In Vitro Studies of Piperacillin, a New Semisynthetic Penicillin

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Piperacillin, a new semisynthetic penicillin, was compared with other semisynthetic penicillins, cephalosporins, and aminoglycosides by the agar dilution method against 3,600 isolates of facultative gram-negative bacilli, *Bacteroides fragilis*, and enterococci. At 64 µg/ml, piperacillin inhibited 90% of the isolates in each group of organisms tested except for *Escherichia coli* (83% inhibited by 64 µg/ml). Compared with carbenicillin, piperacillin had a 16-fold increase in activity by weight against *Pseudomonas aeruginosa* and the enterococcus, an 8-fold increase against *Serratia marcescens*, and a 4-fold increase against *B. fragilis* and *Enterobacter* species. Piperacillin was highly active against carbenicillin-resistant *Klebsiella pneumoniae* and inhibited many aminoglycoside-resistant organisms. Except for *P. aeruginosa*, the minimum bactericidal concentration of piperacillin was usually within one tube dilution of the minimum inhibitory concentration. Approximately one-third of the gram-negative bacilli were inhibited synergistically by piperacillin plus amikacin, but no synergy could be demonstrated against enterococci. Piperacillin's in vitro activity against gram-negative bacilli was similar to gentamicin's except that it also included *B. fragilis*, and piperacillin was decidedly superior to presently available penicillins against *K. pneumoniae*.

Piperacillin sodium, a new semisynthetic penicillin developed by Ueo et al. (12), has a broad spectrum of in vitro activity against gram-negative bacteria, including *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Its major potential clinical usefulness would appear to be related to its wider spectrum of activity than other beta-lactam antimicrobial agents and its lower toxicity than the aminoglycoside drugs. Thus, in vitro testing was conducted at the UCLA Center for the Health Sciences against 3,600 clinical isolates of gram-negative bacilli and enterococci, and the in vitro activity of piperacillin was compared with that of other semisynthetic penicillins (carbenicillin, ampicillin), cephalosporins (cephalothin, cefoxitin), and aminoglycosides (gentamicin, amikacin).

MATERIALS AND METHODS

Organisms. The in vitro susceptibilities of 3,549 clinical isolates of facultative gram-negative bacilli, 35 isolates of *Bacteroides fragilis*, and 15 strains of gram-positive enterococci were evaluated between April and September 1977. All organisms were cultured from clinical material in the Clinical Microbiology Laboratories of the UCLA Center for the Health Sciences and identified by standard criteria (3, 4, 7, 10, 11). The standard reference strains of *Escherichia coli* ATCC

25922, *Pseudomonas aeruginosa* ATCC 27853 ("Boston" strain), and *Staphylococcus aureus* ATCC 25923 were always tested as internal controls with the facultative organisms. Two control strains of *B. fragilis* (UCLA no. 55073 and 55711) were tested with each group of anaerobic isolates. The susceptibilities of 27 gentamicin-resistant (minimum inhibitory concentration [MIC] ≥ 16 µg/ml) organisms and 13 amikacin-resistant (MIC ≥ 32 µg/ml) organisms to piperacillin and carbenicillin were also evaluated. The gentamicin-resistant organisms were isolated from patients at the UCLA Center for the Health Sciences and the Wadsworth Veterans Administration Hospital, Los Angeles; the amikacin-resistant organisms were all permeability mutants obtained from Kenneth Price of Bristol Laboratories, Syracuse, N.Y.

Antimicrobial agents. Laboratory-standard powders were provided as follows: piperacillin (lot no. 722385; potency, 910 µg/ml) from Martin Forbes of Lederle Laboratories, Pearl River, N.Y.; carbenicillin (lot no. 6J077-36QCS; potency, 869 µg/ml) from Pfizer Laboratories, New York, N.Y.; gentamicin (lot no. GMC-4-M-6044; potency, 571 µg/mg) from the Schering Corp., Bloomfield, N.J.; cephalothin (lot no. P-86614; potency, 946 µg/mg) from Eli Lilly and Co., Indianapolis, Ind.; ampicillin (lot no. 75F1836; potency, 849 µg/mg) and amikacin (lot no. 75F1598; potency, 850 µg/mg) from Bristol Laboratories; and cefoxitin (lot no. C-E 126; potency, 949 µg/mg) from Merck, Sharp, and Dohme, West Point, Pa.

Procedures. Antimicrobial susceptibility testing of facultative organisms by the agar dilution method was performed by methods as previously described (1, 13). Mueller-Hinton medium (control no. 640657, Difco Laboratories, Detroit, Mich.) with 1.7 g of agar per liter (pH 7.4) was prepared to contain twofold increments of each antimicrobial agent. Susceptibility testing of the enterococci also incorporated human blood in agar at a concentration of 3%. The following ranges of drug concentrations were tested: piperacillin, 8.0 to 256 $\mu\text{g/ml}$; carbenicillin, 16 to 256 $\mu\text{g/ml}$; gentamicin, 0.25 to 8.0 $\mu\text{g/ml}$; ampicillin, 2.0 to 16 $\mu\text{g/ml}$; and cephalothin, 0.5 to 16 $\mu\text{g/ml}$. An inoculum of approximately 10^4 organisms diluted from broth cultures in the logarithmic phase of growth was used.

Anaerobic bacteria were tested by the agar dilution method, using Wilkins-Chalgren medium (pH 7.2) (15) and the following ranges of drug concentrations: piperacillin, 0.25 to 128 $\mu\text{g/ml}$; carbenicillin, 8.0 to 128 $\mu\text{g/ml}$; and cefoxitin, 0.5 to 64 $\mu\text{g/ml}$. An inoculum of 10^5 organisms was used after a 72-h incubation on blood agar and an 18- to 24-h incubation in Schaedler broth at 37°C. The agar plates were read after incubation at 37°C in a GasPak jar (BioQuest, Cockeysville, Md.) for 48 h.

Broth dilution susceptibility tests were performed with Mueller-Hinton broth medium (pH 7.4) (control no. 631999, Difco Laboratories) and an inoculum of 5×10^5 organisms (1, 2). The influence of the inoculum size on the MIC of piperacillin and carbenicillin was also determined by the broth dilution method. Twenty isolates were tested at inocula of 1×10^3 , 1×10^5 , and 1×10^7 colony-forming units (CFU)/ml.

The synergistic activity of piperacillin plus amikacin against gram-negative bacilli and enterococci was assessed by the agar dilution method described by Sabbath and co-workers (9). Synergism was defined as a fourfold reduction in the MIC for each drug by the combination of antimicrobial agents (14).

Criteria for susceptibility. Isolates were considered susceptible if they were inhibited at or below the peak serum level usually achieved during administration of each antimicrobial agent to hospitalized patients or normal volunteers. In our laboratories, the following mean peak serum levels are usually obtained after intravenous administration: carbenicillin, 128 $\mu\text{g/ml}$ at a dose of 5.0 g; ampicillin, 16 $\mu\text{g/ml}$ at a dose of 2.0 g; cephalothin, 16 $\mu\text{g/ml}$ at a dose of 2.0 g; gentamicin, 4 $\mu\text{g/ml}$ at a dose of 1.5 mg/kg; and cefoxitin, 32 $\mu\text{g/ml}$ at a dose of 2.0 g. Data made available from Lederle Laboratories (private communication from Vincent A. Joy) indicate that peak serum levels achieved after intravenous administration of 4 g of piperacillin are at least 128 $\mu\text{g/ml}$. To relate results of susceptibility tests to achievable blood levels, the inhibitory indexes were calculated for each antimicrobial agent tested. The inhibitory index is defined as the ratio between the mean peak serum level of each antimicrobial agent and the MIC in agar required to inhibit two-thirds of each group of organisms (16).

RESULTS

Table 1 summarizes the MICs and minimum bactericidal concentrations (MBCs) for the standard reference strains as determined by agar and

TABLE 1. Comparative activity of antimicrobial agents against standard reference organisms^a

Organism	Piperacillin			Carbenicillin			Gentamicin			Cephalothin			Ampicillin, MIC in agar	Chloram- phenicol, MIC in agar	Clinda- mycin, MIC in agar
	Agar, MIC	Broth		Agar, MIC	Broth		Agar, MIC	Broth		Agar, MIC	Broth				
		MIC	MBC		MIC	MBC		MIC	MBC		MIC	MBC			
<i>Escherichia coli</i> ATCC 25922	8	2	4	16	16	32	1	1	2	8	16	32	8		
<i>Pseudomonas aeruginosa</i> ATCC 27853	8	8	512	64	64	512	4	0.25	0.5						
<i>Staphylococcus aureus</i> ATCC 25923	8	1	1	16	4	4	0.25	0.25	1	0.5	0.5	0.5	2		
<i>Bactroides fragilis</i> UCLA 55073	32			128						4 ^b			8	0.5	
<i>B. fragilis</i> UCLA 55711	2			16						8 ^b			4	<0.03	

^a All values are expressed as micrograms per milliliter.

^b Cefoxitin.

broth dilution methods. These values differed by no more than one dilution on repetitive testing. For *E. coli* ATCC 25922, the MBC was usually one dilution higher than the MIC for each antimicrobial agent tested. However, for *P. aeruginosa* ATCC 27853, the MBCs of piperacillin and carbenicillin were several dilutions higher than the MICs. Such a spread between the MIC and MBC was not seen with gentamicin. Except for gentamicin, the MIC and MBC of each antimicrobial agent tested against *S. aureus* 25923 were identical.

The results of the in vitro susceptibility testing of isolates to piperacillin alone are summarized in Table 2. Except for *Acinetobacter calcoaceticus*, 50% of the isolates in each group of organisms tested were inhibited by 8 µg/ml. Similarly, 90% or more of the isolates in each group were inhibited by 64 µg/ml except for *E. coli*.

The comparative activities of piperacillin, carbenicillin, and other antimicrobial agents against specific gram-negative bacilli and the enterococcus are also summarized in Table 2. Generally, piperacillin was more active on a weight basis than carbenicillin against all gram-negative and enterococcal organisms tested except *E. coli*. This increased activity of piperacillin over carbenicillin was most notable for *K. pneumoniae* (32-fold), *P. aeruginosa* (more than 8-fold), enterococcus (16-fold), and *Serratia marcescens*

(more than 4-fold). Compared with gentamicin, piperacillin was more active against *S. marcescens* but less active against *E. coli*. Gentamicin inhibited only 50% of the *P. aeruginosa* isolates at 4 µg/ml, but susceptibility results for *P. aeruginosa* against aminoglycosides are known to be highly medium dependent (5).

Since only piperacillin and gentamicin showed in vitro activity against all the facultative isolates tested (excluding enterococcus), these two antimicrobial agents are compared in Table 3. As shown, piperacillin's in vitro activity was similar to or better than gentamicin's for all organisms except *E. coli* (14% resistant to piperacillin but only 3% resistant to gentamicin).

Susceptibility results using Mueller-Hinton broth correlated well with the agar dilution method for piperacillin against all organisms except *Proteus* (Table 4).

The MBC was within one dilution of the MIC for approximately 80% of the isolates tested against piperacillin, carbenicillin, and gentamicin (Table 5). Ninety-two percent of the organisms tested against cephalothin had a MBC within one dilution of the MIC. For *P. aeruginosa*, only 32 µg of piperacillin per ml was needed to inhibit all 16 isolates tested compared with 512 µg/ml for carbenicillin. On the other hand, both piperacillin and carbenicillin required 512 µg/ml to kill all 16 isolates of *P.*

TABLE 2. Comparison of piperacillin and other antimicrobial agents against clinical isolates

Organism	% strains inhibited	Concn (µg/ml) required to inhibit given % of strains					
		Piperacillin	Carbenicillin	Gentamicin	Ampicillin	Cephalothin	Cefoxitin
<i>Klebsiella pneumoniae</i> (549) ^a	50	8	256	1		8	
	90	64	>256	1		16	
<i>Pseudomonas aeruginosa</i> (656)	50	8	64	4			
	90	32	>256	>8			
Enterococcus (15)	50	8	64				
	90	8	128				
<i>Escherichia coli</i> (1,496)	50	8	16	1	8	8	
	90	256	>256	4	>16	16	
<i>Serratia marcescens</i> (73)	50	8	32	1			
	90	64	>256	8			
<i>Bacteroides fragilis</i> (35)	50	4	16				8
	90	32	128				16
<i>Enterobacter cloacae</i> (202)	50	8	16	1			
	90	16	128	1			
<i>Enterobacter aerogenes</i> (62)	50	8	16	1			
	90	32	128	1			
<i>Proteus mirabilis</i> (338)	50	8	16	1	2	8	
	90	8	64	4	16	16	
Indole-positive <i>Proteus</i> (84)	50	8	16	1			
	90	8	32	1			
<i>Citrobacter freundii</i> (51)	50	8	16	1			
	90	8	256	1			
<i>Acinetobacter calcoaceticus</i> (38)	50	16	64	4			
	90	32	256	>8			

^a Number of clinical isolates tested.

TABLE 3. Comparative activity of piperacillin and gentamicin as determined by agar dilution

Organism	No.	% inhibited by:	
		Piperacillin (128 µg/ml) ^a	Gentamicin (4 µg/ml) ^a
<i>Escherichia coli</i>	1,496	86	97
<i>Pseudomonas aeruginosa</i>	656	97	44
<i>Klebsiella pneumoniae</i>	549	94	98
<i>Enterobacter cloacae</i>	202	96	99
<i>Enterobacter aerogenes</i>	62	100	95
<i>Serratia marcescens</i>	73	95	81
<i>Proteus mirabilis</i>	338	99	97
Indole-positive <i>Proteus</i>	84	100	100
<i>Citrobacter freundii</i>	51	100	100
<i>Acinetobacter calcoaceticus</i>	38	100	61

^a Mean peak serum level.

TABLE 4. Comparison of piperacillin agar and broth MICs

Organism	% of strains inhibited	Concn (µg/ml) required to inhibit given % of strains	
		Agar	Broth
<i>Klebsiella pneumoniae</i> (31) ^a	50	8	8
	90	16	16
<i>Pseudomonas aeruginosa</i> (36)	50	8	8
	90	32	32
<i>Enterococcus</i> (6)	50	8	8
	90	8	8
<i>Escherichia coli</i> (29)	50	8	8
	90	32	64
<i>Serratia marcescens</i> (23)	50	8	8
	90	8	8
<i>Enterobacter cloacae</i> (11)	50	8	16
	90	16	32
<i>Enterobacter aerogenes</i> (6)	50	32	16
	90	64	32
<i>Proteus mirabilis</i> (14)	50	8	16
	90	32	256
Indole-positive <i>Proteus</i> (17)	50	8	8
	90	8	256

^a Number of isolates tested.

aeruginosa. Thus, a 4- to 32-fold difference between the MIC and MBC was observed for piperacillin against 56% of the *P. aeruginosa* isolates tested, whereas for carbenicillin, because of the higher MICs, a 4- to 8-fold difference between MIC and MBC was seen for only 12% of the same isolates. Such a large spread between the MIC and MBC for piperacillin was seen infrequently with other organisms. Sixty percent of the *E. coli* tested against carbenicillin had an MBC-to-MIC ratio of 1 or 2, whereas only 40% of the indole-positive *Proteus* species had an MBC-to-MIC ratio of 1 or 2 for gentamicin.

The effect of inoculum size on the broth MICs of piperacillin and carbenicillin is summarized in Table 6. For piperacillin, the inoculum effect was relatively small at low inocula (100% of the isolates inhibited by 128 µg/ml at both 10⁵ and 10⁶ CFU/ml) but much greater at higher inocula (only 25% of the isolates inhibited by 128 µg/ml at 10⁷ CFU/ml). For carbenicillin, a similar inoculum effect was observed (25, 20, and 10% of the isolates inhibited by 128 µg/ml at inocula of 10³, 10⁵, and 10⁷ CFU/ml, respectively).

Since the activity by weight of an antimicrobial agent may be less meaningful than the relationship between potency by weight and the achievable peak serum levels, the inhibitory index as previously defined was calculated for each group of organisms and each antimicrobial agent. Piperacillin had a significantly higher inhibitory index than carbenicillin for *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *S. marcescens*, *B. fragilis*, and the enterococcus (Table 7). For *P. mirabilis*, indole-positive *Proteus*, and the *Enterobacter* species, the indexes for piperacillin and carbenicillin were similar. Piperacillin also had a significantly higher index than cephalothin against *K. pneumoniae*. For *B. fragilis*, the piperacillin index was markedly higher than the cefoxitin index.

Only 5 of 27 gentamicin-resistant organisms were inhibited by both carbenicillin and piperacillin.

TABLE 5. Comparison of MIC and MBC

Organism	No. tested	No. of isolates having MBC/MIC ratio of 1 or 2			
		Piperacillin	Carbenicillin	Gentamicin	Cephalothin
<i>Pseudomonas aeruginosa</i>	16	7 (44) ^a	14 (88)	14 (88)	
<i>Klebsiella pneumoniae</i>	7	7 (100)	6 (86)	7 (100)	5 (71)
<i>Escherichia coli</i>	15	14 (93)	9 (60)	14 (93)	12 (80)
<i>Serratia marcescens</i>	17	15 (88)	17 (100)	12 (71)	
<i>Enterococcus</i>	6	6 (100)	6 (100)		
<i>Enterobacter cloacae</i>	9	8 (89)	6 (67)	7 (78)	
<i>Enterobacter aerogenes</i>	5	5 (100)	5 (100)	3 (60)	
<i>Proteus mirabilis</i>	7	5 (71)	5 (71)	5 (71)	7 (100)
Indole-positive <i>Proteus</i>	13	11 (85)	11 (85)	6 (46)	
Total	95	79/95 (83)	78/95 (82)	68/89 (76)	24/26 (92)

^a Numbers in parentheses are percentages.

TABLE 6. Effect of inoculum size on MIC of piperacillin and carbenicillin

Organism	MIC ($\mu\text{g/ml}$)					
	Piperacillin			Carbenicillin		
	10^7 ^a	10^5	10^3	10^7	10^5	10^3
<i>Klebsiella pneumoniae</i>						
6064	>512	16	4	>512	>512	512
9296	>512	16	4	>512	>512	512
7111	>512	4	2	>512	>512	>512
7942	>512	16	8	>512	>512	>512
<i>Pseudomonas aeruginosa</i>						
7005	>512	8	4	>512	>512	512
6691	>512	8	4	>512	512	128
6506	16	4	4	>512	512	256
5460	>512	32	4	>512	32	16
<i>Escherichia coli</i>						
9603	256	8	8	>512	>512	>512
7700	>512	128	32	>512	>512	>512
7487	>512	64	32	>512	>512	>512
6454	>512	32	4	>512	>512	512
<i>Serratia marcescens</i>						
4798	256	4	2	128	32	16
5096	>512	4	1	>512	32	16
0549	512	4	4	>512	>512	>512
5904	>512	2	1	64	32	4
<i>Enterococcus</i>						
1711	4	4	2	512	512	512
1630	8	4	2	>512	512	512
2433	8	4	4	>512	512	512
2411	8	4	4	>512	512	256

^a Inoculum size of organisms (CFU/milliter) prepared from 18-h culture in Mueller-Hinton broth.

TABLE 7. Inhibitory index^a

Agent	Mean peak level ($\mu\text{g/ml}$)	Inhibitory index								
		<i>Pseudo-</i> <i>monas</i> <i>aeruginosa</i>	<i>Escher-</i> <i>ichia</i> <i>coli</i>	<i>Klebsiella</i> <i>pneumoniae</i>	<i>Serratia</i> <i>mar-</i> <i>cescens</i>	<i>Proteus</i> <i>mira-</i> <i>bilis</i>	Indole- positive <i>Proteus</i>	<i>Enter-</i> <i>obacter</i> sp.	<i>Bacter-</i> <i>oides</i> <i>fragilis</i>	<i>Enter-</i> <i>ococcus</i>
Piperacillin	128	16	16	16	16	16	16	16	16	16
Carbenicillin	128	1	4	<0.5	4	16	16	8	4	2
Gentamicin	4	0.5	4	4	4	4	4	4		
Cephalothin	16		2	2		2			2 ^b	
Ampicillin	16		2			8				

^a Ratio between the mean peak serum level of each antimicrobial agent and the MIC required to inhibit two-thirds of each group of organisms.

^b Cefoxitin (mean peak level = 32 $\mu\text{g/ml}$).

cillin (Table 8). All 5 were *P. aeruginosa* isolates. Of the 13 amikacin-resistant organisms, 11 were inhibited by piperacillin and 8 were inhibited by carbenicillin. However, the MICs of aminoglycoside-resistant organisms susceptible to both piperacillin and carbenicillin were usually 2- to 16-fold lower for piperacillin than for carbenicillin.

Approximately one-third of the gram-negative bacilli tested were inhibited synergistically by the combination of piperacillin plus amikacin (Table 9). No synergy could be demonstrated against the six isolates of enterococci.

DISCUSSION

The results of these studies document piperacillin's broad spectrum of activity against gram-negative bacilli and the enterococcus. Except for *E. coli*, piperacillin inhibited 90% or more of the isolates in each group of organisms tested at 64 $\mu\text{g/ml}$ in agar, a clinically achievable blood level (private communication from Vincent A. Joy, Lederle Laboratories). Ueo et al. (12) also have reported recently on the in vitro effectiveness of piperacillin, but observed lesser activity against *Escherichia coli*, *K. pneumoniae*, *S. marces-*

TABLE 8. Comparative activity of carbenicillin and piperacillin against aminoglycoside-resistant organisms

Organism	No.	No. of strains growing with:			
		Gentamicin (≥ 16) ^a	Amikacin (≥ 32)	Carbenicillin (≥ 256)	Piperacillin (≥ 256)
<i>Pseudomonas aeruginosa</i>	9	9		4	4
	6		6	1	0
<i>Serratia marcescens</i>	9	9		9	9
	1		1	1	1
<i>Providencia stuartii</i>	9	9		9	9
<i>Escherichia coli</i>	3		3	1	1
<i>Enterobacter</i> sp.	3		3	1	1

^a MIC.

TABLE 9. Synergistic activity of amikacin and piperacillin

Organism	No.	Synergy (%)
<i>Pseudomonas aeruginosa</i>	14	5 (36)
<i>Klebsiella pneumoniae</i>	9	3 (33)
<i>Serratia marcescens</i>	8	3 (38)
<i>Escherichia coli</i>	5	2 (40)
<i>Enterobacter</i> sp.	6	2 (33)
Indole-positive <i>Proteus</i>	4	1 (25)
<i>Proteus mirabilis</i>	3	0 (0)
<i>Enterococcus</i>	6	0 (0)
Total	55	16 (29)

cens, and *Enterobacter cloacae*. The use of a different medium (heart infusion agar) and a slightly heavier inoculum (5×10^5 CFU) by the latter investigators may account for these differences. Except for the enterococcus, gram-positive organisms were not evaluated in our studies, but other investigators have noted piperacillin's in vitro activity against *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes* (12; K. P. Fu and H. C. Neu, 10th Int. Cong. Chemother., Abstr. 71, 1977; T. C. Eickhoff and J. M. Ehret, 10th Int. Cong. Chemother., Abstr. 72, 1977; R. Jones, P. Fuchs, and A. Barry, Program Abstr. 17th Intersci. Conf. Antimicrob. Agents Chemother., Abstr. 318, 1977). Like penicillin G, piperacillin is subject to hydrolysis in the presence of penicillinase and thus is less active than the cephalosporins against *Staphylococcus aureus* (12).

Piperacillin's in vitro activity compared favorably with that of other antimicrobial agents commonly used to treat infections caused by gram-negative bacillary organisms. Either on a weight basis or in terms of the inhibitory index, piperacillin was more active than carbenicillin against all organisms tested, especially *K. pneumoniae*, *P. aeruginosa*, the enterococcus, *Serratia marcescens*, and *B. fragilis* (Table 2). For these five organisms, piperacillin had a 4- (*B. fragilis*) to 32-fold (*K. pneumoniae*) increase in

activity by weight over carbenicillin. Piperacillin's inhibitory activity was also similar to gentamicin's for most of the organisms tested (Table 3). Piperacillin had a higher inhibitory index than cephalothin against *K. pneumoniae*, *E. coli*, and *Proteus mirabilis* and than cefoxitin against *B. fragilis* (Table 7). Finally, relative to ampicillin, piperacillin had greater inhibitory activity against *E. coli* and *P. mirabilis* (Table 2). Ueo et al. also observed greater activity for piperacillin compared with carbenicillin and ampicillin against gram-negative organisms susceptible to all three antimicrobial agents (12).

The specific mechanisms responsible for piperacillin's wide spectrum of antimicrobial activity have not been completely elucidated. As observed in this study and by other investigators (Fu and Neu, 10th Int. Congr. Chemother., Abstr. 71, 1977; N. A. Kuck and M. Forbes, 10th Int. Congr. Chemother., Abstr. 73, 1977), piperacillin's in vitro activity is influenced by the inoculum size, especially at higher inocula. Inactivation of piperacillin by large amounts of beta-lactamase is a likely explanation for this phenomenon (S. Mitsuhashi, Y. Fukuoka, T. Hayashi, and N. Matsubara, 10th Int. Congr. Chemother., Abstr. 75, 1977). At the same time, piperacillin is stable to the beta-lactamases produced by certain organisms resistant to carbenicillin, ampicillin, and cephalosporins (12). Piperacillin's increased accessibility to the target sites of beta-lactamase antibiotics (Mitsuhashi et al., 10th Int. Congr. Chemother., Abstr. 75, 1977) and inhibition of the induction of autolysins (K. Iida, S. Hirata, S. Nakamuta, and M. Koike, 10th Int. Congr. Chemother., Abstr. 70, 1977) may be additional factors contributing to its antibacterial activity. Further studies defining the mechanism of action of piperacillin are needed.

A number of potential clinical uses for piperacillin seem possible in view of the findings reported here. In terms of its antibacterial spectrum, piperacillin is similar to gentamicin. How-

ever, gentamicin's usage has been limited by its potential nephrotoxicity and ototoxicity. Piperacillin, on the other hand, has been used in over 1,200 patients in Japan without significant renal or ototoxicity (private communication from Vincent A. Joy, Lederle Laboratories). Most side effects observed with piperacillin were skin rashes or gastrointestinal symptoms such as nausea, vomiting, and diarrhea. Thus, if further clinical trials can confirm its therapeutic efficacy with lessened toxicity, piperacillin could become an acceptable single-agent alternative to the aminoglycosides in the treatment of gram-negative infections. Relative to carbenicillin, piperacillin's greater activity on a weight basis may make it possible to use lower doses and thus avoid the untoward effects of sodium overload and hypokalemia observed with carbenicillin (6, 8). At the same time, piperacillin has the added advantage of significant activity against carbenicillin-resistant *K. pneumoniae* and amikacin-resistant permeability mutants. It may also be possible to take advantage of the potential synergistic activity of the piperacillin-aminoglycoside combination against *K. pneumoniae* as well as *P. aeruginosa* in the treatment of severe infections caused by these organisms. Thus, further clinical trials and comparative studies in humans to establish piperacillin's role in the treatment of bacterial infections are warranted.

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LITERATURE CITED

1. Dhawan, V., E. Marso, W. J. Martin, and L. S. Young. 1977. In vitro studies with netilmicin compared with amikacin, gentamicin, and tobramycin. *Antimicrob. Agents Chemother.* 11:64-73.
2. Ericsson, H. M., and J. C. Sherris. 1971. Antibiotic sensitivity testing. Report of an international collaborative study. *Acta Pathol. Microbiol. Scand. Sect. B, Suppl.* 217, p. 90.
3. Ewing, W. H., and W. J. Martin. 1974. *Enterobacteriaceae*, p. 189-221. In E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), *Manual of clinical microbiology*, 2nd ed. American Society for Microbiology, Washington, D.C.
4. Facklam, R. R. 1974. Streptococci, p. 96-108. In E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), *Manual of clinical microbiology*, 2nd ed. American Society for Microbiology, Washington, D.C.
5. Gilbert, D. N., E. Kutscher, R. Ireland, J. A. Barnett, and J. P. Sanford. 1971. Effect of the concentrations of magnesium and calcium on the in vitro susceptibility of *Pseudomonas aeruginosa* to gentamicin. *J. Infect. Dis.* 124(Suppl.):S37-S45.
6. Hoffman, T. A., and W. E. Bullock. 1970. Carbenicillin therapy of *Pseudomonas* and other gram-negative bacillary infections. *Ann. Intern. Med.* 73:178-187.
7. Hugh, R., and G. L. Gilardi. 1974. *Pseudomonas*, p. 250-269. In E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), *Manual of clinical microbiology*, 2nd ed. American Society for Microbiology, Washington, D.C.
8. Klustersky, J., B. Vanderkelen, D. Danean, and M. Mathieu. 1973. Carbenicillin and hypokalemia. *Ann. Intern. Med.* 78:774-775.
9. Sabath, L. D., C. E. McCall, N. H. Steigbigel, and M. Finland. 1967. Synergistic penicillin combinations for treatment of human urinary tract infections, p. 149-155. *Antimicrob. Agents Chemother.* 1966.
10. Sutter, V. L., H. R. Attebery, and S. M. Finegold. 1974. Gram-negative nonsporeforming anaerobic bacilli, p. 388-395. In E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), *Manual of clinical microbiology*, 2nd ed. American Society for Microbiology, Washington, D.C.
11. Tatum, H. W., W. H. Ewing, and R. E. Weaver. 1974. Miscellaneous gram-negative bacteria, p. 270-294. In E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), *Manual of clinical microbiology*, 2nd ed. American Society for Microbiology, Washington, D.C.
12. Ueo, K., Y. Fukuoka, T. Hayashi, T. Yasuda, H. Taki, H. Tai, M. Tai, Y. Watanabe, I. Saikawa, and S. Mitsuhashi. 1977. In vitro and in vivo antibacterial activity of T-1220, a new semisynthetic penicillin. *Antimicrob. Agents Chemother.* 12:455-460.
13. Washington, J. A., and A. L. Barry. 1974. Dilution test procedures, p. 410-417. In E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), *Manual of clinical microbiology*, 2nd ed. American Society for Microbiology, Washington, D.C.
14. Weinstein, R. J., L. S. Young, and W. L. Hewitt. 1975. Comparison of methods for assessing in vitro antibiotic synergism against *Pseudomonas* and *Serratia*. *J. Lab. Clin. Med.* 86:853-862.
15. Wilkins, T. D., and S. Chalgren. 1976. Medium for use in antibiotic susceptibility testing of anaerobic bacteria. *Antimicrob. Agents Chemother.* 10:926-928.
16. Young, L. S., and W. L. Hewitt. 1973. Activity of five aminoglycoside antibiotics in vitro against gram-negative bacilli and *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 7:172-178.