In vitro activities of piperacillin or cefoperazone alone and in combination with β-lactamase inhibitors against Gram-negative bacilli

Han-Yueh Kuo[1], Fu-Der Wang[2], Yung-Feng Yen[3], Mei-Lin Lin[2], Cheng-Yi Liu[2]

[1]Division of Infectious Diseases, Department of Medicine, Hsin-Chu General Hospital, Department of Health, Hsinchu, Taiwan;
[2]Division of Infectious Diseases, Department of Medicine, Taipei Veterans General Hospital and National Yang-Ming University, Taipei, Taiwan;
[3]Division of Infectious Diseases, Department of Medicine, Taipei Municipal Yangming Hospital, Taipei, Taiwan

SUMMARY

We investigated in vitro activities of piperacillin or cefoperazone alone and in combination with β-lactamase inhibitors against Gram-negative bacilli. Piperacillin/tazobactam 8:1 lowered resistance for *Escherichia coli*, *Serratia marcescens*, *Enterobactaer cloacae*, *Klebsiella pneumoniae* and imipenem-susceptible *Acinetobacter baumannii*. When piperacillin was combined with sulbactam 2:1 or 4:1, resistance against *E. coli*, *S. marcescens*, *E. cloacae*, extended spectrum β-lactamase (ESBL)-*K. pneumoniae* and *A. baumannii* were reduced. MIC_{90} of cefoperazone against *S. marcescens*, *E. cloacae*, ESBL-*K. pneumoniae* and *A. baumannii* were >128 mg/L. Addition of sulbactam 1:1 or 2:1 enhanced antimicrobial activities. Addition of sulbactam to piperacillin or cefoperazone enhanced antimicrobial activities of GNB.

KEY WORDS: Cefoperazone, Gram-negative bacilli, In vitro activities, β-Lactamase inhibitors, Piperacillin

Received April 28, 2008 *Accepted* June 12, 2008

INTRODUCTION

The β-lactam antibiotics, including penicillins, cephalosporins, carbapenems and monobactams, were widely used because of reliable clinical efficacy, safety and their spectrum of activity. However, bacteria develop resistance to β-lactam antibiotics by a variety of mechanisms, including the production of β-lactamase (Medeiros, 1997). The β-lactamase is capable of hydrolyzing the amide bond of the β-lactam ring and thus inactivate the antibiotic (Livermore, 1995; Sensakovic and Smith, 1995). Inducible β-lactamases have developed in most Gram-negative bacteria (Joly-Guillou *et al.*, 1995; Mealey, 2001), which are important causes of nosocomial infection. 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, which has been developed (Mealey, 2001; Miller *et al.*, 2001; Mohanty *et al.*, 2005).

The present study was carried out to evaluate the in vitro activity of β-lactam antibiotics alone, β-lactam antibiotic/tazobactam and β-lactam antibiotics/sulbactam combination.

Corresponding author
Fu-Der Wang
Division of Infectious Diseases
Department of Medicine
Taipei Veterans General Hospital, No. 201
Sec. 2, Shih-Pai Road, Taipei, Taiwan
E-mail: fdwang@vghtpe.gov.tw

MATERIALS AND METHODS

Organisms tested were isolated from various clinical specimens. They included 50 strains of *Escherichia coli*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Steno-*

TABLE 1 - *In vitro activities of clinical isolates to β-lactam antibiotics.*

Microorganism/Drugs[a]	*MIC*[b] *(mg/L)*			*No. (%) of isolates*
	Range	*MIC50*[c]	*MIC*$_{90}$[d]	*Resistant*
Escherichia coli				
PIP	0.5 to >128	32	>128	14 (28)
PIP/taz (8:1)	0.5-128	4	32	1 (2)
PIP/sul (2:1)	0.5-128	8	64	2 (4)
PIP/sul (4:1)	0.5 to >128	8	64	3 (6)
CAZ	0.03 to >128	0.12	8	4 (8)
FEP	<0.015-128	0.03	0.25	2 (4)
IMP	0.03-0.5	0.12	0.25	0 (0)
CFP	0.06 to >128	1	16	2 (4)
CFP/sul (1:1)	0.03-64	0.5	8	1 (2)
CFP/sul (2:1)	0.03-128	1	8	1 (2)
Serratia marcescens				
PIP	1 to >128	64	>128	22 (44)
PIP/taz (8:1)	1-128	16	128	6 (12)
PIP/sul (2:1)	1-128	16	64	2 (4)
PIP/sul (4:1)	2-128	32	128	6 (12)
CAZ	0.25-64	0.5	16	4 (8)
FEP	0.06-128	0.25	32	5 (10)
IMP	0.06-1	0.5	0.5	0 (0)
CFP	1 to >128	8	>128	21 (42)
CFP/sul (1:1)	0.5-128	8	32	3 (6)
CFP/sul (2:1)	1 to >128	8	64	9 (18)
Pseudomnas aeruginosa				
PIP	4 to >128	8	64	4 (8)
PIP/taz (8:1)	4 to >128	4	64	2 (4)
PIP/sul (2:1)	2-128	4	32	1 (2)
PIP/sul (4:1)	4-128	8	64	1 (2)
CAZ	0.5 to >128	2	32	5 (10)
FEP	0.25-32	2	16	2 (4)
IMP	1-32	2	4	1 (2)
CFP	2 to >128	4	64	7 (14)
CFP/sul (1:1)	1-128	4	32	1 (2)
CFP/sul (2:1)	2-128	4	32	2 (4)
Enterobacter cloacae				
PIP	2 to >128	32	>128	20 (40)
PIP/taz (8:1)	1 to >128	4	64	3 (6)
PIP/sul (2:1)	1-128	4	64	1 (2)
PIP/sul (4:1)	1-128	4	64	4 (8)
CAZ	0.25 to >128	1	>128	20 (40)
FEP	0.03-128	0.12	16	3 (6)
IMP	0.06-2	0.25	1	0 (0)
CFP	0.25 to >128	2	>128	18 (36)
CFP/sul (1:1)	0.12-64	1	32	1 (2)
CFP/sul (2:1)	0.12-64	1	32	3 (6)
Stenotrophomonas maltophilia				
PIP	32 to >128	>128	>128	49 (98)
PIP/taz (8:1)	16 to >128	>128	>128	48 (96)
PIP/sul (2:1)	16 to >128	>128	>128	45 (90)
PIP/sul (4:1)	16 to >128	>128	>128	46 (92)
CAZ	2 to >128	64	>128	32 (64)

TABLE 1 - *In vitro activities of clinical isolates to β-lactam antibiotics (continued).*

Microorganism/Drugs[a]	*MIC*[b] *(mg/L)*			*No. (%) of isolates*
	Range	*MIC50*[c]	*MIC$_{90}$*[d]	*Resistant*
FEP 2-128	32	64		42 (84)
IMP	>128	>128	>128	50 (100)
CFP	4 to >128	128	>128	32 (64)
CFP/sul (1:1)	2 to >128	32	128	15 (30)
CFP/sul (2:1)	2 to >128	32	>128	24 (48)
Klebsiella pneumoniae, ESBL[e]-producers				
PIP	128 to >128	>128	>128	50 (100)
PIP/taz (8:1)	2 to >128	32	64	3 (6)
PIP/sul (2:1)	4 to >128	16	64	2 (4)
PIP/sul (4:1)	8 to >128	64	128	9 (18)
CAZ	64 to >128	>128	>128	50 (100)
FEP	2 to >128	16	64	14 (28)
IMP	0.03-2	0.12	0.25	0 (0)
CFP	16 to >128	128	>128	38 (76)
CFP/sul (1:1)	2 to >128	16	32	4 (8)
CFP/sul (2:1)	2 to >128	32	64	15 (30)
Klebsiella pneumoniae, non-ESBL-producers				
PIP	4 to >128	8	>128	8 (16)
PIP/taz (8:1)	2-128	2	16	1 (2)
PIP/sul (2:1)	2-128	2	16	2 (4)
PIP/sul (4:1)	2 to >128	4	32	2 (4)
CAZ	0.03 to >128	0.25	32	4 (8)
FEP	0.015-16	0.06	4	0 (0)
IMP	0.03-1	0.12	0.25	0 (0)
CFP	0.12 to >128	0.5	64	5 (10)
CFP/sul (1:1)	0.06-32	0.25	4	0 (0)
CFP/sul (2:1)	0.12-32	0.5	8	0 (0)
Acinetobacter baumannii, imipenem-resistant isolates				
PIP	64 to >128	>128	>128	43 (86)
PIP/taz (8:1)	16 to >128	>128	>128	38 (76)
PIP/sul (2:1)	4-128	32	64	3 (6)
PIP/sul (4:1)	8 to >128	64	128	11 (22)
CAZ	16 to >128	128	>128	49 (98)
FEP	16 to >128	32	>128	41 (82)
IMP	16-128	32	64	50 (100)
CFP	128 to >128	>128	>128	50 (100)
CFP/sul (1:1)	1-64	8	32	4 (8)
CFP/sul (2:1)	2 to >128	32	64	12 (24)
Acinetobacter baumannii, imipenem-susceptible isolates				
PIP	16 to >128	64	>128	22 (44)
PIP/taz (8:1)	4 to >128	16	128	5 (10)
PIP/sul (2:1)	1-64	4	32	0 (0)
PIP/sul (4:1)	2-64	4	64	0 (0)
CAZ	2 to >128	4	128	12 (24)
FEP	1 to >128	4	16	4 (8)
IMP	0.12-2	0.25	2	0 (0)
CFP	32 to >128	64	>128	32 (64)
CFP/sul (1:1)	0.5-16	1	16	0 (0)
CFP/sul (2:1)	1-32	2	32	0 (0)

[a]Drugs: PIP (piperacillin), taz (tazobactam), sul (sulbactam), CAZ (ceftazidime), FEP (cefepime), IMP (imipenem), CFP (cefoperazone). [b]MIC: minimum inhibitory concentration. [c]MIC50: MIC for 50% of the organisms. [d]MIC90: MIC for 90% of the organisms. [e]ESBL: extended-spectrum β-lactamase.

trophomonas maltophilia, extended spectrum β-lactamase (ESBL) - *Klebsiella pneumoniae*, non-ESBL - *K. pneumoniae*, imipenem-resistant *Acinetobacter baumannii* and imipenem-susceptible *A. baumannii*.

The following antimicrobial agents were tested in this study: piperacillin (Wyeth-Ayerst, Pearl River, NY), tazobactam (Wyeth-Ayerst, Pearl River, NY), sulbactam (USP, MD, USA), ceftazidime (GSK, PA, USA), cefepime (Bristol-Myers Squibb, Princeton, NJ), imipenem (Merck, Whitehouse Station, NJ), cefoperazone (USP, MD, USA).

The minimum inhibitory concentration (MIC) of each antibiotic against each isolate was determined by the agar dilution method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2006a) . These antibiotics were tested alone and in combination with tazobactam at the ratio of 8:1, sulbactam at the ratio of 1:1, 2:1 or 4:1. Using a replicate-inoculating device, an organism density of 10^4 colony-forming units/spot was inoculated onto the appropriate plates with various concentrations of antimicrobial agents ranging from 0.015 to 128 mg/L. The agar plates were examined after incubation at 35^0C for 18-24 hours. *E. coli* ATCC 25922 and 35218, *P. aeruginosa* ATCC 27853 were used as control strains. The MIC was determined as the lowest concentration of antimicrobial agent visibly inhibiting the growth of the organism on the agar. The MIC_{90} and MIC_{50} were defined as the MIC at which ≥90% and ≥50% of the isolates were inhibited.

The break points for interpreting resistance to antibiotics were ≥128 mg/L for piperacillin, 32 mg/L for ceftazidime, 64 mg/L for cefoperazone, 32 mg/L for cefepime and 16 mg/L for imipenem (CLSI, 2006b).

RESULTS

The in vitro activities of β-lactam alone and in combination with tazobactam or sulbactam are shown in Table 1, Table 2 summarizes the statistical analysis. Piperacillin showed high MICs against most isolates with MIC_{90} >128 mg/L, except *P. aeruginosa* (MIC_{90} 64 mg/L). Addition of tazobactam lowered the resistant rates significantly for *E. coli*, *S. marcescens*, *E. cloacae*, *K. pneumoniae* and imipenem-susceptible *A. baumannii*. When piperacillin was combined with sulbactam, the resistant rates against *E. coli*, *S. marcescens*, *E. cloacae*, ESBL-*K. pneumoniae* and *A. baumannii* were significantly reduced using both antibiotic ratios. Piperacillin/sulbactam 2:1 also exhibited better in vitro activities against most isolates than piperacillin/sulbactam 4:1.

Ceftazidime showed high MICs against *E. cloacae*, *S. maltophilia*, ESBL-*K. pneumoniae* and imipenem-resistant *A. baumannii* with MIC_{90} >128 mg/L. Cefepime was active activities against most strains with MIC_{90} 0.25-32 mg/L but *S. maltophilia*, ESBL - *K. pneumoniae* and imipenem-resistant *A. baumannii* were less susceptible. Imipenem was also active activities with MIC_{90} 0.25-4 mg/L for most isolates except *S. maltophilia* (MIC_{90} >128 mg/L) and imipenem-resistant *A. baumannii* (MIC_{90} 64 mg/L). The agents tested had almost poor activity against *S. maltophilia*.

MIC_{90} of cefoperazone against *S. marcescens*, *E. cloacae*, ESBL - *K. pneumoniae* and *A. baumannii* were >128 mg/L. Addition of sulbactam 1:1 or 2:1 all enhanced the antimicrobial activities significantly, particularly against *A. baumannii*.

DISCUSSION

Tazobactam and sulbactam were the competitive, irreversible inhibitors of the wide variety of β-lactamase produced by common Gram-positive and Gram-negative aerobes and anaerobes. Currently, these two β-lactamase inhibitors (tazobactam and sulbactam) are in clinical use, and in combination with β-lactam antibiotics represent a successful strategy to combat a specific resistant mechanism (Finegold, 1999; Miller *et al.*, 2001). Tazobactam is active against a broad spectrum of plasmid and chromosomally mediated enzymes and has minimal ability to induce class I chromosomally mediated β-lactamase enzymes (Gutmann *et al.*, 1986). Against plasmid-mediated β-lactamases the potency of tazobactam is greater than that of sulbactam (Bush *et al.*, 1993; Sanders and Sanders, 1996).

The combination of tazobactam with piperacillin results in an antimicrobial agent with enhanced activity against most β-lactamase-producing organisms (Higashitani *et al.*, 1990). In our study,

piperacillin/tazobactam 8:1 also exhibited excellent antimicrobial activities against most clinical strains studied. Piperacillin/sulbactam combination was also studied to exhibit the active activities against most Gram-negative organisms (Lister *et al.*, 1997; Seewald, 2000; Frank *et al.*, 2003; Hung *et al.*, 2007) and piperacillin/sulbactam 2:1 had better activities against most Gram-negative bacteria than piperacillin/sulbactam 4:1 (Hung *et al.*, 2007). In our study, piperacillin/sulbactam also presented activities against clinical isolates of most Gram-negative organisms studied, particularly piperacillin/sulbactam 2:1.

The present study demonstrated that in vitro piperacillin/tazobactam 8:1 exhibited greater in vitro activity against *E. coli* than piperacillin/sulbactam 2:1 or 4:1. This finding corresponds to data provided by other investigators (Lister *et al.*, 1997 Schubert and Ullmann, 2000).

As demonstrated previously (Seewald, 2000;

TABLE 2 - *Statistical analysis of resistance.*

Drugs[a] *Microorganism*	*PIP*	*PIP/taz (8:1)*	*PIP/sul (2:1)*	*PIP/sul (4:1)*	*CFP*	*CFP/sul (1:1)*	*CFP/sul (2:1)*
Escherichia coli							
OR[b]	1.00	19.06***	9.33**	6.09**	1.00	2.04	2.04
95% CI[c]	–	2.42-405.91	1.84-63.67	1.47-29.06	–	0.14-58.95	0.14-58.96
Serratia marcescens							
OR	1.00	5.76***	18.86***	5.76***	1.00	11.34***	3.3*
95% CI	–	1.90-18.27	3.83-125.79	1.90-18.27	–	2.84-52.74	1.21-9.14
Pseudomnas aeruginosa							
OR	1.00	2.09	4.26	4.26	1.00	7.98	3.91
95% CI	–	0.31-17.36	0.42-103.95	0.42-103.96	–	0.92-179.48	0.69-28.92
Enterobacter cloacae							
OR	1.00	10.44***	32.67***	7.67***	1.00	27.56***	8.81***
95% CI	–	2.61-48.63	4.24-686.72	2.17-29.63	–	3.56-581.08	2.18-41.25
Stenotrophomonas maltophilia							
OR	1.00	2.04	5.44	4.26	1.00	4.15**	1.93
95% CI	–	0.14-58.95	0.58-127.99	0.42-103.95	–	1.66-10.50	0.80-4.65
Klebsiella pneumoniae, ESBL[d]-producers							
OR	1.00	–***	–***	–***	1.00	36.42***	7.39***
95% CI	–	–	–	–	–	9.74-150.41	2.80-19.96
Klebsiella pneumoniae, non-ESBL-producers							
OR	1.00	9.33*	4.57	4.57	1.00	–	–
95% CI	–	1.10-207.10	0.83-33.15	0.83-33.16	–	–	–
Acinetobacter baumannii, imipenem-resistant isolates							
OR	1.00	1.94	96.24***	21.78***	1.00	–***	–***
% CI	–	0.63-6.15	20.45-534.72	6.94-72.08	–	–	–
Acinetobacter baumannii, imipenem-susceptible isolates							
OR	1.00	7.07***	–***	–***	1.00	–***	–***
95% CI	–	2.19-24.27	–	–	–	–	–

[a]Drugs: PIP (piperacillin), taz (tazobactam), sul (sulbactam), CAZ (ceftazidime), FEP (cefepime), IMP (imipenem), CFP (cefoperazone). [b]OR: odds ratio. [c]CI: confidence interval. [d]ESBL: extended-spectrum β-lactamase. *P<0.05. **P<0.01. ***P<0.001.

Frank *et al.*, 2003; Hung *et al.*, 2007), piperacillin alone, piperacillin/tazobactam and piperacillin/sulbctam presented almost the same degree of activity against *P. aeruginosa*.

Many studies have investigated the in vitro activity of cefoperazone/sulbactam combination and shown it to be superior to that of cefoperazone alone against clinical isolates of many Gram-negative bacilli, particularly against *Acinetobacter* species (Fass *et al.*, 1990; Williams, 1997). An in vitro study showed that cefoperazone/sulbactam was also more active than some individual β-lactam agents against *Acinetobacter* species (Yamaguchi *et al.*, 1999). In our study, the in vitro activity of cefoperazone/sulbactam combination was significantly superior to that of cefoperazone alone against clinical isolates of most Gram-negative bacteria studied, particularly against *Acinetobacter* species. Piperacillin/sulbactam combination also exhibited active activities against imipenem-resistant and imipenem-susceptible *A. baumannii*, a finding also made by other investigators (Finegold, 1999; Seewald, 2000; Frank *et al.*, 2003). This can be explained by the intrinsic activity of sulbactam against *Acietobacter* species (Higgins *et al.*, 2004).

In this study, we can conclude that tazobactam and sulbactam could enhance the in vitro activities of piperacillin or cefoperazone against many resistant Gram-negative bacteria, the important causes of nosocomial infections. Although resistance to β-lactam antibiotics have developed, β-lactam antibiotic/β-lactamase inhibitor combinations can provide an approach to the widespread problem of antibiotic resistance due to β-lactamase-producing organisms and may represent an alternative treatment for these bacterial infections.

ACKNOWLEDGMENT

None of the collaborating authors has any commercial or other association that might pose a conflict of interest.

REFERENCES

Bush K., Macalintal C., Rasmussen B.A., Lee V.J., Yang Y. (1993). Kinetic interactions of tazobactam with β-lactamases from all major structural classes. *Antimicrob Agents Chemother.* **37**, 851-858.

Clinical and Laboratory Standards Institute. (2006). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically-seventh edition; approved standard M7-A7. Clinical and Laboratory Standards Institute.

Clinical and Laboratory Standards Institute. (2006). Performance standards for antimicrobial susceptibility testing; sixteenth informational supplement. Approved standard M100-S16. Clinical and Laboratory Standards Institute, Wayne, PA.

Fass R.J., Gregory W.W., D'Amato R.F., Matsen J.M., Wright D.N., Young L.S. (1990). In vitro activities of cefoperazone and sulbactam singly and in combination against cefoperazone-resistant members of the family Enterobacteriaceae and nonfermenters. *Antimicrob Agents Chemother.* **34**, 2256-2259.

Finegold S.M. (1999). In vitro efficacy of β-lactam/beta-lactamase inhibitor combinations against bacteria involved in mixed infections. *Int. J. Antimicrob. Agents.* **12** (Suppl 1), S9-14; S26-27.

Frank U., Mutter J., Schmidt-Eisenlohr E., Daschner F.D. (2003). Comparative in vitro activity of piperacillin, piperacillin-sulbactam and piperacillin-tazobactam against nosocomial pathogens isolated from intensive care patients. *Clin. Microbiol. Infect.* **9**, 1128-1132.

Gutmann L., Kitzis M.D., Yamabe S., Acar J.F. (1986). Comparative evaluation of a new β-lactamase inhibitor, YTR 830, combined with different β -lactam antibiotics against bacteria harboring known β-lactamases. *Antimicrob. Agents Chemother.* **29**, 955-957.

Higashitani F., Hyodo A., Ishida N., Inoue M., Mitsuhashi S. (1990). Inhibition of β-lactamases by tazobactam and in-vitro antibacterial activity of tazobactam combined with piperacillin. *J. Antimicrob. Chemother.* **25**, 567-574.

Higgins P.G., Wisplinghoff H., Stefanik D., Seifert H. (2004). In vitro activities of the β-lactamase inhibitors clavulanic acid, sulbactam, and tazobactam alone or in combination with β-lactams against epidemiologically characterized multidrug-resistant *Acinetobacter baumannii* strains. *Antimicrob. Agents Chemother.* **48**, 1586-1592.

Hung M.N., Hsueh P.R., Chang H.T., Lee W.S., Chou M.Y., Chen I.S., Wang J.H., Lin C.F., Shyr J.M., Ko W.C., Wu J.J., Liu Y.C., Huang W.K., Teng L.J., Liu C.Y., Luh K.T. (2007). In vitro activities of various piperacillin and sulbactam combinations against bacterial pathogens isolated from Intensive Care Unites in Taiwan: SMART 2004 programme data. *Int. J. Antimicrob. Agents.* **29**, 145-152.

Joly-Guillou M.L., Decré D., Herrman J.L., Bourdelier E., Bergogne-Bérézin E. (1995). Bactericidal in-vitro activity of β-lactams and β-lactamase inhibitors, alone or associated, against clinical strains of *Acinetobacter baumannii*: effect of

combination with aminoglycosides. *J. Antimicrob. Chemother.* **36**, 619-629.

LISTER P.D., PREVAN A.M., SANDERS C.C. (199). Importance of β-lactamase inhibitor pharmacokinetics in the pharmacodynamics of inhibitor-drug combinations: studies with piperacillin-tazobactam and piperacillin-sulbactam. *Antimicrob. Agents Chemother.* **41**, 721-727.

LIVERMORE D.M. (1995). β-Lactamases in laboratory and clinical resistance. *Clin. Microbiol. Rev.* **8**, 557-584.

MEALEY K.L. (2001). Penicillins and β-lactamase inhibitor combinations. *J. Am. Vet. Med. Assoc.* **218**, 1893-1896.

MEDEIROS A.A. (1997). Evolution and dissemination of β-lactamases accelerated by generations of β-lactam antibiotics. *Clin. Infect. Dis.* **24** (Suppl 1), S19-45.

MILLER L.A, RATNAM K., PAYNE D.J. (2001). β-Lactamase-inhibitor combinations in the 21st century: current agents and new developments. *Curr. Opin. Pharmacol.* **1**, 451-458.

MOHANTY S., SINGHAL R., SOOD S., DHAWAN B., DAS B.K., KAPIL A. (2005). Comparative in vitro activity of β -lactam/β-lactamase inhibitor combinations against gram negative bacteria. *Indian J. Med. Res.* **122**, 425-428.

SANDERS W.E. JR., SANDERS C.C. (1996). Piperacillin/tazobactam: a critical review of the evolving clinical literature. *Clin. Infect. Dis.* **22**, 107-123.

SCHUBERT S., ULLMANN U. (2000). Comparative bactericidal activity of piperacillin-tazobactam and peperacillin-sulbactam against piperacillin-resistant *E. coli* strains [abstract WeP85]. *Clin. Microbiol. Infect.* **6** (suppl 1), 84.

SEEWALD M. (2000). In-vitro activity of piperacillin alone and combined with sulbactam or tazobactam against hospital-derived pathogens [abstract WeP60]. *Clin. Microbiol. Infect.* **6** (suppl 1), 77.

SENSAKOVIC J.W., SMITH L.G. (1995). β-Lactamase inhibitor combinations. *Med. Clin. North. Am.* **79**, 695-704.

WILLIAMS J.D. (1997). β-Lactamase inhibition and in vitro activity of sulbactam and sulbactam/cefoperazone. *Clin. Infect. Dis.* **24**, 494-497.

YAMAGUCHI K., MATHAI D., BIEDENBACH D.J., LEWIS M.T., GALES A.C., JONES R.N. (1999). Evaluation of the in vitro activity of six broad-spectrum β-lactam antimicrobial agents tested against over 2,000 clinical isolates from 22 medical centers in Japan. Japan Antimicrobial Resistance Study Group. *Diagn. Microbiol. Infect. Dis.* **34**, 123-134.