AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

MECHANISMS OF RESISTANCE

Impact of Acquired Broad-Spectrum β-Lactamases on Susceptibility to Cefiderocol and Newly Developed β-Lactam/β-Lactamase Inhibitor Combinations in *Escherichia coli* and *Pseudomonas aeruginosa*

Laurent Poirel,[a,c,d] Jose-Manuel Ortiz de la Rosa,[a] Mustafa Sadek,[a,b] Patrice Nordmann[a,c,d,e]

[a]Medical and Molecular Microbiology, Faculty of Science and Medicine, University of Fribourg, Fribourg, Switzerland
[b]Department of Food Hygiene and Control, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt
[c]INSERM European Unit (IAME), University of Fribourg, Fribourg, Switzerland
[d]Swiss National Reference Center for Emerging Antibiotic Resistance (NARA), University of Fribourg, Fribourg, Switzerland
[e]Institute for Microbiology, University of Lausanne and University Hospital Centre, Lausanne, Switzerland

ABSTRACT The ability of broad-spectrum β-lactamases to reduce the susceptibility to ceftazidime-avibactam (CZA), ceftolozane-tazobactam (C/T), imipenem-relebactam, meropenem-vaborbactam, aztreonam-avibactam (AZA), and cefiderocol (FDC) was evaluated both in *Pseudomonas aeruginosa* and in *Escherichia coli* using isogenic backgrounds. Although metallo-β-lactamases conferred resistance in most cases, except for AZA, several clavulanic-acid-inhibited extended-spectrum β-lactamases (PER, BEL, SHV) had a significant impact on the susceptibility to CZA, C/T, and FDC.

KEYWORDS cefiderocol, *Pseudomonas aeruginosa*, susceptibility testing, β-lactamase

The novel siderophore cephalosporin cefiderocol (FDC) and the novel β-lactam/β-lactamase inhibitor (BL/BLI) combinations (ceftazidime-avibactam [CZA], ceftolozane-tazobactam[C/T], meropenem-vaborbactam [MVB], imipenem-relebactam [I/R], aztreonam-avibactam [AZA]) possess a broad-spectrum activity against most multidrug-resistant Gram-negative bacteria (1, 2).

We previously evaluated the activity of FDC and comparators against a collection of 753 well-characterized Gram-negative isolates including 164 *Escherichia coli*, 45 *Pseudomonas aeruginosa*, and 87 *Acinetobacter baumannii* isolates (mostly multidrug-resistant) and showed that FDC exhibited potent activity against most of the isolates (3). In another recent study focusing on *A. baumannii*, we showed that some β-lactamases with broad-spectrum activity might confer significantly reduced susceptibility to FDC, namely, the Ambler class A PER-type extended-spectrum β-lactamases, and to a lesser extent the class B NDM-type metallo-β-lactamases (4).

In addition, and even though production of the widespread KPC-2/KPC-3 carbapenemases has been demonstrated to not significantly impact the susceptibility to FDC, some *in vitro* obtained mutants such as KPC-31 (5), or mutants identified from CZA-resistant clinical isolates, were shown to confer reduced susceptibility (KPC-50) or even resistance (KPC-41) to FDC once produced in *E. coli* (6).

Considering that the activity of FDC was previously shown to be (at least partially) compromised by the hydrolytic activity of some broad-spectrum β-lactamases in *A. baumannii*, our aim here was first to evaluate the impact of a large series of broad-spectrum β-lactamases on susceptibility to FDC in *E. coli* and *P. aeruginosa*. Indeed, FDC is being considered as a very interesting therapeutic option for treating infections caused by multidrug-resistant *P. aeruginosa* strains, and very little is known about the

Address correspondence to Laurent Poirel, laurent.poirel@unifr.ch.

The authors declare no conflict of interest.

Received 10 January 2022
Returned for modification 14 February 2022
Accepted 18 February 2022
Published 22 March 2022

potential effect broad-spectrum β-lactamases might have on FDC efficacy with respect to those bacterial species.

In parallel, we aimed to evaluate the impact of the different broad-spectrum β-lactamases on resistance to BL/BLI. Resistance to CZA has been mostly associated with mutations in the KPC carbapenemases in Enterobacterales, or overproduction of AmpC β-lactamases possessing specific amino acid substitutions in *P. aeruginosa*. Resistance to C/T is mediated mostly by amino acid substitutions, insertions, and deletions in the chromosomal AmpC cephalosporinases of *Pseudomonas aeruginosa*, many of those changes conferring cross-resistance to CZA. Finally, permeability defect and efflux overproduction are the primary bases for resistance to MVB and I/R (7).

In order to evaluate the impact of a large series of β-lactamases on FDC and BL/BLI susceptibility both in *E. coli* and in *P. aeruginosa*, corresponding genes were amplified by PCR with a large series of primers specific for β-lactamase genes (8), and corresponding amplicons were cloned into plasmid pUCp24, which is a shuttle vector able to replicate in both of these species. Our focus was mainly on broad-spectrum β-lactamases, namely ESBLs and carbapenemases of different classes. Nevertheless, narrow-spectrum β-lactamases were also included as comparison. Hence, the different β-lactamases included class A penicillinases (TEM-1, TEM-3), class A ESBLs (OXY-2 [a natural and chromosomally-encoded ESBL from *Klebsiella oxytoca*], CTX-M-3, CTX-M-15, GES-1, BEL-1, BEL-2, SHV-2a, SHV-12, PER-1, PER-2, PER-6, PER-7), class A ESBLs with weak carbapenemase activity (CTX-M-33, GES-2, GES-5), class A carbapenemases (IMI-1, KPC-2, GES-6), class B carbapenemases (AIM-1, DIM-1, VIM-2, GIM-1, NDM-1, NDM-5, NDM-7, NDM-9, SPM-1), class C cephalosporinase (DHA-1), narrow-spectrum class D β-lactamase (OXA-1), and carbapenem-hydrolyzing class D β-lactamases (OXA-23, OXA-48, OXA-58, OXA-427). Noteworthy, those enzymes were chosen as representatives mostly of clinically relevant β-lactamases being sources of resistance to broad-spectrum β-lactams either in *E. coli* or in *P. aeruginosa*. Among the class D β-lactamases, OXA-1, OXA-23, OXA-48, and OXA-427 (a peculiar oxacillinase) may be identified in Enterobacterales, OXA-2 in *P. aeruginosa* and OXA-23 and OXA-58 mostly in *A. baumannii*. In addition, some of these enzymes have been either mainly (and even sometimes exclusively) identified in *P. aeruginosa* (e.g., BEL, AIM, GIM, SPM). Nevertheless, our approach was comparative by expressing corresponding genes either in *E. coli* or *P. aeruginosa*, giving rise to a total number of 35 *E. coli* and 35 *P. aeruginosa* recombinant strains.

MICs were determined by broth microdilution for FDC, C/T, CZA, I/R, MVB, and AZA, and the corresponding β-lactams of the combinations alone. Additionally, susceptibility testing was also evaluated by broth microdilution for the FDC + avibactam (FDCA) combination with AVI at a fixed concentration of 4 μg/mL (4), in order to evaluate whether any resistance or reduced susceptibility to FDC could be antagonized by this recently developed inhibitor (potentially inhibiting most Ambler class A and class C β-lactamases) (9).

MICs were determined in triplicate using broth microdilution in cation adjusted Mueller-Hinton (MH) broth (Bio-Rad, Marnes-la-Coquette, France) for all antibiotics or antibiotic combinations listed above, except for FDC and FDC-containing combinations, for which iron-depleted cation-adjusted MH broth was used, according to the EUCAST guidelines. Results were interpreted according to the latest EUCAST breakpoints (https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_11.0_Breakpoint_Tables.pdf). The reference strain *E. coli* ATCC 25922 was used as quality control for all testing.

Results obtained for recombinant *E. coli* strains highlighted a series of important features (Table 1). Overall, the most efficient combinations were MVB, FDCA, and AZA. As expected, neither the CZA nor the C/T combos were active against MBL producers, although FDC proved to be a very interesting option, even when used without AVI. Noteworthy, the impact of some ESBLs (especially the PER-type enzymes) was particularly significant on susceptibility to C/T, CZA, FDC, and AZA. A significant drop (≤0.5 μg/mL) was observed in term of MICs of FDC for those strains producing serine-type β-lactamases

TABLE 1 Susceptibility testing of recombinant *E. coli* and *P. aeruginosa* strains[a]

Strain	Ambler class	Minimal inhibitory concentrations (μg/mL)											
		COZ	C/T	CAZ	CZA	IMP	I-R	MEM	MVB	FDC	FDCA	ATM	AZA
E. coli TOP10		0.5	0.25	0.25	≤0.125	0.25	0.25	0.03	0.03	≤0.125	≤0.125	≤0.125	≤0.125
E. coli TOP10 + OXY-2	A	1	0.5	1	0.25	0.5	0.25	0.03	0.03	≤0.125	≤0.125	**16**	≤0.125
E. coli TOP10 + TEM-1	A	0.5	0.5	0.5	≤0.125	0.25	0.25	0.03	0.03	≤0.125	≤0.125	≤0.125	≤0.125
E. coli TOP10 + TEM-3	A	0.5	0.25	4	≤0.125	0.25	0.25	0.03	0.03	≤0.125	≤0.125	2	0.25
E. coli TOP10 + CTX-M-15	A	256	0.5	>**256**	≤0.125	0.5	0.25	0.03	0.03	≤0.125	≤0.125	**128**	≤0.125
E. coli TOP10 + CTX-M-3	A	64	0.5	**32**	0.5	0.5	0.25	0.03	0.03	≤0.125	≤0.125	**128**	≤0.125
E. coli TOP10 + CTX-M-33	A	32	**4**	**16**	0.25	1	0.5	0.12	0.03	≤0.125	≤0.125	**64**	≤0.125
E. coli TOP10 + GES-1	A	32	**8**	**16**	0.25	0.25	0.25	0.06	0.03	≤0.125	≤0.125	0.25	≤0.125
E. coli TOP10 + GES-2	A	4	0.5	**8**	0.5	0.5	0.25	0.03	0.03	≤0.125	≤0.125	0.25	≤0.125
E. coli TOP10 + GES-5	A	8	**8**	4	0.25	1	0.25	0.125	0.03	≤0.125	≤0.125	0.25	≤0.125
E. coli TOP10 + GES-6	A	128	**32**	**64**	2	2	0.5	0.25	0.03	≤0.125	≤0.125	0.5	≤0.125
E. coli TOP10 + IMI-1	A	1	0.5	2	0.25	**32**	0.25	4	0.03	≤0.125	≤0.125	**64**	0.25
E. coli TOP10 + BEL-1	A	8	2	**32**	0.25	0.5	0.25	0.06	0.03	0.25	≤0.125	**32**	≤0.125
E. coli TOP10 + BEL-2	A	32	**8**	**64**	1	0.5	0.25	0.03	0.03	0.5	0.125	**32**	≤0.125
E. coli TOP10 + SHV-2a	A	0.25	0.25	**8**	0.25	0.25	0.25	0.03	0.03	0.25	≤0.125	**16**	≤0.125
E. coli TOP10 + SHV-12	A	64	**32**	**256**	0.5	0.25	0.25	0.03	0.03	**4**	≤0.125	**256**	0.25
E. coli TOP10 + PER-1	A	>256	**64**	**256**	**16**	1	0.5	0.06	0.03	**4**	0.25	>**256**	**8**
E. coli TOP10 + PER-2	A	>256	**8**	**64**	4	1	0.25	0.03	0.03	1	≤0.125	>**256**	2
E. coli TOP10 + PER-6	A	>256	**256**	**128**	**64**	0.5	0.25	0.06	0.03	**4**	0.25	>**256**	**32**
E. coli TOP10 + PER-7	A	>256	**256**	**128**	**32**	0.5	0.25	0.06	0.03	**4**	0.125	>**256**	**16**
E. coli TOP10 + KPC-2	A	16	**16**	**32**	0.25	**16**	0.5	**4**	0.03	≤0.125	≤0.125	**64**	≤0.125
E. coli TOP10 + DIM-1	B	64	**64**	**16**	**16**	2	2	0.25	0.25	≤0.125	≤0.125	0.125	≤0.125
E. coli TOP10 + VIM-2	B	256	**256**	**16**	**16**	**16**	**16**	2	2	≤0.125	≤0.125	≤0.125	≤0.125
E. coli TOP10 + AIM-1	B	32	**32**	**16**	**16**	4	**4**	4	4	≤0.125	≤0.125	≤0.125	≤0.125
E. coli TOP10 + GIM-1	B	16	**16**	**16**	**16**	0.5	0.5	0.25	0.25	≤0.125	≤0.125	≤0.125	≤0.125
E. coli TOP10 + NDM-1	B	>256	>**256**	**128**	**128**	**16**	**16**	8	8	1	1	0.125	≤0.125
E. coli TOP10 + NDM-5	B	>256	>**256**	**256**	**128**	**32**	**32**	**16**	**16**	1	1	≤0.125	≤0.125
E. coli TOP10 + NDM-7	B	>256	>**256**	**128**	**128**	4	**4**	4	4	1	1	≤0.125	≤0.125
E. coli TOP10 + NDM-9	B	>256	>**256**	**64**	**64**	**8**	**8**	8	8	2	2	≤0.125	≤0.125
E. coli TOP10 + SPM-1	B	>256	>**256**	**64**	**64**	2	2	8	8	2	2	≤0.125	≤0.125
E. coli TOP10 + DHA-1	C	32	2	**128**	0.25	0.5	0.25	0.03	0.015	≤0.125	≤0.125	**16**	≤0.125
E. coli TOP10 + OXA-1	D	0.5	0.25	0.5	0.25	0.5	0.25	0.03	0.03	≤0.125	≤0.125	0.25	≤0.125
E. coli TOP10 + OXA-23	D	0.25	0.25	0.5	0.5	1	0.5	0.5	0.5	≤0.125	≤0.125	0.25	≤0.125
E. coli TOP10 + OXA-48	D	0.5	0.5	0.5	0.25	1	0.5	0.5	0.5	≤0.125	≤0.125	0.125	≤0.125
E. coli TOP10 + OXA-58	D	0.5	0.25	0.5	≤0.125	1	0.5	0.5	0.5	≤0.125	≤0.125	0.125	≤0.125
E. coli TOP10 + OXA-427	D	32	**8**	**32**	0.5	1	0.5	0.5	0.5	0.25	≤0.125	**16**	0.25
P. aeruginosa PAO1		0.5	0.25	1	1	0.5	0.25	0.5	0.25	0.5	0.25	2	1
P. aeruginosa PAO1 + OXY-2	A	1	0.5	2	1	1	0.25	1	0.25	0.5	0.25	**64**	2
P. aeruginosa PAO1 + TEM-1	A	0.5	0.25	1	1	0.5	0.25	0.5	0.5	0.5	0.25	2	2
P. aeruginosa PAO1 + TEM-3	A	2	0.5	8	1	0.5	0.5	1	0.25	0.5	0.25	2	2
P. aeruginosa PAO1 + CTX-M-15	A	256	0.5	**256**	1	0.5	0.25	0.5	0.25	0.5	0.25	**128**	2
P. aeruginosa PAO1 + CTX-M-3	A	64	0.5	**32**	2	0.5	0.25	0.5	0.25	0.5	0.25	**128**	2
P. aeruginosa PAO1 + CTX-M-33	A	32	4	**32**	2	2	0.25	4	0.5	0.5	0.25	**64**	1
P. aeruginosa PAO1 + GES-1	A	32	**16**	**64**	1	0.5	0.25	1	0.25	0.5	0.5	2	1
P. aeruginosa PAO1 + GES-2	A	8	2	**16**	1	0.5	0.25	1	0.25	0.5	0.25	2	2
P. aeruginosa PAO1 + GES-5	A	8	**8**	**16**	2	2	1	8	1	0.5	0.25	2	2
P. aeruginosa PAO1 + GES-6	A	128	**64**	**64**	2	4	1	8	2	2	0.5	4	2
P. aeruginosa PAO1 + IMI-1	A	4	1	2	1	**32**	0.5	**32**	1	0.5	0.25	**128**	2
P. aeruginosa PAO1 + BEL-1	A	32	**16**	**32**	2	1	0.25	1	0.25	2	0.25	**128**	1
P. aeruginosa PAO1 + BEL-2	A	128	**32**	**256**	4	1	0.25	1	0.25	**4**	0.5	**64**	1
P. aeruginosa PAO1 + SHV-2a	A	16	**8**	**64**	2	1	0.25	0.5	0.25	**4**	0.5	**64**	1
P. aeruginosa PAO1 + SHV-12	A	128	**32**	**256**	4	0.5	0.25	2	0.25	**8**	0.5	>**256**	**8**
P. aeruginosa PAO1 + PER-1	A	>256	>**256**	>**256**	**32**	1	0.25	1	0.5	**16**	0.5	>**256**	**16**
P. aeruginosa PAO1 + PER-2	A	>256	**8**	**128**	4	1	0.25	1	0.25	2	0.5	>**256**	4
P. aeruginosa PAO1 + PER-6	A	>256	**256**	**256**	**32**	1	0.25	0.5	0.25	**8**	1	>**256**	**32**
P. aeruginosa PAO1 + PER-7	A	>256	>**256**	**256**	**32**	1	0.25	1	0.25	**8**	1	>**256**	**32**
P. aeruginosa PAO1 + KPC-2	A	32	**32**	**128**	2	>**32**	2	>**32**	8	1	0.25	**256**	4
P. aeruginosa PAO1 + DIM-1	B	64	**64**	**32**	**32**	2	1	**16**	**16**	0.5	0.25	2	2
P. aeruginosa PAO1 + VIM-2	B	>256	>**256**	**128**	**128**	>**32**	>**32**	>**32**	>**32**	1	0.5	2	2
P. aeruginosa PAO1 + AIM-1	B	64	**64**	**64**	**64**	>**32**	>**32**	>**32**	>**32**	2	1	2	2
P. aeruginosa PAO1 + GIM-1	B	16	**16**	**64**	**64**	4	**4**	**32**	**32**	2	1	2	2

(Continued on next page)

TABLE 1 (Continued)

Strain	Ambler class	Minimal inhibitory concentrations (μg/mL)											
		COZ	C/T	CAZ	CZA	IMP	I-R	MEM	MVB	FDC	FDCA	ATM	AZA
P. aeruginosa PAO1 + NDM-1	B	>256	**>256**	**256**	**256**	**32**	**32**	**>32**	**>32**	**4**	4	2	2
P. aeruginosa PAO1 + NDM-5	B	>256	**>256**	**256**	**256**	**32**	**32**	**>32**	**>32**	2	2	2	2
P. aeruginosa PAO1 + NDM-7	B	>256	**>256**	**256**	**256**	**32**	**32**	**>32**	**>32**	**4**	2	2	2
P. aeruginosa PAO1 + NDM-9	B	>256	**>256**	**256**	**256**	**32**	**32**	**>32**	**>32**	**4**	4	2	2
P. aeruginosa PAO1 + SPM-1	B	>256	**>256**	**128**	**128**	4	**4**	**32**	**32**	**8**	8	2	2
P. aeruginosa PAO1 + DHA-1	C	32	4	**256**	1	0.5	0.25	0.5	0.25	0.5	0.25	**32**	2
P. aeruginosa PAO1 + OXA-1	D	0.5	0.5	2	1	0.5	0.25	0.5	0.25	0.5	0.25	2	2
P. aeruginosa PAO1 + OXA-23	D	1	0.5	4	1	4	**4**	8	8	0.5	0.25	4	2
P. aeruginosa PAO1 + OXA-48	D	0.5	0.5	1	0.5	2	2	2	2	0.5	0.25	2	2
P. aeruginosa PAO1 + OXA-58	D	0.5	0.25	1	0.5	2	2	4	4	0.5	0.5	2	2
P. aeruginosa PAO1 + OXA-427	D	>256	**>256**	**256**	**128**	2	2	**16**	4	**4**	2	**128**	**16**

[a]COZ, ceftolozane; C/T, ceftolozane-tazobactam; CAZ, ceftazidime; CZA, ceftazidime-avibactam; IPM, imipenem; I-R, imipenem/relebactam; MEM, meropenem; MVB, meropenem-vaborbactam; FDC, cefiderocol; FDCA, cefiderocol/avibactam; ATM, aztreonam; AZA, aztreonam/avibactam. MIC values indicated in bold are those corresponding to a categorization of resistance. Shaded MIC values are those corresponding to a significantly elevated MIC value, though not categorized as resistant, compared to wild-type *E. coli* TOP10 or *P. aeruginosa* PAO1.

for which elevated MICs of FDC were observed. This further highlighted that such FDC + AVI combo might represent a potent therapeutic option for such isolates producing those enzymes.

A detailed analysis of the respective MIC values for all β-lactamases allowed to underscore several so far unpredictable features (Table 1). Interestingly, the GES-2 enzyme possessing some carbapenemase activity had much lower impact on the MIC of C/T, by contrast to GES-1 or GES-5, the latter also possessing carbapenemase activity. The carbapenemase IMI-1 had very limited impact on almost all novel therapeutic options, despite its high impact on carbapenem MIC values. Production of PER-type enzymes led to resistance to AZA, although this drug combination remained very effective against all other *E. coli* recombinant strains. Within the class B β-lactamase group, only NDM-type enzymes along with SPM-1 exhibited a capacity to reduce the activity of FDC, whereas VIM-2, AIM-1, and GIM-1 did not confer any significant decreased susceptibility to this antibiotic.

Analysis of the MICs determined with the recombinant *P. aeruginosa* strains overall mirrored what was observed in *E. coli* but with more marked effects. As expected, considering the intrinsic reduced susceptibility of *P. aeruginosa* to antibiotics as a consequence of a lower permeability, MIC values were found to be higher than those obtained for *E. coli* (Table 2). Noteworthy, resistance to FDC was observed only for a quite limited number of β-lactamases, namely, the class A BEL-2, SHV-2a, SHV-12, PER-1, PER-6, and

TABLE 2 Specific β-lactamase activities

β-lactamase	Molecular class	sp act (μmol min^{-1} mg^{-1})	
		Penicillin G	Cefiderocol
CTX-M-15	A	1.06	<0.005
BEL-1	A	5.94	<0.005
BEL-2	A	2.15	<0.005
SHV-2	A	4.51	0.01
SHV-12	A	7.83	0.33
PER-1	A	1.53	0.80
PER-2	A	0.52	0.36
PER-6	A	3.21	1.69
PER-7	A	1.31	0.84
NDM-1	B	3.85	0.05
NDM-5	B	1.06	0.02
NDM-7	B	2.05	0.04
NDM-9	B	3.92	0.10
SPM-1	B	3.30	0.04
OXA-427	D	0.75	0.006

PER-7, and the class B NDM-type and SPM-1. In addition, resistance was observed for the OXA-427-producing *P. aeruginosa*; nevertheless, that enzyme has so far been identified only among Enterobacterales. Regarding AZA, the number of recombinant strains being susceptible to that combination was even higher than FDC, only SHV-12, PER-type, and OXA-427 β-lactamases conferring resistance to it.

In order to further assess the respective impacts of the different β-lactamases included in our study on the activity of FDC, cultures of *E. coli* TOP10 strains producing enzymes presumably hydrolyzing this antibiotic were performed, with the goal to measure the respective specific hydrolytic activities. β-lactamase crude enzymatic extracts were extracted by sonication, and specific activities were measured with a GENESYS 10S UV-Vis Spectrophotometer (Thermo Scientific) as previously described (8), using either benzylpenicillin or FDC as substrates (Table 3). Interestingly, PER-type enzymes showed the higher hydrolytic activity against FDC, as previously reported (4). By contrast, no activity was observed for CTX-M-type and other minor ESBLs, mirroring the results of susceptibility testing. Noteworthy, although most carbapenem-hydrolyzing class D β-lactamases did not confer reduced susceptibility to FDC as previously published (10), hydrolysis assays further confirmed that the recently identified carbapenemase OXA-427 possesses significant activity against this substrate. In fact, 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 (10).

When excluding the β-lactam/β-lactamase inhibitor combinations that do not correspond to clinically available therapeutic options so far (FDCA and AZA) and considering mainly C/T, CZA, I-R, MVB, and FDC as last-resort alternatives, it is important to underscore the excellent performances of I-R and MVB by comparison to CZA in *P. aeruginosa*, that latter combo being just poorly efficient when MBLs are actually produced.

Noteworthy, and to the best of our knowledge, this study firstly investigated the respective abilities of different NDM-type β-lactamases to impact the activity of FDC, while there is currently a debate about these relative effects. Of note, the impact of all the different NDM variants tested was almost identical, respective MICs differing by no more than 1-fold dilution, which may be considered as not significant. Finally, our results indicate that the continuous spread of NDM but also the PER enzymes may be a source of concern when considering treatment with novel available therapeutics. Actually, PER enzymes are extensively distributed in Gram negatives in particular in South America, the Middle East, Turkey, and Asia (11–13).

ACKNOWLEDGMENTS

This work was financed by the University of Fribourg, Switzerland, the NARA, and by the Swiss National Science Foundation (grant FNS 310030_1888801).

REFERENCES

1. Zhanel GG, Golden AR, Zelenitsky S, Wiebe K, Lawrence CK, Adam HJ, Idowu T, Domalaon R, Schweizer F, Zhanel MA, Lagacé-Wiens PRS, Walkty AJ, Noreddin A, Lynch Iii JP, Karlowsky JA. 2019. Cefiderocol: a siderophore cephalosporin with activity against carbapenem-resistant and multidrug-resistant Gram-negative bacilli. Drugs 79:271–289. https://doi.org/10.1007/s40265-019-1055-2.
2. Doi Y. 2019. Treatment options for carbapenem-resistant Gram-negative bacterial infections. Clin Infect Dis 69:S565–S575. https://doi.org/10.1093/cid/ciz830.
3. Dobias J, Dénervaud-Tendon V, Poirel L, Nordmann P. 2017. Activity of the novel siderophore cephalosporin cefiderocol against multidrug-resistant Gram-negative pathogens. Eur J Clin Microbiol Infect Dis 36:2319–2327. https://doi.org/10.1007/s10096-017-3063-z.
4. Poirel L, Sadek M, Nordmann P. 2021. Contribution of PER-type and NDM-type β-lactamases to cefiderocol resistance in *Acinetobacter baumannii*. Antimicrob Agents Chemother 65:e0087721. https://doi.org/10.1128/AAC.00877-21.
5. Hobson CA, Cointe A, Jacquier H, Choudhury A, Magnan M, Courroux C, Tenaillon O, Bonacorsi S, Birgy A. 2021. Cross-resistance to cefiderocol and ceftazidime-avibactam in KPC β-lactamase mutants and the inoculum effect. Clin Microbiol Infect 27:1172.e7–1172.e10. https://doi.org/10.1016/j.cmi.2021.04.016.
6. Poirel L, Sadek M, Kusaksizoglu A, Nordmann P. 2022. Co-resistance to ceftazidime-avibactam and cefiderocol in clinical isolates producing KPC variants. Eur J Clin Microbiol Infect Dis. https://doi.org/10.1007/s10096-021-04397-x.
7. Papp-Wallace KM, Mack AR, Taracila M, Bonomo RA. 2020. Resistance to novel β-lactam-β-lactamase inhibitor combinations: the "price of "progress." Infect Dis Clin North Am 34:773–819. https://doi.org/10.1016/j.idc.2020.05.001.
8. Ortiz de la Rosa JM, Nordmann P, Poirel L. 2019. ESBLs and resistance to ceftazidime/avibactam and ceftolozane/tazobactam combinations in *Escherichia coli* and *Pseudomonas aeruginosa*. J Antimicrob Chemother 74:1934–1939. https://doi.org/10.1093/jac/dkz149.

9. Nichols WW, Newell P, Critchley IA, Riccobene T, Das S. 2018. Avibactam pharmacokinetic/pharmacodynamic targets. Antimicrob Agents Chemother 62:e02446-17. https://doi.org/10.1128/AAC.02446-17.
10. Poirel L, Kieffer N, Nordmann P. 2018. Stability of cefiderocol against clinically significant broad-spectrum oxacillinases. Int J Antimicrob Agents 52:866–867. https://doi.org/10.1016/j.ijantimicag.2018.11.005.
11. Villegas MV, Kattan JN, Quinteros MG, Casellas JM. 2008. Prevalence of extended-spectrum β-lactamases in South America. Clin Microbiol Infect 14(Suppl 1):154–158. https://doi.org/10.1111/j.1469-0691.2007.01869.x.
12. Potron A, Poirel L, Nordmann P. 2015. Emerging broad-spectrum resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: mechanisms and epidemiology. Int J Antimicrob Agents 45:568–585. https://doi.org/10.1016/j.ijantimicag.2015.03.001.
13. Mazraeh FN, Hasani A, Sadeghi J, Kafil HS, Barhaghi MHS, Sefidan FY, Sharabiani HR, Hematyar Y, Rezaee MA. 2021. High frequency of bla_{PER-1} gene in clinical strains of *Acinetobacter baumannii* and its association with quorum sensing and virulence factors. Gene Rep 24:101232. https://doi.org/10.1016/j.genrep.2021.101232.