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Role of efflux pumps on the antimicrobial activity of representative penicillins, aminoglycosides and macrolides against laboratory strains of *Achromobacter insuavis* and clinical isolates of *Achromobacter xylosoxidans* from patients with cystic fibrosis

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Introduction & Purpose

***Achromobacter xylosoxidans* (*Ax*)**, which is highly resistant to a wide range of antibiotics, becomes increasingly prevalent in patients with cystic fibrosis (CF) [1,2]. One resistance mechanism in *Ax* is active efflux through AxyABM and AxyXY-OprZ efflux pumps, which share homology with MexAB-OprM and MexXY-OprM in *Pseudomonas aeruginosa*, respectively [3,4].

Here, we explore the role of AxyABM and AxyXY-OprZ in the resistance of *Ax* to antibiotics previously described as substrates for MexAB-OprM (ticarcillin and temocillin [6-α-methoxy-ticarcillin; resistant to most ESBLs]), MexXY-OprM (aminoglycosides) or both (macrolides) [5,6].

Materials & Methods

Strains: 3 *Achromobacter insuavis* strains [*Ai:* clinical isolate, *AiΔB/ΔY: Ai* with deletions in *axyB* [3] or *axyY* [4] efflux genes; 99% identity with corresponding genes in *Ax*], 1 *Ax* ATCC27061 and 19 isolates (identified as *Ax* by MALDI-TOF) recovered from sputum samples of chronically colonized patients with CF at the Münster University Hospital and Clemenshospital, Münster, Germany.

MICs of temocillin (TMO), ticarcillin (TIC), amikacin (AMK), and azithromycin (AZI) were determined by broth microdilution with appropriate QC strains according to CLSI guidelines (2018) for non-*Enterobacteriaceae*, with or without a β-lactamase inhibitor (tazobactam ([TZB]), or an efflux inhibitor (berberine [BER] [7]).

NPN uptake: The uptake of the lipophilic probe N-phenyl-1-naphtylamine [8] (NPN at 10 µM, a potential substrate for AxyXY-OprZ) after 10 minutes of incubation was used to follow the AxyXY pumping activity in reference strains and CF bacterial isolates.

Results

MICs (n=3) in broth (microdilution method)

Strain	MIC (mg/L)									
	BER alone	TMO			TIC		AMK		AZI	
		alone	+TZB (32 mg/L)	+ BER (128 mg/L)	alone	+TZB (32 mg/L)	Alone	+BER (128 mg/L)	alone	+BER[a]
Ai	>256	512-1024	512	512	0.5[b]	0.5	128	16	64	32
AiΔB	>256	128-256	128-256	128-256	512[b]	0.25	128	16	64	32
AiΔY	>256	1024-2048	2048	2048	512[b]	2	32	4	16	16
Ax ATCC	>256	512	512	512	2	4	1024	64	128	16
Ax (CF) MIC_{50}	ND	1024	ND	2048[c]	512	256	512	32	128	16
MIC range	ND	256->2048			2->2048		8->2048		16->2048	

[a] BER concentration: 4 mg/L in *Ai* and 128 mg/L in *Ax* (ATCC and clinical isolates) [concentration causing the maximal decrease in MIC in each case]

[b] Ai is innately susceptible to ticarcillin. Ai ΔB and ΔY mutants harbor pUC19 plasmid which encodes a carboxy-penicillinase. TZB was thus added to inhibit this beta-lactamase when testing for beta-lactam MICs (note however that TMO resists to carboxy-penicillinase).

[c] Tested on 7 CF isolates, no effect of berberine [128 mg/L] was observed.

ND: Not determined. CLSI breakpoints (mg/L) for AMK: S≤16, I=32, R≥64; TIC(+CLAV) S≤16, I=32-64, R≥128. No breakpoints are set for TMO or AZI.

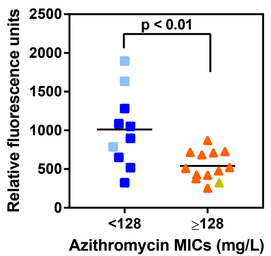
Reference strains:

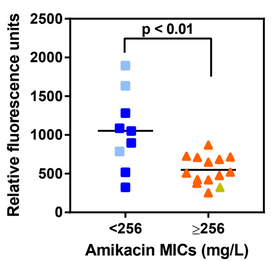
- As compared to the parental strain, TMO and TMO-TZB MICs were decreased by 1-2 doubling dilutions in *AiΔB*, but that of TIC-TZB by 1 doubling dilution only; AMK and AZI MICs were decreased by 2 doubling dilutions in *AiΔX*.
- BER markedly reduced the MICs of AMK and of AZI to a lower extent; it did not affect the MICs of β-lactams.

Clinical isolates:

- The MIC_{50} of all drugs were high, and significantly reduced by BER for AMK and AZI only.
- Based on CLSI interpretive criteria, up to 30% of the clinical isolates were susceptible to AMK.

NPN accumulation in reference strains and clinical CF isolates of *Achromobacter* as a function of their AZI/AMK MIC





Clinical isolates of *Ax* with low AZI / AMK MICs (< 128 / < 256 mg/L) also showed significantly higher NPN accumulation, suggestive of lower efflux activity.

Light colors: reference strains; dark colors: clinical isolates

Statistical analysis: partition tree to determine the MIC value splitting distribution in 2 with the highest Logworth value (-log p-value), using JMP-Pro 14.

Main Messages

- While TIC, TMO, and AZI are substrates for MexAB-OprM in *P. aeruginosa* [5,6], they are not or only marginally affected by AxyABM efflux pump in *Achromobacter.*
- AMK and AZI are both substrates for AxyXY-OprZ efflux pump.
- The high MICs of beta-lactams in clinical isolates and the poor effect of TZB / absence of effect of BER on these MICs suggest the presence of other resistance mechanisms to these drugs beside production of TZB-inhibitable β-lactamases or efflux.
- Susceptibility to AMK in some clinical isolates was unexpected (*Ax* being considered innately resistant to aminoglycosides [9]) and needs to be further explored.

References

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