Comparative *in vitro* activity of piperacillin/tazobactam against Gram-negative bacilli

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Objective. To describe the *in vitro* activity of piperacillin/tazobactam against clinical isolates of Gram-negative bacteria, compared with other antibacterial agents.

Design. Survey of susceptibility of clinical isolates of Gram-negative bacilli.

Setting. Academic hospitals of the University of the Witwatersrand teaching complex.

Bacterial strains. 180 selected clinical isolates of Gram-negative bacilli.

Main outcome measures. Minimum inhibitory concentrations (MICs) determined by agar dilution using techniques according to the recommendations of the National Committee for Clinical Laboratory Standards.

Results. Ciprofloxacin, biapenem, imipenem, cefepime and cefpirome were all highly active against most of the Enterobacteriaceae. All the ampicillin-resistant strains of Enterobacteriaceae were susceptible to piperacillin/tazobactam, MIC_{90} values being 4/4 mg/l for *Klebsiella* and *Proteus/Providencia* spp., 8/4 mg/l for *Citrobacter* and *Serratia* spp., and 16/4 mg/l for *Escherichia coli.* All the agents, with the exception of ampicillin (MIC_{90} 4 mg/l) and chloramphenicol (MIC_{90} 4 mg/l), were highly active against the *Haemophilus influenzae* isolates tested. All *Bacteroides fragilis* strains were susceptible to piperacillin/tazobactam (MIC_{90} 8/4 mg/l), as well as to co-amoxiclav (MIC_{90} 4/2 mg/l), biapenem and imipenem (MIC_{90}s 0.5 mg/l). The *Pseudomonas* spp. tested included strains resistant to piperacillin/tazobactam, ceftazidime, biapenem, gentamicin, tobramycin and ciprofloxacin. Cefepime was the most active agent against *Pseudomonas* isolates, with 90% of the strains being susceptible to this agent, while biapenem was the most active agent against the *Acinetobacter* isolates investigated.

Conclusions. The *in vitro* spectrum of activity of piperacillin/tazobactam against the majority of isolates was comparable to those of the other new agents tested.

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Piperacillin is an ureidopenicillin that is active against *Pseudomonas aeruginosa,* other Gram-negative bacteria and some Gram-positive bacteria. However, it is inactivated by many β-lactamases and bacteria with acquired resistance to ampicillin are therefore also piperacillin-resistant. Piperacillin is now being marketed in combination with the β-lactamase antagonist tazobactam. This drug is active against plasmid-mediated TEM, SHV and extended-spectrum β-lactamases, as well as the β-lactamases produced by staphylococci and *Bacteroides fragilis.* It is not very active against class I chromosomally mediated β-lactamases produced by *Enterobacter cloacae, Citrobacter freundii,* indole-positive *Proteus* spp., *Serratia marcescens*, and *P. aeruginosa.* In this study the *in vitro* activity of piperacillin/tazobactam was compared with that of ampicillin, co-amoxiclav, cefoxitin, ceftriaxone, cefpirome, cefepime, biapenem, imipenem, gentamicin, tobramycin and ciprofloxacin against selected clinical isolates likely to cause infections in the hospital setting.

Materials and methods

This study was performed in late 1993/early 1994 on clinical isolates from patients attending Johannesburg, Hillbrow and Baragwanath hospitals. The strains were collected and stored in liquid nitrogen until used. Antibiotic reference powders and their sources were as follows: piperacillin/tazobactam, biapenem (Lederle); ampicillin, co-amoxiclav (SmithKline Beecham); cefoxitin, imipenem (Logos); ceftriaxone (Roche); cefpirome (Roussel); cefepime (Bristol-Myers Squibb); gentamicin, tobramycin (Eli Lilly); azithromycin (Pfizer); ceftazidime (Glaxo); clindamycin (Upjohn) and ciprofloxacin (Bayer). Minimum inhibitory concentrations (MICs) were determined by an agar dilution method, according to the National Committee for Clinical Laboratory Standards (NCCLS) recommendations, using Mueller-Hinton agar (Oxoid) for the Enterobacteriaceae and non-fermentors; *Haemophilus* Test Medium (Oxoid) for *H. influenzae;* and Wilkins-Chalgren agar (Oxoid) for *B. fragilis.*[1] For the determination of piperacillin/tazobactam MICs, the concentration of tazobactam was maintained at 4 mg/l, while that of piperacillin was diluted as normal.

The significance of differences in proportions of susceptible isolates was calculated using Yates' corrected chi-square or Fisher's exact test where appropriate, by means of Epi-Info version 6.

Results

The results of the MIC determinations and percentage susceptibility based on NCCLS breakpoints for the various organisms are listed in Tables I - IV. The significance of the difference in the proportion of all the Enterobacteriaceae and non-fermentors susceptible to piperacillin/tazobactam in comparison with the other antimicrobial agents is given in Tables V and VI.

Against the Enterobacteriaceae tested, which excluded *Enterobacter* spp., the most active agents overall were ciprofloxacin, the carbapenems (biapenem and imipenem), and the new cephalosporins (cefepime and cefpirome). Piperacillin/tazobactam demonstrated good activity against most of the Enterobacteriaceae isolates tested, including the ampicillin-resistant strains. The MIC_{90} values were 4/4 mg/l for *Klebsiella* and *Providencia* spp., 8/4 mg/l for *Citrobacter, Proteus* and *Serratia* spp., and 16/4 for *Escherichia coli.* However, when the percentages of Enterobacteriaceae strains falling into the sensitive, moderately sensitive or resistant ranges based on NCCLS breakpoints were compared, none of the isolates tested fell into the resistant range for piperacillin/tazobactam, cefepime and biapenem. One *Serratia* isolate was resistant to ciprofloxacin, and 1 *E. coli* isolate was resistant to cefpirome and the third-generation cephalosporins tested. There was no significant difference between the percentage of Enterobacteriaceae isolates susceptible to piperacillin/tazobactam and the third- and fourth-generation cephalosporins, carbapenems and ciprofloxacin. All these agents were superior to ampicillin and co-amoxiclav ($P < 0.001$).

Apart from 3 β-lactamase-producing strains of *H. influenzae* which were resistant to ampicillin, and 1 chloramphenicol-resistant isolate, 100% of the *H. influenzae* strains tested were extremely sensitive to all other agents.

A number of multiply-resistant *Pseudomonas* and *Acinetobacter* spp. were selected for the study. When MIC_{90} values and percentage sensitivity were compared, cefepime was the most active agent against the *Pseudomonas* spp. tested, with only 2 of the isolates being resistant to this agent. The next most active agents were ciprofloxacin and piperacillin/tazobactam with 25% of strains being resistant to ciprofloxacin and 30% resistant to piperacillin/tazobactam. A different pattern was observed with the *Acinetobacter* isolates. All strains were fully sensitive to the carbapenems, biapenem and imipenem, and either fully sensitive or moderately susceptible to cefepime. Twenty-five per cent of the isolates were resistant to piperacillin/tazobactam and ciprofloxacin. In respect of all the non-fermentors (*Pseudomonas* and *Acinetobacter* spp.), there was a significantly larger percentage of strains sensitive to cefepime than any of the other agents tested ($P < 0.001$ in comparison with piperacillin/tazobactam and $P = 0.04$ in comparison with imipenem). Ceftazidime and cefpirome had significantly fewer strains susceptible than piperacillin/tazobactam, cefepime, the carbapenems and ciprofloxacin.

All the *B. fragilis* isolates were resistant to ciprofloxacin, and 1 to clindamycin. The remaining strains were sensitive to all the other antibiotics tested with the most active agents being metronidazole and the carbapenems.

Discussion

Piperacillin, in combination with tazobactam, demonstrated good activity against most of the isolates tested. The MIC_{50}s, MIC_{90}s and MIC ranges of piperacillin/tazobactam and the other agents tested against the Enterobacteriaceae were similar to those previously reported.[2-6] An interesting observation was that there were considerably more *Citrobacter* spp. susceptible to piperacillin/tazobactam but resistant to co-amoxiclav. Clavulanic acid does not

Table I. Comparative *in vitro* sensitivity of piperacillin/tazobactam against Enterobacteriaceae

Organism	Antimicrobial agent	MIC_{50} (mg/l)	MIC_{90} (mg/l)	MIC range (mg/l)	Percentage susceptibility S	MS	R	*P*-value
Escherichia coli	Piperacillin/tazobactam	2/4	16/4	0.5/4 - 64/4	90	10	0	—
(*N* = 20)	Ampicillin	> 128	> 128	1 - > 128	25	5	70	< 0.001
	Co-amoxiclav	8/4	16/8	1/0.5 - 64/32	75	15	10	NS
	Cefoxitin	4	8	1 - > 128	90	0	10	NS
	Ceftriaxone	0.06	0.5	≤ 0.007 - 128	95	0	5	NS
	Cefpirome	0.03	0.5	0.015 - 64	95	0	5	NS
	Cefepime	0.06	0.5	≤ 0.007 - 16	95	5	0	NS
	Biapenem	≤ 0.015	≤ 0.015	≤ 0.015 - 1	100	0	0	NS
	Imipenem	0.25	0.25	0.06 - 8	95	5	0	NS
	Gentamicin	1	8	0.5 - > 128	85	—	15	NS
	Tobramycin	1	8	0.5 - 32	90	—	10	NS
	Ciprofloxacin	0.015	0.25	≤ 0.007 - 1	100	0	0	NS
Klebsiella spp.	Piperacillin/tazobactam	2/4	4/4	1/4 - 8/4	100	0	0	—
(*N* = 20)	Ampicillin	> 128	> 128	32 - > 128	0	0	100	< 0.001
	Co-amoxiclav	4/2	8/4	2/1 - 16/8	90	10	0	NS
	Cefoxitin	2	8	1 - 128	95	0	5	NS
	Ceftriaxone	2	8	0.03 - 32	90	5	5	NS
	Cefpirome	0.5	2	0.03 - 4	100	0	0	NS
	Cefepime	0.25	2	0.015 - 4	100	0	0	NS
	Biapenem	0.06	0.06	≤ 0.03 - 0.12	100	0	0	NS
	Imipenem	0.25	0.5	0.12 - 0.5	100	0	0	NS
	Gentamicin	4	16	0.12 - 128	50	—	50	0.001
	Tobramycin	16	32	0.25 - 64	35	—	65	< 0.001
	Ciprofloxacin	0.06	0.12	0.015 - 2	95	5	0	NS
Citrobacter spp.	Piperacillin/tazobactam	4/4	8/4	2/4 - 32/4	95	5	0	—
(*N* = 20)	Ampicillin	64	> 128	8 - > 128	10	15	75	< 0.001
	Co-amoxiclav	16/8	64/32	2/1 - 128/64	45	10	45	0.002
	Cefoxitin	16	128	1 - > 128	45	10	45	NS
	Ceftriaxone	0.06	0.5	0.03 - 64	95	0	5	NS
	Cefpirome	0.06	0.12	0.03 - 1	100	0	0	NS
	Cefepime	0.06	0.12	0.015 -0.5	100	0	0	NS
	Biapenem	0.06	0.25	0.03 - 0.25	100	0	0	NS
	Gentamicin	0.5	8	0.12 - 16	80	—	20	NS
	Tobramycin	0.25	8	≤ 0.06 - 64	85	—	15	NS
	Ciprofloxacin	0.015	0.03	≤ 0.007 - 0.06	100	0	0	NS
Proteus/	Piperacillin/tazobactam	1/4	4/4	0.5/4 - 32/4	95	5	0	—
Providencia spp.	Ampicillin	128	> 128	8 - > 128	10	25	65	< 0.001
(*N* = 20)	Co-amoxiclav	16/8	32/16	1/0.5 - 64/32	30	30	40	< 0.001
	Cefoxitin	64	> 128	1 - > 128	30	5	65	<0.001
	Ceftriaxone	0.06	0.5	0.03 - 16	95	5	0	NS
	Cefpirome	0.06	0.06	≤ 0.03 - 0.25	100	0	0	NS
	Cefepime	0.06	0.12	0.015 - 0.25	100	0	0	NS
	Biapenem	0.06	0.12	0.03 - 0.25	100	0	0	NS
	Imipenem	0.5	2	0.12 - 4	100	0	0	NS
	Gentamicin	2	16	1 - 128	60	—	40	0.02
	Tobramycin	0.5	64	0.12 - 64	75	—	25	NS
	Ciprofloxacin	≤ 0.003	0.015	≤ 0.003 - 0.06	100	0	0	NS
Serratia spp.	Piperacillin/tazobactam	2/4	8/4	0.25/4 - 16/4	100	0	0	—
(*N* = 20)	Ampicillin	64	> 128	8 - > 128	5	5	90	< 0.001
	Co-amoxiclav	32/16	64/32	2/1 - > 128/64	5	5	90	< 0.001
	Cefoxitin	16	32	4 - 64	45	25	30	< 0.001
	Ceftriaxone	0.12	2	0.06 - 8	100	0	0	NS
	Cefpirome	0.06	0.12	≤ 0.03 - 1	100	0	0	NS
	Cefepime	0.06	0.12	0.03 - 2	100	0	0	NS
	Biapenem	1	1	0.5 - 2	100	0	0	NS
	Imipenem	1	4	0.5 - 4	100	0	0	NS
	Gentamicin	4	8	1 - 128	80	—	20	NS
	Tobramycin	4	16	0.5 - 64	55	—	45	< 0.001
	Ciprofloxacin	0.25	0.5	0.06 - 4	95	0	5	NS

S = susceptible; MS = moderately susceptible; R = resistant; *P*-value = significance of the difference of the proportion of isolates susceptible in comparison with piperacillin/tazobactam; NS = no significant difference.

Table II. *In vitro* activity of agents against *H. influenzae*

Organism	Antimicrobial agent	MIC_{50} (mg/l)	MIC_{90} (mg/l)	MIC range (mg/l)	Percentage susceptibility S	MS	R	*P*-value
H. influenzae	Piperacillin/tazobactam	≤ 0.007/4	≤ 0.007/4	≤ 0.007/4 - 0.015/4	100	0	0	—
(*N* = 20)	Ampicillin	0.25	4	≤ 0.03 - 4	85	—	15	NS
	Co-amoxiclav	0.06/0.03	0.25/0.12	≤ 0.007/0.003 - 0.5/0.25	100	0	0	NS
	Ceftriaxone	≤ 0.0015	≤ 0.0015	≤ 0.0015 - 0.07	100	0	0	NS
	Cefpirome	≤ 0.007	0.03	≤ 0.007 - 0.03	100	0	0	NS
	Cefepime	0.015	0.015	≤ 0.007 - 0.6	100	0	0	NS
	Biapenem	0.015	0.06	≤ 0.007 - 0.25	100	0	0	NS
	Imipenem	0.03	0.06	≤ 0.015 - 0.12	100	0	0	NS
	Azithromycin	0.015	0.25	≤ 0.003 - 0.12	100	0	0	NS
	Chloramphenicol	0.25	4	0.06 - 8	95	—	5	NS
	Ciprofloxacin	≤ 0.015	0.06	≤ 0.015 - 0.06	100	0	0	NS

Table III. *In vitro* activity of agents against non-fermentors

Organism	Antimicrobial agent	MIC_{50} (mg/l)	MIC_{90} (mg/l)	MIC range (mg/l)	Percentage susceptibility S	MS	R	*P*-value
Pseudomonas spp.	Piperacillin/tazobactam	16/4	>128/4	1/4 - >128/4	70	—	30	—
(*N* = 20)	Ceftazidime	16	> 128	2 - > 128	40	10	50	NS
	Cefpirome	32	64	4 - > 128	5	20	75	< 0.001
	Cefepime	4	8	0.5 - 16	90	10	0	NS
	Biapenem	8	> 128	1 - > 128	15	25	60	0.01
	Imipenem	32	> 128	2 - > 128	35	25	45	0.06
	Gentamicin	16	> 128	2 - > 128	40	—	60	NS
	Tobramycin	2	> 128	0.25 - > 128	55	—	45	NS
	Ciprofloxacin	1	16	0.12 - 64	55	20	25	NS
Acinetobacter spp.	Piperacillin/tazobactam	16/4	> 128/4	≤ 0.06/4 - > 128/4	50	25	25	—
(*N* = 20)	Ceftazidime	32	> 128	2 - > 128	25	15	60	NS
	Cefpirome	16	64	1 - > 128	20	20	60	NS
	Cefepime	2	8	0.25 - 8	100	0	0	0.01
	Biapenem	0.5	1	≤ 0.12 - 4	100	0	0	0.01
	Imipenem	1	4	0.25 - 4	100	0	0	0.01
	Gentamicin	8	> 128	0.5 - > 128	50	—	50	NS
	Tobramycin	2	8	0.25 - 128	55	—	45	NS
	Ciprofloxacin	1	128	0.12 - > 128	65	10	25	NS

Table IV. *In vitro* activity of agents against *B. fragilis*

Organism	Antimicrobial agent	MIC_{50} (mg/l)	MIC_{90} (mg/l)	MIC range (mg/l)	Percentage susceptibility S - MS	R	*P*-value
B. fragilis	Piperacillin/tazobactam	2/4	8/4	0.25/4 - 8/4	100	0	—
(*N* = 20)	Co-amoxiclav	1/0.5	4/2	0.25/4 - 4/2	100	0	NS
	Cefoxitin	16	16	4 - 32	100	0	NS
	Biapenem	0.25	0.5	0.12 - 1	100	0	NS
	Imipenem	0.12	0.5	0.03 - 1	100	0	NS
	Ciprofloxacin	64	128	32 - > 128	0	100	< 0.001
	Metronidazole	0.5	0.5	0.06 - 1	100	0	NS
	Clindamycin	0.25	1	0.03 - 8	95	5	NS
	Chloramphenicol	4	8	2 - 8	100	0	NS

Table V. Significance of difference in proportion of susceptible Enterobacteriaceae isolates in comparison with piperacillin/tazobactam

Antimicrobial agent	*P*-value
Piperacillin/tazobactam	—
Ampicillin	< 0.001
Co-amoxiclav	< 0.001
Cefoxitin	< 0.001
Ceftriaxone	NS
Cefpirome	NS
Cefepime	NS
Biapenem	NS
Imipenem	NS
Ciprofloxacin	NS

Table VI. Significance of difference in proportion of susceptible non-fermentors in comparison with piperacillin/tazobactam

Antimicrobial agent	*P*-value
Piperacillin/tazobactam	—
Ceftazidime	0.02
Cefpirome	< 0.001
Cefepime	< 0.001
Biapenem	NS
Imipenem	NS
Gentamicin	NS
Tobramycin	NS
Ciprofloxacin	NS

effectively inhibit the class I chromosomal enzymes produced by these organisms, and so does not potentiate the activity of amoxycillin against strains that are stably derepressed for these enzymes.[7-9] Tazobactam is a weaker inducer of β-lactamases than clavulanic acid, and has been shown to enhance the action of piperacillin against *C. freundii* isolates.[10] Presumably the strains in our study produced chromosomal β-lactamases which were induced by clavulanic acid but not by tazobactam. Although *Enterobacter* spp., which are known to produce class 1 chromosomal β-lactamases, were not included in this study, in a multicentre study which evaluated 978 *E. aerogenes* and 1 789 *E. cloacae* isolates, their susceptibility rates to piperacillin/tazobactam were 70.7% and 69% respectively.[6] The fourth-generation cephalosporins, cefepime and cefpirome, were also highly active against the Enterobacteriaceae, a finding confirmed in previous reports.[11,12]

Multiply-resistant strains of *P. aeruginosa* and *Acinetobacter* spp. were included in the study. *P. aeruginosa* typically does not produce the plasmid-mediated β-lactamases susceptible to tazobactam/clavulanic acid, or may be resistant to penicillin-β-lactamase inhibitor combinations based on impermeability.[6,7,10] However, piperacillin itself has good antipseudomonal activity, and this was observed with piperacillin/tazobactam. Cefpirome has been reported to be less active than ceftazidime against *P. aeruginosa*, which was also observed in this study.[11] In accordance with other reports, cefepime and ciprofloxacin were the most active agents against the *P. aeruginosa* isolates,[12] and the carbapenems most active against *Acinetobacter* spp.[12,13] When the activity of the carbapenems, biapenem and imipenem was compared in respect of all the Gram-negative bacilli tested, biapenem was at least as active as and often more active than imipenem, a finding which is in accordance with previous reports.[13]

Tazobactam and clavulanic acid have previously been shown to enhance the activity of β-lactams against β-lactamase-producing anaerobic Gram-negative bacilli.[3,14] Although cefoxitin-resistant isolates were not included in this study, the MICs of these sensitive strains were similar to those previously reported, with the carbapenems being the most active agents.[13-15]

Conclusion

Piperacillin/tazobactam may be included in the group of agents useful against most Gram-negative pathogens that cause nosocomial infections in South African patients.

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