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Original Article

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



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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 MIC₅₀ and MIC₉₀ 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.

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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.

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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 led to the development of resistance to these antibiotics. The production of β -lactamases is the major mechanism that causes acquired β -lactam antibiotic resistance¹⁰; thus, the use of β -lactamase 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 <i>E. coli</i>	ESBL-producing <i>K. pneumoniae</i>	Carbapenem-resistant <i>E. coli</i>	Carbapenem-resistant <i>P. aeruginosa</i>	Carbapenem-resistant <i>A. baumannii</i>	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, MIC₅₀ and MIC₉₀ 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 <i>E. coli</i> (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 <i>K. pneumoniae</i> (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 <i>E. coli</i> in normal inoculum (n = 33)	16~>64	>64	>64	1~>64	16	>64	4~>64	32	>64
Carbapenem-resistant <i>P. aeruginosa</i> in normal inoculum (n = 33)	4~>64	>64	>64	4~>64	32	>64	4~>64	64	>64
Carbapenem-resistant <i>A. baumannii</i> 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 previously²¹ 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 MIC₅₀ and MIC₉₀ > 64 mg/L, except ESBL-producing *K. pneumoniae* (MIC₅₀ = 64 mg/L). After the addition of sulbactam at a 1:1 ratio, all of MIC₅₀ and most of MIC₉₀ values decreased, except ESBL-producing *K. pneumoniae* at a high inoculum, carbapenem-resistant *E. coli* and carbapenem-resistant *P. aeruginosa* (MIC₉₀ values remain >64 mg/L). Furthermore, we tested the different compositions of cefoperazone-sulbactam at a 2:1 ratio. Most of the MIC₅₀ values were higher than cefoperazone-sulbactam

at a 1:1 ratio, except ESBL-producing *E. coli* at normal inoculum. For MIC₉₀, 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 MIC₅₀) than ESBL-producing *E. coli* (1–2 fold increase of MIC₅₀).

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; MIC₅₀: >64 mg/L vs 16 mg/L; MIC₉₀: >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 <i>E. coli</i> (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 <i>K. pneumoniae</i> (n = 33)			
Normal inoculum	9.1	75.8 ^c	63.6 ^c
High inoculum	0	51.5 ^c	42.4 ^c
Carbapenem-resistant <i>E. coli</i> (n = 33)	3.0	67.6 ^c	44.1 ^{b,c}
Carbapenem-resistant <i>P. aeruginosa</i> (n = 33)	27.3	33.3	34.4
Carbapenem-resistant <i>A. baumannii</i> 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.

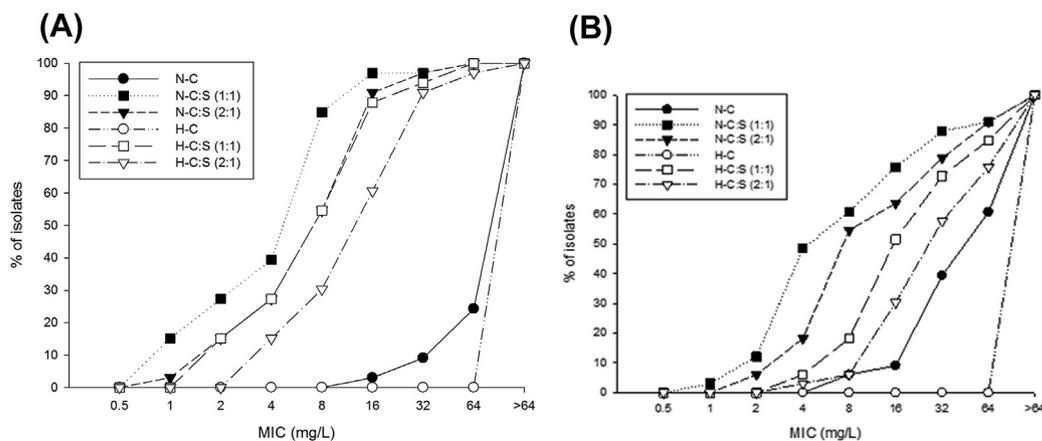


Figure 1. Cumulative distributions of minimum inhibitory concentrations (MICs) to cefoperazone alone and cefoperazone-sulbactam at a 1:1 and 2:1 ratio against ESBL-producing *E. coli* (A) and ESBL-producing *K. pneumoniae* (B) at normal (N) and high (H) inoculum sizes.

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 study²⁵ 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 study²⁶ 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. coli*, 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 study²⁷ 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 study²⁷ 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 study²⁸ 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 MIC₅₀ 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 MIC₅₀) than ESBL-producing *E. coli* (1–2-fold increase of MIC₅₀). 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

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Appendix A. Supplementary data

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