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Laboratory Studies with a New Broad-Spectrum Penicillin, Pirbenicillin

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Pirbenicillin {6-[D-2-phenyl-2(*N*-4-pyridylformimidoylaminoacetamido)-acetamido]-penicillanic acid} showed broad-spectrum antibacterial activity in vitro and also in the treatment of experimental infections after parenteral administration to mice. Against *Pseudomonas aeruginosa*, a three- to fourfold potency advantage over carbenicillin was seen both in vitro and in vivo. The in vitro antibacterial spectrum of pirbenicillin includes *Escherichia coli*, *Serratia*, *Citrobacter*, and *Enterobacter* isolates, against which it exhibited minimal inhibitory concentration values comparable to those of carbenicillin. However, mice infected with *E. coli* and *Serratia* were protected at doses of pirbenicillin that were two to four times lower than those required of carbenicillin. Pirbenicillin was more active than carbenicillin against gram-positive bacteria, especially *Streptococcus faecalis*. It was less active than carbenicillin against *Proteus* spp. and was inactive against ampicillin-resistant *E. coli* strains. Pirbenicillin was bactericidal at concentrations generally equal to or only twofold higher than the minimal inhibitory concentration. With appropriately buffered media, pirbenicillin demonstrated eight- and fourfold better minimal bactericidal concentration values towards *Pseudomonas* isolates than those of carbenicillin and ticarcillin, respectively.

The discovery of carbenicillin, with its extended spectrum of clinical activity, has encouraged the search for more potent members in the penicillin series. Laboratory studies presented in this communication demonstrate that pirbenicillin (Fig. 1) has certain potency advantages over carbenicillin in both in vitro and in vivo studies. Pirbenicillin is currently undergoing clinical evaluation as a broad-spectrum chemotherapeutic agent.

MATERIALS AND METHODS

The cultures used were recent clinical isolates obtained from several regions in the eastern United States. Media were products of BBL, Cockeysville, Md. With the exception of ticarcillin, which was a gift of Beecham Pharmaceuticals, all antibiotics other than pirbenicillin were commercial products. β-Lactam antibiotics were stored in a desiccator or a freezer; solutions were discarded after a single use. The synthetic M9 medium was that of Witkin (17). Phosphate-buffered (0.1 M) Mueller-Hinton (MH) medium was prepared by adding equal amounts of sterile 1 M KH_2PO_4 and 1 M K_2HPO_4 to the medium, with the final pH being 6.9.

In vitro studies. Minimal inhibitory concentration (MIC) values were determined according to the method of Ericsson and Sherris (6) using approximately 20,000 cells/strain as inoculum. Brain heart infusion (BHI) agar was employed (20 ml per petri dish) unless indicated otherwise. Single colonies were disregarded in determining MIC values after an 18-h incubation. The procedure for broth MIC determinations, utilizing an inoculum of $\approx 1 \times 10^6$ cells/ml, has been described previously (5). Synergy tests were performed in BHI broth using a seven-by-seven checkerboard dilution arrangement as described by Sabath et al. (13). All incubations were at 37 C.

Killing-curve experiments were initiated by diluting an overnight culture, grown on a rotary shaker, 100-fold in BHI broth containing an appropriate concentration of antibiotic. During incubation on a shaker, aliquots were removed and diluted at 2-log intervals, and 0.1 ml was plated out in triplicate on BHI agar plates. After overnight incubation, colonies were counted and recorded as viable colony-forming units.

Minimum bactericidal concentrations (MBCs) were measured by first determining MICs in broth medium. Clear broth cups were subcultured by streaking a loop calibrated to deliver 0.01 ml over the surface of agar medium. Since the time required for subculturing was substantial, the MIC trays not in actual use were kept refrigerated. The agar plates were incubated overnight. The MBC was defined as the lowest concentration of antibiotic permitting growth on subculture of ≤ 5 colonies. Thus, the MBC indicates ≤ 500 colony-forming units per ml, or as is recommended (3), $\geq 99.9\%$ lysis. Colonies observed at the MBC, or if necessary at the next lower antibiotic concentration, were sub-

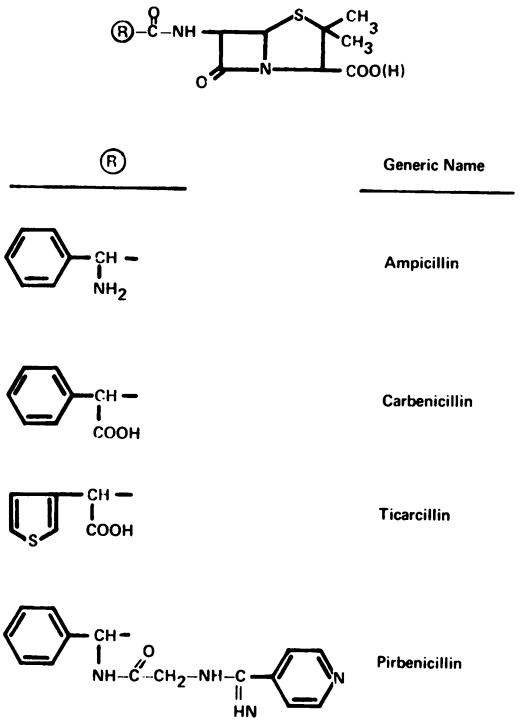

FIG. 1. *Chemical structures of ampicillin, carbenicillin, ticarcillin, and pirbenicillin.*

cultured in broth and incubated overnight. These cultures were then assayed against their respective antibiotics in a broth or agar MIC determination. The observed values were recorded as the resistance determination MIC (R-MIC). MBCs for pseudomonal species must be determined in media buffered to ≤pH 7.5 (see Results and Discussion). The pH of broth media was read directly on a pH meter. The pH of agar medium was determined by placing the agar (20 ml) in a test tube, adding 10 ml of water, mixing, and reading the pH.

Systemic protection tests. Acute systemic infections in mice were produced by intraperitoneal inoculation of standardized cultures suspended in 5% hog gastric mucin. The severity of infection was generally 1 to 10 LD_{100} (1 to 10 times the number of organisms needed to kill 100% of the mice within a 4-day period). In the instance of experimental *Pseudomonas* infections, treatment was administered at 1, 3, 5, 7, and 24 h postchallenge. The dosage regimen for infections other than *Pseudomonas* was initiated 0.5 h postchallenge; a second dose was administered at 4 h, and a third dose was given at 24 h, postchallenge. After a holding period of 4 days, PD_{50} values in milligrams per kilogram were calculated by means of a probit method (4) (PD_{50} is defined as the dose of antibiotic in milligrams per kilogram required to protect 50% of the treated mice against the otherwise lethal infection).

RESULTS AND DISCUSSION

As indicated by MIC values presented in Table 1, pirbenicillin was four to eight times more active than carbenicillin and two times more active than ticarcillin against *Pseudomonas aeruginosa* isolates, when determined in either BHI or in MH agar, or in BHI broth (Table 1). A 10-fold increase in inoculum was without significant effect on the MIC values obtained on agar for pirbenicillin and the other two antibiotics. Based on a comparison of the 50 and 90% cumulative MIC values for pirbenicillin and carbenicillin with respect to carbenicillin-susceptible and resistant *Pseudomonas* isolates, it is evident that there is only partial cross-resistance between these antibiotics. Initial experience with *P. putida* (Table 1), shows

TABLE 1. *Comparative in vitro activity of pirbenicillin and other semisynthetic β-lactam antibiotics against members of the family Pseudomonadaceae*

Species, growth medium, and penicillin	No. of strains	Cumulative % inhibited by indicated MIC (μg/ml)[a]	
		50%	90%
Pseudomonas aeruginosa			
Carbenicillin susceptible			
BHI agar	32		
Pirbenicillin		12.5	25
Ticarcillin		25	50
Carbenicillin		100	100
Ampicillin		>200	
MH agar	32		
Pirbenicillin		12.5	25
Ticarcillin		25	50
Carbenicillin		50	100
BHI broth			
Pirbenicillin	26	7.8	15.6
Ticarcillin	16	15.6	62.5
Carbenicillin	26	62.5	125
Ampicillin	26	>200	
Carbenicillin resistant			
BHI agar[b]	32		
Pirbenicillin		50	200
Ticarcillin		100	>200
Carbenicillin		>200	>200
P. putida	5		
Pirbenicillin			50
Carbenicillin			>200

[a] Mean value of three determinations.

[b] When testing was performed on MH agar, the same values for the 50% and 90% levels were obtained.

that pirbenicillin has a fourfold in vitro advantage over carbenicillin.

In general, the spectrum and degree of activity of pirbenicillin were most similar to those of carbenicillin against the *Enterobacteriaceae* (Table 2). Only two exceptions were noted: (i) pirbenicillin was less active than carbenicillin against all species of *Proteus*, and (ii) pirbenicillin was slightly less active than ampicillin against *Proteus mirabilis*. Compared with ampicillin, the broader spectrum of pirbenicillin was particularly noticeable with *Enterobacter, Citrobacter, Serratia,* and indole-positive *Proteus* species. Pirbenicillin was markedly more active than representative cephalosporins against all genera and species tested except *Escherichia coli* and *Klebsiella pneumoniae*.

MIC values for pirbenicillin against other representative gram-negative bacteria are pre-

TABLE 2. *Comparative in vitro activity of pirbenicillin and other β-lactam antibiotics against members of the family Enterobacteriaceae*

Genera, species, and antibiotic	No. of strains	Cumulative % inhibited by indicated MIC (μg/ml)[a]			
		50%	75%	90%	100%
Escherichia coli					
Ampicillin sensitive	22				
Pirbenicillin		3.12	6.25	6.25	12.5
Carbenicillin		6.25	6.25	12.5	12.5
Ampicillin		6.25	6.25	6.25	12.5
Cephaloridine		3.12	3.12	3.12	3.12
Ampicillin resistant	10				
Pirbenicillin		>200			
Carbenicillin		>200			
Ampicillin		>200			
Cephaloridine		6.25	100	100	100
Enterobacter aerogenes	21				
Pirbenicillin		6.25	6.25	50	50
Carbenicillin		6.25	6.25	25	>200
Ampicillin		6.25	12.5	50	>200
Cefazolin		3.12	100	>200	
E. cloacae	34				
Pirbenicillin		6.25	12.5	>200	
Carbenicillin		12.5	12.5	>200	
Ampicillin		25	50	>200	
Cefazolin		100	200	>200	
E. hafnia	4				
Pirbenicillin					6.25
Carbenicillin					50
E. agglomerans	3				
Pirbenicillin					3.12
Carbenicillin					50
Citrobacter freundii	28				
Pirbenicillin		3.12	6.25	100	>200
Carbenicillin		6.25	12.5	50	>200
Ampicillin		25	25	>200	
Cefazolin		6.25	50	100	>200
Serratia marcescens	29				
Pirbenicillin		12.5	25	>200	
Carbenicillin		3.12	>200		
Ampicillin		25	200	>200	
Cefazolin		>200			
Salmonella typhimurium	14				
Pirbenicillin		3.12	6.25	200	<200
Carbenicillin		6.25	12.5	>200	
S. arizona	2				
Pirbenicillin					3.12
Carbenicillin					6.25
Shigella flexneri	3				
Pirbenicillin					0.39
Carbenicillin					3.12
Edwardsiella tarda	2				
Pirbenicillin					0.39
Carbenicillin					0.78
Proteus mirabilis[b]	20				
Pirbenicillin		3.12	6.25	12.5	12.5
Carbenicillin		0.78	0.78	0.78	0.78
Ampicillin		1.56	1.56	3.12	3.12
Cefazolin		6.25	6.25	12.5	12.5
P. rettgeri	11				
Pirbenicillin		6.25	6.25	200	200
Carbenicillin		0.78	0.78	200	>200
Ampicillin		1.56	50	>200	
Cefazolin		25	100	>200	
P. morganii	16				
Pirbenicillin		3.12	6.25	50	>200
Carbenicillin		0.39	0.78	0.78	>200
Ampicillin		50	200	200	>200
Cefazolin		>200			
P. vulgaris[b]	20				
Pirbenicillin		3.12	12.5	25	50
Carbenicillin		0.78	1.56	25	100
Ampicillin		100	200	>200	
P. inconstants (*Providencia* sp.)	18				
Pirbenicillin		25	>200		
Carbenicillin		3.12	>200		
Ampicillin		50	>200		
Klebsiella pneumoniae	30				
Pirbenicillin		50	>200		
Carbenicillin		>200			
Ampicillin		50	>200		
Cephaloridine		3.12	6.25	6.25	50

[a] Mean value of three determinations.

[b] A heated stainless-steel ring was placed over the inoculum of *Proteus mirabilis* and *P. vulgaris* to prevent swarming.

sented in Table 3. Although pirbenicillin has activity against ampicillin-susceptible *Haemophilus influenzae*, it is ineffective against ampicillin-resistant *H. influenzae* isolates. Pirbenicillin demonstrated good activity against *Aeromonas hydrophilia* in comparison with the lesser activities of carbenicillin or cefazolin.

Pirbenicillin is active against gram-positive microorganisms, although less active than ampicillin, except for penicillin-resistant *Staphylococcus aureus* isolates (Table 4). Pirbenicillin inhibited all *Streptococcus faecalis* isolates at 3.12 μg/ml, whereas 100 μg of carbenicillin per ml was required (Table 4) for similar inhibition.

The synergistic effect observed with combinations of carbenicillin and gentamicin in vitro against *P. aeruginosa* is well documented and appears to hold in vivo (1, 2, 8, 9, 10). Pirbenicillin also demonstrated in vitro synergy with gentamicin against the strains tested (Table 5). This effect was pronounced when the isolates were resistant to both carbenicillin and gentamicin; the MIC of gentamicin was lowered 16-fold, and the MIC of pirbenicillin was lowered eightfold. The combination MIC was well within the clinically observed blood levels for gentamicin (11) and pirbenicillin (R. P. Luthy, E. D. Ralph, R. D. Libke, J. T. Clarke, and W. M. M. Kirby, *Clinical Pharmacokinetics of Pirbenicillin Compared to Carbenicillin*, in press). When the data in Table 5 are plotted in an isobologram (12), all the combination MIC ratios can be demonstrated to be synergistic. Carbenicillin and gentamicin combinations were also synergistic against the strains included in Table 5.

The synergistic relationship was further defined by a killing-curve experiment employing one of the strains listed in Table 5 that is resistant to both carbenicillin and gentamicin (Fig. 2). Starting with a high initial inoculum (>3 × 10^7 cells/ml), gentamicin alone (10 μg/ml) only slowed the growth of the culture, as compared with the control. Pirbenicillin alone (50 μg/ml) reduced the viable

TABLE 3. *Activity of pirbenicillin against other representative gram-negative bacteria in vitro*

Species and antibiotic	No. of strains	Cumulative % inhibited by indicated MIC (μg/ml)	
		50%	90%
Pasteurella multocida	1		
Pirbenicillin			0.10
Carbenicillin			0.39
Aeromonas hydrophilia	8		
Pirbenicillin		3.12	12.5
Carbenicillin		200	>200
Cefazolin		>200	
Acinetobacter calcoaceticus	14		
Pirbenicillin		50	200
Carbenicillin		25	100
Cefazolin		>200	
Alcaligenes species	5		
Pirbenicillin		12.5	100
Carbenicillin		12.5	>200
Cefazolin		100	>200
Haemophilus influenzae[a]			
Ampicillin sensitive	10		
Pirbenicillin		0.78	6.25
Carbenicillin		0.78	1.56
Ampicillin		0.39	3.12
Cefazolin		6.25	25

[a] BHI broth and agar were enriched with 5% Fildes plus 2% Isovitalex (both from BBL). The inoculum was a 10-fold dilution of a culture grown overnight; MIC values were read after 40 h at 37 C.

TABLE 4. *Comparative in vitro activity of pirbenicillin and other semisynthetic β-lactam antibiotics against gram-positive bacteria*

Species and antibiotic	No. of strains	Cumulative % inhibited by indicated MIC (μg/ml)[a]	
		50%	100%
Streptococcus pyogenes[b]	5		
Pirbenicillin			0.39
Ampicillin			0.0078
Streptococcus faecalis	29		
Pirbenicillin		3.12	3.12
Carbenicillin		50	100
Ampicillin		0.78	1.56
Cephalothin		12,5	12.5
Staphylococcus aureus			
Ampicillin sensitive	14		
Pirbenicillin		0.78	3.12
Carbenicillin		0.78	6.25
Ampicillin		≤0.10	0.39
Ampicillin resistant	11		
Pirbenicillin		12.5	50
Carbenicillin		12.5	100
Ampicillin		50	100
Methicillin		3.12	25
Staphylococcus epidermidis	18		
Pirbenicillin		0.30	3.12
Ampicillin		0.10	3.12

[a] Mean value of three determinations.

[b] The growth medium employed was BHI agar enriched with 5% blood. Cultures of *S. pyogenes* were diluted 10-fold for use as inoculum; otherwise, procedures were as described in Materials and Methods.

TABLE 5. *Synergistic effect obtained by combining pirbenicillin and gentamicin against susceptible and resistant Pseudomonas aeruginosa in vitro*[a]

Strain no.	Antibiotic and MIC (μg/ml)[b]		
	Pirbenicillin	Gentamicin	Pirbenicillin and gentamicin
52A104	12.5	0.5	3.12 + 0.125
52A173	12.5	1.0	1.56 + 0.25
52A399^{R} [c]	50	0.5	6.25 + 0.125
52A520^{R}	100	25	12.5 + 1.56
52A513^{R}	100	50	12.5 + 3.12
52A514^{R}	100	50	12.5 + 3.12
52A508^{R}	800	12.5	50 + 1.56

[a] Synergy is defined as occurring when the MIC of each of the antibiotics in combination is one-quarter or less of the MIC of each antibiotic alone.

[b] Mean value of three determinations.

[c] Denotes carbenicillin-resistant strains, MIC of 200 μg/ml for 52A220 and 52A399, 400 μg/ml for 52A513 and 52A514, and 1600 μg/ml for 52A508. The strains resistant to both carbenicillin and gentamicin represent two different geographical sources.

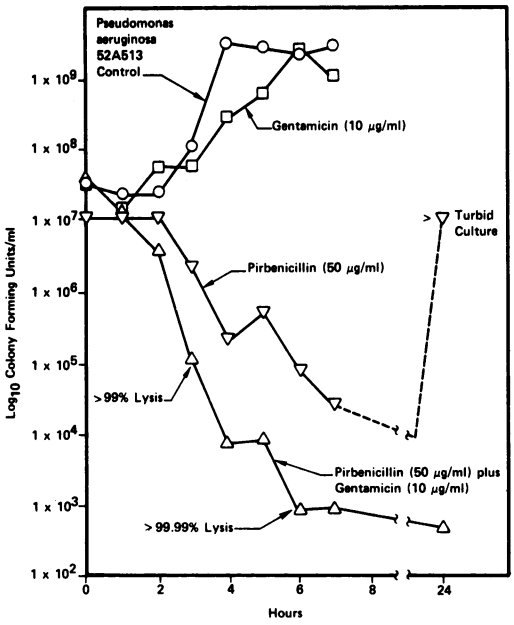

FIG. 2. *Synergy between pirbenicillin and gentamicin against Pseudomonas aeruginosa 52A513 resistant to both gentamicin and carbenicillin.*

colony-forming units by over 99% within 6 h. However, after 24 h the culture had regrown. The combination of the two antibiotics resulted in >99.9% lysis in 6 h, and after 24 h the viable cell count was even lower.

Table 6 demonstrates that pirbenicillin produced MIC-MBC values in BHI broth with a variety of gram-negative and gram-positive bacteria in the same manner as other β-lactam antibiotics. Pirbenicillin was particularly superior to carbenicillin against *S. faecalis*, but inferior against *Proteus*. The lower broth MIC-MBC values obtained for pirbenicillin in comparison to carbenicillin against *E. coli* and *Serratia marcescens* correlates well with the increased activity observed with pirbenicillin in protecting mice infected with *E. coli* and *S. marcescens* (Table 9). In most cases the pirbenicillin MBC equals or is only twofold higher than the MIC. MBC values against *Enterobacteriaceae* are equivalent in MH and BHI.

P. aeruginosa, growing in nitrogen-rich MH medium, elevates media pH values (8.2 to 8.6), presumably by release of NH_4^+ as a waste product (7, 14, 15). Pirbenicillin is chemically unstable at alkaline pH, and true MBC values against pseudomonal species are therefore only obtained when the pH is maintained below 7.5. No problem is presented using BHI broth (already buffered), and is obviated with MH broth by buffering with 0.1 M phosphate buffer (Table 7). In BHI broth, buffered MH, or Trypticase soy broth (another buffered medium containing free glucose), pirbenicillin (Table 7) gave median MBC values (15.6 μg/ml) equal to one-eighth the median carbenicillin MBC value (125 μg/ml).

In M9 medium, pirbenicillin exhibited an MBC value of ≤31.2 μg/ml against 90% of the strains, in comparison to 250 μg/ml for carbenicillin (Table 8). Carbenicillin MBC values in buffered media are usually 1 dilution higher than the MIC (Tables 7 and 8). In the case of pirbenicillin, the MBCs are within 2 to 4 dilutions of the MIC. These MBC values are well within achievable human serum levels of pirbenicillin.

A 10-fold increase in *Pseudomonas* inoculum (from ≈1 × 10^6 to ≈1 × 10^7 cells/ml) caused, on the average, a twofold increase in MIC values for pirbenicillin, carbenicillin, and ticarcillin. The MBCs, however, increased drastically to median values of >1,000 μg/ml even in BHI media for the three penicillins. At the higher inoculum the criteria for the MBC increased to ≥99.99% kill.

The R-MIC values presented in Tables 6 through 8 show that one-step high-level resistance did not emerge with any of the test penicillins. Neither the *Enterobacteriaceae* nor the *Pseudomonas* strains harbored highly resistant variants within the population (high-level resistance is defined as an R-MIC of ≥10 × MIC).

TABLE 6. *Comparative MIC-MBC values (μg/ml) for gram-negative-active β-lactam antibiotics*[a]

Genera, species, and strain no.	Pirbenicillin			Carbenicillin			Ampicillin			Cefazolin		
	MIC	MBC	R-MIC	MIC	MBC	R-MIC	MIC	MBC	R-MIC	MIC	MBC	R-MIC
Gram-positive												
Staphylococcus aureus												
01A009	≤2.0	≤2.0	≤2.0	≤2.0	≤2.0	≤2.0	≤2.0	≤2.0	≤2.0	≤2.0	≤2.0	≤2.0
01A400[R]	3.9	62.5	7.8	3.9	62.6	7.8	125	>250	62.5	≤2.0	62.5	≤2.0
01A087[R]	7.8	15.6	31.2	250	>250	>250	7.8	15.6	62.5	15.6	250	125
Streptococcus faecalis (median value of 7 strains)	0.5	1.0	2.0	62.5	125	62.5	0.5	0.5	1.0	15.6	>250	15.6
Gram-negative												
Escherichia coli (median value of 6 strains)	2.0	2.0	7.8	7.8	15.6	31.2	3.9	7.8	15.6	1.0	1.0	3.9
Serratia marcescens (median value of 7 strains)	3.9	7.8	15.6	31.2	31.2	62.5	62.5	125	125	>250		
63A103[R]	>250			>250			>250			>250		
63A109[R]	125	250	125	>250			>250					
Enterobacter aerogenes (median value of 4 strains)	2.0	2.0	2.0	7.8	15.6	62.5	125	250	>250	>250		
E. cloacae (median value of 4 strains)	3.9	7.8	3.9	31.2	62.5	62.5	>250			>250		
Citrobacter freundii												
70B007	31.2	31.2	62.5	31.2	31.2	62.5	62.5	250	>250	250	>250	>250
Salmonella typhimurium												
58D009	2.0	2.0	2.0	7.8	7.8	15.6	1.0	1.0	3.9	0.5	1.0	2.0
58D013	3.9	3.9	7.8	15.6	31.2	31.2	1.0	1.0	7.8	0.5	0.5	7.8
Proteus mirabilis												
57CO22	1.0	1.0	7.8	≤0.12	≤0.12	≤12.12	0.5	0.5	3.9	1.0	3.9	62.5
57CO64	7.8	15.6	31.2	≤0.12	0.25	2.0	3.9	7.8	15.6	31.2	62.5	125
P. morganii												
57G001	62.5	>250	250	1.0	1.0	3.9	250	>250	>250	>250		
P. inconstants												
77A009	125	125	250	7.8	7.8	2.0	250	250	>250	>250		
Klebsiella pneumoniae												
53A009	62.5	62.5	31.2	125	250	250	62.5	125	62.5	0.5	0.5	2.0
53A079	250	>250	125	>250			250	>250	125	2.0	2.0	3.9

[a] Results of one determination, except for *E. coli*, which is a mean of three determinations. MIC values were determined in BHI broth; the resistance determination MIC (R-MIC) was determined on BHI agar. Strains tested are clinical isolates; known resistant isolates are indicated by an R after the strain number.

TABLE 7. *Pirbenicillin: median MIC-MBC values for 18 Pseudomonas aeruginosa isolates in buffered and nonbuffered media*

Medium	Penicillin and value (μg/ml)[a]								
	Pirbenicillin			Carbenicillin			Ticarcillin		
	MIC	MBC	R-MIC	MIC	MBC	R-MIC	MIC	MBC	R-MIC
BHI[b]	3.9	15.6	15.6	62.5	125	125	15.6	62.5	62.5
MH[c]	3.9	250	15.6	31.2	500	62.5	15.6	125	31.2
MH plus 0.1 M phosphate[d]	2.0	15.6	15.6	62.5	250	250	ND[e]		
Trypticase soy broth[f]	3.9	15.6	ND	62.5	125	ND	ND		

[a] Ampicillin MIC values were >200 μg/ml for all but three strains. Results of one determination with 18 strains.

[b] The R-MIC was determined on BHI agar.

[c] The MBC value encompassing 75% of the isolates in MH broth was >1,000 μg/ml for all three penicillins. The R-MIC was determined in MH broth.

[d] The R-MIC was determined in 0.1 M potassium phosphate-buffered MH broth (pH 6.9).

[e] ND, Not done.

[f] MBC was determined as described by Wald et al. (16); i.e., the MBC corresponds to ≥1,000 colony-forming units/ml or ≈99.9% lysis of the initial inoculum. The agar medium employed in the MBC determination was Trypticase soy broth plus 2% agar.

The activity of pirbenicillin against experimental infections in mice produced by a variety of microorganisms is presented in Table 9. Comparative data, including agar MIC values, are also presented for carbenicillin and ampicillin. Pirbenicillin has outstanding activity against *P. aeruginosa* infections, being about three to four times more active than carbenicillin. Thus, there is a general agreement between the activity ratio of pirbenicillin to carbenicillin in vitro and in vivo. Ampicillin, as was expected, was inactive against all the *Pseudomonas* isolates.

Pirbenicillin proved to be two to four times more active than carbenicillin against experimental infections produced by *S. marcescens* and *E. coli* (Table 9). In contrast to the observations with *Pseudomonas*, the in vitro susceptibility of the *S. marcescens* and *E. coli* isolates to pirbenicillin and carbenicillin were nearly equivalent. In vivo, ampicillin was inactive against *S. marcescens* and was about half as active against *E. coli* isolates as was pirbenicillin. However, ampicillin was about five times more active against infections produced by *S. aureus* than pirbenicillin. This probably reflects the lower MIC for ampicillin. Pirbenicillin demonstrated little activity against an infection produced by *K. pneumoniae* 53A009, compared with cephaloridine, which was used as a positive control in place of carbenicillin.

The data presented in this report demonstrate that pirbenicillin has a significant antibacterial spectrum. In vitro and in vivo results

TABLE 8. *MIC-MBC values for anti-Pseudomonas aeruginosa penicillins determined in M9 growth medium*

% of 18 strains	Antibiotic and value (μg/ml)[a]					
	Pirbenicillin			Carbenicillin		
	MIC	MBC	R-MIC	MIC	MBC	R-MIC
50%	≤2.0			31.2		
50%		3.9			62.5	
50%			3.9			62.5
75%	≤2.0			62.5		
75%		15.6			125	
75%			15.6			62.5
90%	7.8			62.5		
90%		31.2			250	
90%			31.2			125
100%	15.6			500		
100%		125			500	
100%			62.5			250

[a] Ampicillin MICs were >200 μg/ml to inhibit all except three of the isolates in both M9 broth and M9 agar. The R-MIC was determined on M9 agar.

TABLE 9. *Pirbenicillin activity against experimental infections in mice*

Expt infection	PD_{50}[a] with:		
	Pirbenicillin	Carbenicillin	Ampicillin
Pseudomonas aeruginosa			
52A104	69.8 ± 10.4 (6.25)[b]	158 ± 42 (25)[b]	>400 (>200)[b]
52A249	33 ± 12 (6.25)	115 ± 48 (25)	>400 (>200)
52A260	68 ± 34 (12.5)	235 ± 66 (100)	>400 (>200)
52A264	50 ± 24 (6.25)	220 ± 78 (100)	>400 (>200)
52A475	110 ± 46 (12.5)	200 estimated (50)	>400 (200)
52A476	63 ± 19 (6.25)	170 ± 49 (50)	>400 (>200)
Serratia marcescens			
63A017	50 ± 19 (12.5)	128 ± 11 (12.5)	>400 (50)
63A026	74 ± 19 (6.25)	120 ± 17 (12.5)	>400 (50)
63A033	57 ± 20 (12.5)	125 ± 16 (12.5)	>400 (50)
Escherichia coli			
51A266	6.0 ± 1.4 (3.12)	27 ± 3.6 (3.12)	14 ± 2 (3.12)
51A010	5.7 ± 1.4 (6.25)	26 ± 4.8 (6.25)	14 ± 2 (6.25)
Klebsiella pneumoniae			
53A009	280 estimated (12.5)	25 ± 5[c] (1.56)	>400 (25)
Staphylococcus aureus			
01A005	10.7 ± 3.5 (1.56)	12.6 ± 2.4 (3.13)	2.0 ± 0.9 (≤0.1)

[a] PD_{50} given as milligrams per kilogram, with 95% confidence limits; subcutaneous route was used. Numbers in parentheses show MICs in micrograms per milliliter.

[b] Median value of three determinations on BHI agar.

[c] Cephaloridine value. Carbenicillin not studied.

show that this new antibiotic has a bactericidal action and certain potency advantages over carbenicillin. When measuring bactericidal activity against *Pseudomonas* isolates, it is necessary to use a buffered medium to maintain the pH within the range at which pirbenicillin shows no chemical instability.

LITERATURE CITED

1. **Andriole, V. T.** 1971. Synergy of carbenicillin and gentamicin in experimental infection with *Pseudomonas*. J. Infect. Dis. 124(Suppl.):S46-S55.
2. **Andriole, V. T.** 1974. Antibiotic synergy in experimental infection with *Pseudomonas*. II. The effect of carbenicillin, cephalothin, or cephanone combined with tobramycin or gentamicin. J. Infect. Dis. 129: 124-133.
3. **Barry, A. L., and L. D. Sabath.** 1974. Special tests: bactericidal activity and activity of antibiotics in combination, p. 431-432. *In* E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), Manual of clinical microbiology. American Society for Microbiology, Washington, D.C.
4. **Batson, H. D.** 1957. An introduction to statistics in the medical sciences, p. 64-67. Burgess Publishing Co., Minneapolis, Minn.
5. **English, A. R., J. A. Retsema, V. A. Ray, and J. E. Lynch.** 1972. Carbenicillin indanyl sodium, an orally active derivative of carbenicillin. Antimicrob. Agents Chemother. 1:185-191.
6. **Ericsson, H. M., and J. C. Sherris.** 1971. Antibiotic sensitivity testing—report of international collaborative study. Acta Pathol. Microbiol. Scand. Suppl. 217B:64-68.
7. **Hugh, R., and G. L. Gilardi.** 1974. *Pseudomonas*, p. 250-255. *In* E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), Manual of clinical microbiology. American Society for Microbiology, Washington, D.C.
8. **Klastersky, J., R. Cappel, and D. Daneau.** 1972. Clinical significance of in vitro synergism between antibiotics in gram-negative infections. Antimicrob. Agents Chemother. 2:470-475.
9. **Klastersky, J., R. Chappel, and L. Debusscher.** 1971. Evaluation of gentamicin with carbenicillin in infections due to gram-negative bacilli. Curr. Ther. Res. Clin. Exp. 13:174-181.
10. **Phair, J. P., C. Watanakunakorn, and T. Bannister.** 1969. In vitro susceptibility of *Pseudomonas aeruginosa* to carbenicillin and the combination of carbenicillin and gentamicin. Appl. Microbiol. 18: 303-306.
11. **Physicians Desk Reference,** 29th ed., p. 1321. 1975. Medical Economics Co., Oradell, N.J.
12. **Sabath, L. D.** 1968. Synergy of antibacterial substances by apparently known mechanisms, p. 210-217. Antimicrob. Agents Chemother. 1967.
13. **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.
14. **Sherris, J. C., J. G. Shoesmith, M. T. Parker, and D. Breckon.** 1959. Tests for rapid breakdown of arginine by bacteria: their use in the identification of *Pseudomonas*. J. Gen. Microbiol. 21:389-396.
15. **Sokatch, J. R.** 1969. Bacterial physiology and metabolism, p. 175-193. Academic Press Inc., New York.
16. **Wald, E. R., H. C. Standiford, B. A. Tatem, F. M. Calia, and R. B. Hornick.** 1975. BL-P1654, ticarcillin, and carbenicillin: in vitro comparison alone and in combination with gentamicin against *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. 7:336-340.
17. **Witkin, E. M.** 1947. Mutations in *Escherichia coli* induced by chemical agents. Cold Spring Harbor Symp. Quant. Biol. 12:256-269.