P1604

Dual AmpC and AmpD Mutations in Pseudomonas aeruginosa: Unraveling the Mechanisms of Resistance to Ceftazidime and Avibactam

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Background · Ceftazidime/avibactam (CZA) is commonly used against multidrug resistant *Pseudomonas aeruginosa* (Pa) although resistances are frequently reported subsequent to treatment. Two isogenic clinical isolates were collected during bacterial infection: one susceptible and one resistant to CZA, which exhibited mutation in the chromosomic beta-lactamase ($AmpC^{G183D}$) and its negative regulator ($AmpD^{H157Y}$). **This study assesses the respective impact of each mutations on ceftazidime (CAZ) and avibactam (AVI).**

Materials and methods · Single and double mutants $AmpC^{G183D}$ and $AmpD^{H157Y}$ were previously generated in a PAO1 reference strain by scarless directed mutagenesis (L. Deroche *et al.,* 2023*)*. Minimum inhibitory concentrations (MIC) of CAZ, AVI, and CZA were assessed by broth microdilution (EUCAST, 2022). Time-kill curves (TKC) were performed (n = 3) in MHB II on exponential phase cultures of PAO1 and its mutant with starting inoculum at 1.10^6 CFU/mL. CAZ (½*CMI to 4*CMI) and AVI (0,125 mg/L to 64 mg/L) were separately added to obtain different concentrations of the combination in each well. One well without addition of antibiotic was included in each experiment as growth control. Bacteria were sampled and plated at 0, 2, 4, 6, 24, and 30 h on MHA plate using spotting method and CFU was counted after a 16-24 h incubation using a semi-automatic plate reader.

Results · *Minimal inhibitory Concentrations*

- For all strains, **AVI MIC exceeded 256 mg/L** (Table 1).
- **No difference** was observed in CAZ and CZA MIC of $AmpC^{G183D}$ mutant compared to PAO1.
- $AmpD^{H157Y}$ single mutant showed increased CAZ MIC and moderately increased CZA MIC.
- Elevated CZA MICs occurred only in the double mutant.

Table 1 : Minimal inhibitory concentrations of PAO1 and mutant-derived strains. EUCAST breakpoint is established at 8 mg/L. (1) Avibactam concentration is fixed at 4 mg/L.

Strain	MIC (mg/L)		
	CAZ	AVI	CZA(1)
PAO1	2	>256	2
PAO1 - $AmpC^{G183D}$	2	>256	2
PAO1 - $AmpD^{H157Y}$	16	>256	4
PAO1 - $AmpC^{G183D}$ / $AmpD^{H157Y}$	32	>256	16

· *Time-Kill Curves*

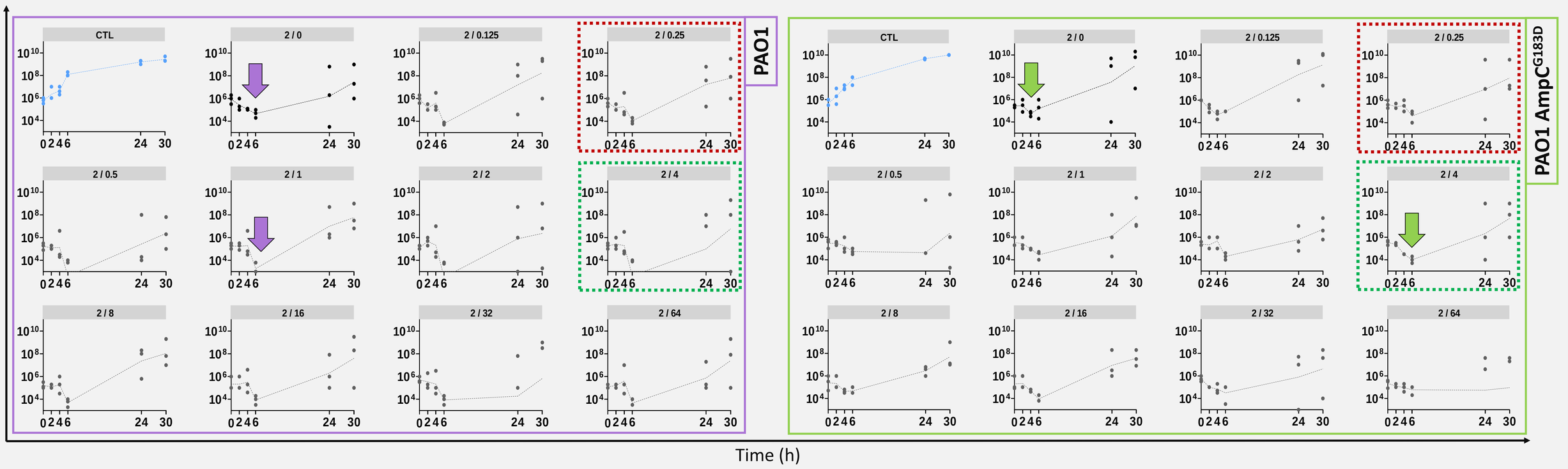

Figure 2: Colony-forming units monitored under static conditions over time during time-kill curves against CAZ and AVI on PAO1 and PAO1 $AmpC^{G183D}$. On each gray box upon graphs, left number represent CAZ concentration (MIC) and right number represent AVI concentration (from 0 to 64 mg/L). Orange boxes denotes the Zavicefta® fixed 4:1 ceftazidime-to-avibactam ratio. Green boxes denotes the fixed concentration of avibactam (4 mg/L) used *in vitro*.

- PAO1 showed an initial CFU decay followed by regrowth for all concentrations, but **AVI seems to potentiate the effect on initial decay even at low concentrations** (purple arrows).
- $AmpC^{G183D}$ mutant, displaying basal expression of *ampC*, seemed to show an initial CFU decay as PAO1 without influence of AVI (green arrows).

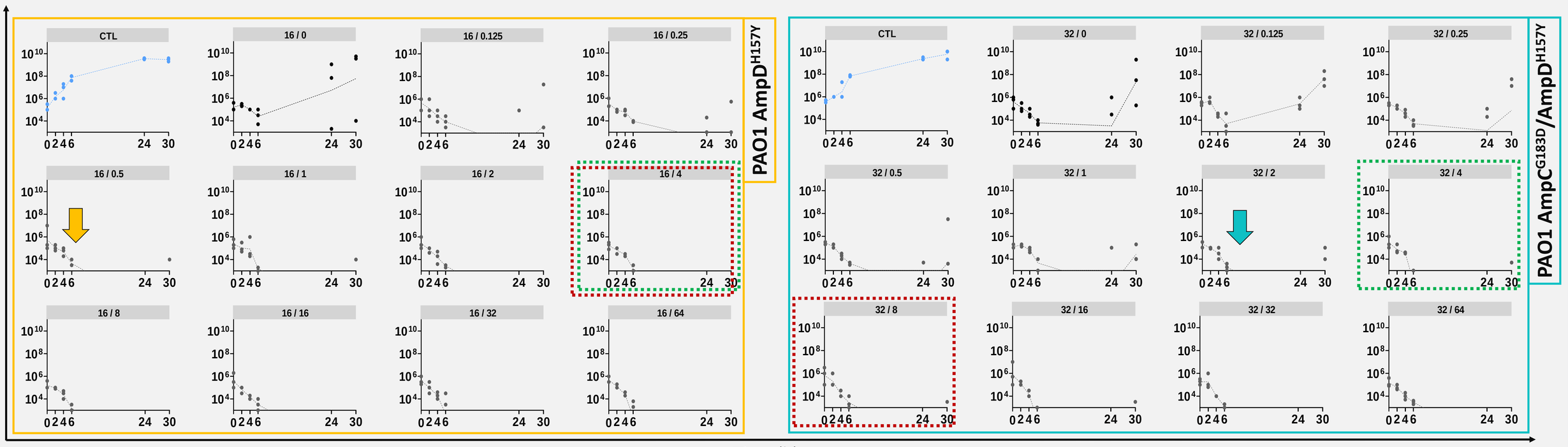

Figure 3: Colony-forming units monitored under static conditions over time during time-kill curves against CAZ and AVI on PAO1 $AmpD^{H157Y}$ and PAO1 $AmpD^{H157Y}$/$AmpC^{G183D}$. On each gray box upon graphs, left number represent CAZ concentration (MIC) and right number represent AVI concentration (from 0 to 64 mg/L). Orange boxes denotes the Zavicefta® fixed 4:1 ceftazidime-to-avibactam ratio. Green boxes denotes the fixed concentration of avibactam (4 mg/L) used *in vitro*.

- $AmpD^{H157Y}$ mutant, overexpressing *ampC*, showed **no regrowth for weak concentrations of AVI from 0.5 mg/L** (yellow arrow).
- The double mutant, overexpressing mutated *ampC*, showed **no regrowth for higher concentrations of AVI (from 2 mg/L) than $AmpD^{H157Y}$ mutant** (blue arrow).

Conclusion · Our results demonstrated that the presence of the $AmpC^{G183D}$ mutation alone is not sufficient to confer resistance to CAZ and CZA in *Pseudomonas aeruginosa*. Moreover, the inhibitory effect of avibactam is different between strains either on the initial decay or regrowth. A semi-mechanistic PK/PD model is being developed to precisely characterize the respective contributions of these mutations on the efficacy of CAZ and AVI.

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