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MECHANISMS OF RESISTANCE

Contribution of PER-Type and NDM-Type β-Lactamases to Cefiderocol Resistance in *Acinetobacter baumannii*

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ABSTRACT Cefiderocol (FDC) is a recently developed siderophore cephalosporin showing excellent antibacterial activity against Gram-negative bacteria, including *Acinetobacter baumannii*. By investigating a series of *A. baumannii* clinical isolates with elevated MICs of FDC, we showed that PER-like β-lactamases and, to a lesser extent, NDM-like β-lactamases, significantly contributed to reduced susceptibility to that antibiotic. Interestingly, we showed that combination of FDC with avibactam exhibited excellent activity against all multidrug-resistant isolates coproducing OXA-23 and PER-type β-lactamases.

KEYWORDS cefiderocol, *Acinetobacter baumannii*, susceptibility testing, PER-type β-lactamase, NDM, PER, beta-lactamase

Cefiderocol (FDC) is a novel siderophore cephalosporin with broad-spectrum activity against a large variety of Gram-negative bacteria, including carbapenem-resistant *Enterobacterales* and nonfermenters such as *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, and *Acinetobacter baumannii* (1–3). Cefiderocol uses a unique and so-called "Trojan horse" strategy for actively penetrating into Gram-negative bacterial cells. It binds to iron and uses the bacterial iron transport system to enter the bacterial periplasmic space. Once across the outer membrane, the iron dissociates and FDC binds to penicillin-binding proteins (PBPs), mainly PBP3, to disrupt cell wall synthesis, eventually leading to cell death (4–6).

We previously evaluated the activity of FDC and comparators against a collection of 753 well-characterized Gram-negative isolates, including 87 *A. baumannii* isolates (mostly multidrug resistant), and showed that FDC exhibited potent activity against most of the isolates (with MICs of ≤4 μg/ml) (7). MIC values of FDC were determined by using the two commercially available broth microdilution methods, namely, Sensititre EUMDROXF BMD panels (Thermo Fisher, Basel, Switzerland) and Sensititre cefiderocol MIC panel CMP1SHIH (Thermo Fisher) according to the manufacturer's instructions (8). Noteworthy, higher MICs of FDC were observed for a total of 8 isolates (MICs of ≥8 μg/ml or ≥16 μg/ml depending on the panel used, respectively). Those eight clinical isolates were all positive for the bla_{OXA-23} carbapenemase gene.

Considering that FDC was previously shown not to be significantly hydrolyzed by OXA-23 (9), our aim here was therefore to decipher the genetic bases of such elevated MICs of FDC in those *A. baumannii* isolates.

To evaluate whether this resistance to FDC could be related to class A or C β-lactamase activity, MICs of FDC were determined in combination with avibactam (AVI). Avibactam is a non-β-lactam β-lactamase inhibitor that inhibits the activities of Ambler class A (including extended-spectrum β-lactamases), class C, and some class D β-lactamases, including carbapenemases (e.g., KPC and OXA-48) (10). Hence, MICs of FDC

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dropped to ≤0.5 μg/ml when combined with AVI at a fixed concentration of 4 μg/ml. This suggested that a serine-type β-lactamase was likely involved in the increased MICs of FDC in those 8 isolates. PCR experiments with a large series of primers specific for β-lactamase genes (11) were then performed in order to detect putative genes encoding β-lactamases that might be responsible for FDC hydrolysis. Surprisingly, all of the *A. baumannii* isolates were found positive for bla_{PER}-like genes in addition to bla_{OXA-23}, corresponding either to bla_{PER-1} or bla_{PER-7} after sequencing of the corresponding amplicons.

To clarify whether the expression of bla_{PER}-like genes might be involved in reduced susceptibility to FDC, cloning of this β-lactamase gene was performed into the shuttle and broad-host range pUCp24, and the recombinant plasmid was then electrotransformed into *Escherichia coli* strain TOP10, as previously described (11). The same approach was used to clone a series of other β-lactamase genes in parallel, with the goal to obtain a relative comparison of the different β-lactamase-related impacts with respect to susceptibility to FDC in an isogenic background. Cloning of the bla_{NDM-1} gene was also performed as a "positive" control, considering that metallo-β-lactamases are known to weakly hydrolyze FDC (12, 13). MIC determinations showed that *E. coli* recombinant strains producing either PER-1 or NDM-1 had MIC values of FDC ≥16-fold compared to that of the recipient strain but still remaining in the susceptibility range (Table 1). As expected, when supplemented with AVI (4 μg/ml), MIC of FDC was significantly reduced for the PER-producing *E. coli* recombinant strain (Table 1). Interestingly, elevation of the MICs of FDC was also observed for other *E. coli* recombinant strains producing SHV-2, BEL-2, NDM-1, and NDM-5, but remaining in the susceptibility range when considering the EUCAST breakpoint for *Enterobacterales* (less than or equal to 2 μg/ml) (Table 1). Conversely, no change was observed in term of MICs of FDC for recombinant *E. coli* strains producing the class A β-lactamases CTX-M-2, CTX-M-15, CTX-M-33, VEB-1, OXY-2, GES-1, GES-2, GES-5, and GES-6, the class B β-lactamase VIM-2, and the class C β-lactamase DHA-1. In a previous study (9), we showed that all carbapenem-hydrolyzing class D β-lactamase (CHDL)-producing *E. coli* clones (OXA-23, OXA-40, and OXA-48) remained fully susceptible to FDC, with no change in the MICs (respective values being all at 0.03 μg/ml), which is consistent with findings of the present study (Table 1). Previous studies highlighted that SHV- and PER-type β-lactamases might be associated with higher MICs of FDC (12, 14–16). As expected, addition of AVI did not modify the MIC value of FDC for the NDM-producing recombinant strains (Table 1). In contrast, addition of the metallo-β-lactamase inhibitor dipicolinic acid (DPA) at 100 μg/ml recovery of the full susceptibility to FDC, while it did not impact the MIC of FDC for those isolates coproducing OXA-23 and PER enzymes, as expected (Table 1).

To evaluate whether the occurrence of bla_{PER} genes is significant in that genus, a blast genome search of the bla_{PER-1} sequence was performed against *Acinetobacter* taxonomic identifier (taxid) 469 (https://www.ncbi.nlm.nih.gov/genome/403). Hence, a total of 37 positive isolates were identified over 1,567 complete genomes. The bla_{PER-1} gene was actually the most commonly encountered extended-spectrum β-lactamase (ESBL) gene among *A. baumannii* isolates. Analysis of the literature revealed that PER-1-producing *A. baumannii* isolates have been reported from many different countries worldwide, including in Europe, the United States, Asia, and the Middle East (17). A recent epidemiological study performed in Iran reported a prevalence of PER-1-producing *A. baumannii* isolates, representing 64.2% of clinical isolates of multidrug-resistant *A. baumannii* (18).

All PER-positive *A. baumannii* isolates from our collection were typed using Pasteur multilocus sequence typing (MLST) scheme to evaluate whether such occurrence of PER-producing strains might be due to few or multiple clones. Three sequence types (STs) were identified, including ST25 ($n = 5$), ST2 ($n = 2$), and ST10 ($n = 1$). ST2 is known as an endemic strain in European countries, including Italy and Spain, as previously described (19, 20). In another study in Kuwait, the predominant ST identified was ST2,

TABLE 1 Susceptibility testing of cefiderocol nonsusceptible *Acinetobacter baumannii* isolates and *A. baumannii* or *E. coli* recombinant strains

		MIC (μg/ml)[a]		
Species or strain	**Resistance determinant**	**FDC (EUMDROXF [CMP1SHIH panel])[b]**	**FDC-AVIc**	**FDC-DPAc**
A. baumannii	OXA-23+PER-7	>8 (>16)	0.5	>16
	OXA-23+PER-7	>8 (>16)	0.25	>16
	OXA-23+PER-7	>8 (>32)	0.5	>32
	OXA-23+PER-1	>8 (16)	0.5	16
	OXA-23+PER-1	>8 (32)	0.5	32
	OXA-23+PER-1	>8 (>32)	0.25	>32
	OXA-23+PER-7	>8 (>32)	0.25	>32
	OXA-23+PER-7	>8 (>32)	2	>32
	NDM-1	2	ND	≤0.125
	NDM-1	8	ND	2
	NDM-1	4	ND	0.25
	NDM-1	(>8) 16	ND	2
	NDM-1	2	ND	1
	NDM-1	4	ND	0.5
	NDM-5	8	ND	1
	NDM-9	(>8) 16	ND	0.5
A. baumannii CIP70.10	PER-1	2	≤0.03	ND
	NDM-1	1	ND	≤0.125
	NDM-5	0.5	ND	≤0.125
	NDM-9	1	ND	≤0.125
	OXA-23	≤0.125	≤0.125	ND
	OXA-40	≤0.125	≤0.125	ND
	OXA-48	≤0.125	≤0.125	ND
	OXA-58	≤0.125	≤0.125	ND
	CTX-M-2	≤0.125	≤0.125	ND
		≤0.125	≤0.125	ND
E. coli TOP10+PER-1	PER-1	2 (4)	≤0.03	ND
E. coli TOP10+SHV-2	SHV-2	0.25	≤0.03	ND
E. coli TOP10+NDM-1	NDM-1	1	1	ND
E. coli TOP10+NDM-5	NDM-5	1	1	ND
E. coli TOP10+NDM-9	NDM-9	2	2	ND
E. coli TOP10+OXA-23	OXA-23	0.03	≤0.03	ND
E. coli TOP10+OXA-40	OXA-40	0.03	≤0.03	ND
E. coli TOP10+OXA-48	OXA-48	0.03	≤0.03	ND
E. coli TOP10+OXA-58	OXA-58	0.03	≤0.03	ND
E. coli TOP10+BEL-2	BEL-2	0.5	≤0.03	ND
E. coli TOP10		0.03	≤0.03	ND

[a]FDC, cefiderocol; AVI, avibactam at 4 μg/ml; DPA, dipicolinic acid at 100 μg/ml; ND, not determined.
[b]Commercial cefiderocol MIC panels used in this study. If both MICs were the same value, then only one value is indicated.

followed by ST25 and ST32 (21). Moreover, those STs have also been identified in Saudi Arabia (22) and in Yemen (23).

Considering that PER-type β-lactamases are frequently described in *A. baumannii* (17), the bla_{PER-1} gene was subsequently cloned into the shuttle plasmid (pVRL1), and the recombinant plasmid was then electroporated into an *A. baumannii* CIP70.10 recipient strain. The resulting PER-1-producing *A. baumannii* strain showed a ≥16-fold increase in the MIC of FDC compared to that of the recipient counterpart (from ≤0.125 to 2 μg/ml) (Table 1), further confirming the significant impact of PER-1 on susceptibility to that antibiotic.

Since those different experiments indicated the impact of PER enzymes on susceptibility to FDC, cultures of *E. coli* TOP10 harboring recombinant plasmids producing PER-1 and other selected β-lactamases were performed in order to measure specific hydrolytic activities. β-Lactamase crude enzymatic extracts were extracted by sonication, and specific activities were measured with a GENESYS 10S UV-visible (UV-Vis) spectrophotometer (Thermo Scientific) as previously described (11), using either benzylpenicillin or FDC as the substrate. The following wavelengths and molar extinction coefficient ($\Delta\varepsilon$) values were used: benzylpenicillin, 232 nm and $-1{,}100\ M^{-1}\cdot cm^{-1}$; FDC, 259 nm and $-9{,}430\ M^{-1}\cdot cm^{-1}$, respectively. Interestingly, PER-1-β-lactamase showed much

TABLE 2 Specific β-lactamase activities of PER-1 and other selected β-lactamases

Enzyme	Sp act (μmol·min^{-1}·mg^{-1})	
	Benzylpenicillin	Cefiderocol
PER-1	6.72	2.14
NDM-1	13.30	0.28
CTXM-2	26.28	NH[a]
VEB-1	19.07	NH
VIM-2	8.5	NH
CTX-M-15	2.65	NH

[a]NH, no hydrolysis detected.

higher hydrolytic activity against FDC (2.14 μmol·min^{-1}·mg^{-1}) than NDM-1 β-lactamase (0.28 μmol·min^{-1}·mg^{-1}), even though the latter was also significant, while hydrolysis by SHV-2 was very weak. In contrast, no activity was observed for CTXM-2, CTX-M-15, VIM-2, and VEB-1, mirroring the results of susceptibility testing (Table 2). Noteworthy, we previously showed the stability of cefiderocol against carbapenem-hydrolyzing class D β-lactamases, such as OXA-48, OXA-23, and OXA-40, regardless of their bacterial hosts (9). Hydrolysis experiments were performed using purified β-lactamases (OXA-48, OXA-23, and OXA-40), and kinetic parameters showed a significant hydrolysis rate of penicillins and carbapenems but no activity against FDC, mirroring the susceptibility test results of the present study (9).

The hydrolysis of FDC observed with the NDM-1-producing recombinant *E. coli* strain prompted us to further evaluate the impact of NDM enzymes on FDC susceptibility, using a collection of bla_{NDM}-positive *A. baumannii* isolates. By testing eight NDM producers (among which there were 6 NDM-1, 1 NDM-5, and 1 NDM-9 producer) that were negative for PER-encoding genes, MICs ranging from 2 to 16 μg/ml were obtained for FDC, those MICs being diminished in the presence of DPA (Table 1). Some of NDM variants (NDM-1, -5, and -9) were subsequently cloned into the shuttle plasmid (pVRL1), and the recombinant plasmid was then electroporated into the *A. baumannii* CIP70.10 recipient strain. Notably, elevation of the MICs of FDC was observed for the *A. baumannii* recombinant strains producing different NDM variants (NDM-1, NDM-5, and NDM-9) (Table 1). Those data confirmed that NDM enzymes partially contributed to a reduced susceptibility to FDC in *A. baumannii*.

Our results showed that PER-like β-lactamases and, to a lesser extent, NDM β-lactamases contribute to a decreased susceptibility to FDC, especially in *A. baumannii*, although production of the PER enzyme alone in that species does not lead to MICs being higher than the pharmacokinetic/pharmacodynamic (PK/PD) concentrations obtained clinically (>2 μg/ml), as established by EUCAST (24). This suggests that combined factors such as modification of the target (PBP-3), modification of the ion transport (25), permeability defects or/and efflux overexpression (25), and additional β-lactamases such as NDM-like enzymes might contribute to resistance to FDC in *A. baumannii*. Interestingly, we also evidenced that supplementation of FDC with AVI may inhibit the activity of PER-type β-lactamases. This *in vitro* observation is noteworthy, since the FDC-AVI combo might therefore be considered an effective therapeutic alternative to be considered against carbapenem and FDC-resistant *A. baumannii* isolates.

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