

Appropriate composites of cefoperazone–sulbactam against multidrug-resistant organisms

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Objectives: This study aims to assess the in vitro activity of different cefoperazone–sulbactam ratios against different multidrug-resistant organisms (MDROs).

Materials and methods: Minimum inhibitory concentrations (MICs) and susceptibility rates of cefoperazone, sulbactam and cefoperazone–sulbactam at fixed ratios of 2:1, 1:1 and 1:2 against 344 MDRO clinical isolates, including extended-spectrum β -lactamase (ESBL)-producing *Escherichia coli* (n=58), ESBL-producing *Klebsiella pneumoniae* (n=58), carbapenem-resistant *Enterobacteriaceae* (n=57), carbapenem-resistant *Pseudomonas aeruginosa* (n=49) and carbapenem-resistant *Acinetobacter baumannii* (n=122), were measured.

Results: Combined treatment with sulbactam and cefoperazone resulted in decreased MIC₅₀ values across all MDROs, as well as decreases in most MIC₉₀ values, except for carbapenem-resistant *Enterobacteriaceae* and carbapenem-resistant *P. aeruginosa* (MIC₉₀ values remained >64 mg/L). Susceptibility rates of treatment with cefoperazone alone against all MDROs were much lower than that of cefoperazone–sulbactam combination (all $P < 0.05$), except in carbapenem-resistant *P. aeruginosa*. Additionally, the susceptibility rate gradually increased as the ratio of cefoperazone–sulbactam was adjusted from 2:1 to 1:1 and to 1:2 for carbapenem-resistant *Enterobacteriaceae*, ESBL-producing *K. pneumoniae* and carbapenem-resistant *A. baumannii*. There were no significant ratio-dependent changes in susceptibility rates with cefoperazone–sulbactam in carbapenem-resistant *P. aeruginosa*.

Conclusion: Adding sulbactam enhances cefoperazone activity against most MDROs excluding carbapenem-resistant *P. aeruginosa*, and the activity of cefoperazone–sulbactam against these MDROs is greatest at a ratio of 1:2, followed by ratios of 1:1 and 2:1.

Keywords: cefoperazone–sulbactam, extended-spectrum β -lactamases, *Escherichia coli*, *Klebsiella pneumoniae*, multidrug-resistant organisms

Introduction

β -Lactam antibiotics, which include penicillin, cephalosporin, monobactam and carbapenem, are the most commonly used antibiotics in the world. However, the increased use of β -lactam antibiotics has led to the development of various types of antibiotic resistance, with the production of β -lactamases as one of the primary mechanisms.¹ Therefore, β -lactamase inhibitors, such as sulbactam, tazobactam, clavulanic acid, avibactam, relebactam and vaborbactam, have been developed and combined with β -lactam antibiotics to overcome this mechanism.^{2–5} To date, several β -lactam/ β -lactamase inhibitor antibiotics have been shown to exhibit synergistic in vitro activities against multidrug-resistant organisms (MDROs), including amoxicillin–clavulanate,

ampicillin–sulbactam, piperacillin–tazobactam, cefoperazone–sulbactam, ceftolozane–tazobactam, ceftazidime–avibactam and meropenem–vaborbactam.^{6–9} However, the ratio of β -lactam to β -lactamase inhibitor that exerts the greatest inhibitory activity against MDROs is not known, and it is unclear whether the present formula of β -lactam/ β -lactamase inhibitor is the best composite. Our previous study⁹ demonstrated that cefoperazone–sulbactam at a 1:1 ratio had a higher susceptibility rate against MDROs such as extended-spectrum β -lactamase (ESBL)-producing *Escherichia coli*, carbapenem-resistant *E. coli* and carbapenem-resistant *Acinetobacter baumannii*, compared with cefoperazone–sulbactam at a 2:1 ratio. In this study, we hypothesize that a higher ratio of sulbactam in the cefoperazone–sulbactam combined antibiotic may lead to greater in vitro activity against MDROs. Therefore, in this study, we test the efficacy of cefoperazone–sulbactam at ratios of 2:1, 1:1 and 1:2 against MDROs in vitro.

Materials and methods

Collection of clinical isolates

Clinical isolates of ESBL-producing *E. coli*, ESBL-producing *Klebsiella pneumoniae*, carbapenem-resistant Enterobacteriaceae, carbapenem-resistant *Pseudomonas aeruginosa* and carbapenem-resistant *A. baumannii* were collected during the period of 2008–2015. These isolates were obtained from the Department of Bacteriology at three hospitals including one medical center (1,273 beds), one regional hospital (876 beds) and one district hospital (263 beds). ESBL-producing *E. coli* and *K. pneumoniae* isolates were confirmed as previously described.¹⁰ Carbapenem resistance is classified as resistance to either imipenem, meropenem, doripenem or ertapenem.

In vitro susceptibility

The minimum inhibitory concentrations (MICs) of the drugs were measured by broth microdilution as described in a previous study.¹¹ Standard powders of cefoperazone and sulbactam were provided by TTY (TTY Biopharm, Taipei, Taiwan), and MIC and susceptibility interpretation criteria were determined according to previous guidelines.^{10,12} Doubling dilutions of cefoperazone ranged from 0.25 to 64 mg/L, and four different sets of dilutions were tested. The first series of cefoperazone dilutions were tested without added sulbactam. The second, third and fourth series contained cefoperazone combined with sulbactam at a 2:1 ratio (two parts cefoperazone to one part sulbactam), a 1:1 ratio (one part cefoperazone to one part sulbactam) and a 1:2 ratio (one part cefoperazone to two parts sulbactam). Finally, we tested sulbactam without cefoperazone. Susceptibilities of

cefoperazone alone and cefoperazone–sulbactam at 2:1, 1:1 and 1:2 ratios were determined using the criterion of MIC of cefoperazone ≤ 16 mg/L.¹¹ *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were treated as quality control strains.

Statistical analysis

A chi-squared test was used for the analysis, with *P*-values of <0.05 considered statistically significant.

Results

In this study, a total of 344 MDROs from clinical isolates, including ESBL-producing *E. coli* ($n=58$), ESBL-producing *K. pneumoniae* ($n=58$), carbapenem-resistant Enterobacteriaceae ($n=57$), carbapenem-resistant *P. aeruginosa* ($n=49$) and carbapenem-resistant *A. baumannii* ($n=122$), were enrolled for testing. The MIC values of cefoperazone alone and in combination with sulbactam against ESBL-producing *E. coli*, ESBL-producing *K. pneumoniae*, carbapenem-resistant Enterobacteriaceae, carbapenem-resistant *P. aeruginosa* and carbapenem-resistant *A. baumannii* are shown in Table 1. Cefoperazone alone showed high MICs against most isolates, with MIC₅₀ and MIC₉₀ >64 mg/L, except ESBL-producing *K. pneumoniae* (MIC₅₀=64 mg/L). MIC₅₀ values decreased for all of MDROs after the addition of sulbactam, and most MIC₉₀ values decreased, except of carbapenem-resistant Enterobacteriaceae and carbapenem-resistant *P. aeruginosa* (MIC₉₀ values remained >64 mg/L). We then tested the different combinations of cefoperazone–sulbactam at 2:1, 1:1 and 1:2 ratios. For ESBL-producing *E. coli*, *K. pneumoniae* and carbapenem-resistant *A. baumannii*, MIC₅₀ and MIC₉₀ values decreased as the ratio of cefoperazone–sulbactam changed from 2:1 to 1:1 and to 1:2. For carbapenem-resistant Enterobacteriaceae, only MIC₅₀ values decreased as the ratio of cefoperazone–sulbactam changed from 2:1 to 1:1 and to 1:2, with all of MIC₉₀ values >64 mg/L. For carbapenem-resistant *P. aeruginosa*, no significant change in MIC values was noted for various ratios of cefoperazone–sulbactam combinations.

Table 2 shows the antibiotic susceptibility rate of cefoperazone alone and in combination with different ratios of sulbactam against MDROs. The susceptibility rates of cefoperazone alone against all MDROs were much lower than cefoperazone–sulbactam combinations (all $P<0.05$), excluding carbapenem-resistant *P. aeruginosa*. For the different ratios of cefoperazone–sulbactam combinations, the susceptibility rate gradually increased as the ratio of cefoperazone–sulbactam was changed from 2:1 to 1:1 and to 1:2 for carbapenem-resistant Enterobacteriaceae, ESBL-producing *K. pneumoniae* and carbapenem-resistant *A. baumannii*. For

Table 1 MIC range, MIC₅₀ and MIC₉₀ of cefoperazone alone, cefoperazone–sulbactam (2:1), cefoperazone–sulbactam (1:1) and cefoperazone–sulbactam (1:2) against different drug-resistant organisms

MIC,	Carbapenem-resistant Enterobacteriaceae (n=57)	ESBL <i>Escherichia coli</i> (n=58)	ESBL <i>Klebsiella pneumoniae</i> (n=58)	Carbapenem-resistant <i>Pseudomonas aeruginosa</i> (n=49)	Carbapenem-resistant <i>Acinetobacter baumannii</i> (n=122)
Cefoperazone					
MIC ₅₀	>64	>64	64	>64	>64
MIC ₉₀	>64	>64	>64	>64	>64
Range	8–>64	16–>64	16–>64	4–>64	64–>64
Sulbactam					
MIC ₅₀	64	64	32	>64	16
MIC ₉₀	>64	>64	64	>64	32
Range	32–>64	32–>64	16–>64	8–>64	2–>64
Cefoperazone–sulbactam (2:1)					
MIC ₅₀	32	8	16	64	32
MIC ₉₀	>64	32	64	>64	64
Range	4–>64	2–>64	4–>64	4–>64	4–>64
Cefoperazone–sulbactam (1:1)					
MIC ₅₀	32	8	8	32	16
MIC ₉₀	>64	16	32	>64	32
Range	2–>64	1–>64	2–>64	4–>64	2–>64
Cefoperazone–sulbactam (1:2)					
MIC ₅₀	16	4	4	32	8
MIC ₉₀	>64	16	16	>64	16
Range	2–>64	0.5–64	1–64	2–>64	1–>64

Abbreviations: ESBL, extended-spectrum β-lactamase; MIC, minimum inhibitory concentration.

Table 2 Antibiotic susceptibility rates of cefoperazone alone, cefoperazone–sulbactam (2:1), cefoperazone–sulbactam (1:1) and cefoperazone–sulbactam (1:2) against different drug-resistant organisms

	Susceptibility rate (%)				
	Carbapenem-resistant Enterobacteriaceae (n=57)	ESBL <i>Escherichia coli</i> (n=58)	ESBL <i>Klebsiella pneumoniae</i> (n=58)	Carbapenem-resistant <i>Pseudomonas aeruginosa</i> (n=49)	Carbapenem-resistant <i>Acinetobacter baumannii</i> (n=122)
Cefoperazone	3.5	3.4	1.7	24.5	0.0
Cefoperazone–sulbactam (2:1)	33.3 ^a	84.5 ^a	67.2 ^a	30.6	41.0 ^a
Cefoperazone–sulbactam (1:1)	49.1 ^a	91.4 ^a	75.9 ^a	30.6	76.2 ^{a,b}
Cefoperazone–sulbactam (1:2)	68.4 ^{a,c}	91.4 ^a	89.7 ^{a,b}	34.7	92.6 ^{a,c}

Notes: Susceptibilities of cefoperazone alone and cefoperazone–sulbactam at 2:1, 1:1 and 1:2 ratios were classified according to the MIC of cefoperazone ≤16 mg/L. ^aP-value <0.05 compared to cefoperazone. ^bP-value <0.05 compared to cefoperazone–sulbactam (2:1). ^cP-value <0.05 compared to cefoperazone–sulbactam (1:1).

Abbreviations: ESBL, extended-spectrum β-lactamase; MIC, minimum inhibitory concentration.

these three MDROs, cefoperazone–sulbactam at a 1:2 ratio had a higher susceptibility rate than at a 2:1 ratio ($P<0.05$). For carbapenem-resistant Enterobacteriaceae and carbapenem-resistant *A. baumannii*, cefoperazone–sulbactam at a 1:2 ratio had a higher susceptibility rate than at a 1:1 ratio ($P<0.05$). For ESBL-producing *E. coli*, the susceptibility rates were the same for cefoperazone–sulbactam at 1:1 and 1:2 ratios, but both were higher than the susceptibility at a 2:1 ratio. For carbapenem-resistant *P. aeruginosa*, no significant changes in susceptibility rates were observed across different ratios of cefoperazone–sulbactam.

Discussion

This study investigated the in vitro activity of different ratios of cefoperazone–sulbactam and of cefoperazone alone against various MDROs and identified several significant findings. First, both MIC and antibiotic susceptibility tests show that the in vitro activity of cefoperazone against MDROs, even carbapenem-resistant *A. baumannii*, can be enhanced after adding sulbactam, with carbapenem-resistant *P. aeruginosa* being the only exception. This is consistent with a previous study by Kuo et al,¹³ which demonstrated that the addition of sulbactam to cefoperazone can significantly enhance the antimicrobial

activities against *Serratia marcescens*, *Enterobacter cloacae*, ESBL-*K. pneumoniae* and *A. baumannii*. In addition to Kuo et al's finding,¹³ our study showed this combination can also enhance the antibiotic activity against carbapenem-resistant Enterobacteriaceae and ESBL-*E. coli*. Overall, our study and several other in vitro studies^{9,13,14} indicated that the addition of sulbactam can improve cefoperazone's activity against MDR-Enterobacteriaceae and *A. baumannii*. However, further study will be needed to see if the result on planktonic bacteria also applies to biofilm-embedded bacteria, which is more likely to correspond to clinical antibiotic failure.

Second, the impact of sulbactam on the activity of cefoperazone-sulbactam against MDROs varies according to the ratio of sulbactam and the type of MDRO. For most MDRO isolates, we observed that the inhibitory activity of the cefoperazone-sulbactam combination would increase with increased ratios of sulbactam (1:2>1:1>2:1). These findings expand upon the previous knowledge⁹ that cefoperazone-sulbactam at a 1:1 ratio has 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 $P<0.05$). In contrast, this additional effect of sulbactam was not observed for carbapenem-resistant *P. aeruginosa*. Current commercial products containing cefoperazone-sulbactam are made using the fixed ratio of 1:1. Our findings indicate that adding more sulbactam to the current cefoperazone-sulbactam formulations could enhance their in vitro activity against some MDROs, including carbapenem-resistant Enterobacteriaceae, ESBL-producing *E. coli*, ESBL-producing *K. pneumoniae* and carbapenem-resistant *A. baumannii*. Similar findings report that piperacillin-sulbactam at a 2:1 ratio has improved activity against most Gram-negative bacteria, compared to piperacillin-sulbactam at a 4:1 ratio.¹⁵ Even for *Mycobacterium tuberculosis*, a 1:1 ratio of ampicillin to β -lactamase inhibitor was more active than a 2:1 ratio.¹⁶ However, in vitro activity may not translate into clinical efficacy, and further studies are required to confirm this effect.

Third, we found that the in vitro activity of sulbactam alone against MDROs was poor. While 17 carbapenem-resistant *A. baumannii* isolates had MIC ≤ 4 mg/L, all other organisms had MIC ≥ 8 mg/L. Temocin et al¹⁷ showed that 2 (6.7%) out of 30 MDR-*A. baumannii* were susceptible to sulbactam. Fass et al¹⁸ showed that among 28,000 isolates of the family Enterobacteriaceae, sulbactam alone was inactive against 99.6% of the isolates with the exception of *Acinetobacter calcoaceticus* and *Pseudomonas cepacia*. These data suggest that sulbactam alone may not be a good choice for treating MDROs, except in the case of *A. baumannii*.

In conclusion, the addition of sulbactam can enhance cefoperazone's activity against most MDROs, except carbapenem-resistant *P. aeruginosa*, and the activity of cefoperazone-sulbactam against these MDROs is greatest at a 1: two ratio, followed by 1:1 and 2:1 ratios.

Disclosure

The authors report no conflicts of interest in this work.

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