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Sulbactam Combined with the Novel β-lactamase Inhibitor ETX2514 for the Treatment of Multidrug-Resistant *Acinetobacter baumannii* Infections

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Abstract

Background
Multidrug resistant (MDR) *Acinetobacter baumannii* infections are of great concern due to high mortality rates and limited number of treatment options. β-lactamase (BLA) expression, especially Class D, is an important resistance mechanism in this organism. The novel BLA inhibitor ETX2514 has potent activity against Class A, C and D serine BLAs. The MIC_{90} of sulbactam (SUL) in the presence of ETX2514 is 4 mg/L against a large, globally diverse set of MDR *A. baumannii* clinical isolates from 2014. In this study, we characterize the mechanism of synergy of this combination alone or in the presence of imipenem (IPM) or meropenem (MEM), whose spectra of target inhibition vary across bacterial species.

Methods
MICs were performed according to CLSI guidelines. The frequency of resistance (FOR) to SUL/ETX2514 was determined in several clinical isolates of *A. baumannii*. Resistant mutants were analyzed by whole genome sequencing (WGS). Morphological changes were examined by microscopy. PBP acylation rates were determined by competition with BOCILLIN FL in fluorescence polarization assays.

Results
MIC_{90}s of relevant combinations against 598 contemporary isolates of *A. baumannii* are shown below.

Agent(s)	MIC_{90}
SUL	>32
IPM	>32
MEM	>8
SUL/ETX2514*	2
IPM/ETX2514*	16
IPM/SUL*	>32
IPM/SUL*/ETX2514*	≤0.03
MEM/SUL*/ETX2514*	4

*held constant at 4 mg/L

SUL-treated *A. baumannii* strains had elongated cell morphologies which became spherical when ETX2514 was added, a phenotype associated with PBP2 inhibition. The FOR to SUL-ETX2514 at 4X MIC was 7.6 x 10^{-10}. Resistance mapped to residues S390, V505 or V511 in PBP3 or mutations in tRNA synthetase genes (*aspS* and *gltX*). The latter are predicted to play a role in resistance to PBP2 inhibitors in *E. coli*. Purified mutant PBP3 proteins had reduced affinity for SUL and variable affinity for IPM and MEM in BOCILLIN FL competition assays.

Conclusions
Resistance mapping plus morphological changes suggest that, in addition to BLA inhibition, ETX2514 enhances antibacterial activity through PBP2 inhibition. The remarkable synergy observed with the triple combination IMP/SUL/ETX2514 likely reflects superior antibacterial potency achieved through a combination of BLA inhibition and enhanced PBP activity.

Introduction

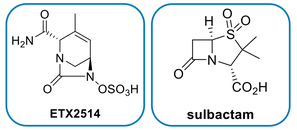
The emergence of bacteria resistant to multiple antibiotics is of great concern. The non-fermenting Gram-negative pathogen *Acinetobacter baumannii* is increasingly reported as multi-drug or pan-drug resistant and has been classified as a "serious threat" pathogen by the CDC (1). *A. baumannii* can cause severe infections, particularly in immunocompromised patients. The wide-spread acquisition by *A. baumannii* of β-lactamases conferring resistance to broad-spectrum cephalosporins and carbapenems is an alarming trend. In *A. baumannii* one of the most prevalent mechanisms of carbapenem resistance is expression of Class D β-lactamases (2).

Currently marketed β-lactamase inhibitors (BLI) cover Class A, C and only a small subset of Class D enzymes (OXA-48-like). Thus these BLIs offer little therapeutic value for the treatment of carbapenem-resistant *A. baumannii*. Sulbactam-ETX2514 is a novel combination currently being investigated for the treatment of resistant *A. baumannii* infections.

The Sulbactam-ETX2514 Combination

Sulbactam is a class A β-lactamase inhibitor with intrinsic antibacterial activity against *A. baumannii*; however, the prevalence of β-lactamases limits its activity.

ETX2514 is a novel BLI from a series of diazabicyclooctanones with best-in-class, broad-spectrum activity against Class A, C and D β-lactamases. ETX2514 also has intrinsic antibacterial activity vs. some species of *Enterobacteriaceae* due to inhibition of PBP2.



Activity of ETX2514 Combinations Against *A. baumannii*

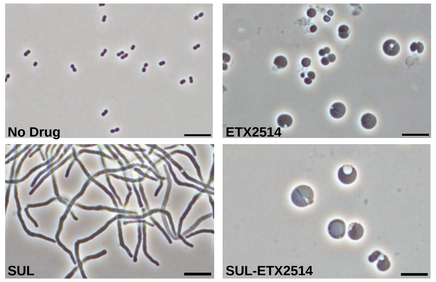
The antibacterial activity of sulbactam-ETX2514 combinations was measured against ~600 *A. baumannii* clinical isolates from 2012, 2013 and 2014.

Agent(s)	n	Range	MIC_{50}	MIC_{90}
SUL	199*	0.25 - >128	16	64
IPM	600	0.06 - >32	32	>32
MEM	600	≤0.03 - >8	>8	>8
SUL + 4 mg/L ETX2514	199*	≤0.06 - 4	1	2
IPM + 4 mg/L ETX2514	600	≤0.03 - >32	4	16
MEM + 4 mg/L ETX2514	598	0.06 - >32	16	>32
IPM + 4 mg/L SUL	598	≤0.03 - >32	32	>32
IPM + 4 mg/L SUL + 4 mg/L ETX2514	600	≤0.03 - >32	≤0.03	≤0.03
MEM + 4 mg/L SUL + 4 mg/L ETX2514	598	≤0.03 - 32	≤0.03	4

*Isolates from year 2014 only

- The addition of ETX2514 at 4 mg/L to sulbactam decreased the MIC_{90} by 32-fold to 2 mg/L
- A triple combination of imipenem, sulbactam and ETX2514 improved activity even further to an MIC_{90} of ≤0.03 mg/L.
- Combinations with imipenem were more potent than those with meropenem.

Morphology of *A. baumannii* in the Presence of SUL-ETX2514



A. baumannii ATCC 17978 was exposed to 1/2x MIC of drug for 3 hrs at 35° C and examined by light microscopy. Scale bar = 5 μm.

- *A. baumannii* became rounded and enlarged in the presence of ETX2514, consistent with the ability of ETX2514 to inhibit PBP2 (3).
- *A. baumannii* became filamentous in the presence of sulbactam, typical of PBP3 inhibition.
- In the presence of both sulbactam and ETX2514, the cells were also rounded, suggesting that ETX2514 contributed to the antibacterial activity of the combination, despite its weak intrinsic antibacterial activity against *A. baumannii*.

Frequency of Resistance to Sulbactam - ETX2514 in *A. baumannii*

The *in vitro* frequency of spontaneous resistance to sulbactam + 4 mg/L ETX2514 was measured against four clinical isolates of *A. baumannii*.

Strain No.	β-lactamase content[a]	SUL MIC (mg/L)[b]	SUL-ETX2514 MIC (mg/L)[b]	Selection (Fold MIC)[c]	Frequency of Resistance
A. baumannii ARC2058	ADC-99-like; OXA-95	2	1/4	2	2.1×10^{-8}
				4	$<9.0 \times 10^{-10}$
				8	$<9.0 \times 10^{-10}$
A. baumannii ARC2681	ADC-42-like; TEM-1; OXA-40; OXA-132	16	1/4	2	$<7.6 \times 10^{-10}$
				4	7.6×10^{-10}
				8	$<7.6 \times 10^{-10}$
A. baumannii ARC2782	ADC-79; TEM-1; PER-1; OXA-23; OXA-66	32	0.5/4	2	9.0×10^{-10}
				4	$<9.0 \times 10^{-10}$
				8	$<9.0 \times 10^{-10}$
A. baumannii ARC3488	ADC-76; OXA-68; OXA-235-like	16	0.5/4	2	$<7.6 \times 10^{-10}$
				4	$<7.6 \times 10^{-10}$
				8	$<7.6 \times 10^{-10}$

[a]Determined by whole genome sequencing. [b]Broth MIC. [c]Concentrations represent multiples of the agar dilution MIC for sulbactam with ETX2514 held constant at 4 mg/L

- The frequency of spontaneous resistance to sulbactam-ETX2514 was very low against clinical isolates of *A. baumannii*.

Characterization of Resistance to Sulbactam - ETX2514 in *A. baumannii*

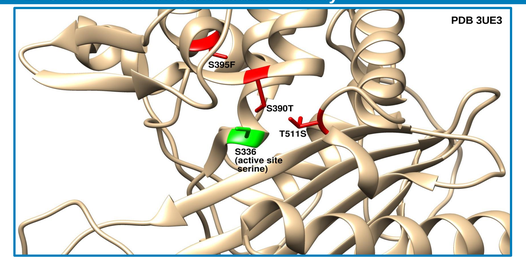
Representative colonies from frequency of resistance studies were profiled for susceptibility to sulbactam-ETX2514 and cross-resistance to other antibiotics. Sulbactam-ETX2514-resistant isolates were subjected to whole genome sequencing.

Strain	Variant	Protein affected	SUL-ETX2514 MIC (mg/L)	SUL MIC (mg/L)	MEM MIC (mg/L)	CAZ MIC (mg/L)	CIP MIC (mg/L)
ARC2058	Parent		1/4	4	0.5	4	0.5
	2X-1	AspS [Q47P]	16/4	4	16	16	1
	2X-2	GltX [M240I]	16/4	4	8	4	0.5
	2X-3	GltX [R117S]	64/4	4	32	8	0.5
	2XL-1	PBP3 [V505L]	16/4	16	0.25	4	0.25
ARC2681	Parent		2/4	8	32	256	>64
	4X-1	PBP3 [S390T]	>64/4	>64	32	128	>64
ARC2782	Parent		0.5/4	32	16	>512	8
	2X-1	PBP3 [T511A]	4/4	64	16	>512	16

CAZ = ceftazidime; CIP = ciprofloxacin

- Mutations in resistant isolates mapped to genes encoding tRNA synthetases and PBP3.
- Resistant mutations in tRNA synthetases are commonly seen with PBP2 inhibitors and are presumed to induce the stringent response (4). These mutations did not affect the MIC of sulbactam alone but some of these isolates had shifts in MIC to other β-lactams.
- Mutations in PBP3 affected the MIC of SUL-ETX2514 and sulbactam alone.
- Resistant mutants isolated here are consistent with morphology results which suggested sulbactam-ETX2514 works by inhibiting PBP2 and PBP3.
- PBP3 [S390T] and [S395F] mutations were associated previously with *in vitro* sulbactam resistance and had fitness costs (5).
- Strains with the PBP3 [S395T] mutation that were isolated in sulbactam resistance studies also had elevated MICs to sulbactam-ETX2514 (data not shown).

The Effect of PBP3 Mutations on Sulbactam Activity



The structure of *A. baumannii* PBP3 (FtsI) (6)
The active site serine (S336) is shown in green. Mutations which mapped to PBP3 in sulbactam-ETX2514-resistant isolates are shown in red

PBP3 mutations associated with sulbactam-ETX2514 resistance were engineered into *A. baumannii* PBP3. The effects of these mutations in PBP3 on reactivity with BOCILLIN FL, sulbactam, aztreonam, imipenem and meropenem were measured with purified proteins.

	k_{on} ($M^{-1}s^{-1}$) (% of WT)				
PBP3 Variant	BOCILLIN	Sulbactam	Aztreonam	Imipenem	Meropenem
Wild-type	18,000	14	700	470	5000
S390T	250 (1.4%)	0.1 (0.7%)	900 (129%)	540 (115%)	16,000 (320%)
S395F	740 (4.1%)	0.6 (4.3%)	110 (16%)	9 (1.9%)	1500 (30%)
T511S	4200 (23%)	1.5 (11%)	450 (64%)	220 (47%)	3400 (68%)

- All three PBP3 substitutions impaired the *A. baumannii* PBP3 on-rates for BOCILLIN FL and sulbactam.
- The S390T mutation increased or had no significant effect on the k_{on} for aztreonam, imipenem or meropenem.
- These data suggest that PBP3 mutations cause resistance to sulbactam and sulbactam-ETX2514 due to reduced binding of sulbactam to PBP3.
- BLAST analysis of 1,537 whole genome sequenced strains of *A. baumannii* showed no variation in PBP3 at S390, S395 or V505. Nine strains were found to have a T511S substitution but no T511A variants were found. This suggests that pre-existing target-mediated resistance to sulbactam-ETX2514 is not a significant resistance mechanism in the clinical setting.

Conclusions

- **The novel β-lactamase inhibitor ETX2514 restores sulbactam antibacterial activity across a large collection of contemporary *A. baumannii* clinical isolates from around the world.**
- **Addition of imipenem to the sulbactam-ETX2514 combination improves activity even further.**
- **The frequency of spontaneous resistance to sulbactam-ETX2514 is low.**
- **Resistance to sulbactam-ETX2514 maps to PBP3, the target of sulbactam antibacterial activity.**
- **Additionally, some sulbactam-ETX2514 resistant isolates carried mutations in genes encoding tRNA synthetases, which are commonly associated with PBP2 inhibition and an induction of the stringent response.**
- **Resistance mapping and cellular morphological changes that occur in the presence of ETX2514 and sulbactam-ETX2514 suggest that ETX2514 enhances sulbactam antibacterial activity through inhibition of PBP2, in addition to its role as a β-lactamase inhibitor.**

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Acknowledgements
We thank Sam Bouchillon, Meredith Hackel and their teams at International Health Management Associates, Inc. (IHMA) for conducting susceptibility testing.

Presented at IDWeek October 26-30, 2016