Appropriate composites of cefoperazone–sulbactam against multidrug-resistant organisms

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, cefopera-
zone–sulbactam, ceftolozane–tazobactam, ceftazidime–avi-
bactam 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 demon-
strated 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 baum-
nannii*, 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 Enterobacteri-
aceae, 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.13 *E. coli* ATCC 25922 and *K. pneu-
moniae* 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 Enterobacteria-
cae (*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
Enterobacteria, carbapenem-resistant *P. aeruginosa* and
carbapenem-resistant *A. baumannii* are shown in Table 1.
Cefoperazone alone showed high MICs against most isolates,
with MIC<sub>50</sub> and MIC<sub>90</sub> > 64 mg/L, except ESBL-producing
*K. pneumoniae* (MIC<sub>50</sub>=64 mg/L). MIC<sub>50</sub> values decreased
for all of MDROs after the addition of sulbactam, and most
MIC<sub>90</sub> values decreased, except of carbapenem-resistant
Enterobacteria and carbapenem-resistant *P. aeruginosa*
(MIC<sub>50</sub> values remained > 64 mg/L). We then tested the differ-
ent 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*, MIC<sub>50</sub> and MIC<sub>90</sub> values
decreased as the ratio of cefoperazone–sulbactam changed
from 2 : 1 to 1 : 1 and to 1 : 2. For carbapenem-resistant Entero-
bacteria, only MIC<sub>50</sub> values decreased as the ratio of
cefoperazone–sulbactam changed from 2:1 to 1:1 and to 1:2.
For carbapenem-resistant Enterobacteriaceae, only MIC<sub>50</sub>
values decreased as the ratio of cefoperazone–sulbactam changed
from 2:1 to 1:1 and to 1:2, with all of MIC<sub>90</sub> 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 cefo-
perazone 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 cefopera-
zone–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
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., which demonstrated that the addition of sulbactam to cefoperazone can significantly enhance the antimicrobial activity against various MDROs.

### Table 1

<table>
<thead>
<tr>
<th>MIC, Carbenpenem-resistant Enterobacteriaceae (n=57)</th>
<th>ESBL Escherichia coli (n=58)</th>
<th>ESBL Klebsiella pneumoniae (n=58)</th>
<th>Carbenpenem-resistant Pseudomonas aeruginosa (n=49)</th>
<th>Carbenpenem-resistant Acinetobacter baumannii (n=122)</th>
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<tbody>
<tr>
<td>Cefoperazone</td>
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<tr>
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<td>64</td>
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<tr>
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<tr>
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<td>&gt;64</td>
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<tr>
<td>MIC$_{50}$ 32</td>
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<td>2--&gt;64</td>
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</table>

### Abbreviations:

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

### Table 2

<table>
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<tr>
<th>Susceptibility rate (%)</th>
<th>Carbopenem-resistant Enterobacteriaceae (n=57)</th>
<th>ESBL Escherichia coli (n=58)</th>
<th>ESBL Klebsiella pneumoniae (n=58)</th>
<th>Carbenpenem-resistant Pseudomonas aeruginosa (n=49)</th>
<th>Carbenpenem-resistant Acinetobacter baumannii (n=122)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefoperazone</td>
<td>3.5</td>
<td>3.4</td>
<td>1.7</td>
<td>24.5</td>
<td>0.0</td>
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<tr>
<td>Cefoperazone–sulbactam (2:1)</td>
<td>33.3*</td>
<td>84.5*</td>
<td>67.2*</td>
<td>30.6</td>
<td>41.0*</td>
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<tr>
<td>Cefoperazone–sulbactam (1:1)</td>
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<td>91.4*</td>
<td>75.9*</td>
<td>30.6</td>
<td>76.2*</td>
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<tr>
<td>Cefoperazone–sulbactam (1:2)</td>
<td>68.4*</td>
<td>91.4*</td>
<td>89.7*</td>
<td>34.7</td>
<td>92.6*</td>
</tr>
</tbody>
</table>

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.

*P-value <0.05 compared to cefoperazone. **P-value <0.05 compared to cefoperazone–sulbactam (2:1). ***P-value <0.05 compared to cefoperazone–sulbactam (1:1).

Abbreviations: ESBL, extended-spectrum β-lactamase; MIC, minimum inhibitory concentration.
activities against *Serratia marcescens*, *Enterobacter cloacae*, ESBL- *K. pneumoniae* and *A. baumannii*. In addition to Kuo et al’s finding, 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 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>2:1:1>2:1). These findings expand upon the previous knowledge 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. Even for *Pseudomonas aeruginosa*, a 1:1 ratio of ampicillin to β-lactamase inhibitor was more active than a 2:1 ratio. 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 al showed that 2 (6.7%) out of 30 MDR-*A. baumannii* were susceptible to sulbactam. Fass et al 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 1:2 ratio, followed by 1:1 and 2:1 ratios.

**Disclosure**

The authors report no conflicts of interest in this work.

**References**
