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International Journal of Antimicrobial Agents 29 (2007) 145–152

INTERNATIONAL JOURNAL OF Antimicrobial Agents

www.ischemo.org

In vitro activities of various piperacillin and sulbactam combinations against bacterial pathogens isolated from Intensive Care Units in Taiwan: SMART 2004 programme data

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Received 29 December 2005; accepted 13 February 2006

Abstract

We investigated the in vitro activity of various piperacillin and sulbactam combinations against Gram-negative bacterial isolates from Intensive Care Units (ICUs) in Taiwan. Antimicrobial susceptibility testing of 1030 bacterial isolates recovered from ICUs of nine major teaching hospitals was performed using the agar dilution method. Sulbactam was added to piperacillin either at a fixed sulbactam concentration of 4 mg/L and 8 mg/L or at a piperacillin:sulbactam ratio of 2:1 and 4:1. Piperacillin/sulbactam at a ratio of 2:1 or a fixed 8 mg/L concentration of sulbactam had better activities against *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Serratia marcescens* than other piperacillin/sulbactam formulations. For *Pseudomonas aeruginosa*, piperacillin/sulbactam (2:1 or 4:1 ratios) had MIC_{90} values (minimum inhibitory concentration for 90% of the organisms) of 64 mg/L (>90% susceptibility) compared with 64 mg/L for cefoperazone/sulbactam (68% susceptibility) and 128 mg/L for piperacillin/tazobactam (82% susceptibility). For *Acinetobacter baumannii*, both piperacillin/sulbactam (either 2:1 ratio or a fixed 8 mg/L sulbactam) and cefoperazone/sulbactam were the most potent agents. Adding sulbactam to piperacillin resulted in increased susceptibility rates among piperacillin-resistant *P. aeruginosa* (53–57% in either 2:1 or 4:1 ratios) and *A. baumannii* (38–46% in either 2:1 ratio or a fixed 8 mg/L concentration of sulbactam) isolates. Results of susceptibility tests with piperacillin/sulbactam are dependent on the method used. Piperacillin/sulbactam combinations possessed better in vitro activities than piperacillin alone or piperacillin/tazobactam against *P. aeruginosa* and *A. baumannii*.

Keywords: Piperacillin; Sulbactam; Piperacillin/tazobactam; *P. aeruginosa*; *A. baumannii*

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doi:10.1016/j.ijantimicag.2006.02.017

1. Introduction

The β-lactam antibiotics are widely used owing to their reliable clinical efficacy and safety. However, bacteria develop resistance to β-lactam antibiotics by a variety of mechanisms, including the production of β-lactamases [1]. One strategy that has been devised for circumventing resistance mediated by β-lactamases is to combine the β-lactam agent with a β-lactamase inhibitor [2,3]. These β-lactamase inhibitors include clavulanic acid, sulbactam and tazobactam. Sulbactam is a penicillanic sulfone obtained by oxidation of the thiazolidine sulphur of penicillanic acid and is currently combined with ampicillin or cefoperazone in clinical use [4]. More recently, because of its specific in vitro activity against *Acinetobacter* species, sulbactam has been approved in some countries (Germany, Australia, Switzerland, China and Taiwan) as a single substance to be combined with piperacillin or other β-lactam antibiotics [5–7]. The combination of piperacillin and tazobactam has been demonstrated to be highly active against pathogens associated with nosocomial infections and is also active against many piperacillin-resistant isolates of staphylococci, Enterobacteriaceae and *Bacteroides* spp. [8]. The in vitro activity and in vivo efficacy of sulbactam in combination with piperacillin at a fixed sulbactam concentration or a fixed piperacillin:sulbactam ratio against nosocomial pathogens, especially *Acinetobacter* spp., have been evaluated [9–12].

The objective of this study was to investigate the antimicrobial activity of sulbactam and piperacillin alone, cefoperazone/sulbactam and piperacillin/tazobactam, as well as piperacillin in combination with different ratios or concentrations of sulbactam against Gram-negative bacterial pathogens isolated from nine Intensive Care Units (ICUs) from nine major teaching hospitals in Taiwan. This study was part of the Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART) 2004 programme.

2. Materials and methods

2.1. Hospitals

A nationwide surveillance programme (SMART) involving major teaching hospitals has tracked the trends in antimicrobial resistance in Taiwan annually since 2000. From August to December 2003, nine medical centres located in the northern, central and southern regions of Taiwan participated in this study. The nine hospitals were National Taiwan University Hospital, Far Eastern Memorial Hospital, Taipei Municipal Wan-Fang Hospital, Cheng Hsin Rehabilitation Medical Center and Cardinal Tien Hospital in northern Taiwan; Taichung Veterans General Hospital and China Medical College Hospital in central Taiwan; and National Cheng-Kung University Hospital and Kaohsiung Veterans General Hospital in southern Taiwan.

2.2. Bacterial isolates

A total of 1030 consecutive, non-duplicate isolates associated with various nosocomial infections were collected, including *Escherichia coli* ($n = 160$), *Klebsiella pneumoniae* ($n = 162$), *Enterobacter cloacae* ($n = 75$), *Morganella morganii* ($n = 33$), *Citrobacter freundii* ($n = 12$), *Serratia marcescens* ($n = 68$), *Proteus mirabilis* ($n = 64$), *Stenotrophomonas maltophilia* ($n = 85$), *Pseudomonas aeruginosa* ($n = 164$), *Acinetobacter baumannii* ($n = 167$) and *Burkholderia cepacia* ($n = 40$). Among these isolates, 206 (20%) were recovered from blood specimens and the rest were from sputum, bronchial washings, urine, wound pus and bile samples. The isolates were submitted to National Taiwan University Hospital for identification confirmation by colonial morphology, biochemical reactions, Vitek ID cards (bioMérieux, Hazelwood, MO) and the Phoenix System (Becton Dickinson, Cockeysville, MD). Isolates were stored at −70 °C prior to susceptibility testing.

2.3. Antimicrobial agents

The following antimicrobial agents were provided by their manufacturers for use in this study: ceftazidime (GlaxoSmithKline, Greenford, UK); cefotaxime (Aventis Pharma, Romainville, France); cefoperazone/sulbactam (Pfizer Inc., New York, NY); piperacillin and piperacillin/tazobactam (Wyeth-Ayerst, Pearl River, NY); and sulbactam (TTY Biopharm Company Ltd., Taiwan).

2.4. Susceptibility testing

Minimum inhibitory concentrations (MICs) were determined for all of the isolates using the agar dilution method according to the guidelines established by the National Committee for Clinical Laboratory Standards (NCCLS) [13]. The isolates were grown overnight on trypticase soy agar plates supplemented with 5% sheep blood (BBL Microbiology Systems, Cockeysville, MD) at 37 °C. Bacterial inocula were prepared by suspending the freshly grown bacteria in sterile normal saline and adjusting to a 0.5 McFarland standard. Using a Steers replicator, an organism density of 10^4 colony-forming units/spot was inoculated onto the appropriate plates with various concentrations of antimicrobial agents ranging from 0.03 mg/L to 128 mg/L and plates were incubated at 35 °C for 20–24 h in ambient air. The presence of extended-spectrum β-lactamase (ESBL) phenotype among *E. coli* and *K. pneumoniae* isolates was determined by subjecting isolates with cefotaxime or ceftazidime MICs ≥ 2 mg/L to the ESBL confirmation method using the following four antimicrobial disks: cefotaxime, cefotaxime/clavulanic acid, ceftazidime and ceftazidime/clavulanic acid (BBL Microbiology Systems). The results were interpreted based on NCCLS criteria [13].

Piperacillin/tazobactam with a fixed concentration of tazobactam (4 mg/L) and cefoperazone/sulbactam with a

Table 1
Susceptibility of bacterial pathogens isolated from nine Intensive Care Units in Taiwan to sulbactam, piperacillin, cefoperazone/sulbactam, piperacillin/tazobactam and various concentrations of piperacillin/sulbactam [a]

Microorganism (no. of isolates)/Antimicrobial agent	MIC (mg/L)			No. (%) of isolates		
	Range	MIC_{50}	MIC_{90}	Susceptible	Intermediate	Resistant
Escherichia coli, ESBL-producers (22)						
PIP	32 to >128	>128	>128	0 (0)	3 (14)	19 (86)
SUL	32 to >128	32	128	–	–	–
PIP/TAZ	1 to >128	2	32	19 (86)	1 (5)	2 (9)
CFP/SUL	2 to >61	16	>64	12 (55)	4 (18)	6 (27)
PIP/SUL (2:1)	4 to >128	16	64	11 (50)	9 (41)	2 (9)
PIP/SUL (4:1)	4 to >128	32	128	5 (23)	12 (55)	5 (23)
PIP/SUL (4)	2 to >128	64	>128	6 (27)	7 (32)	9 (41)
PIP/SUL (8)	1 to >128	16	>128	11 (50)	4 (18)	7 (32)
Escherichia coli, non-ESBL-producers (138)						
PIP	0.25 to >128	32	>128	51 (37)	51 (37)	36 (26)
SUL	8 to >128	32	64	–	–	–
PIP/TAZ	0.03 to >128	2	16	124 (90)	7 (5)	7 (5)
CFP/SUL	0.03 to >64	1	16	125 (91)	9 (7)	4 (3)
PIP/SUL (2:1)	0.25 to >128	8	64	112 (81)	19 (14)	7 (5)
PIP/SUL (4:1)	0.5 to >128	8	64	106 (77)	20 (14)	12 (9)
PIP/SUL (4)	0.25 to >128	2	128	105 (76)	13 (9)	20 (14)
PIP/SUL (8)	0.03 to >128	2	64	112 (81)	12 (9)	14 (10)
Klebsiella pneumoniae, ESBL-producers (43)						
PIP	32 to >128	>128	>128	0 (0)	2 (5)	41 (95)
SUL	4 to >128	128	>128	–	–	–
PIP/TAZ	2 to >128	64	>128	18 (42)	4 (9)	21 (49)
CFP/SUL	1 to >64	32	>64	17 (40)	11 (26)	15 (35)
PIP/SUL (2:1)	4 to >128	64	>128	13 (30)	14 (33)	16 (37)
PIP/SUL (4:1)	8 to >128	64	>128	6 (14)	17 (40)	20 (47)
PIP/SUL (4)	0.03 to >128	>128	>128	7 (16)	0 (0)	36 (84)
PIP/SUL (8)	0.03 to >128	>128	>128	13 (30)	1 (2)	29 (67)
Klebsiella pneumoniae, non-ESBL-producers (119)						
PIP	1 to >128	4	128	101 (85)	4 (3)	14 (12)
SUL	32 to >128	32	64	–	–	–
PIP/TAZ	0.03 to >128	4	8	112 (94)	2 (2)	5 (4)
CFP/SUL	0.06 to >64	0.25	4	114 (96)	1 (1)	4 (3)
PIP/SUL (2:1)	1 to >128	4	32	106 (89)	6 (5)	7 (6)
PIP/SUL (4:1)	0.5 to >128	4	64	105 (88)	5 (4)	9 (8)
PIP/SUL (4)	0.25 to >128	2	64	105 (88)	2 (2)	12 (10)
PIP/SUL (8)	0.06 to >128	2	64	106 (89)	2 (2)	11 (9)
Enterobacter cloacae (75)						
PIP	1 to >128	16	>128	39 (52)	10 (13)	26 (35)
SUL	32 to 128	64	128	–	–	–
PIP/TAZ	0.5 to >128	4	128	55 (73)	10 (13)	10 (13)
CFP/SUL	0.03 to >64	4	64	56 (75)	9 (12)	10 (13)
PIP/SUL (2:1)	1 to 128	4	64	55 (73)	19 (25)	1 (1)
PIP/SUL (4:1)	1 to 128	8	64	52 (69)	19 (25)	4 (5)
PIP/SUL (4)	0.5 to >128	4	>128	52 (69)	7 (9)	16 (21)
PIP/SUL (8)	0.5 to >128	4	>128	55 (73)	7 (9)	13 (17)
Morganella morganii (33)						
PIP	0.5 to >128	2	64	24 (73)	6 (18)	3 (9)
SUL	64 to 128	128	128	–	–	–
PIP/TAZ	0.12 to >128	5	2	32 (97)	0 (0)	1 (3)
CFP/SUL	1 to 64	2	8	32 (97)	0 (0)	1 (3)
PIP/SUL (2:1)	0.25 to 64	1	4	32 (97)	1 (3)	0 (0)
PIP/SUL (4:1)	0.25 to 128	1	8	32 (97)	0 (0)	1 (3)
PIP/SUL (4)	0.12 to >128	0.5	4	32 (97)	0 (0)	1 (3)
PIP/SUL (8)	0.12 to >128	0.5	1	32 (97)	0 (0)	1 (3)
Citrobacter freundii (12)						
PIP	2 to 128	64	>128	5 (42)	2 (17)	5 (42)
SUL	32 to >128	32	128	–	–	–
PIP/TAZ	2 to >128	4	>128	8 (67)	0 (0)	4 (33)
CFP/SUL	0.5 to >64	1	>64	9 (75)	1 (8)	2 (17)

Table 1 (*Continued*)

Microorganism (no. of isolates)/Antimicrobial agent	MIC (mg/L)			No. (%) of isolates		
	Range	MIC_{50}	MIC_{90}	Susceptible	Intermediate	Resistant
PIP/SUL (2:1)	2 to 128	4	128	6 (50)	2 (17)	4 (33)
PIP/SUL (4:1)	2 to >128	8	>128	6 (50)	2 (17)	4 (33)
PIP/SUL (4)	1 to >128	2	>128	6 (50)	1 (8)	5 (42)
PIP/SUL (8)	1 to >128	2	>128	6 (50)	1 (8)	5 (42)
Serratia marcescens (68)						
PIP	1 to >128	32	>128	32 (47)	19 (28)	17 (25)
SUL	4 to >128	128	128	–	–	–
PIP/TAZ	1 to >128	8	64	43 (63)	23 (34)	2 (3)
CFP/SUL	0.25 to >64	8	>64	41 (60)	13 (19)	14 (21)
PIP/SUL (2:1)	1 to 128	8	32	50 (74)	16 (24)	2 (3)
PIP/SUL (4:1)	1 to 128	16	64	45 (66)	19 (28)	4 (6)
PIP/SUL (4)	0.03 to >128	8	>128	44 (65)	11 (16)	13 (19)
PIP/SUL (8)	0.03 to >128	8	128	50 (74)	10 (15)	8 (12)
Proteus mirabilis (64)						
PIP	0.25 to >128	8	>128	36 (56)	19 (30)	9 (14)
SUL	32 to >128	128	128	–	–	–
PIP/TAZ	0.25 to 16	0.5	1	64 (100)	0 (0)	0 (0)
CFP/SUL	0.5 to 32	2	16	62 (97)	2 (3)	0 (0)
PIP/SUL (2:1)	0.25 to 64	2	8	61 (95)	3 (5)	0 (0)
PIP/SUL (4:1)	0.25 to 128	2	16	61 (95)	2 (3)	1 (2)
PIP/SUL (4)	0.25 to >128	1	4	60 (94)	2 (3)	2 (3)
PIP/SUL (8)	0.12 to >128	1	4	62 (97)	0 (0)	2 (3)
Stenotrophomonas maltophilia (85)						
PIP	8 to >128	>128	>128	1 (1)	2 (2)	82 (96)
SUL	32 to >128	>128	>128	–	–	–
PIP/TAZ	8 to >128	>128	>128	1 (1)	7 (8)	77 (91)
CFP/SUL	16 to >64	64	>64	8 (9)	18 (21)	59 (69)
PIP/SUL (2:1)	8 to >128	>128	>128	1 (1)	11 (13)	73 (86)
PIP/SUL (4:1)	8 to >128	>128	>128	1 (1)	11 (13)	73 (86)
PIP/SUL (4)	8 to >128	>128	>128	1 (1)	4 (5)	80 (94)
PIP/SUL (8)	8 to >128	>128	>128	1 (1)	8 (9)	76 (89)
Pseudomonas aeruginosa (164)						
PIP	2 to >128	8	>128	134 (82)	–	30 (18)
SUL	2 to 32	>128	>128	–	–	–
PIP/TAZ	2 to >128	8	128	135 (82)	–	29 (18)
CFP/SUL	2 to >64	16	64	112 (68)	17 (10)	35 (21)
PIP/SUL (2:1)	2 to >128	8	64	151 (92)	–	13 (8)
PIP/SUL (4:1)	2 to 128	8	64	150 (91)	–	14 (9)
PIP/SUL (4)	2 to >128	8	128	138 (84)	–	26 (16)
PIP/SUL (8)	2 to >128	8	128	141 (86)	–	23 (14)
Acinetobacter baumannii (167)						
PIP	8 to >128	>128	>128	35 (21)	22 (13)	110 (66)
SUL	0.5 to >128	4	32	–	–	–
PIP/TAZ	0.03 to >128	128	>128	54 (32)	19 (11)	94 (56)
CFP/SUL	2 to >64	16	64	95 (57)	49 (29)	23 (14)
PIP/SUL (2:1)	1 to >128	8	64	96 (57)	62 (37)	9 (5)
PIP/SUL (4:1)	2 to >128	16	128	85 (51)	61 (37)	21 (13)
PIP/SUL (4)	0.03 to >128	0.06	>128	87 (52)	5 (3)	75 (45)
PIP/SUL (8)	0.03 to >128	0.03	>128	95 (57)	8 (5)	64 (38)
Burkholderia cepacia (40)						
PIP	2 to 128	8	16	38 (95)	1 (3)	1 (3)
SUL	4 to >128	32	64	–	–	–
PIP/TAZ	0.03 to >128	4	8	37 (93)	2 (5)	1 (3)
CFP/SUL	4 to >64	32	64	9 (23)	15 (38)	16 (40)
PIP/SUL (2:1)	2 to 128	8	16	38 (95)	1 (3)	1 (3)
PIP/SUL (4:1)	2 to 128	8	16	37 (93)	2 (5)	1 (3)
PIP/SUL (4)	0.03 to 128	8	16	38 (95)	1 (3)	1 (3)
PIP/SUL (8)	0.03 to 128	4	16	38 (95)	1 (3)	1 (3)

MIC, minimum inhibitory concentration (MIC_{50} and MIC_{90}, MIC for 50% and 90% of the organisms, respectively); PIP, piperacillin; SUL, sulbactam; TAZ, tazobactam; CFP, cefoperazone; ESBL, extended-spectrum β-lactamase.

[a] At a fixed piperacillin:sulbactam ratio (2:1 or 4:1) or a fixed sulbactam concentration (4 μg/mL or 8 μg/mL).

concentration ratio of 2:1 were tested. For piperacillin/sulbactam combinations, a fixed sulbactam concentration of either 4 mg/L or 8 mg/L and two fixed piperacillin:sulbactam concentration ratios (2:1 and 4:1) were used. Breakpoints for interpreting susceptibilities of piperacillin and piperacillin/tazobactam followed NCCLS guidelines [13]. For various combinations of piperacillin and sulbactam, interpretation of susceptibility was in accordance with the interpretive breakpoints of piperacillin (susceptible ≤64 mg/L and resistant ≥128 mg/L for *P. aeruginosa*; and susceptible ≤16 mg/L, intermediate 32–64 mg/L and resistant ≥ 128 mg/L for the other species tested).

Regular quality assurance was performed among the isolates with American Type Culture Collection (ATCC) strains: *E. coli* ATCC 25922 and ATCC 35218, *K. pneumoniae* ATCC 700603 and *P. aeruginosa* ATCC 27853. Isolates were classified as susceptible, intermediate or resistant according to NCCLS criteria [13].

3. Results

The in vitro activities of sulbactam and piperacillin alone, cefoperazone/sulbactam and piperacillin/tazobactam, as well as sulbactam in combination with piperacillin against the 1030 bacterial isolates from ICUs are shown in Table 1. Piperacillin alone had poor activities against bacteria tested in this study except for *P. aeruginosa* and *B. cepacia* (>80% susceptibility). Cefoperazone/sulbactam had potent activities (97% susceptibility) against *M. morganii* and *P. mirabilis* isolates. Sulbactam had poor activities against all isolates tested except for *A. baumannii* (MIC_{50} and MIC_{90}, 4 mg/L and 32 mg/L, respectively). The agents tested had almost no activity against *S. maltophilia*. Except for cefoperazone/sulbactam, the agents tested were all active against *B. cepacia* isolates.

ESBL phenotype was found in 22 (14%) isolates of *E. coli* and 43 (27%) isolates of *K. pneumoniae*. None of the ESBL-producing *E. coli* and *K. pneumoniae* isolates were susceptible to piperacillin alone, but 86% and 42%, respectively, were susceptible to piperacillin/tazobactam. With the addition of sulbactam (2:1 ratio or 8 mg/L sulbactam), 50% of the ESBL-producing *E. coli* isolates and 30% of the ESBL-producing *K. pneumoniae* isolates became susceptible.

Fig. 1 shows the susceptibility rates to piperacillin/tazobactam and various combinations of piperacillin and sulbactam among piperacillin-resistant Enterobacteriaceae isolates. In vitro activity of piperacillin/tazobactam was better than all combinations of piperacillin and sulbactam against Enterobacteriaceae except for *S. marcescens*, for which piperacillin/sulbactam combinations were more active, particularly with a fixed 8 mg/L concentration of sulbactam or a 2:1 ratio.

Piperacillin/sulbactam had better activities than piperacillin/tazobactam both against *P. aeruginosa* and *A. baumannii* (Fig. 2). More than 50% of piperacillin-resistant *P. aeruginosa* isolates ($n = 30$) were susceptible to piperacillin/sulbactam (2:1 or 4:1 ratio); however, their MIC distribution was 32–64 mg/L. By contrast, although ≥38% of piperacillin-resistant *A. baumannii* isolates ($n = 132$) were susceptible to any combination of piperacillin/sulbactam, 36% and 41% of these isolates exhibited MICs of ≤0.03 mg/L at a fixed 4 mg/L or 8 mg/L sulbactam concentration, respectively (Fig. 3). The bimodal distribution of MICs was more prominent with a fixed 4 mg/L or 8 mg/L of sulbactam than that of piperacillin/sulbactam at ratios of 2:1 or 4:1.

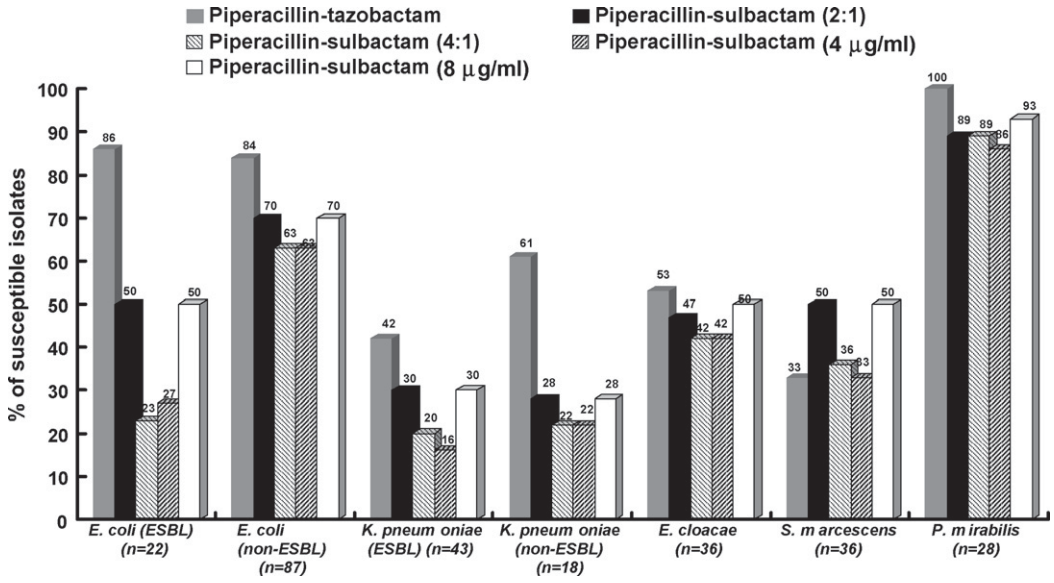

Fig. 1. Susceptibility rates of piperacillin-resistant Enterobacteriaceae isolates to piperacillin/tazobactam and various combinations of piperacillin and sulbactam at a fixed piperacillin:sulbactam ratio (2:1 or 4:1) or a fixed sulbactam concentration (4 μg/mL or 8 μg/mL). ESBL, extended-spectrum β-lactamase.

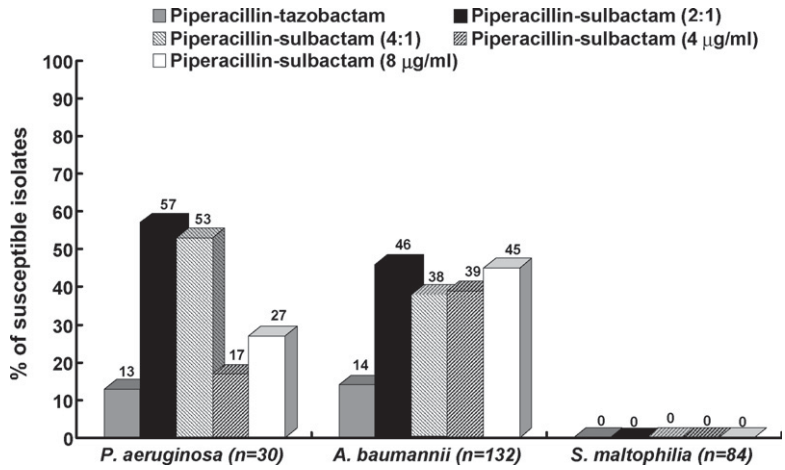

Fig. 2. Susceptibility rates of piperacillin-resistant *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* isolates to piperacillin/tazobactam and various combinations of piperacillin and sulbactam at a fixed piperacillin:sulbactam ratio (2:1 or 4:1) or a fixed sulbactam concentration (4 μg/mL or 8 μg/mL).

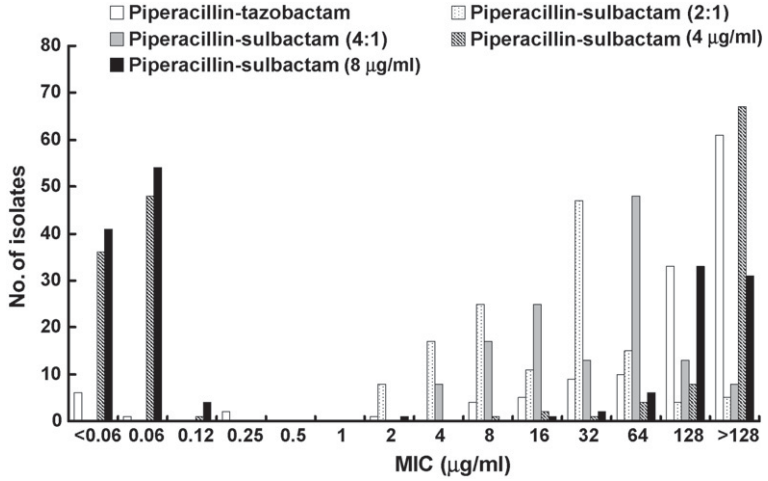

Fig. 3. Minimum inhibitory concentration (MIC) distribution of piperacillin/tazobactam and various combinations of piperacillin and sulbactam at a fixed piperacillin:sulbactam ratio (2:1 or 4:1) or a fixed sulbactam concentration (4 μg/mL or 8 μg/mL) against 132 piperacillin-resistant *Acinetobacter baumannii* isolates.

4. Discussion

This study revealed several important points. First, among the single agents and combinations tested, piperacillin in combination with sulbactam had the most potent activity against *A. baumannii* isolates. Adding sulbactam to piperacillin decreased the MICs of piperacillin by ca. 40%. These MICs were below the susceptibility breakpoints (≤16 mg/L) of piperacillin-resistant *A. baumannii* and 40% of isolates had a decrease in MIC to ≤0.03 mg/L at a fixed 4 mg/L or 8 mg/L of sulbactam. Second, piperacillin in combination with sulbactam, particularly a fixed ratio of 2:1 or 4:1, had in vitro activities that were slightly higher than those of piperacillin alone and piperacillin/tazobactam against *P. aeruginosa* isolates. These observations are partly in accordance with findings by previous investigators [9,10].

At present, sulbactam is the only β-lactamase inhibitor available as a freely combinable single substance. In addition to being a weaker inducer of group I β-lactamases than clavulanic acid [2,14], it is unique from other β-lactamase inhibitors for its high level of antimicrobial activity against *Bacteroides fragilis* and *Acinetobacter* spp. [15–17]. Sulbactam showed degrees of activity against β-lactamase-producing members of the Enterobacteriaceae family which were different from tazobactam [18]. A higher therapy success rate of sulbactam compared with piperacillin/tazobactam was found in a recent study of paediatric cancer patients with febrile neutropenia, and a comparable clinical success rate of sulbactam compared with piperacillin/tazobactam was found in the treatment of community-acquired respiratory and urinary tract infections [12,19]. Piperacillin/sulbactam could be a suitable replacement for piperacillin/tazobactam in the therapy of

bacterial infections caused by susceptible nosocomial pathogens [12,19].

Although the effectiveness of combinations of antimicrobial agents has been the subject of several studies [20–22], establishing the optimal sulbactam concentration or ratio to piperacillin in such formulations will require further study. Although doses of 0.375 g tazobactam and 0.5 g sulbactam provided similar pharmacokinetic profiles in humans [23,24], in testing with sulbactam combinations the NCCLS guidelines require a ratio of β-lactam to sulbactam of 2:1, but require a fixed tazobactam concentration of 4 mg/L when testing with piperacillin/tazobactam. It has been suggested that the maintenance of a critical ratio between the components is essential for optimal bactericidal activity. However, according to an in vitro study [25], the antibacterial activity of drug–inhibitor combinations when dosed at their currently recommended ratios is more dependent on the pharmacokinetics of the inhibitor than on that of the β-lactam drug. The antibacterial activity of combinations appeared to be lost when the amount of inhibitor available fell below a critical concentration that varied depending upon the type and amount of enzyme produced as well as the specific inhibitor used.

In this study, comparison of the MICs obtained with piperacillin/sulbactam at a fixed 4:1 ratio with those obtained at a fixed 2:1 ratio revealed that the sulbactam component of the MIC_{90} remained constant at both ratios for most strains, including ESBL-producing *E. coli*, *E. cloacae*, *C. freundii* and *S. marcescens*, whereas the piperacillin component consistently differed by two-fold between the two ratios. This suggests that the sulbactam component may be more important than the piperacillin component for determining the MIC at these ratios, and that some minimal critical concentration of sulbactam was identified by tests using both of the fixed dose ratios. Because the NCCLS breakpoints for the interpretation of susceptibility to ampicillin and piperacillin are 8 mg/L and 16 mg/L, respectively, testing sulbactam combinations at the 2:1 ratio of β-lactam to sulbactam recommended by the NCCLS would be comparable with testing a fixed concentration of sulbactam of 4 mg/L and 8 mg/L, respectively, although the latter method usually gave lower MIC_{50} but higher MIC_{90} values [13].

Previous in vitro and in vivo studies of a β-lactam antibiotic and sulbactam combination on bacteria producing ESBLs showed variable responses [6,7,11,26–34]. In this study, piperacillin/sulbactam at a ratio of 2:1 significantly decreased the rate of ESBL-producing non-susceptible *E. coli* from 100% to 50% and decreased the MIC_{50} from >128 mg/L to 16 mg/L compared with piperacillin alone. The difference in response between *E. coli* and *K. pneumoniae* might be dependent upon the type and amount of enzymes produced, although we did not perform molecular typing. Since therapeutic options for multiresistant Enterobacteriaceae are limited, combinations of piperacillin and sulbactam appear to represent an important alternative for treating infections caused by ESBL-producing Enterobacteriaceae.

In conclusion, piperacillin/sulbactam combinations possessed better in vitro activities than piperacillin alone or piperacillin/tazobactam against *P. aeruginosa* and *A. baumannii*. Further clinical studies to determine the optimal formulation of piperacillin and sulbactam combinations for in vitro testing to provide the best correlation with therapeutic outcome are warranted.

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