Population analysis, mutant prevention concentrations and characterization of resistance mutations to novel β-lactams in wild-type, mutator, and high-risk clones of *Pseudomonas aeruginosa*

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BACKGROUND AND OBJECTIVES

Pseudomonas aeruginosa is one of the main causes of nosocomial and chronic infections, which possesses exceptional abilities for developing antimicrobial resistance. The objectives of this work were to determine the mutant prevention concentration (MPC) and to characterize the one-step mutants to novel β-lactams in wild-type, mutator and XDR high-risk clones of *P. aeruginosa*.

METHODOLOGY

Resistant populations analysis was performed for imipenem/relebactam (IMR), ceftazidime/avibactam (CZA), ceftolozane/tazobactam (CTZ) and cefiderocol (FDC) in PAO1, PAOMS (Δ*mutS*) and three XDR clinical strains belonging to high-risk clones ST111 (Δ*oprD*, ↑AmpC, CARB-2), ST175 (Δ*oprD*, ↑AmpC) and ST235 (Δ*oprD*, OXA-2). Approximately 10^9 UFCs and serial dilutions were plated in Müller-Hinton (MH) agar plates with increasing concentrations (0-64mg/L) of IMR, CZA, CTZ and FDC for each strain in duplicate independent experiments. MPC was defined as the lowest concentration of antibiotic yielding no growth. Six mutants from each experiment obtained from the highest antibiotic concentration showing growth were characterized through the determination of the susceptibility profiles (EUCAST MH broth microdilution, iron-depleted for FDC) and whole genome sequencing.

RESULTS

Figure 1. Resistant population analysis of CTZ, CZA, FDC and IMR.

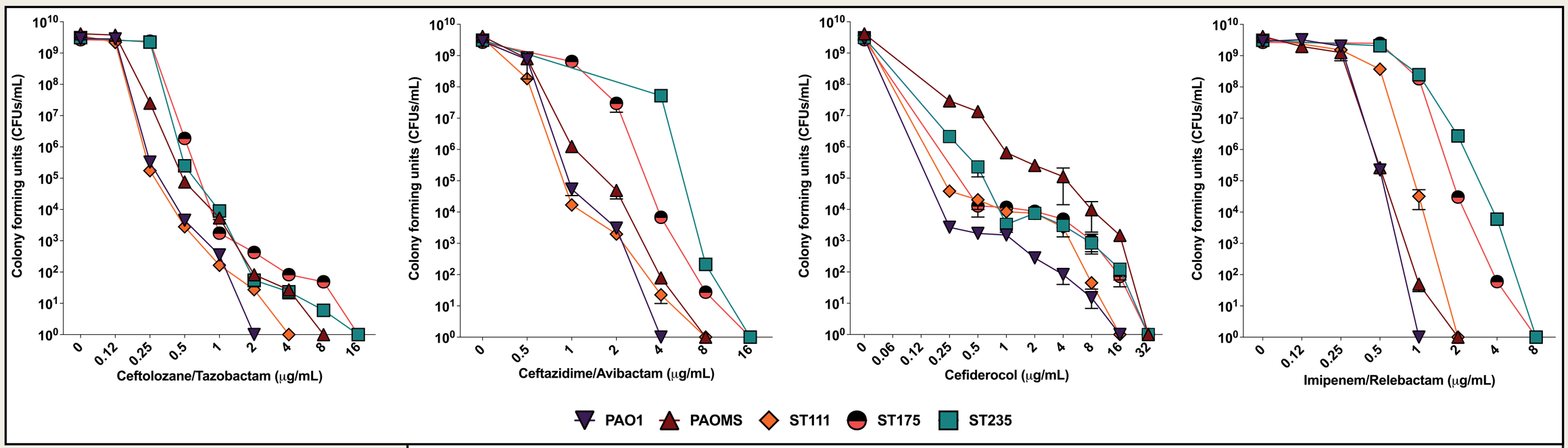

Figure 2. MIC and MPC of parental strains, phenotype and genes mutated in one-step resistant mutants.

IMR presented the lowest MPC values (1-8 mg/L), followed by CTZ (2-16 mg/L), CZA (4-16 mg/L) and FDC (16-32 mg/L). Overall, the MICs of the mutants were consistent with the antibiotic selection concentration, except for FDC that were much lower. Moreover, Gradient MIC and disk diffusion revealed FDC heteroresistance in all FDC mutants. Crossresistance was highest between CTZ and CZA and lowest for IMR. CZA and CTZ derivatives presented mutations in *ampC*, *galU*, *cpxRS* and, when present (ST235), in OXA-2. FDC mutants were defective in iron-uptake systems, particularly *piuA/DC*. IMR derivatives showed changes in *oprD* (when functional), *mexB*, *mexF*, and/or *aroB*.

Antibiotic	Strain	MIC	MPC	One-step resistant mutants: CTZ	CZA	FDC	IMR	Genes mutated **(number of mutants)**
CTZ	PAO1	0.25	2					*cpxS* (**5**), *cpxR* (**1**)
	PAOMS*	0.25	8					*ampC* (**3** G183D), *ampR* (**3**), *mpl* (**3**), *nalD* (**2**)
	ST111	0.5	4					*ampC* (**3** G183D, **2** aa234Δ7, **1** E247K)
	ST175	0.5	16					*ampC* (**4** aa246Δ3, **2** G183D)
	ST235	0.5	16					OXA2 (**5** N148K, **1** W159R), PA2333 (**2**)
CZA	PAO1	1	4					Δ*mexXY-galU* region (**6**)
	PAOMS*	1	8					*mexY* (**3**), *galU* (**1**), *oprN* (**1**)
	ST111	1	8					*ampC* (**3** G183D, **3** aa234Δ7)
	ST175	2	16					*ampC* (**6** G183D)
	ST235	2	16					OXA2 (**3** G162D, **3** W159R), *oprQ* (**3**)
FDC	PAO1	0.06	8-16					*piuA* (**1**), *phuV* (**1**)
	PAOMS*	0.12-0.25	32					*piuA* (**3**), *piuC* (**2**), *cpxS* (**2**), *pirR* (**2**), *pirA* (**1**), *dacC* (**1**)
	ST111	0.06	16					PA0819 (**2**), *fecI* upstream (**1**), *piuD* (**1**), *piuC* (**1**), *ynbD* (**1**)
	ST175	0.12	32					ND
	ST235	0.5	32					*piuD* (**4**), *piuC* (**1**), *oprN* (**1**), PA2108 (**1**), PA2163 (**1**)
IMR	PAO1	0.25	1					*oprD* (**6**)
	PAOMS*	0.25	2					*oprD* (**5**), *mexF* (**1**)
	ST111	0.5	2					*mexB* (**6**)
	ST175	1	8					*aroB* (**5**), PA1068 (**1**), *purB* (**1**), *hmgA* (**1**)
	ST235	1	8					*aroC* (**3**), *aroB* (**3**), *oprQ* (**1**), PA0479 (**1**), PA1960 (**1**), PA0562 (**1**)

-6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6

MIC change (2-fold dilution)

*: Up to 19-30 mutations detected per derivative, only those genes that have been previously associated with β-lactam resistance are shown.
ND: not detected.

CONCLUSIONS

This work first describes the MPCs and first-step resistance mechanisms for novel β-lactams in *P. aeruginosa*, including mutators and XDR high-risk clones. Lowest MPC values and crossresistance was documented for IMR, consistently with the resistance mechanisms involved. On the other hand, FDC had high MPC values, but mutants, frequently defective in iron-transport systems, showed heteroresistance in agar based assays (gradient MIC and disk diffusion) and low MICs in iron-depleted MH broth, adding further complexity to resistance testing for this agent.