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Avibactam is a substrate for MexAB-OprM in *Pseudomonas aeruginosa*

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Introduction & Purpose

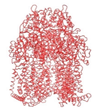
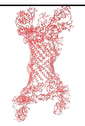
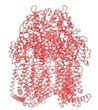
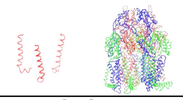
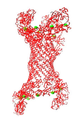
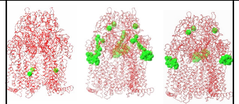
Avibactam (AVI), a novel broad-spectrum inhibitor of beta-lactamases, is marketed in combination with ceftazidime (CAZ) as Zavicefta® in the EU and Avycaz® in the US (henceforth abbreviated as CAZ-AVI) for the treatment of serious Gram-negative infections, including those caused by *Pseudomonas aeruginosa* and AmpC-, KPC- or ESBL producing Enterobacteriaceae. We recently showed that .the susceptibility of *Pseudomonas aeruginosa* isolates from patients with cystic fibrosis to CAZ increases from 36% to 76% (EUCAST breakpoints [1]) when combined with AVI [2]. Both the US label [3] and the European Summary of Product Characteristics [4] of CAZ-AVI mention that active efflux may confer resistance to this combination. We therefore explored whether MexAB-OprM affects the activity of CAZ-AVI in *P. aeruginosa* isolates from cystic fibrosis, taking into account that up to 1/3 of them show mutations inactivating MexAB-OprM [5].

Materials & Methods

We used (i) PAO1 and mutants thereof defective or overexpressing MexAB-OprM, and (ii) 10 clinical isolates from patients with cystic fibrosis that were resistant to CAZ but susceptible or resistant to CAZ-AVI. MICs were determined by broth microdilution (in the presence of 0.25 mg/L imipenem [sub-MIC concentration] for PAO1 and its mutants to induce AmpC expression). *mexA* and *mexB* were sequenced and MexAB-OprM functionality assessed by measuring the kinetics of efflux of the specific fluorescent substrate N-phenyl-1-naphthylamine (NPN). MexA/B proteins were rendered using *Visual Molecular Dynamics* software.

Results

Description of bacterial isolates, MICs, and efflux characteristics.

Strains	MIC (mg/L)		Type of mutations in MexAB-OprM pumps	Molecular Representation*		NPN efflux (V_{max} – units/s)
	CAZ	CAZ-AVI		MexA	MexB	
PAO1	32	0.125	none			-0.48
PAO1 *ΔmexAB-OprM*	32	0.125	no expression			-0.12
PAO1 MexAB-OprM overproducing	32	4	none			-0.72
4 cystic fibrosis isolates	128 to 512	4 to 8	truncated MexA or MexB			-0.02 to -0.10
3 cystic fibrosis isolates	256 to 1024	16	truncated MexA			-0.01 to -0.12
3 cystic fibrosis isolates	128 to 1024	16 to 512	amino acid substitutions in MexA or MexB			-0.25 to -0.58

* Color code: red for encoded parts of MexA and MexB proteins; blue for deleted residues; green for nonsynonymous substitutions of amino acids.

- Overproduction of MexAB-OprM in PAO1 markedly increased CAZ-AVI MICs (5 $\log_2$ dilutions) without increasing the MIC of CAZ, demonstrating a role of this transporter in AVI efflux.
- 4 isolates with truncated MexA or MexB (low efflux activity) became susceptible to CAZ-AVI while 3 other isolates had a MIC of 16 mg/L in the presence of AVI.
- 3 isolates with amino acid substitutions in MexA or B (higher efflux activity) still showed elevated MICs in the presence of AVI.

Conclusions

- Efflux mediated by MexAB-OprM contributes to reducing AVI activity in *P. aeruginosa* (leading to elevated MICs for CAZ-AVI).
- Testing CAZ-AVI in isolates from patients with cystic fibrosis appears useful because of the high prevalence of isolates defective in MexAB-OprM.
- Yet, some of these efflux-defective isolates remain less susceptible to CAZ-AVI than the wild-type reference strain, probably due to expression of other resistance mechanisms that need to be further investigated.

Acknowledgments

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References

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