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ORIGINAL RESEARCH

Sulbactam Enhances in vitro Activity of β-Lactam Antibiotics Against *Acinetobacter baumannii*

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Purpose: To evaluate in vitro activities of β-lactam antibiotics alone and in combination with sulbactam at different ratios against *Acinetobacter baumannii* clinical strains from China.

Methods: A total of 300 clinical isolates of *A. baumannii* were collected from 29 hospitals across China in 2018. Susceptibility to common antibiotics was assessed, and β-lactamase genes were detected. In vitro activity of ampicillin, cefoperazone and imipenem was tested alone and in combination with sulbactam at the ratios of 2:1, 1:1, 1:1.5, 1:2, 1:2.5 and 1:3.

Results: High resistant rates for common antibiotics were observed except tigecycline and polymyxin B. Among carbapenem-resistant *A. baumannii*, 97.3% isolates harbored $bla_{\text{OXA-23}}$. MIC_{50} and MIC_{90} values for sulbactam were 32 mg/L and 64 mg/L, respectively. High resistant rates for ampicillin, cefoperazone and imipenem were observed (92.3%, 93% and 85.3%, respectively). A stepwise increase in the ratio of sulbactam to partner β-lactam antibiotics led to a stepwise decrease in the MICs and a stepwise increase in the susceptible rates. The susceptible rates for imipenem-sulbactam 1:3, ampicillin-sulbactam 1:3 and cefoperazone-sulbactam 1:3 reached 16.3%, 58.3% and 91%, respectively.

Conclusion: The increasing proportion of sulbactam could enhance antimicrobial activities of imipenem-sulbactam, ampicillin-sulbactam and cefoperazone-sulbactam combinations against *A. baumannii* clinical strains in China, with cefoperazone-sulbactam as the most potent compound.

Keywords: sulbactam, ampicillin, cefoperazone, imipenem, *Acinetobacter baumannii*

Introduction

Acinetobacter baumannii is one of the most troublesome pathogens among health-care-associated infections, and it could evolve into multidrug-resistance (MDR) clones, even extensively-drug resistance (XDR) clones, which are often associated with prolonged length and increased cost of hospital stay as well as high mortality.[1] Apparently, intrinsic resistance and acquired resistance due to inappropriate administration of antimicrobial agents could be the critical reasons for the development of MDR bacteria.[2,3]

Carbapenem-resistant *A. baumannii* (CRAB), as a matter of great concern, has been labelled as "critical pathogen" by World Health Organization (WHO) and as "urgent threat" by Centers for Disease Control and Prevention (https://www.cdc.gov/drugresistance/biggest-threats.html).[4] In the China Antimicrobial Surveillance Network (www.chinets.com/Chinet), clinical isolates of *Acinetobacter* in 2019 displayed resistance rates to cefoperazone-sulbactam 2:1, ampicillin-sulbactam, imipenem and meropenem of 46.5%, 69.3%, 73.6% and 75.1%, respectively.

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Additionally, resistance rates to cefoperazone-sulbactam 1:1 and meropenem were 40.4% and 75.3%, respectively, for *Acinetobacter* isolates collected from China in 2013–2014.[5] Despite potent in vitro antimicrobial activities, tigecycline and polymyxin demonstrate conspicuous disadvantages, such as poor distribution in tissue, low plasma concentration and obvious adverse reactions, which could preclude their clinical use.[6] Hence, few treatment options are available against MDR *A. baumannii*.

Sulbactam, a semisynthetic penicillanic acid sulfone, acts as an inhibitor of β-lactamase and has intrinsic activity against the *Acinetobacter* genus.[7] Previous study has found excellent antimicrobial activity of cefoperazone-sulbactam, ampicillin-sulbactam and carbapenem-sulbactam combinations against MDR *Acinetobacter* in vitro and in vivo.[8–11] In China, commercially available are sulbactam alone, ampicillin-sulbactam in proportion of 2:1 as well as cefoperazone-sulbactam in proportion of 2:1 and 1:1. As indicated by above resistance data, the current combinations could be insufficiently effective yet. Notably, adding more sulbactam to cefoperazone-sulbactam combinations enhanced the in vitro antimicrobial activity against *Acinetobacter*.[12] Consequently, sulbactam-based combinations, as one of alternative therapeutics, are attracting attention.

Therefore, we evaluated in vitro activity of ampicillin-sulbactam, cefoperazone-sulbactam and imipenem-sulbactam at different ratios against *A. baumannii* clinical isolates in China so as to find better antimicrobial regimens.

Materials and Methods

Bacterial Strains

A total of 300 clinical isolates of *A. baumannii* were collected from 29 hospitals in 26 cities across China in 2018, including carbapenem-susceptible *A. baumannii* (CSAB) and CRAB. They were isolated from respiratory tract (199, 66.3%), skin and skin structure (21, 7.0%), blood (12, 4.0%), urine tract (8, 2.7%), cerebrospinal fluid (4, 1.3%), bile (3, 1.0%) and others (53, 17.7%). Bacteria were preliminarily identified in the isolated hospitals, and confirmed by Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) Vitek mass spectrometry (VMS) in our hospital. The study protocol was approved by the Institutional Review Board of Huashan Hospital, Fudan University (No. 2018-408).

Antimicrobial Susceptibility Testing

The minimum inhibitory concentrations (MICs) of antimicrobial agents were determined using broth microdilution method and interpreted on the basis of the Clinical and Laboratory Standards Institute (CLSI) criteria.[13] Sulbactam, β-lactam antibiotics, fluoroquinolones, amikacin, trimethoprim-sulfamethoxazole, tigecycline and polymyxin B were tested for comparison. Ampicillin, cefoperazone and imipenem were tested alone and in combination with sulbactam at the ratios of 2:1, 1:1, 1:1.5, 1:2, 1:2.5 and 1:3, respectively.

Given that neither CLSI nor European Committee on Antimicrobial Susceptibility Testing (EUCAST) provides the breakpoints for sulbactam-based combinations against *A. baumannii*, breakpoints for partner β-lactam antibiotics were referred carefully.[12] Specifically, the MIC breakpoints for ampicillin and ampicillin-sulbactam were as follows: S, ≤8 mg/L; I, 16 mg/L; R, ≥32 mg/L. The MIC breakpoints for cefoperazone and cefoperazone-sulbactam were those for *Enterobacteriaceae*: S, ≤16 mg/L; I, 32 mg/L; R, ≥64 mg/L. The MIC breakpoints for imipenem and imipenem-sulbactam were as follows: S, ≤2 mg/L; I, 4 mg/L; R, ≥8 mg/L.

Detection of β-Lactamase

β-lactamase genes (bla_{TEM}, bla_{SHV}, bla_{VEB}, bla_{PER}, bla_{GES}, bla_{IMP}, bla_{VIM}, bla_{KPC}, bla_{OXA-23} and bla_{OXA-58}) were detected by PCR.[14] Primers sequences were obtained as previously described.[13] Amplification was performed as follows: initial denaturation at 94°C for 5 min; 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and elongation at 72°C for 1 min; and a final elongation step at 72°C for 5 min.

Results

Antimicrobial Susceptibility Testing for Common Antibiotics and Identification of β-Lactamase

Susceptibility rates of 300 *A. baumannii* isolates to most comparator agents were less than 30%, including β-lactams, fluoroquinolones, amikacin and trimethoprim-sulfamethoxazole, while those to tigecycline and polymyxin B were 98.7% and 99.3%, respectively (Table 1).

Among CRAB, 97.3% (256/263) isolates harbored bla_{OXA-23}, and bla_{TEM} was present in 51.7% (136/263) isolates. Neither bla_{TEM} nor bla_{OXA-23} was detected in the CSAB isolates. None of the isolates carried bla_{SHV},

Table 1 Antimicrobial Susceptibilities of *A. baumannii* Determined by the Broth Microdilution Method

Antimicrobial Agents	MIC (mg/L)			Susceptible Rate (%)	Intermediate Rate (%)	Resistant Rate (%)
	Range	MIC_{50}	MIC_{90}			
Ceftazidime	1–>32	>32	>32	11.7	0.7	87.7
Cefepime	0.5–>128	128	>128	12.0	1.7	86.3
Aztreonam	4–>128	64	128	3.3	7.7	89.0
Piperacillin-tazobactam[a]	≤2–>256	>256	>256	10.7	0.7	85.3
Meropenem	0.12–>64	64	>64	12.0	0.7	87.3
Ciprofloxacin	≤0.06–>8	>8	>8	12.0	0.0	88.0
Levofloxacin	≤0.12–>16	8	>64	12.3	3.3	84.3
Amikacin	≤1–>128	>128	>128	17.7	0.7	81.7
Trimethoprim-sulfamethoxazole[b]	≤0.25–32	>32	>32	28.3	/	71.7
Tigecycline	≤0.06–16	1	2	98.7	1.0	0.3
Polymyxin B	0.25–4	1	2	99.3	/	0.7

Notes: [a]For piperacillin-tazobactam, only concentration of piperacillin is listed. [b]For trimethoprim-sulfamethoxazole, only concentration of trimethoprim is listed.

bla$_{VEB}$, *bla*$_{PER}$, *bla*$_{GES}$, *bla*$_{IMP}$, *bla*$_{VIM}$, *bla*$_{KPC}$ or *bla*$_{OXA-58}$.

Antimicrobial Susceptibility Testing for Sulbactam-Based Combinations

In vitro activities of sulbactam, ampicillin, cefoperazone, imipenem and corresponding combinations are shown in Table 2. MIC_{50} and MIC_{90} values for sulbactam were 32 mg/L and 64 mg/L, respectively. High resistant rates for ampicillin, cefoperazone and imipenem were observed (92.3%, 93% and 85.3%, respectively). Overall, a stepwise increase in the ratio of sulbactam to β-lactams led to a stepwise decrease in the β-lactams MIC. Small-scale increase was observed in the susceptibility for imipenem-sulbactam from 13.7% to 16.3%, while remarkable elevations were evidenced in the susceptibility for ampicillin-sulbactam and cefoperazone-sulbactam with the increasing proportion of sulbactam (from 12.3% to 58.3%, from 15% to 91%, respectively). Notably, susceptibility overtly increased from 29.3% for cefoperazone-sulbactam 1:1 to 66.3% for cefoperazone-sulbactam 1:1.5. Among all combinations, cefoperazone-sulbactam 1:3 demonstrated the most effective antibacterial activity with MIC_{90} of 16 mg/L.

Although susceptibility rates of CSAB to ampicillin and cefoperazone were fairly low (only 10.8% and 18.9%, respectively), all CSAB isolates were susceptible to ampicillin-sulbactam combinations and cefoperazone-sulbactam combinations at all ratios (Table 3). For CRAB strains, escalating addition of sulbactam enhanced the in vitro antimicrobial activities of imipenem-sulbactam, ampicillin-sulbactam and cefoperazone-sulbactam combinations gradually, with the susceptibility rate rising from 1.5% to 4.6%, from 0% to 52.5%, from 3.0% to 89.7%, respectively.

Discussion

A. baumannii displayed high resistance rate to imipenem (85.3%) in this study. Meanwhile, *Acinetobacter spp*. in the China Antimicrobial Surveillance Network in 2018 (www.chinets.com/Chinet) also depicted similar resistance rate to imipenem (73.2%). A 10-year-spanning study in Hungary observed an increase in resistance levels of *Acinetobacter spp*. originating from urine samples.[15] Although there are emerging novel antimicrobial agents for gram-negative bacteria, few of them are effective for *A. baumannii* yet. Hence, nowadays combination therapeutics based on existing drugs could be a practical approach to treating infections caused by *A. baumannii*.

This study demonstrated again that the higher ratio of sulbactam, the more potent in vitro antimicrobial activity of imipenem-sulbactam, ampicillin-sulbactam and cefoperazone-sulbactam combinations against *A. baumannii*. Higher susceptibility rate for cefoperazone-sulbactam was also observed compared with those of imipenem-sulbactam and ampicillin-sulbactam. Cefoperazone-sulbactam 1:3 displayed best in vitro activity among all sulbactam-based combinations and showed superior activity to most comparator agents, including other β-lactams, fluoroquinolones, aminoglycosides and trimethoprim-sulfamethoxazole.

Previous study revealed that cefoperazone-sulbactam 1:1 exhibited greatest in vitro activity against CRAB,

Table 2 In vitro Activities of Imipenem-Sulbactam, Ampicillin-Sulbactam and Cefoperazone-Sulbactam Against *A. baumannii*

Drugs	MIC (mg/L)[a]			Susceptible Rate (%)	Intermediate Rate (%)	Resistant Rate (%)
	Range	MIC_{50}	MIC_{90}			
SUL	0.5–256	32	64	/	/	/
IPM	0.12–128	32	64	13.7	1.0	85.3
IPM-SUL 2:1	0.12–256	32	64	13.7	1.0	85.3
IPM-SUL 1:1	0.12–64	16	32	14.3	1.0	84.7
IPM-SUL 1:1.5	0.12–64	16	32	14.3	1.3	84.3
IPM-SUL 1:2	0.12–64	8	16	15.0	3.7	81.3
IPM-SUL 1:2.5	0.12–64	8	16	15.7	9.0	75.3
IPM-SUL 1:3	0.12–32	8	16	16.3	18.3	65.3
AMP	8–>256	>256	>256	1.3	6.3	92.3
AMP-SUL 2:1	1–>256	64	128	12.3	2.0	85.7
AMP-SUL 1:1	0.5–256	32	64	14.3	16.3	69.3
AMP-SUL 1:1.5	0.25–256	16	64	20.3	35.0	44.7
AMP-SUL 1:2	0.25–256	16	32	28.3	46.0	25.7
AMP-SUL 1:2.5	0.12–128	8	32	51.3	35.3	13.3
AMP-SUL 1:3	0.25–128	8	16	58.3	32.7	9.0
CFP	16–>256	>256	>256	2.3	4.7	93.0
CFP-SUL 2:1	1–>256	64	128	15.0	12.7	72.3
CFP-SUL 1:1	0.5–>256	32	64	29.3	47.3	23.3
CFP-SUL 1:1.5	0.5–256	16	32	66.3	24.0	9.7
CFP-SUL 1:2	0.25–256	16	32	78.7	14.0	7.3
CFP-SUL 1:2.5	0.25–256	16	32	86.3	8.0	5.7
CFP-SUL 1:3	0.25–128	8	16	91.0	4.3	4.7

Notes: [a]For MIC of IPM-SUL, AMP-SUL and CFP-SUL combinations, only the concentrations of imipenem, ampicillin and cefoperazone are listed respectively.
Abbreviations: SUL, sulbactam; IPM, imipenem; IPM-SUL, imipenem-sulbactam; AMP, ampicillin; AMP-SUL, ampicillin-sulbactam; CFP, cefoperazone; CFP-SUL, cefoperazone-sulbactam.

Table 3 In vitro Activities of Ampicillin-Sulbactam and Cefoperazone-Sulbactam Against Carbapenem-Susceptible *A. baumannii* and Carbapenem-Resistant *A. baumannii*

Drugs	MIC (mg/L)		Susceptible Rate (%)	Drugs	MIC (mg/L)		Susceptible Rate (%)
CSAB (n=37)	MIC_{50}	MIC_{90}		CRAB (n=263)	MIC_{50}	MIC_{90}	
SUL	1	2	/	SUL	32	64	/
AMP	16	32	10.8	AMP	>256	>256	0.0
AMP-SUL 2:1	2	4	100.0	AMP-SUL 2:1	64	128	0.0
AMP-SUL 1:1	1	2	100.0	AMP-SUL 1:1	32	64	2.3
AMP-SUL 1:1.5	0.5	1	100.0	AMP-SUL 1:1.5	32	64	9.1
AMP-SUL 1:2	0.5	1	100.0	AMP-SUL 1:2	16	32	18.3
AMP-SUL 1:2.5	0.5	0.5	100.0	AMP-SUL 1:2.5	16	32	44.5
AMP-SUL 1:3	0.25	0.5	100.0	AMP-SUL 1:3	8	32	52.5
CFP	32	128	18.9	CFP	>256	>256	0.0
CFP-SUL 2:1	2	4	100.0	CFP-SUL 2:1	64	128	3.0
CFP-SUL 1:1	1	2	100.0	CFP-SUL 1:1	32	64	19.4
CFP-SUL 1:1.5	1	1	100.0	CFP-SUL 1:1.5	16	64	61.6
CFP-SUL 1:2	0.5	1	100.0	CFP-SUL 1:2	16	32	75.7
CFP-SUL 1:2.5	0.5	1	100.0	CFP-SUL 1:2.5	16	32	84.4
CFP-SUL 1:3	0.5	0.5	100.0	CFP-SUL 1:3	8	32	89.7

Abbreviations: SUL, sulbactam; IPM, imipenem; IPM-SUL, imipenem-sulbactam; AMP, ampicillin; AMP-SUL, ampicillin-sulbactam; CFP, cefoperazone; CFP-SUL, cefoperazone-sulbactam; CSAB, carbapenem-susceptible *A. baumannii*; CRAB, carbapenem-resistant *A. baumannii.*

followed by cefoperazone-sulbactam 2:1 and cefoperazone (susceptibility rate, 80.0% vs 40.0% and 0%).[16] Additionally, cefoperazone-sulbactam 1:2 demonstrated antimicrobial benefit, with the susceptibility rates for cefoperazone-sulbactam 1:2, 1:1 and 2:1 of 92.6%, 76.2% and 41.0%, respectively.[12] Similarly, imipenem-sulbactam at a 1:1 ratio was slightly superior to that at a 2:1 ratio (resistance rate, 23.3% vs 26.7%) in inhibiting CRAB isolates.[17] Our findings were basically consistent with these data, which revealed the importance of the ratio of sulbactam. However, there were scarce reports about in vitro activities of imipenem-sulbactam, ampicillin-sulbactam combinations at higher ratios against *A. baumannii* clinical strains.

Growing clinical evidence has suggested sulbactam as a promising treatment option in the management of *Acinetobacter* infections. For the bloodstream infections caused by carbapenem-non-susceptible *Acinetobacter* spp., sulbactam-based regimens demonstrated similar clinical efficacy rates as carbapenem-based regimens (50.0% vs 45.8%).[18] In term of the treatment of XDR *Acinetobacter* infections, sulbactam-based regimens displayed higher clinical efficacy rate instead of carbapenem-based regimens (62.5% vs 47.4%).[19] A significant reduction in the 28-day mortality was observed for those receiving cefoperazone-sulbactam compared with those receiving tigecycline (29.3% vs 51.9%).[20] In a network meta-analysis, combination therapy of colistin with sulbactam was superior to that of colistin with tigecycline and colistin monotherapy in microbiological eradication while no significant differences were noted in all-cause mortality for MDR and XDR *A. baumannii* infections.[21] Early study also reported lower incidence rate of adverse events of high-dose ampicillin-sulbactam (30.7%, including 15.3% nephrotoxicity) than that of colistin (39.6%, including 33% nephrotoxicity) in patients with MDR *A. baumannii* ventilator-associated pneumonia.[22] Additionally, compared with tigecycline and colistin, sulbactam is also characterized by advantages of tissue distribution, tolerability and cost.

CSAB displayed susceptibility to sulbactam alone, ampicillin-sulbactam combinations and cefoperazone-sulbactam combinations, and either bla_{OXA23} or bla_{TEM} gene was undetected among them in this study. Sulbactam possesses the intrinsic activity against *A. baumannii*. It binds to penicillin-binding proteins (PBPs), hinders the synthesis of bacterial cell wall and kills bacteria.[23] Moreover, sulbactam rendered the bacteria more susceptible to phagocytosis as well as to killing by polymorphonuclear leukocytes.[24] Nevertheless, as indicated by in vitro activity, sulbactam alone was insufficient to fight against CRAB, which could be primarily due to hydrolysis of β-lactamases, including class A β-lactamase TEM-1 and Class D β-lactamase OXA-23.[25–27] In fact, sulbactam activity could be potentiated by durlobactam (formerly ETX2514),[28] a novel inhibitor for class A, C and D β-lactamases, with the combination sulbactam/durlobactam currently being assessed in a Phase III clinical trial in patients with *A. baumannii* infections (https://clinicaltrials.gov/ct2/results?cond=&term=ETX2514&cntry=&state=&city=&dist=).

There are few studies on the actual mechanism underpinning enhanced antibacterial activity of β-lactam-sulbactam combinations. The complementary and saturated PBP binding could be one of the mechanisms since β-lactams and sulbactam exhibit different binding affinities for PBPs.[29,30] Another possible explanation could be the alternative shielding hypothesis.[31] Sulbactam binds to the active site of the β-lactamases and acts as a better substrate for β-lactamases, thus preventing the hydrolysis of the partner β-lactam antibiotics and allowing the partner to reach its target PBPs more effectively.[25–27,32] High turnover numbers of sulbactam for TEM-1 and OXA-23 enzyme (sulbactam/enzyme molar ratio required for complete inhibition of each enzyme) as well as the coexistence of other β-lactamases may rationalize high-ratio sulbactam in combinations.[33] In addition, compounds could damage biofilm architecture significantly and exerted superior killing effects against CRAB.[34] Overall, above hypotheses need further confirmations. However, it remains to explain why cefoperazone-sulbactam exhibited superior activity over imipenem-sulbactam and ampicillin-sulbactam.

Nevertheless, this study had some limitations. Firstly, numbers of clinical isolates were relatively small though these isolates were from multiple centers. Secondly, the β-lactamase type is relatively simple, focusing on TEM-1 and OXA-23. Further large-scale studies are warranted to verify our research.

Conclusion

In conclusion, the increasing proportion of sulbactam could enhance antimicrobial activity of imipenem-sulbactam, ampicillin-sulbactam and cefoperazone-sulbactam compound against *A. baumannii* strains, with cefoperazone-sulbactam as the most potent. Our study suggests that high-ratio cefoperazone-sulbactam compound could be a promising antimicrobial regimen for *A. baumannii* infection in China.

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Disclosure

The authors report no conflicts of interest in this work.

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