In vivo Emergence of Cefiderocol Resistance in *Pseudomonas aeruginosa* following prolonged treatment at San Martino Policlinico Hospital (HSM), Genoa, Italy

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Introduction

In recent years, the nosocomial spread of multidrug-resistant (MDR) *Pseudomonas aeruginosa* has become a serious threat for the health care system. Beyond agents suffering from poorer pharmacokinetic properties and increased risk of toxicity, such as aminoglycosides or polymyxins, few antibiotics are effective against MDR *P. aeruginosa*, including ceftazidime/avibactam (CZA), ceftolozane/tazobactam (C/T), cefiderocol (FDC) a novel siderophore cephalosporin[1,2]. Recently, cases of *P. aeruginosa* isolates developing resistance against these agents have been reported, either due to mutations in functionally-diverse housekeeping genes (e.g. genes coding for the resident Amp-C βlactamase, bla_{PDC}, for porins and regulators of efflux pumps, and for siderophore receptors involved in FDC uptake) or to acquisition of genes coding for metallo-β-lactamases (e.g. VIM-/NDM-type) and/or various serine β-lactamases (e.g. PER-, GES-, SHV-types)[2-5]. Here, we report a case of *in vivo* development of FDC resistance in a CZAR/C/TR *P. aeruginosa* isolates from a patient during prolonged treatment.

Materials and methods

Identification and antibiotic susceptibility testing (AST) were performed with Vitek MS MALDI-TOF and reference broth microdilution method, respectively. Carbapenemases production was assesed by lateral flow Immunochromatography (LFIA), RT-PCR and mCIM assays. C/T and FDC susceptibility tests were performed by E-test *(Liofilchem, Teramo, Italy*), and by Kirby Bauer disk-diffusion (DD), respectively; AST results were interpreted according to EUCAST clinical breakpoints v14.0. Genomic DNA was extracted with Qiagen DNeasy Blood and tissue and subjected to whole genome sequencing (WGS) with Illumina NovaSeq. Bioinformatics analysis was performed with QUAST, snippy, using *P. aeruginosa* PAO1 as reference strain, and BLAST, AMRFinder, MLST.

Results

1. Antibiotic susceptibility testing

Three *P. aeruginosa* isolates (PSA1, PSA2 and PSA3) were sequentially collected (14 December, 9 and 19 February) from the respiratory tract of a patient exposed to prolonged treatment with FDC. All isolates were resistant to β-lactams (meropenem (MEM) and ceftazidime (CAZ)) and β-Lactam-β-Lactamase Inhibitor Combinations including CZA and C/T (**Table 1).** Specifically:

- Minimum inhibitory concentrations (MICs) for MEM ranged to 16 mg/L to 64 mg/;
- MICs for CAZ decrease by 8-, 2-, and 2- fold when performed in combination with cloxacillin (CLO) in PSA1, PSA2 and PSA3, respectively;
- PSA1, PSA2 and PSA3 exhibited high-level resistance for CZA (16-64 mg/L);

PSA2 and PSA3, collected several days apart since the treatment with FDC ended, exhibited reduced zone diameter (ZD) to FDC (22, 19 mm) vs PSA1 (31mm).

LFIA and mCIM did not support production of carbapenemase enzymes.

	Broth microdilution				*E-test*	*DD*
	MEM (mg/L)	CAZ (mg/L)	CAZ + CLO (mg/L)	CZA (mg/L)	C/T (mg/L)	FDC (mm)
PSA1	32	128	8	16	8	31
PSA2	16	256	128	64	>256	22
PSA3	64	256	128	32	>256	19

Table 1: MICs and ZD values of P. *aeruginosa* isolates

Resistant
Suscetible

2. Molecular assays

RT-PCR did not detect any carbapenemases (bla_{KPC}, bla_{VIM}, bla_{NDM}, $bla_{OXA-48-LIKE}$, bla_{IMP} and bla_{GES}) genes, consistent with LFIA and mCIM results.

Analysis of WGS data, using *P. aeruginosa* PAO1 as reference genome, revealed several genetic alterations associated with antimicrobial resistance (**Table 2**), including:

- frameshift mutations in *oprD*, affecting MEM resistance;
- amino acid substitution (F148L) in the resident pseudomonas-derived cephalosporinase (PDC) β-lactamase, evolving from PDC-36 to PDC-427
- frameshift mutations in *ampD*, accounting for PDC induction
- Mutations in genes involved in siderophores uptake, that may reduce FDC activity.

Strain

Gene	Product	**PSA1**	**PSA2**	**PSA3**
oprD	Porin	FS*	FS*	FS*
bla_{PDC-36}	Class C β-lactamase	WT	PDC-427 (F148L)	PDC-427 (F148L)
ampD	β-lactamase regulator	FS**	FS**	FS**
fptA	Ferric pyochelin receptor	WT	L24P	L24P
pir	Ferric pseudobactin receptor	WT	WT	FS***

Table 2

* FS at position 229
** FS at position 71
WT: wild type
*** FS at position 133

Conclusions

FDC represents a promising treatment option against MDR *P. aeruginosa*¸ being not impacted by β-lactam resistance mechanisms commonly encountered in this species. Although resistance rates currently appear to be low, on-treatment emergence of FDC-resistant isolates has been reported, raising major concerns due to limited therapeutic alternatives. In our case, the prolonged FDC exposure likely contributed to a stepwise selection of mutant isolates developing FDC resistance. Further investigation of the specific contribution of individual genes involved in FDC resistance, such as cephalosporinases, efflux pumps, porins, and FDC uptake receptors, are needed.

Reference

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