Resistance to Sulbactam-Durlobactam in Clinical Isolates of *Acinetobacter baumannii* is Rare and Maps to PBP3

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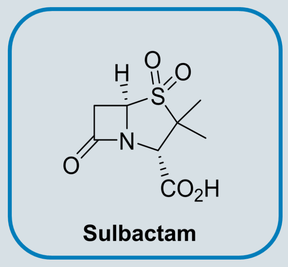
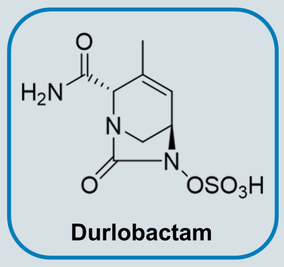
Abstract

Effective treatments for infections caused by *Acinetobacter baumannii* (*Ab*) are desperately needed. These infections are considered a significant public health concern due to high mortality rates associated with multidrug resistance. Historically, sulbactam (SUL), a class A β-lactamase inhibitor (BLI), could be used for the treatment of these infections due to its intrinsic antibacterial activity against *Ab*. However, its therapeutic utility has been severely compromised due to the emergence of resistance. We previously showed that laboratory-derived SUL resistance mapped to residues proximal to the active site of PBP3 at very low frequencies, and were associated with a fitness cost (1). Durlobactam (DUR, previously known as ETX2514), a broad-spectrum inhibitor of Class A, C and D serine β-lactamases (2), is currently in Phase 3 clinical testing in combination with SUL for the treatment of carbapenem-resistant *Ab*. Multiple, unbiased, global surveillance studies have shown that DUR restores SUL susceptibility (MIC ≤ 4 mg/L) in nearly 99% of clinical isolates (>3,600 tested to date). 59% of these were found to be carbapenem-non susceptible (CARB-NS). Whole genome sequencing analyses revealed that the rare SUL-DUR-resistant Ab strains encoded either A515V, T526S or I343F variants of PBP3 (3). These mutant alleles were cloned, purified and compared to wildtype PBP3 for relative binding to SUL and other β-lactams (BLs). The T526S mutant showed an almost complete loss of SUL binding, whereas A515V and I343F mutants showed only ~2-fold reduction. All three mutant PBP3s had a significant reduction in imipenem binding. Meropenem binding to PBP3 was lowered by A515V and T526S but not I343F. Aztreonam binding was modestly affected in all three mutants. Taken together, these results show that: (1) the vast majority (~99%) of SUL resistance in *Ab* is serine β-lactamase-mediated, which can be mitigated by combining with DUR and (2) specific mutations in PBP3, while very rare, can confer resistance to SUL-DUR. This is in contrast with recently approved BL-BLIs such as AvyCaz and Zerbaxa, where pre-existing clinical resistance was reported to be as high as 20% in certain target pathogens, such as CARB-NS *P. aeruginosa* (4).

Introduction

Sulbactam-Durlobactam (SUL-DUR) is a BL/BLI combination currently in Phase 3 clinical testing for the treatment of *A. baumannii* infections.





Durlobactam (previously known as ETX2514) is a novel BLI from a series of diazabicyclooctenones with best-in-class broad spectrum activity against class A, C and D β-lactamases.

Methods

- Broth microdilution susceptibility testing was conducted according to CLSI guidelines using cation-adjusted Mueller-Hinton broth. SUL-DUR was tested by dilution of sulbactam in the presence of a fixed concentration of 4 mg/L durlobactam. Testing of 2648 global *A. baumannii-calcoaceticus* complex isolates from 2016-2018 was performed at IHMA laboratories. Resistant mutants were analyzed by whole genome sequencing at Entasis Therapeutics on an Illumina MiSeq.
- Site-directed mutagenesis of the *A. baumannii* and *A. baylyi* PBP3 genes was performed using standard laboratory protocols. Transformation of ADP1 was performed as previously described (5).
- The *A. baumannii* PBP3 proteins (wildtype, I343F, S390T, S395F, T511S, A515V, and T526S) were purified using a 2-step purification scheme as previously described (1). PBP acylation rate constants were determined by competition with BOCILLIN FL in fluorescence polarization assays (6).

Results

Table 1 Global Surveillance of 2648 *A. baumannii-calcoaceticus* complex (ABC) isolates (2016-2018) tested by IHMA

All ABC (2016-2018)	Range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	% Sensitive*
Sulbactam-Durlobactam	**≤0.03 - >64**	**1**	**2**	**N/A**
Sulbactam	**0.25 - >64**	**8**	**64**	**N/A**
Amikacin	≤0.5 - >64	4	>64	59%
Cefepime	≤0.12 - >16	16	>16	44%
Ciprofloxacin	≤0.12 - >4	>4	>4	43%
Colistin	≤0.25 - >8	0.5	1	96%
Imipenem	0.06 - >64	8	64	49%
Meropenem	≤0.03 - >64	8	>64	48%
Minocycline	≤0.12 - >16	0.5	16	81%
Tigecycline	≤0.015 - 32	0.5	1	N/A

* Based on CLSI breakpoints, M100, 2019.

- Nearly 99% isolates are sensitive to SUL-DUR (based on a preliminary breakpoint of 4 mg/L)
- 38 isolates have an MIC ≥8 mg/L
- 15 of the 38 isolates carry the class B metallo β-lactamase, NDM-1 which durlobactam does not inhibit
- 21 out of the 38 isolates encode a mutation in PBP3, the target of sulbactam, with the T526S allele representing the majority of mutations (n=12), 4 isolates encoding an A515V allele, 2 isolates encoding F548I, and 1 each encoding K235N, Q488K, and G523V.

- Durlobactam restores sulbactam activity against 2648 *A. baumannii-calcoaceticus* complex global isolates collected in 2016-2018.
- The addition of durlobactam at 4 mg/L shifts the MIC_{90} by 32-fold from 64 mg/L to 2 mg/L.

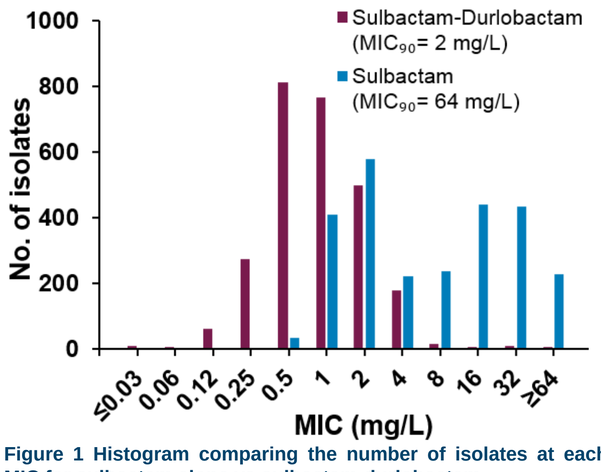


Figure 1 Histogram comparing the number of isolates at each MIC for sulbactam alone vs sulbactam-durlobactam

Results (continued)

To confirm the PBP3 mutations found in the clinical isolates from the 2016-2018 surveillance studies (and other previous studies) indeed confer sulbactam resistance, we used the genetically tractable *Acinetobacter baylyi* ADP1 strain to backcross the PBP3 mutants onto the chromosome. Linear PCR fragments of *A. baylyi* ADP1 PBP3 wild type, A515V, and T526S were used to transform ADP1 and the transformations were plated on agar plates with concentrations of sulbactam from 4 to 32 mg/L (ADP1 sulbactam MIC = 2 mg/L). MICs were determined for these isolates and PBP3 alleles for each resulting mutant were determined by Sanger sequencing.

Table 2 MICs of *A. baylyi* ADP1 strains transformed with wild type or mutant PBP3 alleles and selected at various concentrations of sulbactam

		MIC (mg/L)							
Strain	**Sequenced PBP3 allele**	**SUL**	**DUR**	**SUL-DUR**	**IMI**	**ATM**	**TET**	**COL**	**RIF**
A. baylyi ADP1		2	16	0.25	<0.06	32	1	0.5	2
ADP1 + wild type PCR @ SUL8	S390T	128	64	32	<0.06	16	1	0.25	2
ADP1 + A515V PCR @ SUL32	S390T A515V	128	64	16	0.13	16	1	0.5	2
ADP1 + T526S PCR @ SUL4	T526S	16	32	8	<0.06	32	1	0.25	2
ADP1 + T526S PCR @ SUL8	S395P T526S	32	32	8	0.25	64	1	0.5	2

SUL: Sulbactam; DUR: Durlobactam; IMI: Imipenem; ATM: Aztreonam; TET: Tetracycline; COL: Colistin; RIF: Rifampin

- on increasing concentration of sulbactam had SUL MICs 16-64 fold higher than the parent.
- Wild type PCR transformants resulted in spontaneous resistant mutants previously described (i.e. S390T)
- This MIC shift in sulbactam resulted in increased MICs of the SUL-DUR combination, as was observed in the 2016-2018 surveillance studies.

In addition to genetic confirmation of sulbactam resistance being driven by mutations in PBP3, the wild type and various PBP3 alleles were cloned, expressed, and purified. The effects of these mutations on the reactivity with sulbactam, aztreonam, imipenem, and meropenem were determined.

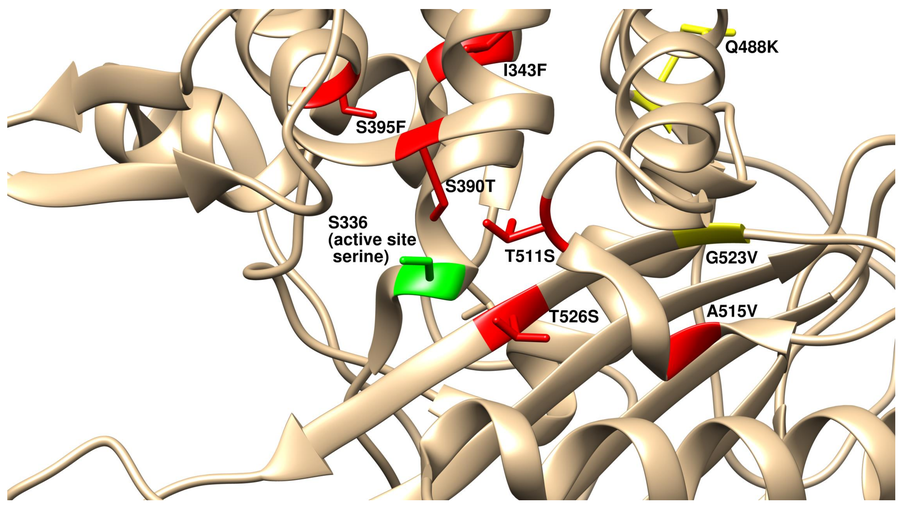


Figure 2 Structure of *A. baumannii* PBP3 with residues of interest highlighted. Green: active site Serine. Red: Mutations associated with SUL resistance and tested *in vitro*. Yellow: Mutations associated with SUL resistance and pending confirmation.

- The identified mutants of PBP3 associated with sulbactam resistance map near the active site of *A. baumannii* PBP3.
- PDB: 3UE3

Table 3 Acylation rate constants of various compounds against purified wild type and mutants associated with sulbactam resistance.

	k_{inact}/K_i ($M^{-1}s^{-1}$) (% of WT)			
PBP3 Variant	**Sulbactam**	**Aztreonam**	**Imipenem**	**Meropenem**
wild type	18	750	490	6000
I343F	9.2 (51%)	900 (120%)	246 (50.2%)	6800 (113%)
S390T	0.1 (0.6%)	900 (120%)	540 (110%)	16,000 (266%)
S395F	0.6 (3.3%)	110 (14.7%)	9 (1.8%)	1500 (25%)
T511S	1.5 (8.3%)	450 (60%)	220 (44.9%)	3400 (56.7%)
A515V	10.8 (60%)	590 (78.7%)	120 (24.5%)	1405 (23.4%)
T526S	0.8 (4.4%)	900 (120%)	38 (7.8%)	1300 (21.7%)

- All mutants of PBP3 had reduced rate constants for sulbactam acylation (0.6 – 60% of wild type) but had variable effects on the other tested compounds.
- The most resistant mutants, at positions S390 and S395, were lab generated but have not yet been found in any clinical isolates, presumably due to fitness defects (1).

Conclusions

- **The novel β-lactamase inhibitor durlobactam restores sulbactam antibacterial activity against a large (n = 2648) and globally diverse set of *A. baumannii* isolates collected from 2016 – 2018.**
- **Genetic and biochemical approaches were employed to confirm the PBP3-driven resistance to sulbactam and sulbactam-durlobactam.**
- **Nearly 99% of tested isolates to date are sensitive to SUL-DUR. Resistance to the combination is rare and driven by the presence of class B metallo β-lactamases (such as NDM-1) or mutations in PBP3, which is the target of sulbactam.**

References

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