ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1979, p. 540-543
0066-4804/79/04-0540/04$02.00/0$02.00/0

Vol. 15, No. 4

Comparative In Vitro Activity of Azlocillin, Ampicillin, Mezlocillin, Piperacillin, and Ticarcillin, Alone and in Combination with an Aminoglycoside

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Received for publication 25 January 1979

The in vitro activities of the newer semisynthetic penicillins azlocillin, mezlocillin, and piperacillin were compared with those of ampicillin and ticarcillin by using 290 clinical laboratory isolates. Piperacillin and mezlocillin were the most active against *Escherichia coli*, *Proteus mirabilis*, *Klebsiella* spp., and *Enterobacter* spp. When *Pseudomonas aeruginosa* was tested, piperacillin and azlocillin were more active than either mezlocillin or ticarcillin. *Streptococcus pneumoniae* and *Haemophilus influenzae* species were highly susceptible to all of the penicillins tested. Ticarcillin had relatively poor activity against enterococci. The rate of bacterial killing with multiples of the minimal inhibitory concentration of azlocillin, ampicillin, or ticarcillin was tested for *E. coli*, *P. mirabilis*, *P. aeruginosa*, and *Klebsiella* spp. Increasing concentrations increased the bactericidal effect. The effect of combining azlocillin, ampicillin, or ticarcillin with an aminoglycoside was studied by using both killing curves and checkerboards. The isobolograms constructed from the checkerboards showed a synergistic pattern for the organisms tested, which included *E. coli*, *P. aeruginosa*, *Klebsiella* spp., *P. mirabilis*, and enterococci. However, the rate of killing was increased by the combination only for *P. aeruginosa* and enterococci.

The need for safer and more active antibiotics has led to the development of several new semisynthetic penicillins, which in preliminary studies appear to have greater activity against a wider range of organisms than currently available antimicrobial agents (3-6). In this report, a piperazine penicillin (piperacillin) and two acylureidopenicillins (azlocillin and mezlocillin) were tested in vitro and compared with ampicillin and ticarcillin. Studies were included to examine the effect of these penicillins against various strains of *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and enterococci, alone and in combination with aminoglycosides.

(These results were presented in part at the 18th Interscience Conference on Antimicrobial Agents and Chemotherapy, Atlanta, Ga., 1-4 October 1978.)

MATERIALS AND METHODS

Susceptibility tests. A total of 290 clinical isolates were tested for antibiotic susceptibility by using the microdilution technique with Mueller-Hinton broth (*Haemophilus influenzae* was tested in Mueller-Hinton broth containing 2% supplement C and incubated in CO_2). The inoculum used was 10^6 organisms per ml. The lowest antibiotic concentration that showed no visible growth after 18 h of incubation at 37°C was defined as the minimal inhibitory concentration (MIC). The minimal bactericidal concentration was the lowest concentration of antibiotic which yielded 99.9% killing of the initial inoculum.

Killing rates with multiples of the MIC. An overnight inoculum of the bacterial strain to be tested was diluted to 10^6 organisms per ml with Mueller-Hinton broth. Azlocillin, ampicillin, or ticarcillin was added to make a final concentration of 1, 4, or 16 times the MIC. Samples were removed initially and at various intervals up to 24 h, diluted, and plated on appropriate agar containing penicillinase to inactivate the penicillin. Killing rates of the penicillins were determined for *Escherichia coli*, *P. aeruginosa*, *Proteus mirabilis*, and *Klebsiella* spp. from the number of colony-forming units per milliliter.

Combination antibiotic studies. Bacterial killing with the combination of a penicillin and an aminoglycoside was evaluated as described above by using four times the MIC of each antibiotic. Bacterial inhibition with the combination of two antibiotics was studied by the microtiter checkerboard technique. Isobolograms were constructed from the results of the checkerboards to observe whether there was a synergistic

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or an additive effect. The MIC of each antibiotic alone was assigned the value of one. Synergy was defined as a combination in which the sum of the fractional inhibitory concentrations (concentration of each agent in combination divided by the concentration of each agent alone) was less than 1; an additive effect was any sum which was equal to 1 (1).

Antibiotics. Azlocillin and mezlocillin were supplied by Delbay Pharmaceuticals Inc.; piperacillin was supplied by Lederle Laboratories; ticarcillin was supplied by Beecham-Massengill Pharmaceuticals; ampicillin was supplied by Bristol Laboratories; and gentamicin was supplied by Schering Corp.

Beta-lactamase production. The analytical isoelectric focusing technique (5) was used to demonstrate beta-lactamase production by azlocillin- and ampicillin-resistant strains.

RESULTS

Table 1 summarizes the activities of five penicillins against different *Enterobacteriaceae*, *P. aeruginosa*, and enterococci. The 26 strains of enterococcus were highly susceptible to all of the penicillins except ticarcillin, with 90% of the organisms being inhibited by 8.0 μg/ml or less.

The 50 strains of *E. coli* could be divided into two groups. A total of 70% were inhibited by 32 μg or less of all the penicillins per ml, with piperacillin having the greatest activity at concentrations lower than 4.0 μg/ml. The other 30% were resistant to the five penicillins, and two of these strains were shown to be beta-lactamase producers.

Ampicillin had little activity when tested against the *Enterobacter* spp. The other four penicillins inhibited 70% of the organisms at 64 μg/ml or less. Again, piperacillin was the most effective at lower concentrations, followed by mezlocillin, azlocillin, and ticarcillin.

The spectrum of activity of these antibiotics against *Klebsiella* spp. was variable. Most of the organisms were resistant to ticarcillin. Among the other four penicillins, piperacillin and mez-

TABLE 1. *Comparative in vitro activity of five penicillins against enterococci and gram-negative bacilli*

Organism	(No. of isolates)	Antibiotic	MIC (μg/ml) for:		
			50% inhibition	70% inhibition	90% inhibition
Enterococcus	26	Azlocillin	2	2	2
		Mezlocillin	1	2	2
		Ticarcillin	64	64	125
		Piperacillin	2	4	8
		Ampicillin	0.5	1	1
E. coli	50	Azlocillin	8	16	>500
		Mezlocillin	2	8	>500
		Ticarcillin	4	32	>500
		Piperacillin	2	8	>500
		Ampicillin	2	8	>500
Enterobacter spp.	40	Azlocillin	16	64	>500
		Mezlocillin	4	8	125
		Ticarcillin	8	64	500
		Piperacillin	4	8	500
		Ampicillin	125	500	>500
Klebsiella spp.	50	Azlocillin	64	64	125
		Mezlocillin	16	64	125
		Ticarcillin	500	>500	>500
		Piperacillin	16	32	64
		Ampicillin	64	125	250
		Cephalothin	4	8	16
P. mirabilis	37	Azlocillin	4	4	8
		Mezlocillin	1	2	2
		Ticarcillin	1	2	8
		Piperacillin	0.5	1	2
		Ampicillin	1	4	32
P. aeruginosa (gentamicin susceptible)	44	Azlocillin	16	16	64
		Mezlocillin	32	64	125
		Ticarcillin	64	64	125
		Piperacillin	8	8	16
P. aeruginosa (gentamicin resistant)	12	Azlocillin	125	500	>500
		Mezlocillin	125	500	>500
		Ticarcillin	250	500	>500
		Piperacillin	125	250	>500

locillin were more active than azlocillin and ampicillin. Cephalothin, included for comparative purposes, exhibited the greatest inhibitory activity.

When 37 strains of *P. mirabilis* were tested, piperacillin was found to inhibit 70% of the organisms at 1.0 μg/ml, whereas the same concentrations of ampicillin, ticarcillin, and mezlocillin inhibited 50%. Azlocillin had the least activity, with only 15% inhibition.

Susceptibility studies with gentamicin-susceptible and -resistant strains of *P. aeruginosa* are shown in Table 1. Piperacillin and azlocillin were considerably more active than mezlocillin and ticarcillin against 44 gentamicin-susceptible strains. A total of 30% of the 12 gentamicin-susceptible strains of *P. aeruginosa* were inhibited at 64 μg/ml by piperacillin, mezlocillin, and azlocillin, but ticarcillin was not effective against these strains at this concentration. However, the other 70% of the strains were resistant to all of the penicillins tested.

Eight strains of *Haemophilus influenzae* tested were highly susceptible to ampicillin (MIC range, 0.01 to 0.8 μg/ml) and azlocillin (MIC range, <0.025 to 0.4 μg/ml). Two strains had MICs of 6.25 and >50 μg/ml for ampicillin and 12.5 and >50 μg/ml for azlocillin, respectively, presumably due to penicillinase production, although they were not tested for this activity. A total of 20 strains of *Streptococcus pneumoniae* were susceptible to ampicillin and azlocillin (MIC range, 0.0025 to 0.32 μg/ml). The minimal bactericidal concentration for all study strains was two to four times greater than the respective MIC.

The rate of bacterial killing by multiples of the MIC of azlocillin is shown in Fig. 1 for four species of gram-negative bacilli. For all of the organisms there was increased killing with increasing concentration of the antibiotic. However, at 6 h the increased bactericidal activity of 4 versus 16 times the MIC was minimal. The same strains were tested concurrently with ampicillin or ticarcillin, which had similar killing rates.

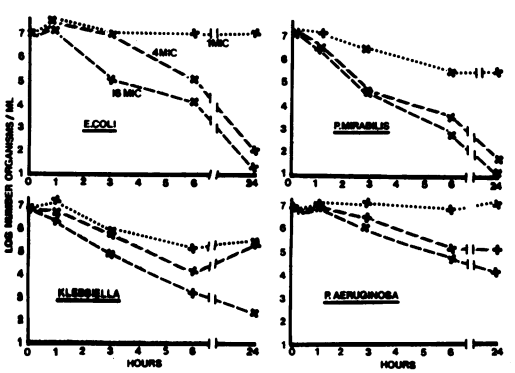

FIG. 1. *Effect of increasing concentrations of azlocillin on bacterial killing of four gram-negative bacilli.*

The increased antibacterial activity of the combination of a penicillin and an aminoglycoside was studied in selected representative strains. Azlocillin plus gentamicin was compared with ampicillin or ticarcillin plus gentamicin. In the presence of azlocillin or ampicillin at a concentration of four times the MIC, the rates of killing of an azlocillin- and ampicillin-susceptible *E. coli* strain (Fig. 2) were similar. Addition of gentamicin to one of these penicillins resulted in more rapid bactericidal action, but this bactericidal rate was the same as when gentamicin was used alone at four times the MIC. The isobologram determined from the inhibitory activity on the azlocillin- and ampicillin-susceptible *E. coli* strain showed an additive effect when gentamicin was combined with azlocillin or ampicillin (Fig. 2). The bactericidal kinetics for *P. mirabilis* and *Klebsiella* spp. were similar to those described for *E. coli*; the isobolograms for these organisms showed a synergistic pattern when either penicillin was combined with gentamicin.

When an azlocillin- and ampicillin-resistant *E. coli* strain was tested, the use of gentamicin alone or in combination with azlocillin or ampicillin caused rapid killing. However, for this beta-lactamase-producing *E. coli* strain synergy between the penicillins and the aminoglycoside was seen on the isobologram.

In contrast to the *Enterobacteriaceae*, there was increased bacterial killing with the combination of gentamicin plus azlocillin or ticarcillin

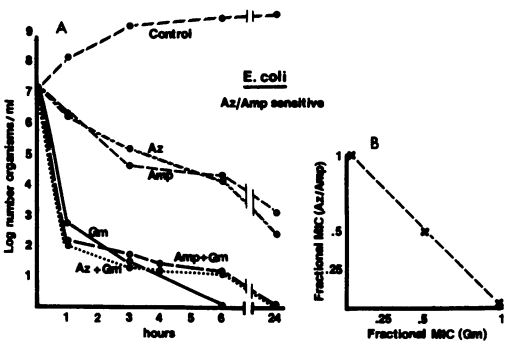

FIG. 2. *(A) Bactericidal effect of ampicillin (Amp), azlocillin (Az), or gentamicin (Gm), alone or in combination on an azlocillin- and ampicillin-susceptible strain of E. coli. (B) Isobologram derived from a checkerboard of the combination of azlocillin or ampicillin plus gentamicin, expressed as fractional inhibitory concentrations.*

for three organisms studied. This effect is seen in Fig. 3 for a gentamicin-susceptible *P. aeruginosa* strain. The bacterial killing rates on a gentamicin-resistant *P. aeruginosa* strain and an enterococcus were markedly increased by the addition of azlocillin or ticarcillin to gentamicin. Isobolograms showed synergy for all of these strains.

DISCUSSION

The limited spectrum of activity and known toxicity of the currently available penicillins has stimulated the development of several new semisynthetic penicillins. Earlier studies have demonstrated an increase in activity of piperacillin, mezlocillin, and azlocillin as compared with that of carbenicillin and ticarcillin against *P. aeruginosa* and the *Enterobacteriaceae* (2, 7). This report confirms previously published studies in that piperacillin was shown to be the most active of the three newer penicillins against all of the gram-negative organisms tested. Mezlocillin was less active than piperacillin, but consistently better than azlocillin, except for strains of *P. aeruginosa*. None of the newer penicillins had an advantage over ampicillin for enterococci. The pattern of resistance for all of the penicillins tested was almost identical, suggesting similar instability to the action of beta-lactamases. A few of the gentamicin-resistant *P. aeruginosa* strains were relatively susceptible to the newer agents.

Studies with multiples of the MIC for various gram-negative bacteria showed that the rate of killing was appreciably increased by increasing the concentration of the antibiotic from one to four times the MIC. However, a further increase to 16 times the MIC did not significantly improve the bactericidal activity.

For the *Enterobacteriaceae* the addition of gentamicin to the penicillin (azlocillin, ticarcillin, or ampicillin) increased the killing rate of the penicillin, but only to the maximum rate of the aminoglycoside. However, the combination (penicillin plus aminoglycoside) accelerated the killing rate for *P. aeruginosa* and enterococci beyond the rate seen with either antibiotic alone.

The isobolograms of all of the organisms tested show that an additive or synergistic effect can be obtained by combining azlocillin, ampicillin, or ticarcillin with gentamicin. Therefore, although the rate of killing was not increased for most of the *Enterobacteriaceae* when combinations were used, the concentrations of both antibiotics could be reduced two- to fourfold and still achieve inhibition of the organisms. Azlocillin, ticarcillin, or ampicillin did not show any significant advantage when compared against each other in these combination studies.

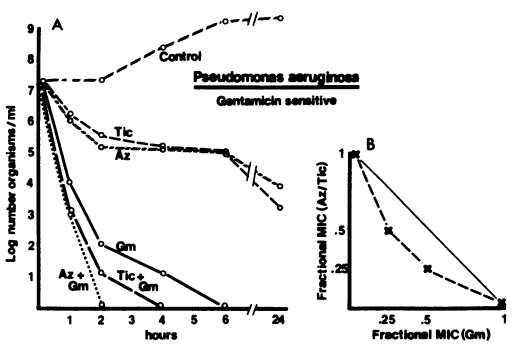

FIG. 3. *(A) Bactericidal effect of ticarcillin (Tic), azlocillin (Az), or gentamicin (Gm), alone or in combination on a gentamicin-susceptible P. aeruginosa strain. (B) Isobologram derived from a checkerboard of the combination of ticarcillin or azlocillin plus gentamicin, expressed as fractional inhibitory concentrations.*

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