Journal of Microbiology, Immunology and Infection (2018) 51, 207–213

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

Original Article

The impact of inoculum size on the activity of cefoperazone-sulbactam against multidrug resistant organisms

Ping-Chin Chang [a], Chi-Chung Chen [b,c], Ying Chen Lu [c], Chih-Cheng Lai [d], Hui-Ling Huang [e], Yin-Ching Chuang [a,b], Hung-Jen Tang [e,f,*]

[a] *Department of Internal Medicine, Chi Mei Medical Center, Liouying, Tainan, Taiwan*
[b] *Department of Medical Research, Chi Mei Medical Center, Tainan, Taiwan*
[c] *Department of Food Science, National Chiayi University, Chiayi, Taiwan*
[d] *Department of Intensive Care Medicine, Chi Mei Medical Center, Liouying, Tainan, Taiwan*
[e] *Department of Health and Nutrition, Chia-Nan University of Pharmacy and Science, Tainan, Taiwan*
[f] *Department of Medicine, Chi Mei Medical Center, Tainan, Taiwan*

Received 31 May 2017; received in revised form 27 August 2017; accepted 31 August 2017
Available online 6 October 2017

KEYWORDS
Cefoperazone-sulbactam;
Extended-spectrum β-lactamases;
Escherichia coli;
Klebsiella pneumoniae;
Inoculum size

Abstract *Objectives:* This study aims to assess the in vitro activity of cefoperazone alone and different cefoperazone-sulbactam ratios against different inoculum sizes of multidrug resistant organisms.
Methods: Minimum inhibitory concentrations (MICs) of cefoperazone, cefoperazone-sulbactam at fixed ratio of 1:1 and 2:1 against a normal inoculum size of 5×10^5 CFU/ml and a high inoculum size of 5×10^7 CFU/ml were measured.
Results: Each 33 isolates of extended-spectrum β-lactamases (ESBL)-producing *Escherichia coli*, ESBL-producing *Klebsiella pneumoniae*, carbapenem-resistant *E. coli*, and carbapenem-resistant *Pseudomonas aeruginosa* and a total of 122 isolates of carbapenem-resistant *Acinetobacter baumannii* were collected. After the addition of sulbactam at a 1:1 ratio, most MIC_{50} and MIC_{90} values decreased. Cefoperazone-sulbactam at a 1:1 ratio had a higher susceptibility rate against ESBL-producing *E. coli*, carbapenem-resistant *E.* coli, and carbapenem-resistant *A. baumannii* than cefoperazone-sulbactam at a 2:1 ratio (all $P < 0.05$). For ESBL-producing *E. coli*, the susceptibility rate of cefoperazone-sulbactam at ratios of (1:1) and (2:1) decreased from 97.0 to 87.9% and 90.9 to 60.6%, for normal to high inoculum, respectively. For ESBL-producing *K. pneumoniae*, both susceptibility rate of cefoperazone-sulbactam at ratios of (1:1) and (2:1) decreased from 75.8%, and 63.6% at normal inoculum to 51.5% and 42.4% at high inoculum.

* Corresponding author. Department of Medicine, Chi Mei Medical Center, Tainan, Taiwan. Fax: +886 6 2832057.
E-mail address: 8409d1@gmail.com (H.-J. Tang).

https://doi.org/10.1016/j.jmii.2017.08.026

Conclusions: Cefoperazone-sulbactam at a 1:1 ratio has greater in vitro activity against most multidrug resistant organisms than cefoperazone-sulbactam at a 2:1 ratio. Such combinations were not influenced by the inoculum size of ESBL-producing *E. coli and K. pneumoniae* and could be a therapeutic option for treating severe infections.

Introduction

Antibiotic resistance has become one of the world's most pressing health issues, and its emergence has weakened the ability of antibiotics to kill pathogenic organisms.[1–5] Although β-lactam antibiotics, including penicillin, cephalosporins, monobactams, and carbapenem, remain the major weapon against bacteria because of their broad-spectrum activity, clinical efficacy and safety,[6–9] widespread use of β-lactam antibiotics has also lead to the development of resistance to these antibiotics. The production of β-lactamases is the major mechanism that causes acquired β-lactam antibiotic resistance[10]; thus, the use of β-lactamases inhibitors in combination with β-lactam antibiotics, such as piperacillin-tazobactam, amoxicillin-clavulanate, and cefoperazone-sulbactam have been developed to overcome this mechanism.[11]

Gram-negative pathogens, including Enterobacteriaceae, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*, are common pathogens that cause nosocomial infections, and these microorganisms carry the broad spectrum of the antibiotic resistance. For Enterobacteriaceae, the emergence of extended-spectrum β-lactamases (ESBL) among *Escherichia coli* and *Klebsiella pneumoniae* are the great threats to the management of infections. Most importantly, serious infections caused by ESBL-producing organisms result in higher mortality rates than non-ESBL producers, especially when the patients did not receive adequate antimicrobial therapy.[12–14] Recently, several studies showed the inoculum effects that the minimal inhibitory concentration (MIC) of an antibiotic would increase as well as the increasing number of the organisms in the inoculum.[15–19] This kind of laboratory phenomenon has been observed for several β-lactam antibiotics, such as piperacillin-tazobactam, amoxicillin-clavulanate, ceftriaxone, ertapenem and imipenem, against *E. coli* or *K. pneumoniae*.[15–19] In this study, the in vitro activity of cefoperazone-sulbactam against ESBL-producing *E. coli* and *K. pneumoniae* clinical isolates were investigated at an inoculum size of 5×10^5 CFU/ml and 5×10^7 CFU/ml. In addition, the in vitro activities of different cefoperazone-sulbactam compositions against ESBL-producing *E. coli*, ESBL-producing *K. pneumoniae*, carbapenem-resistant *E. coli*, carbapenem-resistant *P. aeruginosa*, and carbapenem-resistant *A. baumannii*, were also evaluated.

Materials and methods

Collection of clinical isolates

Thirty-three isolates of ESBL-producing *E. coli*, ESBL-producing *K. pneumoniae*, carbapenem-resistant *E. coli*, and carbapenem-resistant *P. aeruginosa* and 122 isolates of carbapenem-resistant *A. baumannii* were collected from sputum (n = 105), urine (n = 55), blood (n = 16), pus (n = 15), bile (n = 9), ascites (n = 5), and others (n = 8) from patients during the period of 2008–2015 by the department of bacteriology at Chi Mei Medical Center (Table 1). The isolates were stored at −80 °C in Protect Bacterial Preservers (Technical Service Consultants Limited, Heywood, UK) before use. ESBL phenotype among *E. coli* and *K. pneumoniae* isolates are confirmed by the method using the following four antimicrobial disks: cefotaxime, cefotaxime/clavulanic acid, ceftazidime and ceftazidime/clavulanic acid. An increase in the zone diameter by ≥5 mm for either antimicrobial agent tested in combination with clavulanic acid over when tested alone indicates that the isolate is an ESBL producer.[20] Carbapenem resistance is defined as resistant to imipenem, meropenem, doripenem, or ertapenem, and carbapenem-resistant phenotype among *P. aeruginosa* and *A. baumannii* are confirmed by the modified Hodge test. Species confirmation was performed by standard biochemical methods on a VITEK 2 automated system (bioMérieux, Marcy l'Etoile, France).

Table 1 The number of positive specimen for each bacterium.

Number of specimens	ESBL-producing *E. coli*	ESBL-producing *K. pneumoniae*	Carbapenem-resistant *E. coli*	Carbapenem-resistant *P. aeruginosa*	Carbapenem-resistant *A. baumannii*	Total
Blood	9	4	3	0	0	16
Urine	10	8	18	9	10	55
Sputum	4	14	6	22	100	146
Ascites	3	1	1	0	0	5
Bile	4	2	0	1	2	9
Pus	3	1	4	0	7	15
Others	0	3	1	1	3	8

Table 2 MIC range, MIC_{50} and MIC_{90} of cefoperazone alone, cefoperazone-sulbactam (1:1) and cefoperazone-sulbactam (2:1) against different organisms.

Organism	Cefoperazone			Cefoperazone-sulbactam (1:1)			Cefoperazone-sulbactam (2:1)		
	MIC range	MIC_{50}	MIC_{90}	MIC range	MIC_{50}	MIC_{90}	MIC range	MIC_{50}	MIC_{90}
ESBL-producing *E. coli* (n = 33)									
Normal inoculum	16～>64	>64	>64	1—64	8	16	1—64	8	16
High inoculum	>64	>64	>64	2—64	8	32	4～>64	16	32
ESBL-producing *K. pneumoniae* (n = 33)									
Normal inoculum	8～>64	64	>64	1～>64	4	64	2～>64	8	64
High inoculum	>64	>64	>64	4～>64	16	>64	4～>64	32	>64
Carbapenem-resistant *E. coli* in normal inoculum (n = 33)	16～>64	>64	>64	1～>64	16	>64	4～>64	32	>64
Carbapenem-resistant *P. aeruginosa* in normal inoculum (n = 33)	4～>64	>64	>64	4～>64	32	>64	4～>64	64	>64
Carbapenem-resistant *A. baumannii* complex in normal inoculum (n = 122)	64～>64	>64	>64	4～>64	16	32	8～>64	32	64

In vitro susceptibility

The minimum inhibitory concentrations (MICs) of the drugs were measured by broth microdilution in prepared Mueller-Hinton broth (Oxoid, Basingstoke, UK) with 25 μg/mL of calcium and 12.5 μg/mL of magnesium (CAMHB). All experiments were performed in triplicate and the microdilution trays were incubated at 35 °C for 16—20 h.[21] Standard powders of cefoperazone and sulbactam were provided by TTY (TTY Biopharm, Taipei, Taiwan), and MIC determinations and susceptibility interpretation criteria followed CLSI guidelines.[20,22] Antimicrobial susceptibilities were determined using broth microdilution MIC tests at a standard inoculum (5×10^5 CFU/ml) and at a high inoculum (5×10^7 CFU/ml) as previous study.[18] For both microorganisms, doubling dilutions of cefoperazone ranged from 0.25 to 64 mg/L and three different sets of dilutions were prepared. To one series of cefoperazone dilutions was tested without added sulbactam. To the second series contained cefoperazone combined with sulbactam in a 2:1 ratio (2 parts cefoperazone and 1 part sulbactam). The third series contained cefoperazone combined with sulbactam in a 1:1 ratio (1 part cefoperazone and 1 part sulbactam). Susceptibilities of cefoperazone alone, cefoperazone-sulbactam at a 1:1 and 2:1 were classified according to the MIC of cefoperazone ≤16 mg/L.[20] All experiments were performed in triplicate and the microdilution trays were incubated at 35 °C for 16—20 h. We used an ELISA reader for reading. Bacterial growth was detected former by optical density (ELISA reader, Epoch™ Microplate spectrophotometer—BioTek Instruments, Winooski, VT, USA). *E. coli* ATCC 25922, Kp ATCC 700603 were used as quality control strains.

Pulsed-field gel electrophoresis (PFGE)

PFGE was modified as described previously[21] with a CHEF DR II apparatus (Bio-Rad Laboratories, Hercules, Calif.). In brief, the DNA in the plugs was digested with restriction enzyme (Apa1 in *A. baumannii* and Spec1 in *P. aeruginosa*), and electrophoresis was performed in a 1% agarose gel (in 0.5× TBE [Tris-borate-EDTA] buffer). The electrophoretic conditions used were as follows: initial switch time, 5.0 s; final switch time, 35.0 s; run time, 19 h; gradient, 6 V/cm; angle, 120°; and temperature, 14 °C in *A. baumannii* and initial switch time, 5.0 s; final switch time, 40.0 s; run time, 21 h; gradient, 6 V/cm; angle, 120°; and temperature, 14 °C in *P. aeruginosa*. The bacteriophage lambda ladder pulsed-field grade (PFG) and low-range PFG markers were loaded onto all gels. The PFGE patterns were visually examined and interpreted according to the criteria of Tenover et al.[23] The similarities of the PFGE profiles of each strain were compared using a Dice coefficient at 1.0% of tolerance and 1% of optimization.

Statistical analysis

The two-tailed Fisher's exact test was used for analysis, and a P value of <0.05 was considered statistically significant.

Results

The MIC values of cefoperazone alone and in combination with sulbactam against ESBL-producing *E. coli*, ESBL-producing *K. pneumoniae*, carbapenem-resistant *E. coli*, carbapenem-resistant *P. aeruginosa*, and carbapenem-resistant *A. baumannii* are shown in Table 2. For cefoperazone alone, it showed high MICs against most of isolates with a MIC_{50} and MIC_{90} > 64 mg/L, except ESBL-producing *K. pneumoniae* (MIC_{50} = 64 mg/L). After the addition of sulbactam at a 1:1 ratio, all of MIC_{50} and most of MIC_{90} values decreased, except ESBL-producing *K. pneumoniae* at a high inoculum, carbapenem-resistant *E. coli* and carbapenem-resistant *P. aeruginosa* (MIC_{90} values remain >64 mg/L). Furthermore, we tested the different compositions of cefoperazone-sulbactam at a 2:1 ratio. Most of the MIC_{50} values were higher than cefoperazone-sulbactam

at a 1:1 ratio, except ESBL-producing *E. coli* at normal inoculum. For MIC_{90}, all of the values are same with cefoperazone-sulbactam at a 1:1 ratio, except carbapenem-resistant *A. baumannii* (increase from 32 mg/L to 64 mg/L). Finally, the increase of MIC values is more prominent for cefoperazone-sulbactam against ESBL-producing *K. pneumoniae* (2-fold increase in MIC_{50}) than ESBL-producing *E. coli* (1−2 fold increase of MIC_{50}).

Table 3 shows that the antibiotic susceptibility rate of cefoperazone alone and in combination with sulbactam against ESBL-producing *E. coli*, ESBL-producing *K. pneumoniae*, carbapenem-resistant *E. coli*, carbapenem-resistant *P. aeruginosa*, and carbapenem-resistant *A. baumannii*. ESBL-producing *E. coli* at high inoculum, ESBL-producing *K. pneumoniae* at high inoculum, and carbapenem-resistant *A. baumannii* are not susceptible to cefoperazone. In contrast, the susceptible rate of cefoperazone against carbapenem-resistant *P. aeruginosa* is highest (27.3%), followed by ESBL-producing *K. pneumoniae* at normal inoculum (9.1%), and ESBL-producing *E. coli* at normal inoculum (3.0%) and carbapenem-resistant *E. coli* (3%). After the addition of sulbactam at a 1:1 ratio, the susceptibility rates of cefoperazone-sulbactam (1:1) against most of the organisms have significantly increased, except carbapenem-resistant *P. aeruginosa*. While we compared the susceptibility rate of cefoperazone-sulbactam at different ratios (1:1 vs 2:1), we find that cefoperazone-sulbactam at a 1:1 ratio had a higher susceptibility rate against most of organisms, including ESBL-producing *E. coli*, ESBL-producing *K. pneumoniae*, carbapenem-resistant *E. coli*, and carbapenem-resistant *A. baumannii* than cefoperazone-sulbactam at a 2:1 ratio. Among them, the clinical significance was especially great for the high inoculum ESBL-producing *E. coli* (87.9% vs 60.6%), carbapenem-resistant *E. coli* (67.6% vs 44.1%) and carbapenem-resistant *A. baumannii* (68.0% vs 31.1%), respectively (all $P < 0.05$). In contrast, cefoperazone-sulbactam at a 2:1 ratio seems to have a higher susceptibility rate against carbapenem-resistant *P. aeruginosa* than cefoperazone-sulbactam at a 1:1 ratio. However, it did not reach statistical significance. For ESBL-producing *E. coli*, the susceptibility rate of cefoperazone-sulbactam (1:1) decreased from 97.0% at normal inoculum to 87.9% at high inoculum, but cefoperazone-sulbactam (2:1) significantly decreased from 90.9% at normal inoculum to 60.6% at high inoculum ($P < 0.05$). For ESBL-producing *K. pneumoniae*, both susceptibility rates of cefoperazone-sulbactam (1:1) and (2:1) decreased from 75.8%, and 63.6% at normal inoculum to 51.5% and 42.4% at high inoculum. However, these changes did not reach statistical significance.

Fig. 1 shows the cumulative distributions of MICs to cefoperazone alone and cefoperazone-sulbactam at a 1:1 and 2:1 ratio against ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae* at different inoculum sizes. For ESBL-producing *E. coli*, the MIC curves of cefoperazone-sulbactam (1:1) and cefoperazone-sulbactam (2:1) were close, in contrast to the rightward shift of the cefoperazone alone curve at normal and high inoculum sizes. Similar findings were also noted for ESBL-producing *K. pneumoniae* isolates. For ESBL-producing *K. pneumoniae* isolates, the amplitudes of the rightward shift of MIC curves different compositions of cefoperazone-sulbactam between at normal and at high inoculum size were larger than for ESBL-producing *E. coli* isolates. For the carbapenem-resistant *A. baumannii isolates* with different PFGE types (Supplemental Fig. 1), their MICs against cefoperazone was higher than against cefoperazone-sulbactam (1:1) (MIC range: 64−>64 mg/L vs 4−>32 mg/L; MIC_{50}: >64 mg/L vs 16 mg/L; MIC_{90}: >64 mg/L vs 32 mg/L).

Discussion

This study investigating the in vitro activities of different compositions of cefoperazone-sulbactam and cefoperazone

Table 3 Antibiotic susceptible rate of cefoperazone alone, cefoperazone-sulbactam (1:1) and cefoperazone-sulbactam (2:1) against different organisms.

Organism	Susceptible rate (%)		
	Cefoperazone	Cefoperazone-sulbactam (1:1)	Cefoperazone-sulbactam (2:1)
ESBL-producing *E. coli* (n = 33)			
Normal inoculum	3.0	97.0[c]	90.9[c]
High inoculum	0.0	87.9[c]	60.6[a,b,c]
ESBL-producing *K. pneumoniae* (n = 33)			
Normal inoculum	9.1	75.8[c]	63.6[c]
High inoculum	0	51.5[c]	42.4[c]
Carbapenem-resistant *E. coli* (n = 33)	3.0	67.6[c]	44.1[b,c]
Carbapenem-resistant *P. aeruginosa* (n = 33)	27.3	33.3	36.4
Carbapenem-resistant *A. baumannii* complex (n = 122)	0	68[c]	31.1[b,c]

[a] Compared with normal inoculum.
[b] Compared with cefoperazone-sulbactam (1:1).
[c] Compared with cefoperazone.
Susceptibilities of cefoperazone alone, cefoperazone-sulbactam at a 1:1 and 2:1 were classified according to the MIC of cefoperazone ≤ 16 mg/L.
Fisher's exact test was performed and $P < 0.05$ was considered significant.

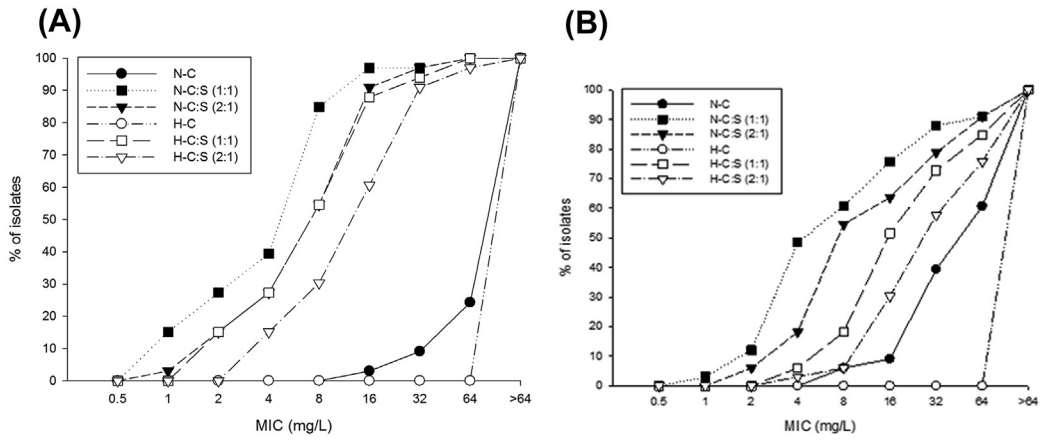

Figure 1. Cumulative distributions of minimum inhibitory concentrations (MICs) to cefoperazone alone and cefoperazone-sulbactam at a 1:1 and 2:1 ratio against ESBL-producing *E. coli* (A) and ESBL-producing *K. pneumoniae* (B) at normal (N) and high (H) inoculum sizes.

alone against various multi-drug resistant organisms has several significant findings. First of all, after the addition of sulbactam, the in vitro activities of cefoperazone against ESBL-producing *E. coli* and *K. pneumoniae* improved, including decreased MIC values and increased antibiotic-susceptibility rates. In our study, we found that 97% of 33 ESBL-producing *E. coli* and 75.8% of 33 ESBL-producing *K. pneumoniae* at normal inoculum were susceptible to cefoperazone-sulbactam at a 1:1 ratio. The good in vitro activity of cefoperazone-sulbactam have been shown in one recent Asia—Pacific Nations resistance surveillance program,[24] where 85.2% of 61 ESBL-producing *E. coli* strains and 74.3% of ESBL-producing *K. pneumoniae* strains were susceptible to cefoperazone-sulbactam. Another study[25] from India found that 94.7% of 57 ESBL-producing *E. coli* and 90% of 10 ESBL-producing *K. pneumoniae* were susceptible to cefoperazone-sulbactam (75/30 mcg). One other study[26] from Taiwan revealed that the resistance rate of cefoperazone-sulbactam against ESBL-producing *K. pneumoniae* was only 4 (for cefoperazone-sulbactam 1:1) and 15% (for cefoperazone-sulbactam 2:1). In contrast, the resistance rate of cefoperazone alone against ESBL-producing *K. pneumoniae* was 76%. For carbapenem-resistant organisms, such as carbapenem-resistant *E. c*oli, and carbapenem-resistant *A. baumannii*, cefoperazone-sulbactam showed better in vitro activities than cefoperazone alone in this study. However, for carbapenem-resistant *P. aeruginosa*, we found that cefoperazone does not show obvious enhanced activities after adding sulbactam. The ratio between cefoperazone and sulbactam does not seem to be influenced by the susceptibility rate to carbapenem-resistant *P aeruginosa*. One study showed the susceptibility rate of cefoperazone-sulbactam against carbapenem-resistant *E. coli* was 63.63% (7/11),[25] and another study showed the resistance rate of cefoperazone-sulbactam against imipenem-resistant *A. baumannii* was 8—24%.[26] Overall, our findings are consistent with previous study[27] in Taiwan that cefoperazone-sulbactam showed better in vitro activity against several multidrug-resistant Gram-negative bacteria, including ESBL-producing *E. coli* and *K. pneumoniae* and carbapenem-resistant *A. baumannii* isolates tested compared to cefoperazone alone. Therefore, sulbactam as an important β-lactamase inhibitor that can augment the activity of cefoperazone against ESBL-producing *E. coli* and *K. pneumoniae* and some carbapenem-resistant *E. coli* and *A. baumannii*, excluding carbapenem-resistant *P. aeruginosa*.

Second, in contrast to previous study[27] about cefoperazone-sulbactam, this study further showed that different compositions of cefoperazone-sulbactam (1:1 vs 2:1) have different in vitro activities against multi-drug resistant organisms. For most of clinical isolates in this study, we observed the trend that cefoperazone-sulbactam at a 1:1 ratio has better in vitro activity based on a higher antibiotic susceptibility rate and lower MIC values than cefoperazone-sulbactam at a 2:1 ratio. Moreover, these differences in antibiotic susceptibility rate between cefoperazone-sulbactam at a 1:1 and 2:1 ratio reach statistical significance for ESBL-producing *E. coli* at high inoculum, carbapenem-resistant *E. coli* and carbapenem-resistant *A. baumannii* (Table 2). In a study from Kuo et al.,[26] cefoperazone-sulbactam at a 1:1 ratio exhibited greater in vitro activity against *Serratia marcescens*, *P. aeruginosa*, *Enterobacter cloacae*, *Stenotrophomonas maltophilia*, and ESBL *K. pneumoniae* than cefoperazone-sulbactam at 2:1. Another study[28] showed similar findings that the resistance rate of cefoperazone-sulbactam at a 2:1 ratio was higher for *E. cloacae* (4% vs 0%), *S. marcescens* (21.6% vs 7.8%), imipenem-resistant *A. baumannii* (16.6% vs 10.0%), and ESBL-producing *K. pneumoniae* (30% vs 6%) than cefoperazone-sulbactam at 1:1. In summary, cefoperazone-sulbactam at 1:1 may pose greater in vitro activity against most gram-negative bacteria than cefoperazone-sulbactam at 2:1. However, further study is warranted to identify the most appropriate composition of cefoperazone-sulbactam with greatest potency.

Previous studies have assessed the effect of inoculum size on the MIC levels for several β-lactam antibiotics such as piperacillin-tazobactam, amoxicillin-clavulanate, ceftriaxone, ertapenem and imipenem against *E. coli* or *K. pneumoniae*.[15—19] For ESBL-producing *E. coli*, Wu et al.[29] determined that the inoculum size affected the activity

of ceftazidime, cefepime and cefotaxime against 35%, 85%, 100% of 80 ESBL-producing *E. coli* strains. In contrast, the inoculum size only affected the activity of piperacillin-tazobactam against 4 (5%) strains. For ESBL-producing *K. pneumoniae*, Harada et al.[17] found that the MIC_{50} of piperacillin-tazobactam, cefotaxime and cefepime increased eight-fold or more and meropenem increased two-fold, in addition to the increasing inoculum size. Our study is the first to evaluate whether there is change of MICs for cefoperazone and cefoperazone-sulbactam against ESBL-producing *E. coli* and *K. pneumoniae* between normal and high inoculum size. Based on our observations, increases in MICs, as well as the increasing inoculum size, are more prominent for cefoperazone-sulbactam against ESBL-producing *K. pneumoniae* (4-fold increase of MIC_{50}) than ESBL-producing *E. coli* (1—2-fold increase of MIC_{50}). Moreover, we found the antibiotic susceptibility rate of cefoperazone-sulbactam (1:1) against ESBL-producing *E. coli* remained high and is not affected by increasing inoculum size. Therefore, the relative stability and in vitro activity of cefoperazone-sulbactam (1:1), even under an increasing bacterial load of ESBL-producing *E. coli*, suggested that cefoperazone-sulbactam (1:1) may be the drug of choice against ESBL-producing *E. coli*, even in the high burden of bacterial load.

In conclusion, carbapenems have been used as the drug of choice in the treatment of ESBL-producing Enterobacteriaceae for years. However, the extensive use of carbapenems trend to induce the production of carbapenem resistant *A. baumannii*. Cefoperazone-sulbactam at a 1:1 ratio, which is a new combination, has greater in vitro activities against most multidrug resistant organisms than cefoperazone-sulbactam at 2:1. Such combinations were not influenced by the inoculum size of ESBL-producing *E. coli* and could be a therapeutic option for treating severe infections due to such pathogens and to avoid the overuse of carbapenems. Further clinical use is expected to document the real role of such a regimen in the future.

Conflict of interest disclosure

None to declare.

Financial disclosures

None.

Acknowledgments

None.

References

1. Grundmann H, Aires-de-Sousa M, Boyce J, Tiemersma E. Emergence and resurgence of meticillin-resistant *Staphylococcus aureus* as a public-health threat. *Lancet* 2006;**368**:874—85.
2. Pitout JD, Laupland KB. Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. *Lancet Infect Dis* 2008;**8**:159—66.
3. Liu HC, Hung YP, Lin HJ, Liu HC, Lee JC, Wu YH, et al. Antimicrobial susceptibility of clinical Enterobacteriaceae isolates at the emergency department in a regional hospital: a threat of extended spectrum beta-lactamase-producers among nursing home residents. *J Microbiol Immunol Infect* 2016;**49**:584—90.
4. Lee CM, Lai CC, Chiang HT, Lu MC, Wang LF, Tsai TL, et al. Presence of multidrug-resistant organisms in the residents and environments of long-term care facilities in Taiwan. *J Microbiol Immunol Infect* 2017;**50**:133—44.
5. Kao CY, Udval U, Huang YT, Wu HM, Huang AH, Bolormaa E, et al. Molecular characterization of extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella* spp. isolates in Mongolia. *J Microbiol Immunol Infect* 2016;**49**:692—700.
6. Jean SS, Lee WS, Yu KW, Liao CH, Hsu CW, Chang FY, et al. Rates of susceptibility of carbapenems, ceftobiprole, and colistin against clinically important bacteria collected from intensive care units in 2007: results from the Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART). *J Microbiol Immunol Infect* 2016;**49**(6):969—76.
7. Lee HS, Loh YX, Lee JJ, Liu CS, Chu C. Antimicrobial consumption and resistance in five Gram-negative bacterial species in a hospital from 2003 to 2011. *J Microbiol Immunol Infect* 2015;**48**:647—54.
8. Chang YY, Chuang YC, Siu LK, Wu TL, Lin JC, Lu PL, et al. Clinical features of patients with carbapenem nonsusceptible *Klebsiella pneumoniae* and *Escherichia coli* in intensive care units: a nationwide multicenter study in Taiwan. *J Microbiol Immunol Infect* 2015;**48**:219—25.
9. Lagace-Wiens P, Rubinstein E. Adverse reactions to beta-lactam antimicrobials. *Expert Opin Drug Saf* 2012;**11**:381—99.
10. Thomson JM, Bonomo RA. The threat of antibiotic resistance in Gram-negative pathogenic bacteria: beta-lactams in peril! *Curr Opin Microbiol* 2005;**8**:518—24.
11. Drawz SM, Bonomo RA. Three decades of beta-lactamase inhibitors. *Clin Microbiol Rev* 2010;**23**:160—201.
12. Paterson DL, Ko WC, Von Gottberg A, Mohapatra S, Casellas JM, Goossens H, et al. Antibiotic therapy for *Klebsiella pneumoniae* bacteremia: implications of production of extended-spectrum beta-lactamases. *Clin Infect Dis* 2004;**39**:31—7.
13. Peralta G, Lamelo M, Alvarez-Garcia P, Velasco M, Delgado A, Horcajada JP, et al. Impact of empirical treatment in extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella* spp. bacteremia. A multicentric cohort study. *BMC Infect Dis* 2012;**12**:245.
14. Tumbarello M, Spanu T, Sanguinetti M, Citton R, Montuori E, Leone F, et al. Bloodstream infections caused by extended-spectrum-beta-lactamase-producing *Klebsiella pneumoniae*: risk factors, molecular epidemiology, and clinical outcome. *Antimicrob Agents Chemother* 2006;**50**:498—504.
15. Docobo-Perez F, Lopez-Cerero L, Lopez-Rojas R, Egea P, Domínguez-Herrera J, Rodríguez-Baño J, et al. Inoculum effect on the efficacies of amoxicillin-clavulanate, piperacillin-tazobactam, and imipenem against extended-spectrum beta-lactamase (ESBL)-producing and non-ESBL-producing *Escherichia coli* in an experimental murine sepsis model. *Antimicrob Agents Chemother* 2013;**57**:2109—13.
16. Goldstein EJ, Citron DM, Cherubin CE. Comparison of the inoculum effect of cefoxitin and other cephalosporins and of beta-lactamase inhibitors and their penicillin-derived components on the *Bacteroides fragilis* group. *Antimicrob Agents Chemother* 1991;**35**:1868—74.
17. Harada Y, Morinaga Y, Kaku N, Nakamura S, Uno N, Hasegawa H, et al. In vitro and in vivo activities of piperacillin-tazobactam and meropenem at different inoculum sizes of ESBL-producing *Klebsiella pneumoniae*. *Clin Microbiol Infect* 2014;**20**:O831—9.

18. Lopez-Cerero L, Picon E, Morillo C, Hernández JR, Docobo F, Pachón J, et al. Comparative assessment of inoculum effects on the antimicrobial activity of amoxycillin-clavulanate and piperacillin-tazobactam with extended-spectrum beta-lactamase-producing and extended-spectrum beta-lactamase-non-producing *Escherichia coli* isolates. *Clin Microbiol Infect* 2010; **16**:132—6.
19. Tam VH, Ledesma KR, Chang KT, Wang TY, Quinn JP. Killing of *Escherichia coli* by beta-lactams at different inocula. *Diagn Microbiol Infect Dis* 2009;**64**:166—71.
20. National Committee for Clinical Laboratory Standards. *Performance standards for antimicrobial susceptibility testing, 9th informational supplement. M100—S24.* Wayne, PA: National Committee for Clinical Laboratory Standards; 2014.
21. Lai CC, Chen CC, Huang HL, Chuang YC, Tang HJ. The role of doxycycline in the therapy of multidrug-resistant *E. coli* — an in vitro study. *Sci Rep* 2016;**6**:31964.
22. Clinical and Laboratory Standards Institute. *Methods for dilution antimicrobial susceptibility testing of bacteria that grow aerobically.* Approved standard, CLSI document M7-A9. 8th ed. Wayne, PA: CLSI; 2012.
23. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995;**33**:2233—9.
24. Mendes RE, Mendoza M, Banga Singh KK, Castanheira M, Bell JM, Turnidge JD, et al. Regional resistance surveillance program results for 12 Asia-Pacific nations (2011). *Antimicrob Agents Chemother* 2013;**57**:5721—6.
25. Sood S. Comparative evaluation of the in-vitro activity of six beta-lactam/beta-lactamase inhibitor combinations against gram negative bacilli. *J Clin Diagn Res* 2013;**7**:224—8.
26. Kuo HY, Wang FD, Yen YF, Lin ML, Liu CY. In vitro activities of piperacillin or cefoperazone alone and in combination with beta-lactamase inhibitors against gram-negative bacilli. *New Microbiol* 2009;**32**:49—55.
27. Chiang TT, Tang HJ, Chiu CH, Chen TL, Ho MW, Lee CH, et al. Antimicrobial activities of cefoperazone-sulbactam in comparison to cefoperazone against clinical organisms from medical centers in Taiwan. *J Med Sci* 2016;**36**:229—33.
28. Wang FD, Lin ML, Lee WS, Liu CY. In vitro activities of beta-lactam antibiotics alone and in combination with sulbactam against Gram-negative bacteria. *Int J Antimicrob Agents* 2004; **23**:590—5.
29. Wu N, Chen BY, Tian SF, Chu YZ. The inoculum effect of antibiotics against CTX-M-extended-spectrum beta-lactamase-producing *Escherichia coli*. *Ann Clin Microbiol Antimicrob* 2014;**13**:45.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jmii.2017.08.026.