Piperacillin and carbenicillin; a collaborative in vitro comparison against 10,838 clinical bacterial isolates

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The clinical efficacy of carbenicillin for treating serious gram-negative infections caused by *Pseudomonas aeruginosa* and some members of the Enterobacteriaceae is well established.[1,2] The synergistic effect of carbenicillin and aminoglycosides[3,4] is of significant value in treating infections caused by *P. aeruginosa*. However, the occurrence of aminoglycoside- and carbenicillin-resistant strains of pseudomonas and enteric gram-negative bacilli[5-6] indicates the need for other antimicrobics with significant activity against these pathogens.

Piperacillin, formerly known as T-1220, is a synthetic derivative of aminobenzyl penicillin (*Fig. 1*). In previous reports this antimicrobic has been shown to be much more active than ticarcillin or carbenicillin against most Enterobacteriaceae, *P. aeruginosa*, *Pseudomonas* species, *Bacteroides fragilis* and *Streptococcus faecalis*.[7-13]

This study presents an in vitro comparison of carbenicillin and piperacillin against 10,838 clinical isolates of bacteria from four institutions in three widely separated geographic regions.

Materials and methods

Bacterial isolates. The bacteria tested were consecutive clinical isolates from the laboratories of St. Francis Hospital, Wichita, Kansas; Kaiser Foundation Hospital and St. Vincent Hospital and Med-

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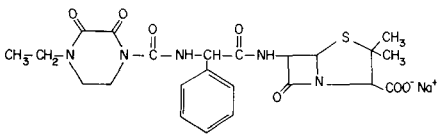

PIPERACILLIN

Fig. 1. Structure of piperacillin.

ical Center, Portland, Oregon; and The Cleveland Clinic Foundation. A total of 10,838 aerobic and the facultative anaerobic bacterial isolates were tested. The isolates were identified by one of the following: the prepackaged reagent kit AnalyTab Products Inc system, the replicator method,[14] and the conventional biochemical and serological methods.[15] Four or five quality control strains were tested daily by each institution. The quality control strains included *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923 or 29213), *P. aeruginosa* (ATCC 27853) and *S. faecalis* (ATCC 29212). The endpoint reproducibility within ±1 dilution of mean values was greater than 99% as demonstrated previously.[16, 17]

Antimicrobic agents. Antimicrobic reference powder for piperacillin was obtained from Lederle Laboratories, Pearl River, New York. Reference powder for carbenicillin was obtained from Beecham Laboratories, Bristol, Tennessee. Stock solutions of the antimicrobics were prepared in water and further diluted in Mueller-Hinton Broth (Difco). Serial 2-fold dilutions of each antimicrobic were prepared. The concentrations prepared for piperacillin were 1 to 64 μg/ml. In testing gram-positive bacteria, the serial concentrations of carbenicillin tested ranged from 1 to 64 μg/ml. When testing gram-negative bacilli, the carbenicillin concentrations ranged from 8 to 512 μg/ml. The antibiotic-containing solutions were dispensed in 100-μl volumes into wells of plastic trays using the MIC 2000 (Cooke Instruments, Alexandria, Virginia) or were commercially dispensed (Micro Media, San Jose, California), after which the trays were stored at −20C.

Susceptibility testing. Plastic trays containing the frozen broth solutions of antimicrobics were thawed to room temperature. Inocula of 1 μl (Cooke) or 5 μl (Micro Media Systems) were simultaneously inoculated into each well. The inocula were adjusted to achieve a final concentration of 5×10^4 colony-forming units (CFU) per well. The trays were then incubated overnight (16 to 18 hours) at 35C in forced-air incubators. The minimal inhibitory concentration (MIC) was read as the lowest concentration of an antimicrobic completely inhibiting growth as evidenced by the lack of turbidity or sediment or both in the wells. When testing the susceptibility of *Haemophilus influenzae*, the Mueller-Hinton broth was enriched with either 5% Fildes reagent or supplement C (Difco). Statistical analysis for the differences in susceptibility patterns of the two drugs was done using the Kalmogorov-Smirnov two sample test.

Results

Table 1 shows the antimicrobial activity of piperacillin and carbenicillin against over 7000 Enterobacteriaceae. Piperacillin generally was more active than carbenicillin, but ranged from near equal inhibition with *Proteus morganii* and *Proteus rettgeri* to 64-fold more active against the *Klebsiella* species. Modal MIC values for piperacillin averaged ≤1 μg/ml, with 91% and 96% of enterics inhibited at 16 and 64 μg/ml respectively. Carbenicillin inhibited only 86% at the 128 μg/ml sensitive breakpoint.

The non-Enterobacteriaceae gram-negative bacilli are tabulated in *Table 2*. Again, piperacillin was more effective than carbenicillin with the single possible exception of *Pseudomonas maltophilia*.

Table 1. Minimum inhibitory concentrations of piperacillin and carbenicillin against 7192 clinical isolates of enterobacteriaceae

Organism (no.)	Agent	Cumulative % inhibited at MIC (μg/ml) of									
		≤1	2	4	8*	16	32	64†	128	256	512
E. coli (3461)	PC	**69**‡	79	82	83	87	90	94			
	CB				**80**	83	84	85	85	85	86
Citrobacter diversus (83)	PC	5	13	**55**	95	96	98	99			
	CB				2	2	2	12	**61**	88	94
freundii (140)	PC	**54**	82	87	91	94	94	98			
	CB				**75**	79	83	88	93	95	97
Klebsiella pneumoniae (1313)	PC	6	27	**74**	88	91	**94**	96			
	CB				2	4	9	23	**52**	78	89
ozaenae (23)	PC	**65**	74	87	91	100					
	CB				22	30	39	**65**	78	91	96
Enterobacter cloacae (245)	PC	**44**	79	86	93	93	94	96			
	CB				**79**	84	88	92	97	99	99
aerogenes (418)	PC	25	**66**	79	84	89	95	98			
	CB				**73**	80	85	89	93	95	96
hafniae (14)	PC	**64**	86	100							
	CB				**86**	86	86	93	100		
agglomerans (43)	PC	**44**	67	77	81	93	95	100			
	CB				**49**	49	66	74	83	83	87
Serratia marcescens (194)	PC	**77**	91	96	97	98	99	100			
	CB				**87**	90	94	97	97	98	99
liquefaciens (21)	PC	4	**79**	98	100						
	CB				**90**	90	90	100			
Proteus vulgaris (47)	PC	**96**	96	96	96	96	98	100			
	CB				**67**	83	91	96	98	100	
mirabilis (1025)	PC	**95**	97	99	99	100					
	CB				**96**	97	99	100			
morganii (119)	PC	**81**	87	91	92	93	96	100			
	CB				**95**	95	98	99	99	100	
rettgeri (23)	PC	**70**	78	87	87	87	91				
	CB				81	81	85	85	89	89	89
Miscellaneous group§ (23)	PC	37	**74**	100							
	CB				**92**	97	97	97	97	100	

* Lowest tested concentration of carbenicillin.
† Highest tested concentration of piperacillin.
‡ Boldface percentage indicates modal MIC value.
§ *Providencia stuartii* (10), *Salmonella enteritidis* (12), *Edwardsiella tarda* (1), and *Yersinia enterocolitica* (1).
PC = piperacillin; CB = carbenicillin.

P. aeruginosa and *Pseudomonas* species were 16- to 32-fold more sensitive to piperacillin (mode, 2 to 4 μg/ml) than carbenicillin (mode, 64 μg/ml). At the concentrations tested both penicillins were equally effective against *H. influenzae*.

Table 3 shows the piperacillin and carbenicillin cumulative susceptibility data for the gram-positive cocci. Carbenicillin appears to be at least 2-fold more active than piperacillin against the staphylococci. Both were effective versus the beta-haemolytic and viridans group streptococci. Piperacillin was 16- to 32-fold more active than carbenicillin against group D streptococci, especially *S. faecalis* and *S. liquefaciens*. This activity was similar to the piperacillin parent compound ampicillin.

Table 2. Minimum inhibitory concentrations of piperacillin and carbenicillin against 2113 clinical isolates of non-Enterobacteriaceae gram-negative bacilli

Organism (no.)	Agent	Cumulative % inhibited at MIC (μg/ml)									
		≤1	2	4	8*	16	32	64†	128	256	512
Acinetobacter calcoaceticus											
var *anitratus* (118)	PC	3	6	19	**53‡**	91	100				
	CB				30	61	**96**	97	97	99	99
var *lwoffi* (23)	PC	22	39	**74**	87	100					
	CB				**88**	88	92	96	96		
Pseudomonas aeruginosa (1562)	PC	10	34	**74**	88	94	97	99			
	CB				5	11	**48**	80	88	97	99
maltophilia (64)	PC			6	**39**	70	91	97			
	CB				**59**	72	80	91	98	100	
species (35)	PC	20	**54**	74	80	91	91	97			
	CB				20	23	**43**	54	66	80	89
Moraxella species (21)	PC	**62**	81	95	100						
	CB				**86**	90				100	
Haemophilus influenzae (279)	PC	**87**	93	94	95	97		99			
	CB				**95**	97	98	100			
Miscellaneous group§ (11)	PC	**82**	91			100					
	CB				36	45	**73**	91		100	

* Lowest tested concentration of carbenicillin.
† Highest tested concentration of piperacillin.
‡ Boldface percentage indicates modal MIC value.
§ *Pasteurella multocida* (6), *P. pneumotropica* (1), *Aeromonas hydrophila* (4).
PC = piperacillin; CB = carbenicillin.

Figure 2 shows the cumulative percentage curves of four bacterial species demonstrating the marked piperacillin/carbenicillin spectrum difference. There is a marked left shift of the piperacillin curve for *Klebsiella pneumoniae*, *P. aeruginosa* and *S. faecalis*. Carbenicillin inhibited more *S. aureus* strains at clinically achievable concentrations. However, both penicillins appear susceptible to gram-positive beta-lactamases.

When using the cumulative percent susceptible curves as susceptibility patterns for the different institutions, true endemic differences emerged. If geographical regions were truly important, the results from the two Portland institutions should have shown the least differences in susceptibility. However, the results with best agreement were those from St. Vincent Hospital (Portland) and the Cleveland Clinic laboratories. This can be illustrated by comparing the cumulative percent susceptible curves for *E. coli* testing piperacillin and carbenicillin (*Fig. 3*). The carbenicillin curves for Kaiser Foundation and St. Francis hospitals were similar and differ significantly from those for St. Vincent and the Cleveland Clinic. These results indicate that institutional factors rather than geographic influences play the major role in determining the antimicrobial susceptibility patterns. These differences were unlikely to be due to technical factors as the results achieved with quality control strains common to each institution were comparable. Each institution had the most susceptible and most resistant pattern for at least one of the clinical species in which significant interlaboratory differences ($p = <0.01$) were noted, regardless of the drug being tested.

Table 3. Minimum inhibitory concentration of piperacillin and carbenicillin against 1533 clinical isolates of gram-positive cocci

Organism (no.)	Agent	Cumulative % inhibited at MIC (μg/ml)						
		≤1	2	4	8	16	32	64
Staphylococcus								
aureus (1051)	PC	25	**46***	63	75	82	88	94
	CB	**51**	60	71	89	96	100	
epidermidis (151)	PC	**66**	72	85	89	93	94	95
	CB	**93**	94	95	96	98	99	100
Streptococcus								
pyogenes (30)	PC	**100**						
	CB	**100**						
pneumoniae (25)	PC	**100**						
	CB	**100**						
viridans groups (23)	PC	**100**						
	CB	**100**						
group D								
not faecalis (80)	PC	**99**			100			
	CB	24	**59**	75	86	89	98	100
faecalis (121)	PC	4	**76**	98	99	100		
	CB	2	7	38	40	43	64	**97**
liquefaciens (52)	PC	4	**79**	98			100	
	CB		1	1	3	8	**80**	93

* Boldface percentage indicates modal MIC value.
PC = piperacillin; CB = carbenicillin.

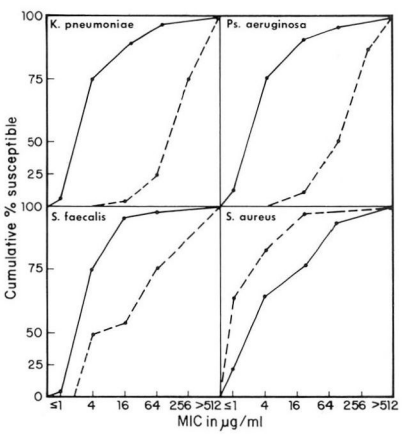

Fig. 2. Susceptibility patterns of four microorganisms to piperacillin and carbenicillin; —— = piperacillin, ----- = carbenicillin.

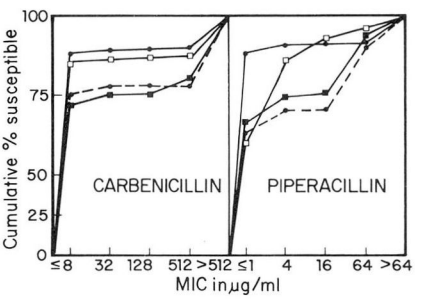

Fig. 3. Cumulative percent susceptibility of *E. coli* to carbenicillin and piperacillin at four institutions; ●—● = Kaiser Foundation, ●- - -● = St. Vincent, ■—■ = St. Francis, □—□ = the Cleveland Clinic.

Discussion

The data for the Enterobacteriaceae show that most species tested had a significantly greater susceptibility to piperacillin than carbenicillin. The increased susceptibility was statistically significant ($p = <0.001$) for *E. coli*, *Citrobacter diversus*, *K. pneumoniae*, *Klebsiella ozaenae*, and *Enterobacter agglomerans*; and less significant ($p = <0.05$) for *Enterobacter cloacae*, *Enterobacter aerogenes*, and *Proteus vulgaris*. There were no statistical differences in susceptibility to

the two penicillins when testing *Serratia marcescens*, *S. liquefaciens*, *Proteus mirabilis*, *P. morganii*, *P. rettgeri*, and the miscellaneous group A strains of gram-negative bacilli. However, these data would suggest a 4- to 8-fold activity advantage for piperacillin. The failure of this study to demonstrate statistical differences in susceptibility of some species may well be due to the two different ranges of antibiotic concentrations tested. The lowest concentration tested for piperacillin was 1 μg/ml compared to 8 μg/ml for carbenicillin.

Many strains were invariably susceptible to the lowest concentrations tested for each drug; thus no differences at the lower concentrations could be detected.

When comparing the susceptibility of the non-Enterobacteriaceae isolates of gram-negative bacilli (*Table 2*), piperacillin again showed greater activity than carbenicillin. This difference in antimicrobial activity was statistically significant ($p = <0.001$) when testing *P. aeruginosa*, *Pseudomonas* species and *Acinetobacter calcoaceticus* var. *anitratus*. The other members of this group were highly susceptible to both penicillins; thus, no differences were evident.

The gram-positive cocci exhibited generic and species differences in susceptibility to the two drugs (*Table 3*). *S. aureus* was much more susceptible to carbenicillin ($p = <0.001$), whereas there were no significant differences in the susceptibility of *Staphylococcus epidermidis*. The streptococci were significantly more susceptible to piperacillin when differences could be detected. However, the majority of *Streptococcus* species were highly susceptible to both drugs. The enterococci were 16-fold more susceptible to piperacillin ($p = <0.001$) than carbenicillin.

The susceptibility patterns suggest other differences in the two penicillins. It was evident that *S. aureus*, a frequent producer of penicillinase, was not significantly inhibited by either drug. Conversely, the strains of *K. pneumoniae* tested were known producers of beta-lactamase, yet these organisms together with enterococci and *P. aeruginosa* were inhibited by piperacillin, but were resistant to carbenicillin. These results suggest that resistance to piperacillin was in part the result of hydrolysis by beta-lactamase, but bacterial intrinsic factors also play important roles in determining susceptibility or resistance.

In conclusion, piperacillin, in direct comparison with carbenicillin, demonstrates definite in vitro superiority in activity and spectrum to any penicillin currently used for treating infections due to gram-negative bacilli and enterococci. Further clinical investigations are warranted, especially in combination with the aminoglycosides.

Summary

By a uniform methodology, the susceptibility to piperacillin and carbenicillin of 10,838 consecutive bacterial isolates at four institutions was determined. Piperacillin exhibited significantly greater activity (4- to 16-fold) than carbenicillin against most Enterobacteriaceae, *P. aeruginosa*, other *Pseudomonas* species, and some streptococci. Its activity against staphylococci, especially *S. aureus*, was 2-fold less than that of carbenicillin. Institutional differences in susceptibility patterns were observed that were statistically significant, not related to technique, and appeared to represent true endemic bacterial species susceptibility differences. The increased antimicrobial spectrum of piperacillin against *K. pneumoniae, K. ozaenae, Citrobacter*, and *S. faecalis* requires further in vivo investigation.

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