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Heteroresistance to cefiderocol among *Pseudomonas aeruginosa* clinical isolates harboring changes in TonB-dependent receptor pathways and AmpC

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Abstract

Background: Cefiderocol (FDC) is an iron siderophore cephalosporin with activity against multi-drug resistant (MDR) Gram-negative bacteria. Despite high rates of in vitro susceptibility, treatment failure with the emergence of resistance is still a concern. Heteroresistance, or the presence of a resistant sub-population in a strain that tests susceptible by standard methods, has been suggested as a potential mechanism for this observation. Previously, we showed that MDR *Pseudomonas aeruginosa* (PA) with changes in the iron siderophore transporters (TBDR) responsible for FDC uptake had decreased zone diameters on Kirby-Bauer (KB) testing as compared to MDR-PA with wild-type TBDRs. Here, we investigated the prevalence of heteroresistance (hR) among TBDR mutants using population analysis profile-area under the curve assay (PAP).

Methods: Previously sequenced MDR-PA isolates were selected for the presence of mutations in TBDR genes within the primary FDC import pathways (*pirA*, *pirRS*, *piuA*, *piuD*). Isolates harboring pre-existing TBDR mutations (n=12) were compared with wild-type *P. aeruginosa* strain PA01 and 5 MDR-clinical isolates harboring wild-type TBDRs. FDC MICs were performed by broth microdilution (BMD) in iron depleted media (IDM). For PAP, isolates were grown overnight in IDM before serial dilution and plating on agar with increasing concentrations of FDC. Agreement between BMD, KB, and PAP-AUC HR detection was assessed.

Results: On BMD, 10 of 12 TBDR isolates tested susceptible to FDC (MIC range ≤0.5-2 µg/mL), and all control isolates had an MIC ≤0.5 µg/mL (**Table 1**). None of the control strains were hR per PAP (**Fig 1**). However, in the TBDR group, 50% of isolates showed either a resistant (n=2) or HR (n=4) phenotype. Resistance occurred in the setting of both TBDR mutations and either an exogenous β-lactamase (OXA-15 variant) or AmpC variant (PDC-205). Of the HR isolates, one had a change in AmpC Ω-loop (E221K), while the other three had only the PirR frameshift mutation. BMD was not able to identify any of the HR isolates.

Conclusions: A greater number of MDR-PA with TBDR mutations showed resistance or HR to FDC than MDR-PA controls with wild-type TBDRs. Mutations in TBDRs could be a marker for FDC non-susceptibility and potential therapeutic failure.

Background/Introduction

Cefiderocol is an iron siderophore cephalosporin with activity against the priority pathogen MDR *Pseudomonas aeruginosa* (PA).[1] However, recent clinical studies suggest some discordance between *in vitro* susceptibility and clinical treatment success.[2,3] Cefiderocol utilizes the Ton-dependent receptors (TBDR)s PiuA and PirA to gain entry to the PA periplasmic space and mutations in this pathway have been associated with loss of cefiderocol susceptibility.[4] We have previously reported the emergence of non-susceptibility to FDC in a clinical isolate with pre-existing TBDR mutations.[5] Further, analysis of a previously sequenced set of PA at our institution found over 20% possessed mutations in the genes *pirA*, *piuA*, or their associated regulatory pathways.[6] A subset of these mutants showed breakthrough colonies on Kirby Bauer (KB) disk diffusion analysis, and subcultures suggested a decrease in FDC susceptibility after only a single drug exposure.[7]

Purpose/Objectives/Hypothesis

- We hypothesized that TBDR mutants would display higher rates of the hR phenotype than non-mutant controls.
- This hR phenotype may be a risk factor for the rapid emergence of FDC resistance.

Methods

- Previously sequenced MDR-PA isolates were selected for the presence of major (e.g. insertion, deletion, frameshift, stop) mutations in the TBDR genes (*pirA*, *pirRS*, *piuA*, and *piuD*) using PAO1 as the reference.
- Isolates harboring pre-existing TBDR mutations (n=12) were compared with wild-type *P. aeruginosa* strain PA01 and 5 MDR-clinical isolates harboring wild-type TBDRs.
- Clinical resistance phenotypes were assessed by performing CLSI gold-standard broth microdilution in iron-depleted Muller Hinton (MH) to obtain MICs for each isolate.
- PAP-AUC was performed for each isolate using growth overnight in iron depleted media followed by subsequent agar dilution on MH agar with FDC at concentrations of 0, 2, 4, 8, and 16 µg/mL.
- Results of BMD and PAP-AUC were compared to prior KB studies.
- Repeat MICs were performed post-FDC exposures on PAP-AUC subpopulations growing at 8 µg/mL and/or 16 µg/mL from agar dilution plates to assess for the emergence of resistance.
  - These isolate underwent 3 serial passages in absence of FDC prior to repeat BMD to assess hR/resistance stability.
- PDC (Amp C) variants were also assessed from the whole genome sequence data.

Results

Table 1. *Pseudomonas aeruginosa* strain characteristics.

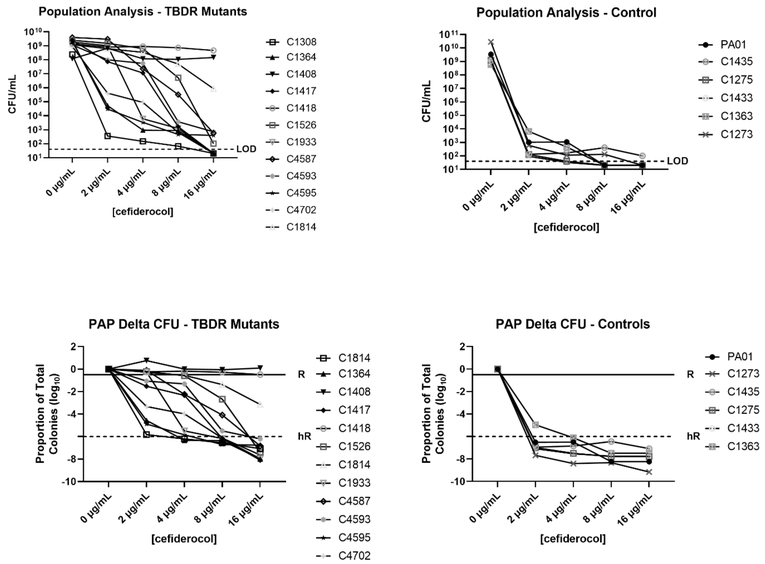
Strain	ST	TBDR mutation	PDC Type	β-lactamase	FDC DD (mm)	ID-CA-MHB MIC (µg/mL)	PAP-AUC	ID-CA-MHB MIC post-FDC (µg/mL) [1]
C1308	ST308	pirR_fs	PDC-37		34	≤0.5	Susceptible	≤0.5
C1364	ST633	piuA_ΔG415	PDC-3		28	≤0.5	Susceptible	2
C1408	ST235	pirR_InsG131	PDC-35	OXA-15	13	32	Resistant	>32
C1417	ST111	pirR_fs	PDC-3		26	≤0.5	Susceptible	≤0.5
C1418	novel ST	pirR_fs	PDC-205		12	8	Resistant	32
C1526	ST111	pirR_fs	novel A46D;T79A;E221K		25	≤0.5	Heteroresistant	≤0.5
C1814	novel ST	pirR_fs	PDC-1		24	1	Heteroresistant	16
C1933	novel ST	pirR_fs	PDC-1		26	≤0.5	Susceptible	1
C4587	ST274	pirR_fs	PDC-24		23	≤0.5	Heteroresistant	2
C4593	ST274	pirR_fs	PDC-24		26	≤0.5	Heteroresistant	8
C4595	ST870	piuA_ΔG415	PDC-3		25	2	Susceptible	1
C4702	novel ST	pirR_fs	PDC-31		25	1	Susceptible	1
Controls								
PA01		WT	PDC-1		30	≤0.5	Susceptible	≤0.5
C1273	ST235	none	PDC-35		33	≤0.5	Susceptible	4
C1435	ST235	none	PDC-35		29	≤0.5	Susceptible	1
C1275	ST111	none	PDC-3		33	≤0.5	Susceptible	≤0.5
C1433	ST111	none	PDC-3		32	≤0.5	Susceptible	-*
C1363	ST235	none	PDC-35		31	≤0.5	Susceptible	-*

DD, disk diffusion; FDC, cefiderocol; ID-CA-MHB, iron-depleted cation adjusted Muller-Hinton broth; PAP-AUC, population analysis profile-area under the curve; PDC, Pseudomonas derived cephalosporinase; ST, sequence type; TBDR, TonB dependent receptor.

[1] MICs obtained after from PAP-AUC breakthrough growth at highest concentrations of FDC where growth was seen (8 or 16 µg/mL). Repeat MICs from growth on 0 µg/mL FDC PAP plates for each isolate were all <=1 fold increase from initial MICs.

* Strains had no growth on agar plates with FDC 8 or 16 µg/mL.

Figure 1. PAP-AUC resistance assessment of CR-PA TBDR mutant clinical isolates compared to non-TBDR controls



Results

PAP-AUC:

- Two TBDR mutants deemed non-susceptible by BMD were found to be resistant per PAP-AUC analysis.
- Four TBDR mutants tested susceptible by BMD but displayed a hR phenotype on PAP-AUC.
- Control group isolates showed no evidence of hR and colonies recovered from PAP plates remained FDC susceptible by BMD.

Repeat BMD MICs of subpopulations:

- Colonies recovered from PAP plates developed a ≥2-fold increase in FDC MIC for 3 of the 4 hR isolates.
- Two of the 3 isolates (C1814 and C4593) were non-susceptible (8 and 16 µg/mL, respectively) on subculture BMD (Table 1, Column 9)

Conclusions/Future Directions

Previously, we have seen clinical isolates of *Pseudomonas aeruginosa* exhibit decreased susceptibility to cefiderocol associated with pre-existing TBDR mutations.[5] Mutations in TBDRs appear to be common, with up to 20% of isolates harboring at least one TBDR mutation in some clinical populations.

- In this study, we demonstrated FDC heteroresistant populations that are not detected with standard iron-depleted broth microdilution susceptibility testing.
- This phenotype was more common in isolates with frameshift mutations leading to a stop codon in pirR or changes in AmpC.
- Further, hR-associated resistant subpopulations can lead to the emergence of cefiderocol resistance *in vitro* after a single FDC exposure.
- We hypothesize that TBDR mutations provide a low barrier for resistance secondary to an increased vulnerability to additional resistance determinants such as exogenous β-lactamases or changes in the intrinsic AmpC enzyme.

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