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ORIGINAL RESEARCH

In vitro activity of cefoperazone and cefoperazone-sulbactam against carbapenem-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*

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Background: This study aimed to investigate the in vitro activity of cefoperazone–sulbactam against carbapenem-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, and to evaluate the antibiotic resistance mechanisms of these bacteria.

Materials and methods: In total, 21 isolates of carbapenem-resistant *P. aeruginosa* and 15 isolates of carbapenem-resistant *A. baumannii* with different pulsed-field gel electrophoresis types were collected for assessment of the in vitro antibacterial activities of cefoperazone and cefoperazone–sulbactam and the associated resistance mechanisms of the bacteria.

Results: For carbapenem-resistant *P. aeruginosa*, the minimum inhibitory concentration (MIC) value and antibiotic susceptibility rate were similar for cefoperazone and cefoperazone–sulbactam (at 1:1 and 2:1 ratios). In contrast, for carbapenem-resistant *A. baumannii*, the MIC values, including the MIC range, MIC that inhibited 50% of isolates (MIC_{50}) and MIC that inhibited 90% of isolates (MIC_{90}), were reduced after treatment with sulbactam and cefoperazone. We screened resistance genes, including VIM-2, OXA-2 and OXA-10, in 21 carbapenem-resistant *P. aeruginosa* isolates. Only one (4.8%) of the isolates showed expression of VIM-2, and neither the OXA-2 nor the OXA-10 gene was detected. However, 20 (95.2%) isolates among the carbapenem-resistant *P. aeruginosa* isolates selected for oprD sequencing showed the phenomenon of nucleotide substitution or deletion. Among 15 carbapenem-resistant *A. baumannii* isolates, we found that ten (66.7%) isolates had concomitant expression of the OXA-23 and ISAba1-OXA-23 genes, and six (40.0%) isolates had expression of the OXA-24-like gene. All 15 isolates had OXA-51-like gene expression, and only 1 (6.7%) isolate had ISAba1-OXA-51-like gene expression. None of the isolates contained the IMP-1, IMP-8, KPC, NDM, VIM-1 or OXA-48 genes.

Conclusion: The in vitro antibacterial activity of cefoperazone against carbapenem-resistant *A. baumannii* can be enhanced by adding sulbactam to cefoperazone, but the addition does not affect carbapenem-resistant *P. aeruginosa*. This significant difference can be explained by the different resistance mechanisms of carbapenem-resistant *A. baumannii* and *P. aeruginosa*.

Keywords: cefoperazone–sulbactam, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*

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Introduction

Both the *Acinetobacter baumannii* complex and *Pseudomonas aeruginosa* belong to the non-fermentative Gram-negative bacilli, and they are common causes of clinical infection. Although carbapenems, including imipenem, meropenem and doripenem, have broad-spectrum antibacterial activity and are commonly used to treat infections

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caused by *A. baumannii* and *P. aeruginosa*, carbapenem resistance among these two pathogens is gradually increasing.[1,2] The list of antibiotics that are effective against these carbapenem-resistant organisms is becoming limited. In vitro studies[3,4] have shown that the addition of sulbactam to cefoperazone can help enhance its antibacterial activity against carbapenem-resistant Enterobacteriaceae or *A. baumannii*. In contrast, two studies[4,5] did not show an additive effect of sulbactam on cefoperazone against carbapenem-resistant *P. aeruginosa*. Therefore, we urgently need a more comprehensive evaluation of the activity of cefoperazone–sulbactam against different carbapenem-resistant organisms. However, knowledge about the antimicrobial activity of cefoperazone–sulbactam against the carbapenem-resistant *A. baumannii* complex and *P. aeruginosa* and their associated antibiotic resistance mechanisms are limited. Therefore, we conducted this study to investigate the in vitro activity of cefoperazone–sulbactam against carbapenem-resistant *A. baumannii* and *P. aeruginosa* and to evaluate their antibiotic resistance mechanisms.

Materials and methods

Collection of clinical isolates

In total, 21 isolates of carbapenem-resistant *P. aeruginosa* and 15 isolates of carbapenem-resistant *A. baumannii* with different pulsed-field gel electrophoresis (PFGE) types were collected for study. Carbapenem resistance is defined as resistance to imipenem, meropenem or doripenem, and the carbapenem-resistant phenotypes of the *P. aeruginosa* and *A. baumannii* isolates were 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).

In vitro susceptibility

The minimum inhibitory concentrations (MICs) of the drugs were measured by broth microdilution in prepared Mueller–Hinton broth (Oxoid, Basingstoke, UK) supplemented 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 hours.[6] Standard powders of cefoperazone and sulbactam were provided by TTY (TTY Biopharm, Taipei, Taiwan), and MIC determinations and susceptibility interpretation criteria followed the Clinical and Laboratory Standards Institute guideline.[7] Antimicrobial susceptibilities were determined using broth microdilution MIC tests with a standard inoculum (5×10^5 CFU/mL). For both microorganisms, doubling dilutions of cefoperazone ranged from 0.25 to 64 μg/mL, and three different sets of dilutions were prepared. The first series of cefoperazone dilutions was created without adding sulbactam. The second series contained cefoperazone combined with sulbactam in a 2:1 ratio (two parts cefoperazone and one part sulbactam). The third series contained cefoperazone combined with sulbactam in a 1:1 ratio (one part cefoperazone and one part sulbactam). Susceptibilities to cefoperazone alone and cefoperazone–sulbactam at 1:1 and 2:1 ratios were classified according to the MIC of cefoperazone ≤16 μg/mL.[7] We used an ELISA plate reader for reading. Bacterial growth was detected by OD (ELISA plate reader, Epoch™ Microplate spectrophotometer; BioTek Instruments, Winooski, VT, USA). *Escherichia coli* ATCC 25922 and Kp ATCC 700603 were used as quality-control strains.

Detection of β-lactamase genes

Gene detection was modified as described previously.[6] In brief, DNA was extracted to be used as a template, and PCR was used to amplify IMP-1, IMP-8, KPC, NDM, OXA-2, OXA-10, OXA-23, ISAba1-OXA-23, OXA-24-like, OXA-48, OXA-51-like, ISAba1-OXA-51-like, oprD, VIM-1 and VIM-2 using specific primers.[6,8,9] PCR products were purified with PCR clean-up kits (Roche Diagnostics, Penzberg, Germany) and sequenced on an ABI Prism 3730 sequencer analyzer (Applied Biosystems, Foster City, CA, USA).

Results

The MIC values of cefoperazone alone and in combination with sulbactam against carbapenem-resistant *P. aeruginosa* and carbapenem-resistant *A. baumannii* are shown in Table 1. For carbapenem-resistant *P. aeruginosa*, the MIC range, MIC_{50} and MIC_{90} values were similar for cefoperazone and cefoperazone–sulbactam (at 1:1 and 2:1 ratios). In contrast, for carbapenem-resistant *A. baumannii*, the MIC values, including the MIC range, MIC_{50} and MIC_{90}, were reduced after adding sulbactam with cefoperazone. The susceptibility rates of carbapenem-resistant *P. aeruginosa* to cefoperazone, cefoperazone–sulbactam (1:1) and cefoperazone–sulbactam (2:1) were 23.8%, 28.6% and 33.3%, respectively. However, the susceptibility rates of carbapenem-resistant *A. baumannii* to cefoperazone, cefoperazone–sulbactam (1:1) and cefoperazone–sulbactam (2:1) were 0.0%, 80.0% and 40.0%, respectively.

Table 1 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}
Carbapenem-resistant *P. aeruginosa* (n=21)	4 to >64	>64	>64	4 to >64	32	>64	4 to >64	64	>64
Carbapenem-resistant *A. baumannii* (n=15)	>64 to >64	>64	>64	8 to 32	16	32	16 to 64	32	64

Abbreviations: MIC, minimum inhibitory concentration; MIC_{50}, MIC that inhibited 50% of isolates; MIC_{90}, MIC that inhibited 90% of isolates; *A. baumannii*, *Acinetobacter baumannii*; *P. aeruginosa*, *Pseudomonas aeruginosa*.

We found that the resistance mechanisms of carbapenem-resistant *P. aeruginosa* and carbapenem-resistant *A. baumannii* isolates were different (Tables 2 and 3). Among 21 carbapenem-resistant *P. aeruginosa* isolates, we screened resistance genes, including VIM-2, OXA-2 and OXA-10. Only one (4.8%) of the isolates had expression of VIM-2, and neither the OXA-2 nor the OXA-10 gene was detected. However, 20 (95.2%) isolates among the carbapenem-resistant *P. aeruginosa* isolates selected for oprD sequencing showed the phenomenon of nucleotide substitution or deletion. Among 15 carbapenem-resistant *A. baumannii* isolates, we found that 10 (66.7%) isolates had concomitant expression of the OXA-23 and ISAba1-OXA-23 genes, and 6 (40.0%) isolates had expression of the OXA-24-like gene. All of the 15 isolates had OXA-51-like expression, and only 1 (6.7%) isolate had ISAba1-OXA-51-like expression. None of them had detectable IMP-1, IMP-8, KPC, NDM, VIM-1 or OXA-48 genes.

Discussion

This study investigated the in vitro activities of different compositions of cefoperazone–sulbactam and cefoperazone alone against carbapenem-resistant *P. aeruginosa* and *A. baumannii*. We also assessed their antibiotic resistance mechanisms to help us better understand the association between antibiotic activity and resistance mechanisms in these two microorganisms. First, after the addition of sulbactam with cefoperazone, the in vitro antibiotic activity, in terms of the MIC values and antibiotic susceptibility rates, against carbapenem-resistant *A. baumannii* improved. In contrast, this changed antibiotic composition did not change the antibiotic activity against carbapenem-resistant *P. aeruginosa*. In summary, after adding sulbactam with cefoperazone, we can enhance the in vitro activity against the carbapenem-resistant *A. baumannii* complex but not against carbapenem-resistant *P. aeruginosa*.

In addition, to explain the different activities of cefoperazone or cefoperazone–sulbactam against carbapenem-resistant *P. aeruginosa* and *A. baumannii* isolates, this study investigated the resistance mechanism of these two bacteria. The resistance mechanism of carbapenem-resistant *P. aeruginosa* was mainly attributed to nucleotide substitution or deletion in the oprD gene, and few isolates carried the VIM-2 gene. This finding is consistent with a previous study of 78 non-duplicated imipenem-resistant *P. aeruginosa* isolates that showed alterations in the oprD protein and the presence of an active efflux pump are the main antibiotic resistance mechanisms of *P. aeruginosa*.[10] Such a resistance mechanism may keep carbapenem-resistant *P. aeruginosa* isolates resistant to β-lactams even after adding β-lactamase inhibitors, such as sulbactam. Therefore, we did not find a synergistic effect between the β-lactam (cefoperazone) and the β-lactamase inhibitor (sulbactam) against carbapenem-resistant *P. aeruginosa* in this study.

Unlike serine-dependent β-lactamases (classes A, C and D), class B β-lactamases are metallo-β-lactamases (MBLs) that require zinc or another heavy metal for catalysis. MBLs are not inhibited by mechanism-based inhibitors, such as clavulanate, sulbactam or tazobactam.[11] The most common families of identified acquired class B MBLs include the VIM and IMP groups, along with the emerging NDM group, none of which was found in our carbapenem-resistant *A. baumannii* isolates. In contrast, most of the carbapenem-resistant *A. baumannii* isolates in this study carried resistance genes, including the OXA-23 gene, ISAba1-OXA-23 gene, OXA-24-like gene and OXA-51-like gene, as previously described.[12] The β-lactamases, OXA-23, OXA-24 and OXA-51, in the carbapenem-resistant *A. baumannii* in this study belonged to class D, and were their major resistance mechanism to sulbactam.[13] Sulbactam is chemically a penicillanic acid sulfone and shows particular activity against class A β-lactamases, whereas its activity against class D enzymes is less potent. Similarly, inhibition of OXA-type enzymes by sulbactam is not as strong as the inhibition produced by TEM-1 and other clinically used inhibitors.[14] In this study, the concentration of

Table 2 Resistance mechanisms of carbapenem-resistant *Pseudomonas aeruginosa* (Pa) isolates

Bacterial isolate	VIM-2	OXA-2-like	OXA-10-like	OprD	oprD, AAG04347.1 (1043983–1045314), compared with *Pseudomonas aeruginosa* PAO1, complete genome AE004091
Pa 09-272	–	–	–	+	8 Amino acid substitutions: T103S, K115T, F170L, E185Q, P186G, V189T, R310E, A315G
Pa 09-274	–	–	–	+	8 Amino acid substitutions: T103S, K115T, F170L, E185Q, P186G, V189T, R310E, A315G
Pa 09-275	–	–	–	+	3 Amino acid substitutions: T103S, K115T, F170L
Pa 09-276	–	–	–	+	D43N, S57E, S59R, 1 bp deletion (G) at nt 169, 1 bp insertion (A) at nt 175, 1 bp insertion (C) at nt 601, stop codon TAA at nt 1093–1095
Pa 09-278	–	–	–	+	V127L, E185Q, P186G, V189T, E202Q, I210A, E230K, S240T, N262T, T276A, A281G, K296Q, Q301E, R310E, G312R, A315G, 1 bp insertion (C) at nt 840, 1 bp deletion (A) at nt 843, 1 bp deletion (C) at nt 1,078
Pa 09-279	–	–	–	+	D43N, S57E, S59R, E202Q, I210A, E230K, S240T, N262T, A267S, A281G, K296Q, Q301E, R310G, V359L, 2 bp deletion (AT) at nt 1,114 (M372V)
Pa 09-283	–	–	–	+	D43N, S57E, S59R, K296Q, Q301E, R310G, 2 bp insertion (AC) at nt 1,051
Pa 09-284	–	–	–	+	T103S, K115T, F170L
Pa 09-288	–	–	–	–	
Pa 09-290	–	–	–	+	S57E, S59R, V127L, E185Q, P186G, V189T, E202Q, I210A, E230K, S240T, N262T, T276A, A281G, K296Q, Q301E, R310E, A315G, L347M, 2 bp deletion (AT) at nt 1,114 (M372V)
Pa 09-291	–	–	–	+	Truncated protein (remain 179 aa)
Pa 09-294	–	–	–	+	S57E, S59R, 11 bp deletion beginning at nt 312
Pa 09-298	+	–	–	+	WT
Pa 09-299	–	–	–	+	S57E, S59R, 1 bp insertion (T) at nt 174, stop codon TGA at nt 505–507
Pa 09-302	–	–	–	+	S57E, S59R, V127L, E185Q, P186G, V189T, E202Q, I210A, 1 bp insertion (C) at nt 633, 4 bp insertion beginning at nt 634
Pa 09-303	–	–	–	+	S57E, S59R, 11 bp deletion beginning at nt 312
Pa 09-309	–	–	–	+	D43N, S57E, S59R, 1 bp insertion (G) at nt 168, 1 bp deletion (A) at nt 175, E202Q, I210A, E230K, S240T, N262T, A267S, A281G, K296Q, Q301E, R310G, V359L, 2 bp deletion (AT) at nt 1,114 (M372V)
Pa 09-311	–	–	–	+	S57E, S59R, V127L, E185Q, P186G, V189T, E202Q, I210A, E230K, S240T, N262T, T276A, A281G, K296Q, Q301E, R310E, A315G, L347M, 2 bp deletion (AT) at nt 1,114 (M372V)
Pa 09-439	–	–	–	+	G60R, T105A, 11 bp deletion beginning at nt 376
Pa 09-445	–	–	–	+	V127L, E185Q, P186G, V189T, E202Q, I210A, E230K, S240T, N262T, T276A, A281G, K296Q, Q301E, R310E, G312R, A315G, L347M, 1 bp insertion (C) at nt 840, 1 bp deletion (A) at nt 843, 1 bp deletion (C) at nt 1,078
Pa 09-464	–	–	–	+	1 bp deletion (T) at nt 209

Table 3 Resistance mechanisms of carbapenem-resistant *Acinetobacter baumannii* (Ab) isolates

Bacterial isolate	KPC	IMP-1	IMP-8	NDM	VIM-1	OXA-23	ISAba1-OXA-23	OXA-24-like	OXA-48	OXA-51-like	ISAba1-OXA-51-like
Ab 02-773	–	–	–	–	–	+	+	–	–	+	–
Ab 02-774	–	–	–	–	–	+	+	+	–	+	–
Ab 02-775	–	–	–	–	–	–	–	+	–	+	–
Ab 02-776	–	–	–	–	–	+	+	–	–	+	–
Ab 02-781	–	–	–	–	–	+	+	–	–	+	–
Ab 02-782	–	–	–	–	–	–	–	+	–	+	–
Ab 02-783	–	–	–	–	–	–	–	+	–	+	–
Ab 02-785	–	–	–	–	–	+	+	–	–	+	–
Ab 02-791	–	–	–	–	–	+	+	–	–	+	–
Ab 02-793	–	–	–	–	–	+	+	–	–	+	–
Ab 02-796	–	–	–	–	–	+	+	–	–	+	–
Ab 02-803	–	–	–	–	–	+	+	–	–	+	–
Ab 02-804	–	–	–	–	–	–	–	+	–	+	–
Ab 02-807	–	–	–	–	–	–	–	+	–	+	+
Ab 02-818	–	–	–	–	–	+	+	–	–	+	–

sulbactam was higher than the conventional concentration, and this concentration may increase the affinity of sulbactam for class D β-lactamases and therefore the activity against these carbapenem-resistant *A. baumannii* isolates. Thus, we found that after adding a relatively high concentration of sulbactam with cefoperazone, the in vitro activity against carbapenem-resistant *A. baumannii* could be enhanced.[15] However, further studies are warranted to elucidate the role of cefoperazone and sulbactam in the treatment of infection and assess β-lactamase gene expression.

This study had one major limitation. Only small numbers of clinical isolates were available in our institution because we wanted to investigate isolates with different PFGE patterns; thus, the clinical utility of the results may be limited. Further large-scale studies are warranted to confirm our findings.

Conclusion

The in vitro activity against carbapenem-resistant *A. baumannii* can be enhanced by adding sulbactam with cefoperazone, but this treatment does not work for carbapenem-resistant *P. aeruginosa*. This significant difference can be explained by the difference in resistance mechanisms between carbapenem-resistant *A. baumannii* and *P. aeruginosa*.

Data sharing statement

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Disclosure

The authors report no conflicts of interest in this work.

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