ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1978, p. 559-565
0066-4804/78/0013-0559$02.00/0

Vol. 13, No. 4
Printed in U.S.A.

Activity of Azlocillin and Mezlocillin Against Gram-Negative Organisms: Comparison with Other Penicillins

R. WISE,* A. P. GILLETT, J. M. ANDREWS, AND K. A. BEDFORD

Department of Medical Microbiology, Dudley Road Hospital, Birmingham, England B18 7QH

Received for publication 26 October 1977

The activities of azlocillin and mezlocillin were compared with those of carbenicillin, ticarcillin, and pirbenicillin against a wide range of gram-negative organisms. The two new drugs were considerably more active than carbenicillin against *Klebsiella* species and *Escherichia coli*. Carbenicillin was twice as active against *Proteus mirabilis* as mezlocillin and four times as active as azlocillin. Against *Pseudomonas aeruginosa*, azlocillin was eight times as active as carbenicillin. Azlocillin and mezlocillin were twice as active as carbenicillin against *Bacteroides fragilis*, and these drugs showed a high degree of activity against *Haemophilus influenzae* and *Neisseria gonorrhoeae*.

Carbenicillin (CAR) is an established antibiotic in the treatment of infections caused by *Pseudomonas aeruginosa, Serratia marcescens,* and the *Proteus* species. The practice of giving large amounts intravenously, which is occasionally associated with toxicity (4, 6), has prompted the search for drugs with a similar spectrum but greater activity. Two new penicillins, azlocillin (AZ) and mezlocillin (MZ), were investigated in this study. Both of these compounds can be considered as α amino-substituted ampicillins, as is pirbenicillin (PB) (2, 11). Their activity against a wide range of gram-negative organisms was studied. The two new drugs were compared with CAR, ticarcillin (TIC), PB, and, where appropriate, benzylpenicillin (PEN) and ampicillin (AMP).

MATERIALS AND METHODS

A wide range of recent clinical isolates of gram-negative organisms was collected. The medium used throughout was Isosensitest agar and broth (Oxoid) at pH 7.2. This was modified for the growth of *Bacteroides fragilis* by the addition of 5% lysed human blood. Chocolate agar and Levinthal agar were used to investigate *Neisseria gonorrhoeae* and *Haemophilus influenzae,* respectively. The organisms were grown overnight in nutrient broth and then diluted to approximately 10^6 colony-forming units (CFU) per ml. In the case of *H. influenzae,* Levinthal broth was used; *N. gonorrhoeae* was grown on chocolate agar plates and the growth was suspended in peptone water just before dilution. A multipoint inoculator delivered 1 μl, or approximately 10^3 CFU, to the antibiotic-containing agar plate. Two inocula were used for investigating the strains of *N. gonorrhoeae.* The plates were incubated at 37°C (with 10% CO_2 in the cases of *H. influenzae* and *N. gonorrhoeae,* using a GasPak system [BBL] for the anaerobes) and examined after 18 h. The minimum inhibitory concentration (MIC) was defined as that concentration of drug which caused a 99% reduction in the initial inoculum.

The production of β-lactamase by certain strains was verified by the chromogenic cephalosporin method (7). The protein binding of CAR, AZ, and MZ in human serum was determined by an ultrafiltration method. The initial concentrations of each drug were 20 and 100 μg/ml. The ultrafiltrate was assayed against standards prepared in phosphate-buffered saline by using *P. aeruginosa* NTCC 10771 as indicator organism.

RESULTS

In Fig. 1, 45 strains that were susceptible to CAR (MIC, <128 μg/ml) are shown. AZ and PB were approximately eight times as active as CAR. Figure 2 shows the activity against 17 strains that were resistant to CAR (MIC, ≥128 μg/ml). All strains (other than those producing β-lactamase) were susceptible to 16 μg or less of AZ per ml and to 64 μg of MZ per ml. For the two strains producing β-lactamase, the MICs of CAR, PB, and TIC were >1,024 μg/ml, and the MICs, of AZ and MZ were 256 and 512 μg/ml, respectively.

The activities of the five drugs against different *Enterobacteriaceae* are shown in Fig. 3 through 7. MZ, TIC, and PB showed a similar degree of activity against *Escherichia coli.* Of the 12 strains of *E. coli* resistant to CAR (MIC, >128 μg/ml and known to be β-lactamase producers), a number were more susceptible to AZ, MZ, and PB. The spectrum of activity of the drugs against the *Klebsiella* species (Fig. 4) was variable, but a number of these organisms were susceptible to these three drugs, yet they were uniformly resistant to TIC and CAR. Against *P. mirabilis* (Fig. 5) AZ, MZ, and PB were two to four times less active than CAR or TIC. This

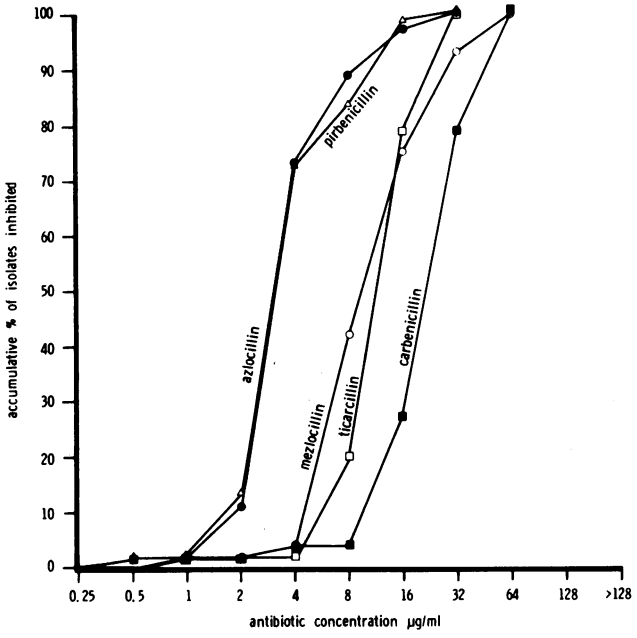

FIG. 1. *Activity of five penicillins against CAR-susceptible strains of P. aeruginosa (45 strains).*

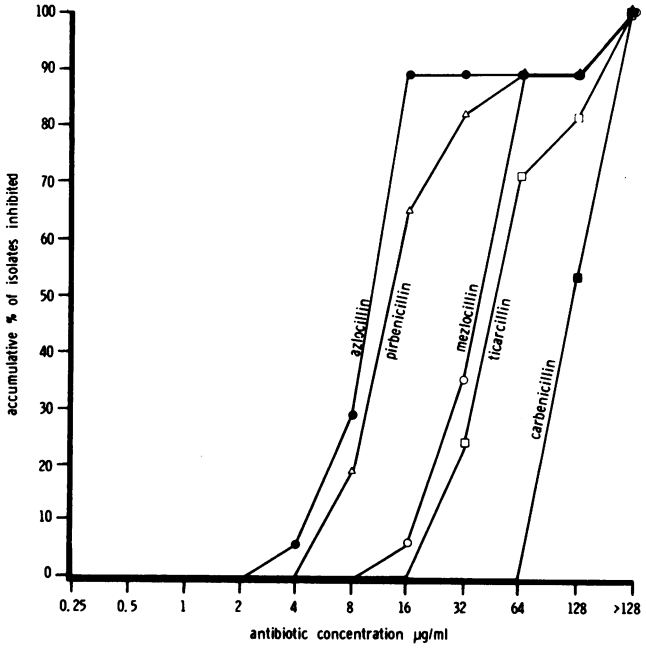

FIG. 2. *Activity of five penicillins against CAR-resistant strains of P. aeruginosa (17 strains).*

was in contrast to the indole-positive *Proteus* species (Fig. 6) where MZ was more active than either CAR or TIC. AZ and PB were two to four times less active than CAR against these indole-positive strains. In the *S. marcescens* strains tested (Fig. 7), MZ and CAR showed the same high degree of activity, and AZ was the least active.

AZ and MZ were about twice as active as CAR and TIC, which were twice as active as PB against the strains of *B. fragilis* tested (Fig. 8).

Against strains of *H. influenzae* (Table 1) that are susceptible to AMP, AZ and MZ were up to 4 times as active as AMP, with the MICs as low as 0.06 μg of AZ per ml in 11 strains. The two strains resistant to AMP and known to be β-

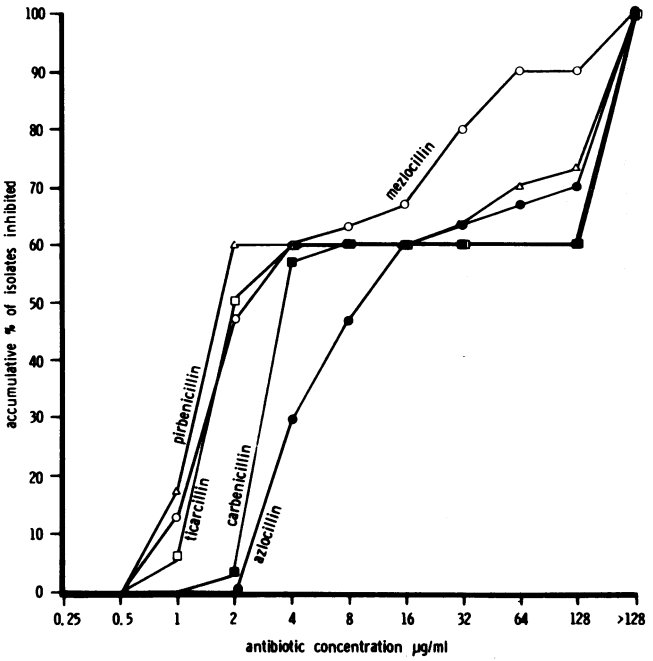

FIG. 3. *Activity of five penicillins against E. coli (30 strains).*

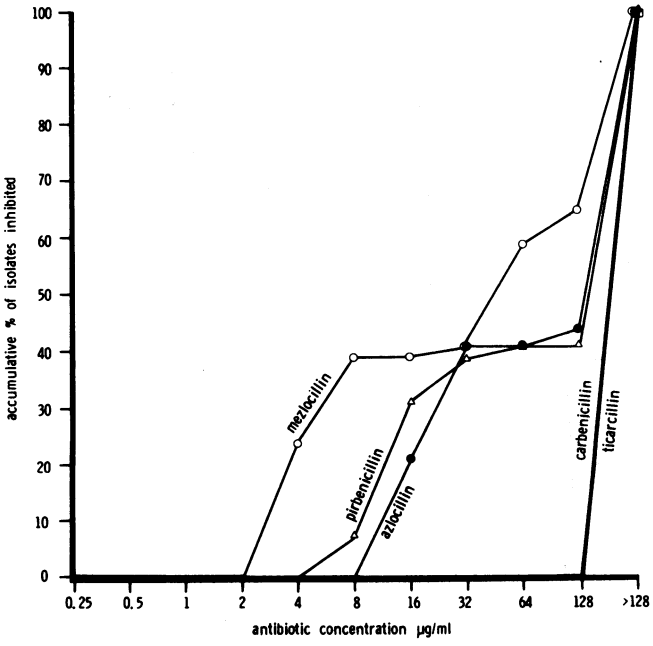

FIG. 4. *Activity of five penicillins against Klebsiella species (29 strains).*

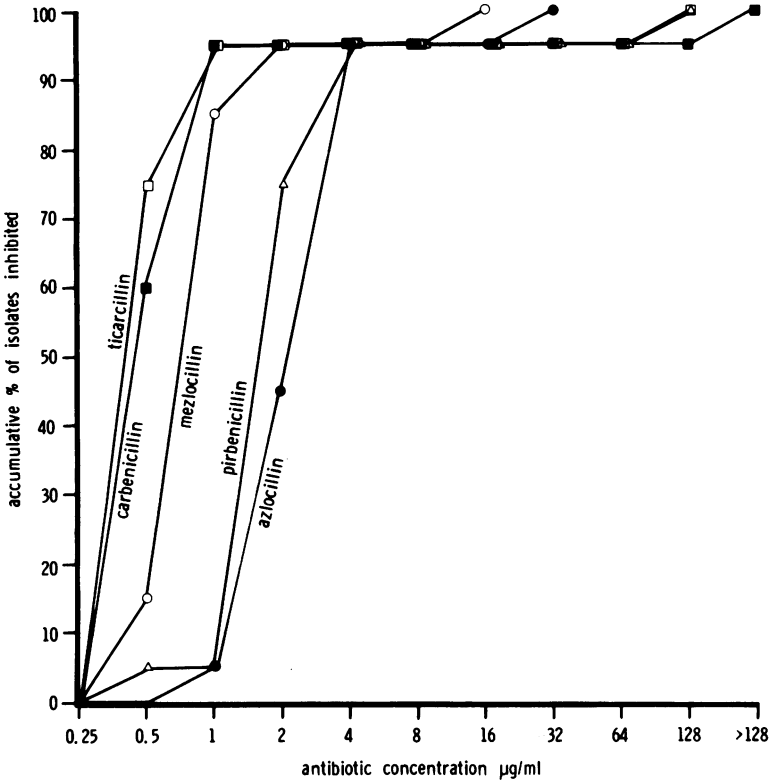

FIG. 5. *Activity of five penicillins against P. mirabilis (20 strains).*

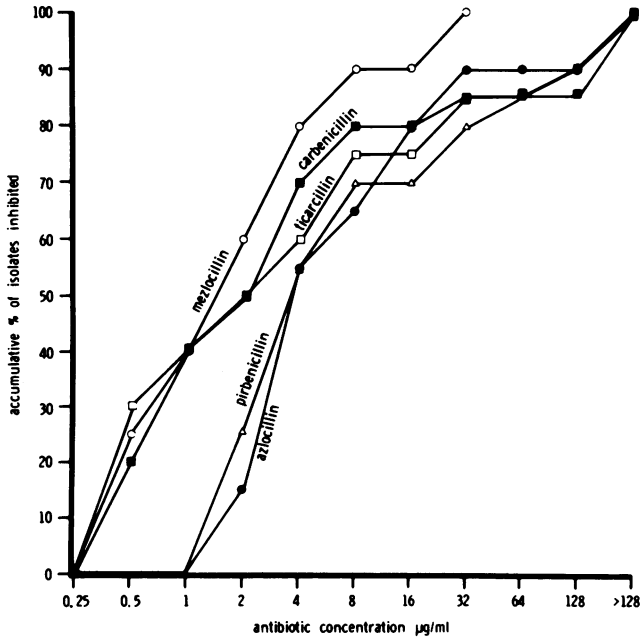

FIG. 6. *Activity of five penicillins against indole-positive Proteus (20 strains).*

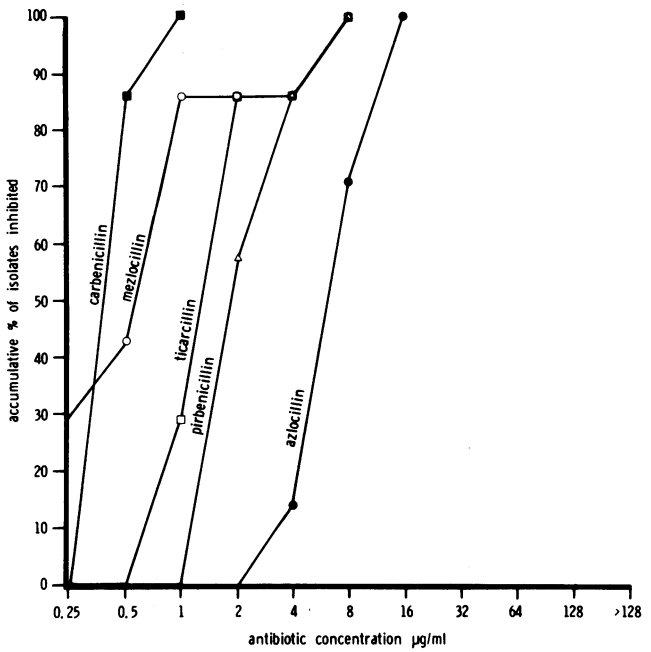

FIG. 7. *Activity of five penicillins against S. marcescens (7 strains).*

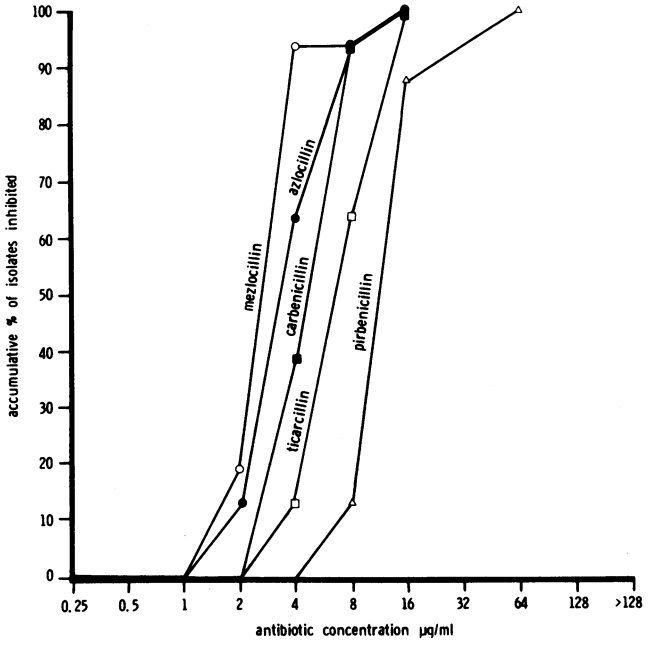

FIG. 8. *Activity of five penicillins against B. fragilis (16 strains).*

lactamase producers showed decreased susceptibility to AZ and MZ.

Table 2 shows the activity of PEN, AZ, and MZ against *N. gonorrhoeae* compared with two inocula. Those strains that were extremely susceptible to PEN (MIC, 0.005 µg/ml) were equally susceptible to the new drugs (4 out of 19 strains). Against 11 strains, AZ and MZ were

TABLE 1. *MIC of 5 penicillins against 13 strains of H. influenzae*

Drug	No. of strains susceptible to MIC equivalent to (μg/ml):									
	0.06	0.12	0.25	0.5	1	2	4	8	16	32
AZ	11			1[a]			1[a]			
MZ	8	3			1[a]			1[a]		
CAR		3	8				2[a]			
TIC		4	6	1			1[a]	1[a]		
PB		1	9	1				2[a]		
AMP		2	7	2				1[a]	1[a]	

[a] Two strains were β-lactamase producers.

TABLE 2. *MIC of PEN, MZ, and AZ against varying inocula of 19 strains of N. gonorrhoeae.*

Strain	PEN[a]		MZ[a]		AZ[a]	
	10^4-10^5	10^2-10^3	10^4-10^5	10^2-10^3	10^4-10^5	10^2-10^3
1103[b]	>16	4	>16	0.12	>16	0.25
1170[b]	4	2	0.25	0.06	0.5	0.12
672[b]	4	0.5	0.5	0.06	1	0.12
US[b]	>16	2	2	0.12	4	0.25
F3262	0.25	0.12	0.03	0.005	0.06	0.03
7	0.12	0.12	0.01	0.005	0.03	0.01
70	0.005	0.005	0.005	0.005	0.005	0.005
30	0.25	0.06	0.01	0.005	0.03	0.01
39	0.06	0.03	0.005	0.005	0.005	0.005
16	0.06	0.03	0.005	0.005	0.005	0.005
71	0.005	0.005	0.005	0.005	0.005	0.005
64	0.12	0.03	0.03	0.005	0.06	0.01
35	0.12	0.12	0.005	0.005	0.03	0.01
42	0.25	0.25	0.03	0.01	0.06	0.06
54	0.005	0.005	0.005	0.005	0.005	0.005
77	0.25	0.12	0.06	0.005	0.06	0.03
82	0.01	0.01	0.005	0.005	0.005	0.005
74	0.25	0.06	0.005	0.005	0.005	0.005
45	0.005	0.005	0.005	0.005	0.005	0.005

[a] Number of CFU per inoculum.
[b] Known β-lactamase-producing strains.

more active than PEN. These strains showed intermediate resistance to PEN (MIC, >0.005; <0.25 μg/ml).

The protein binding of MZ, AZ, and CAR was as follows: MZ—20 μg/ml, 59% bound, 100 μg/ml, 56% bound; AZ—20 μg/ml, 66% bound, 100 μg/ml, 71% bound; CAR—20 μg/ml, 54% bound, 100 μg/ml, 59% bound. Each value represents the mean observed in three experiments.

DISCUSSION

The use of CAR and TIC in the treatment of gram-negative infections, in particular *P. aeruginosa*, is well established. Unlike CAR and TIC, BL-P1654 was a substituted ampicillin or ureido-penicillin that had a marked antipseudomonal activity (8). The further evaluation of BL-P1654 has since been abandoned. Other substituted ampicillins have subsequently been produced, and PB has been shown to be a particularly active compound (2, 11), but no further studies on this compound are to be undertaken. AZ and MZ have a wide spectrum of activity against gram-positive organisms. Both are more active against *P. aeruginosa* than CAR. A total of 89% of the CAR-susceptible strains were inhibited by 8 μg or less of AZ per ml in contrast to CAR, which inhibited 4% of strains when this amount of antibiotic was used. The activity of AZ and MZ against strains of *P. aeruginosa* resistant to CAR was interesting since it could be expected that all but the rare β-lactamase-producing strains might be susceptible to clinically attainable levels of the drugs. Stewart and Bodey (9) have also noticed this effect, although it was more marked in our experience. Against a wide range of *Enterobacteriaceae*, MZ was somewhat more active than AZ.

The activity of β-lactam drugs against *B. fragilis* is an area of interest. It has been known

that CAR has a marginally useful degree of activity against these organisms (3). AZ and MZ were twice as active, which suggests that these drugs should be clinically evaluated. It was of interest to note that against non-β-lactamase-producing strains of *H. influenzae*, AZ and MZ were more active than AMP. The β-lactamase-producing strains were more susceptible to the two new drugs than to AMP. The observation that those strains of *N. gonorrhoeae* showing intermediate resistance to penicillin were more susceptible to AZ and MZ merits further investigation. It is of interest to note that the β-lactamase-producing strains are susceptible to lower levels of AZ and MZ than PEN.

It would be interesting to know whether the increased in vitro activity shown by AZ and MZ against bacteria that are known to produce a β-lactamase is due to greater stability of these drugs to the enzymatic degradation or to a greater facility of these compounds to penetrate the bacterial cell wall. Preliminary work (J. D. Williams, personal communication) would indicate the latter. Bodey and Pan (1) suggest that a population of bacterial cells are not uniformly susceptible to MZ because they noted that inoculum size had a considerable effect on the susceptibility of the cells to this antimicrobial agent.

ACKNOWLEDGMENT

We thank J. Mason from Bayer, United Kingdom, for his help and support.

LITERATURE CITED

1. **Bodey, G. P., and T. Pan.** 1977. Mezlocillin: in vitro studies of a new broad-spectrum penicillin. Antimicrob. Agents Chemother. 11:74–79.
2. **Bodey, G. P., V. Rodriguez, and S. Weaver.** 1976. Pirbenicillin, a new semisynthetic penicillin with broad-spectrum activity. Antimicrob. Agents Chemother. 9:668–674.
3. **Fiedelman, W., and C. C. Webb.** 1975. Clinical evaluation of carbenicillin in the treatment of infections due to anaerobic bacteria. Curr. Ther. Res. 18:441–451.
4. **Klastersky, J., B. Vanderkelen, D. Daneau, and M. Mathieu.** 1973. Carbenicillin and hypokalaemia. Ann. Intern. Med. 78:774–775.
5. **Knudsen, E. T., G. N. Rolinson, and R. Sutherland.** 1967. Carbenicillin: a new semisynthetic penicillin active against *Pseudomonas pyocyanea*. Br. Med. J. 3:75–78.
6. **Lurie, A., M. Ogilvie, C. Townsend, C. Gold, A. M. Meyer, and B. Goldberg.** 1970. Carbenicillin-induced coagulopathy. Lancet i:1114.
7. **O'Callaghan, C. H., A. Morris, S. M. Kirby, and A. H. Shindler.** 1972. Novel method for detection of β-lactamase by using a chromogenic cephalosporin substrate. Antimicrob. Agents Chemother. 1:283–288.
8. **Saunders, C. C., and W. E. Saunders.** 1975. BL-P1654: a bacteriostatic penicillin. Antimicrob. Agents Chemother. 7:435–440.
9. **Stewart, D., and G. P. Bodey.** 1977. Azlocillin: in vitro studies of a new semisynthetic penicillin. Antimicrob. Agents Chemother. 11:865–870.
10. **Sutherland, R., J. Burnett, and G. N. Rolinson.** 1971. α-Carboxy-3-thienylmethylpenicillin (BRL 2288), a new semisynthetic penicillin: in vitro evaluation, p. 390–395. Antimicrob. Agents Chemother. 1970.
11. **Wise, R., J. M. Andrews, and K. A. Bedford.** 1977. Pirbenicillin—a semisynthetic penicillin with antipseudomonal activity. J. Antimicrob. Chemother. 3:175–183.
12. **Wise, R., and D. S. Reeves.** 1974. Clinical and laboratory investigations on ticarcillin an antipseudomonal antibiotic. Chemotherapy (Basle) 20:45–51.