In vitro activity of mezlocillin and azlocillin compared with that of four other penicillins and two aminoglycosides

Arthur L. Barry, Ph.D.*
Clyde Thornsberry, Ph.D.†
Ronald N. Jones, M.D.‡
Thomas L. Gavan, M.D.

Department of Microbiology

Mezlocillin and azlocillin are both semisynthetic penicillins with activity against a broad spectrum of bacterial species, including *Pseudomonas aeruginosa*.[1-10] In this report, we compare mezlocillin and azlocillin with ampicillin, carbenicillin, ticarcillin, and piperacillin. In addition, two aminoglycosides (gentamicin and amikacin) were also tested; they represent antimicrobics that are commonly used because of their broad spectrum of activity. The degree of cross-resistance to the six penicillins was also determined.

Materials and methods

Microdilution susceptibility tests were performed as described previously.[11-14] Drug dilutions were prepared in cation-supplemented Mueller-Hinton broth and then dispensed into wells of microdilution trays. The wells were inoculated with 1×10^5 colony-forming units per milliliter and after 16 to 18 hours at 35 C, minimal inhibitory concentrations (MICs) were determined. The minimal lethal concentrations (MLCs) were determined by subculturing to blood agar plates, with the use of a disposable inoculum replicator that transfers approximately 5 μl from each well. The MLC was recorded as the lowest concentration that yielded no growth upon subculture. Bactericidal end points and inoculum

* *Department of Pathology, University of California, Davis Medical Center, Sacramento, California.*
† *Antimicrobics Investigations Section, Center of Disease Control, Atlanta, Georgia.*
‡ *Department of Pathology, Kaiser Foundation Laboratory, Clackamas, Oregon.*

density studies were performed in Kaiser Foundation Hospitals in Clackamas, Oregon. The remaining tests were performed at the Center for Disease Control, Atlanta, Georgia and at the University of California (Davis), Sacramento Medical Center, Sacramento, California. Tests with control strains confirmed that comparable results were obtained in all three institutions as previously documented.[12–14]

A total of 484 bacterial isolates were studied. Most of the strains were clinical isolates collected from six medical centers located in five separate geographic areas within the United States. A few stock cultures were added to provide representatives of the less common species. The species that were included are identified in *Tables 1 and 2.*

Results

The in vitro activity of each antimicrobial agent is expressed in *Table 1* as the minimal concentration required to inhibit growth of 50% and 90% of the strains in each species subgroup. The activity of mezlocillin was similar to that of piperacillin: both were active against all species tested, but piperacillin was much more active against *P. aeruginosa.* Azlocillin was more active than mezlocillin against *P. aeruginosa*, but less active against the Enterobacteriaceae. The aminoglycosides were also active against most isolates, although some strains of Serratia species and Pseudomonas species were resistant. These data are expressed in *Table 3* as the percentage of strains inhibited by concentrations that can be achieved in the blood during therapy. In that context, the aminoglycosides inhibited a larger proportion of strains than did the penicillins. Piperacillin was the most active penicillin against our isolates.

With these drugs, activity against *P. aeruginosa* is of particular interest. *Table 4* summarizes the results of dilution tests with the 81 *P. aeruginosa* isolates. Data with ampicillin are not included because ampicillin had no activity against this species. Because fairly high blood levels are often achieved during therapy with the penicillins,[3, 8, 15, 16] strains with MICs $\leq$64 μg/ml may be considered susceptible. Strains with MICs $\geq$256 mg/ml may be considered resistant and those with MICs of 128 μg/ml are intermediate (moderately susceptible). If the dosage schedules are reduced, lower blood levels will be achieved and thus the MIC breakpoints for defining susceptible strains must be reduced, i.e., to $\leq$32 μg/ml versus $\leq$64 or $\leq$128 μg/ml. For treating most *P. aeruginosa* infections, the dosage of piperacillin and azlocillin could be reduced because the majority of strains are inhibited by $\leq$16 μg/ml. The antipseudomonas activity of mezlocillin was similar to that of carbenicillin, i.e., modal MICs were 32 μg/ml for both drugs. The MIC mode for ticarcillin was only 16 μg/ml. Our collection of *P. aeruginosa* isolates included strains that were resistant to the aminoglycosides. With both amikacin and gentamicin, modal MICs were near the obtainable blood levels and a considerable proportion of strains was only moderately susceptible (intermediate). With the more active penicillins, MIC modes were well below the obtainable blood levels and fewer strains had MICs in the intermediate range.

The results of in vitro tests with 123 gram-positive cocci are summarized in *Table 2.* Although all eight drugs were effective against penicillin-susceptible strains of *Staphylococcus aureus*, the penicillins were all ineffective against beta lactamase-producing strains of *S. aureus.*

Table 1. In vitro activity of six penicillins and two aminoglycosides against 361 gram-negative bacilli

Antimicrobial agent	*Escherichia coli* (25)	*Salmonella* species (10*)	*Citrobacter* species (20†)	*Klebsiella pneumoniae* (25)	*Enterobacter* species (50‡)	*Serratia marcescens* (25)	*Proteus mirabilis* (25)	*Proteus species* (30§)	*Providencia stuartii* (25)	*Acinetobacter calcoaceticus* (15)	*Pseudomonas* species (30‖)	*Pseudomonas aeruginosa* (81)
	Inhibitory-concentration (μg/ml) for 50% or 90% of strains in each species group											
MIC for 50% of strains												
Ampicillin	4.0	1.0	128	64	256	256	1.0	256	32	16	256	>256
Carbenicillin	4.0	4.0	256	256	4.0	16	0.5	4.0	1.0	8.0	256	32
Ticarcillin	4.0	4.0	256	256	4.0	8.0	0.5	4.0	1.0	8.0	128	16
Piperacillin	2.0	4.0	8.0	8.0	4.0	4.0	0.5	2.0	2.0	16	4.0	4.0
Mezlocillin	2.0	4.0	16	16	4.0	8.0	0.5	2.0	4.0	32	16	32
Azlocillin	8.0	16	64	64	16	64	2.0	4.0	16	32	16	8.0
Amikacin	2.0	2.0	2.0	1.0	2.0	2.0	4.0	1.0	2.0	2.0	2.0	8.0
Gentamicin	0.5	0.5	0.5	0.2	0.2	0.5	1.0	0.5	4.0	0.5	0.5	4.0
MIC for 90% of strains												
Ampicillin	>256	4.0	>256	>256	>256	>256	2.0	256	128	64	>256	>256
Carbenicillin	>256	16	>256	>256	>256	>256	2.0	>256	16	16	>256	256
Ticarcillin	>256	8.0	>256	>256	256	>256	2.0	>256	2.0	8.0	>256	128
Piperacillin	256	4.0	>256	128	16	256	1.0	64	8.0	16	16	32
Mezlocillin	256	8.0	>256	128	16	>256	1.0	64	16	32	64	128
Azlocillin	>256	32	>256	>256	256	>256	4.0	>256	256	64	256	64
Amikacin	8.0	2.0	4.0	4.0	2.0	4.0	8.0	2.0	8.0	4.0	128	32
Gentamicin	1.0	0.5	1.0	1.0	0.5	16	2.0	4.0	8.0	2.0	>64	>64

* 5 *Salmonella typhi* and 5 *Salmonella enteritidis*.
† 10 *Citrobacter diversus* and 10 *Citrobacter freundii*.
‡ 20 *Enterobacter cloacae*, 20 *Enterobacter aerogenes*, and 10 *Enterobacter agglomerans*.
§ 10 *Proteus vulgaris*, 10 *Proteus morganii*, and 10 *Proteus rettgeri*.
‖ 9 *Pseudomonas stutzeri*, 6 *Pseudomonas fluorescens*, 5 *Pseudomonas putida*, 4 *Pseudomonas cepacia*, 3 *Pseudomonas maltophilia*, and 3 *Pseudomonas acidovorans*.

Table 2. In vitro activity of 6 penicillins and 2 aminoglycosides against 123 gram-positive cocci

Antimicrobial agent	Inhibitory concentration (μg/ml) for 50% or 90% of strains					
	Staphylococcus aureus			*Streptococcus*		
	Pen S* 25	Pen R* 24	Meth R* 11	*faecalis* 24	*pyogenes* 19	*pneumoniae* 20
MIC for 50% of strains						
Ampicillin	0.5	1.0	64	1.0	≤0.2	≤0.2
Mezlocillin	1.0	2.0	128	2.0	≤0.2	≤0.2
Azlocillin	0.5	2.0	64	2.0	≤0.2	≤0.2
Piperacillin	0.5	2.0	128	2.0	≤0.2	≤0.2
Ticarcillin	0.5	4.0	64	32	≤0.2	≤0.2
Carbenicillin	0.5	4.0	64	32	≤0.2	≤0.2
Amikacin	1.0	1.0	1.0	256	128	32
Gentamicin	≤0.1	≤0.1	≤0.1	16	8.0	8.0
MIC for 90% of strains						
Ampicillin	0.5	32	128	1.0	≤0.2	≤0.2
Mezlocillin	1.0	64	128	4.0	≤0.2	≤0.2
Azlocillin	0.5	64	128	2.0	≤0.2	≤0.2
Piperacillin	1.0	64	256	4.0	≤0.2	≤0.2
Ticarcillin	1.0	8.0	64	64	≤0.2	1.0
Carbenicillin	1.0	8.0	128	64	≤0.2	2.0
Amikacin	1.0	2.0	4.0	256	128	64
Gentamicin	≤0.1	0.2	0.2	16	8.0	8.0

* Sensitive (S) or resistant (R) to penicillin (Pen) or to methicillin (Meth).

Table 3. In vitro susceptibility of 361 gram-negative bacilli percentage of strains inhibited by clinically achievable concentrations of six penicillins and two aminoglycosides

Genus (no. tested)	Percent of strains inhibited by obtainable concentrations*							
	Ampicillin	Carbenicillin	Ticarcillin	Piperacillin	Mezlocillin	Azlocillin	Amikacin	Gentamicin
Escherichia (25)	76	84	88	88	88	88	100	88
Salmonella (10)	90	90	90	90	90	90	100	100
Citrobacter (20)	0	30	35	75	75	65	100	80
Klebsiella (25)	0	8	12	88	80	56	100	100
Enterobacter (50)	10	82	82	98	96	80	100	100
Serratia (25)	48	76	80	84	84	64	92	80
Providencia (25)	4	92	92	92	98	72	96	68
Proteus								
mirabilis (25)	100	100	100	100	100	100	100	100
other species (30)	7	83	80	90	90	70	100	97
Pseudomonas								
aeruginosa (81)	0	79	85	96	81	95	92	57
other species (30)	30	43	43	97	93	87	70	67
Acinetobacter (15)	20	100	100	100	100	93	93	93

* Ampicillin, 8 μg/ml; 64 μg/ml for the other penicillins; gentamicin, 4 μg/ml, and amikacin, 8 μg/ml.

Table 4. In vitro activity of five penicillins and two aminoglycosides against 81 *Pseudomonas aeruginosa*

Antimicrobial agent	Percent of strains inhibited, μg/ml									
	≤1	2	4	8	16	32	64	128	256	>256
Ticarcillin			1	6	41	16	5	¦*6¦	3	3
Carbenicillin				2	5	38	19	¦ 6¦	6	5
Piperacillin		6	43	18	3	5	3	¦ 3¦		
Azlocillin		2	6	44	8	12	5	¦ 4¦		
Mezlocillin				1	9	45	11	¦ 8¦	7	
Amikacin		1	13	36	¦18¦	8	3	1		3
Gentamicin	1	11	34	¦21¦	5	1	12†			

* Dotted lines represent pharmacological breakpoints, bracketing on intermediate (moderately susceptible) category. Strains with lower MICs are considered susceptible and those with higher MIC values are categorized as being resistant to the drug.

† Highest concentration tested = 64 μg/ml.

The methicillin-resistant strains of *S. aureus* were resistant to all six penicillins but were susceptible to the aminoglycosides. *Streptococcus faecalis* strains were relatively resistant to carbenicillin and ticarcillin, but were susceptible to the other penicillins. *Streptococcus pyogenes* and *Streptococcus pneumoniae* strains were susceptible to all six penicillins. Both aminoglycosides displayed little activity against the streptococci.

All eight drugs were found to be bactericidal against most of the 77 strains that were tested (16 *Escherichia coli*, 10 *Klebsiella pneumoniae*, 10 Enterobacter species, 11 Serratia species, 10 Proteus species, 10 *P. aeruginosa*, and 10 *S. aureus*). The effect of varying the inoculum density was also investigated with the same 77 strains. With the gram-negative bacilli, the MICs were not greatly affected when the inoculum was reduced from 10^5 to 10^3 CFU/ml. However, when the inoculum was increased to 10^7 CFU/ml all of the penicillins appeared to be ineffective. MICs with four penicillin-susceptible strains of *S. aureus* were not significantly influenced by changes in the inoculum density. However, beta lactamase-producing strains of *S. aureus* were greatly affected by the inoculum density: most appeared to be fairly susceptible with a light inoculum, but were resistant when the size of the inoculum was increased.

Data with the five penicillins with antipseudomonas activity were further evaluated to determine whether there was significant cross-resistance among our strains of gram-negative bacilli. *Table 5* lists the percentage of strains that were susceptible to one drug but not susceptible to another (MIC >64 μg/ml to one but ≤64 μg/ml to another). At the same time, the analysis was carried out documenting the percentage of strains that were clearly resistant (MIC ≥256 μg/ml) to one drug but susceptible (MIC ≤64 μg/ml) to another. Both types of analyses were performed because of our previous experience in studying cross-resistance to the cephalosporins.[14, 17] Data with *P. aeruginosa* were separated from those obtained with other gram-negative bacilli because azlocillin and mezlocillin differed in their activity against these two types of microorganisms.

As previously noted,[12] carbenicillin and ticarcillin displayed essentially identical spectra of activity, although ticarcillin is somewhat more active

Table 5. Cross-resistance between five penicillins and 361 gram-negative bacilli

Susceptible (MIC ≤64 μg/ml) to	Percent of strains in different interpretive categories: Not susceptible* (MIC ≥128 μg/ml) to					Resistant (MIC ≥256 μg/ml) to				
	Carbenicillin	Ticarcillin	Mezlocillin	Piperacillin	Azlocillin	Carbenicillin	Ticarcillin	Mezlocillin	Piperacillin	Azlocillin
280 gram-neg bacilli[b]										
Carbenicillin	X	0	0	0	7.9	X	0	0	0	3.9
Ticarcillin	1.1	X	0.4	0	8.9	0	X	0	0	3.9
Mezlocillin	16.8	17.1	X	0	12.5	15.0	13.2	X	0	4.6
Piperacillin	19.6	19.6	0.7	X	13.6	16.4	14.3	0	X	7.1
Azlocillin	12.9	17.5	0	0	X	11.4	10.4	0	0	X
81 *P. aeruginosa*										
Carbenicillin	X	0	1.2	0	0	X	0	0	0	0
Ticarcillin	6.2	X	3.7	0	1.2	1.2	X	0	0	0
Mezlocillin	3.7	0	X	0	0	0	0	X	0	0
Piperacillin	18.5	13.6	16.0	X	3.7	11.1	4.9	6.2	X	1.2
Azlocillin	17.3	11.1	14.8	1.2	X	12.3	4.9	4.9	0	X

* Either moderately susceptible (intermediate) or resistant to one drug but susceptible to another.

† Including all strains listed in *Table 1*, except the 81 *P. aeruginosa*.

against *P. aeruginosa*. Against *P. aeruginosa*, mezlocillin was similar to carbenicillin and ticarcillin, i.e., cross-resistance among our isolates was essentially complete. However, mezlocillin more nearly resembled piperacillin in its activity against the other isolates. Against the non-*P. aeruginosa* isolates, azlocillin appeared to represent a third class of penicillins, with little cross-resistance to other penicillins. But against *P. aeruginosa*, azlocillin and piperacillin were similar.

For the purpose of in vitro testing, it would appear that the class concept is applicable. Tests with ticarcillin and piperacillin might be used to predict mezlocillin susceptibility. With a confidence of 95% or better, one could predict that *P. aeruginosa* isolates susceptible to ticarcillin will also be susceptible to mezlocillin and carbenicillin. In contrast, mezlocillin susceptibility of non-*P. aeruginosa* can be predicted from the results of tests with piperacillin. Piperacillin-susceptible strains of *P. aeruginosa* can be assumed to be susceptible to azlocillin. However, with the other microorganisms separate tests with azlocillin would be necessary if the drug was being considered for therapeutic use. The foregoing conclusions were based on the assumption that a 5% minor discrepancy and 1% major discrepancy would be acceptable for routine susceptibility testing. When dealing with serious life-threatening diseases, separate tests with appropriate drugs might be appropriate.

Discussion

The aminoglycosides are commonly used because of their broad spectrum of activity against a variety of gram-negative bacilli. However, because of the potential toxicity of these drugs the dosage schedule and blood levels should be monitored carefully, especially in patients with impaired renal function. Furthermore, the maximal safe blood level is often close to the MIC of the microorganism being treated. This is especially true when treating infections caused by *P. aeruginosa*. Although the aminoglycosides are often thought of as "broad spectrum" antibacterial agents, they have little activity against the streptococci or against anaerobic bacteria. Consequently, they are often used in conjunction with other antimicrobial agents, when the etiologic agent is not known.

The newer penicillins offer certain advantages over the aminoglycosides. First, they are relatively nontoxic and can be administered in fairly large doses. For treating infections due to *P. aeruginosa* with carbenicillin, ticarcillin, or mezlocillin, rather massive doses are required. The modal MIC for *P. aeruginosa* is close to the maximal blood levels normally obtained during therapy with these penicillins, i.e., there is little margin for error in adjusting dosages to exceed the MIC of the patient's isolate. Piperacillin and azlocillin are much more active against *P. aeruginosa* and thus they may be used with a greater degree of confidence that adequate blood levels are being achieved. With these drugs, reduced dosage schedules might prove to be satisfactory for treating *P. aeruginosa* infections. However, if the dosage of azlocillin is reduced, its effectiveness against microorganisms other than *P. aeruginosa* would be seriously compromised. Unlike the aminoglycosides, the penicillins are active against many anaerobes[4, 6, 9] and most streptococci and pencillin-susceptible *S. aureus*. All of the penicillins appear to be susceptible to inactivation by staphylo-

coccal beta lactamase, and thus they are not effective against penicillin-resistant *S. aureus*.

Cross-resistance analysis of our data suggests that carbenicillin and ticarcillin are essentially identical, although ticarcillin is twice as active as carbenicillin against *P. aeruginosa* (*Table 4*). The antipseudomonal activity of mezlocillin resembles that of carbenicillin. Against the other gram-negative bacilli, mezlocillin resembles piperacillin in terms of their comparative activity and cross-resistance. Azlocillin seems to have a unique spectrum of activity, but against *P. aeruginosa*, azlocillin closely resembles piperacillin.

Despite their broad spectra of activity, we have encountered some strains of gram-negative bacilli that are resistant to one or more of the penicillins studied. Consequently, it is necessary to determine in vitro susceptibility before selecting the most appropriate chemotherapeutic agent. While awaiting such laboratory studies, the type of data included in the present report might be useful. The in vitro activity of several related antimicrobial agents is compared. Such information will help to define the relative merits of the drugs being compared. Other factors that must be considered include the pharmacologic properties, potential side effects of the drug, cost, and ease of administration.

Summary

The in vitro activity of mezlocillin and azlocillin was compared with that of carbenicillin, ticarcillin, piperacillin, ampicillin, amikacin, and gentamicin. Microdilution susceptibility tests were performed with 484 bacterial isolates collected from six separate medical centers. Against *P. aeruginosa*, the activity of mezlocillin resembled that of carbenicillin, and azlocillin resembled the more active drug, piperacillin. Against other gram-negative bacilli, the activity of mezlocillin resembled that of piperacillin, and azlocillin had a unique spectrum of activity. All six penicillins were active against streptococci and penicillin-susceptible *S. aureus* but were ineffective against penicillin-resistant *S. aureus*. Amikacin and gentamicin inhibited a slightly larger proportion of strains that did the "broad spectrum" penicillins included in this study.

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