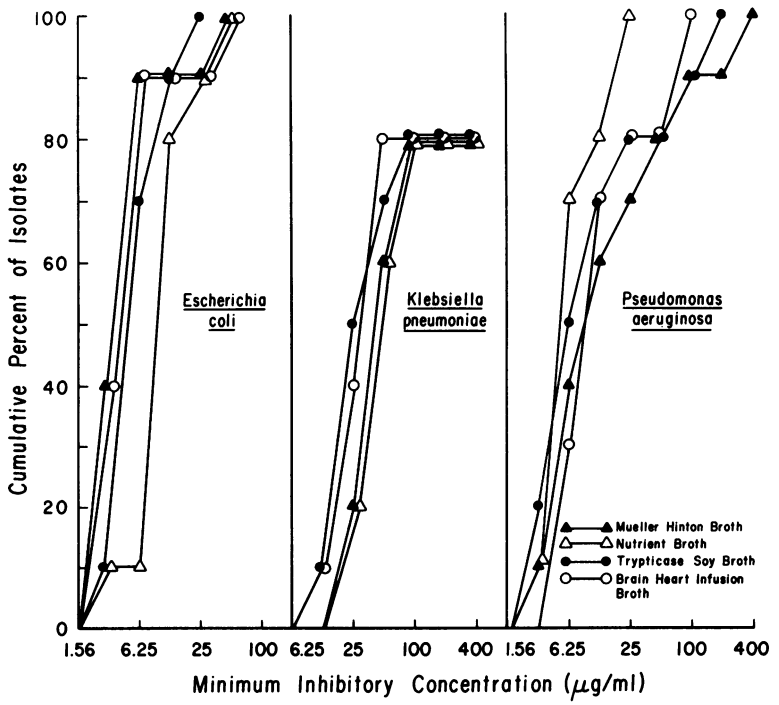


FIG. 4. *Effect of medium on activity of azlocillin.*

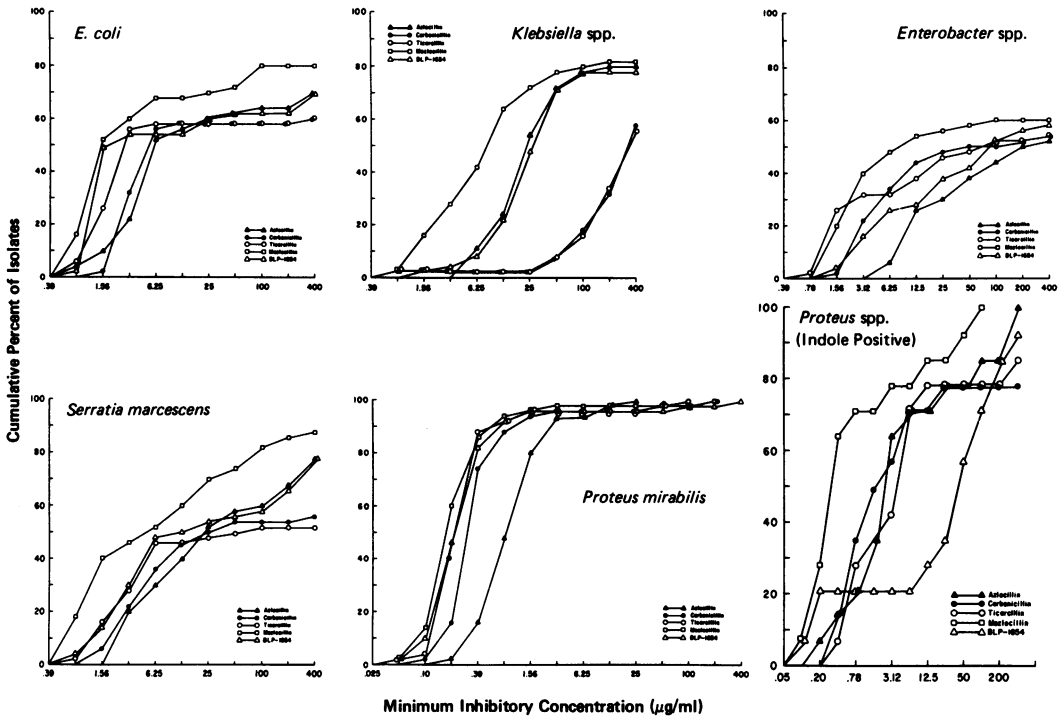


FIG. 5. *Comparative activity of antibiotics against gram-negative bacilli. Fifty isolates each were used except for indole-positive Proteus spp., where only 14 isolates were available.*

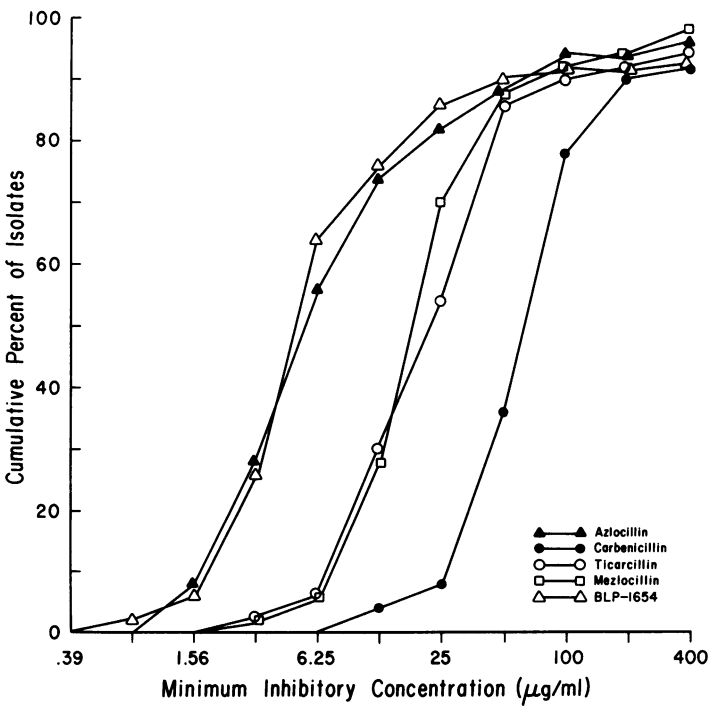


FIG. 6. *Comparative activity of penicillins against P. aeruginosa.*

TABLE 1. *Activity of azlocillin against P. aeruginosa resistant to carbenicillin*

MIC (μg/ml)				
Carbenicillin	Azlocillin	BLP-1654	Mezlocillin	Ticarcillin
400	25	12.5	200	100
400	50	200	200	200
400	50	>400	200	400
400	25	50	50	400
>400	>400	400	>400	>400
>400	>400	>400	>400	>400
>400	50	100	100	>400
>400	>400	400	>400	>400
>400	50	25	100	400
>400	>400	>400	>400	>400
>400	400	>400	>400	>400

does not appear to cause nephrotoxicity (E. F. Murray, personal communication). Our in vitro results are somewhat better than those obtained by Krasemann, who found nearly 60% of isolates of *Klebsiella* spp. and *S. marcescens* resistant to azlocillin (C. Krasemann, Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 16th, Chicago, Ill., Abstr. 346, 1976).

Unfortunately, most isolates of *P. aeruginosa* and other gram-negative bacilli that were resistant to carbenicillin were also resistant to azlocillin. However, a few carbenicillin-resistant isolates of *P. aeruginosa* were susceptible to azlocillin. This may be explained by the fact that ureido-penicillins are resistant to destruction by the β-lactamase produced by these organisms (B. Wiedemann, V. Kremery, and H. Knothe, International Congress of Chemotherapy, London, Abstr. M366, 1975). Azlocillin was active against those isolates of *P. aeruginosa* that were only marginally susceptible to carbenicillin and ticarcillin. This could be of importance clinically, since it is difficult to achieve serum concentrations of carbenicillin of 400 μg/ml without toxicity.

Inoculum size had a major effect on the activity of azlocillin. It was inactive against all isolates of *E. coli*, *K. pneumoniae*, and *P. aeruginosa* when a large inoculum was used. It has been suggested that a larger inoculum contains a greater number of resistant cells. This effect of inoculum variation has been observed by other investigations with other penicillins, including BLP-1654 and pirbenicillin (4, 5, 8, 9). The clinical relevance of this observation is not clear.

The effect of pH on the activity of azlocillin is similar to the results obtained with other semisynthetic penicillins (3). Its activity increases as the pH increases. Media variation had little effect on the activity of azlocillin. However, the activity of other penicillins, like BLP-1654 and pirbenicillin, is influenced by the type of medium utilized (4, 5).

Azlocillin is an interesting semisynthetic penicillin, because it is more active than carbenicillin and ticarcillin against *P. aeruginosa* and has a somewhat broader spectrum of activity. Pharmacology studies indicate that adequate serum concentrations of this drug can be obtained, and preliminary therapeutic studies have been encouraging (H. Lode, U. Niestrath, P. Koeppe, and H. Langmaack, Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 16th, Chicago, Ill., Abstr. 348, 1976). It is deserving of further investigation to ascertain its potential value for clinical therapeutic studies.

ACKNOWLEDGMENTS

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