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MECHANISMS OF RESISTANCE

Impact of Minor Carbapenemases on Susceptibility to Novel β-Lactam/β-Lactamase Inhibitor Combinations and Cefiderocol in *Enterobacterales*

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ABSTRACT The *in vitro* activity of imipenem-relebactam, meropenem-vaborbactam, ceftazidime-avibactam, and cefiderocol was evaluated against both clinical and isogenic enterobacterial isolates producing carbapenemases of the SME, NmcA, FRI, and IMI types. Ceftazidime-avibactam and meropenem-vaborbactam showed the highest activity against all tested isolates; imipenem-relebactam showed only moderate activity. All isolates remained susceptible to cefiderocol. Furthermore, avibactam and vaborbactam have greater inhibitory activity than relebactam against the tested carbapenemases. Overall, ceftazidime-avibactam, meropenem-vaborbactam, and cefiderocol were the most effective therapeutic options for treating infections caused by the tested minor carbapenemase producers.

KEYWORDS minor carbapenemases, SME, *Enterobacterales*, imipenem-relebactam, FRI, NMC-A, carbapenemase

Carbapenemase-producing *Enterobacterales* (CPE) isolates have been increasingly reported worldwide and are considered a matter of great clinical concern (1). The most clinically relevant carbapenemases identified include the serine carbapenemases (e.g., KPC or OXA-48 type enzymes) and metallo-β-lactamases (MBLs) (e.g., NDM, VIM, or IMP enzymes) (2). However, there is a wide diversity of unrelated so-called minor carbapenemases belonging to the β-lactamase Ambler class A that are less frequently detected, such as SME-, NmcA-, IMI-, and the most recently identified FRI-like enzymes (3–5). These enzymes have been identified mostly in *Enterobacter* spp. and *Serratia* spp. as sources of nosocomial infections (5). These enzymes (IMI-, NmcA-, SME-, and FRI-like enzymes) have been identified from not only clinical isolates but also strains recovered from the environment (6–8). Their encoding genes may be chromosomally located (in most cases), plasmid located, or both (5, 9).

Recently, novel β-lactam/β-lactamase inhibitor (BL/BLI) combinations have been successfully developed, such as ceftazidime-avibactam, imipenem-relebactam, and meropenem-vaborbactam (10). Avibactam and relebactam belong to the same group of inhibitors, i.e., the diazabicyclooctane (DBO) group, while vaborbactam is a boronic acid derivative. The efficacy of avibactam, vaborbactam, and relebactam has been shown against different major class A carbapenemases, such as KPC enzymes (10–13). However, little is known about the potential effect minor carbapenemases might have on the efficacy of such newly developed BL/BLI combinations.

On the other hand, cefiderocol, a novel siderophore cephalosporin, is a promising antibiotic with excellent activity against a large variety of multidrug-resistant Gram-negative bacteria, including carbapenem-resistant *Enterobacterales*, using its so-called Trojan horse

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strategy to facilitate cell entry and reach its target (14, 15). The occurrence of reduced susceptibility or even resistance to cefiderocol has been recently reported (16, 17).

Therefore, the objectives of this study were first to evaluate the *in vitro* activity of ceftazidime-avibactam, imipenem-relebactam, meropenem-vaborbactam, and cefiderocol against a series of clinical and isogenic strains producing SME-, NmcA-, IMI-, and FRI-like enzymes and second to compare the inhibitory activity of avibactam, relebactam, and vaborbactam against these enzymes. A representative collection of clinical *Enterobacterales* isolates producing minor carbapenemases ($n = 19$) obtained from the Swiss National Reference Center for Emerging Antibiotic Resistance (University of Fribourg, Switzerland) were analyzed in this study. This collection included various enterobacterial species (*Enterobacter cloacae* [$n = 9$], *Enterobacter asburiae* [$n = 3$], and *Serratia marcescens* [$n = 7$]). The minor carbapenemase types were as follows: SME-1 ($n = 5$), SME-2 ($n = 2$), NmcA ($n = 2$), IMI-1 ($n = 4$), IMI-2 ($n = 4$), FRI-1 ($n = 1$), and FRI-2 ($n = 1$).

The MICs were determined in duplicate using reference broth microdilution in cation-adjusted Mueller-Hinton (MH) broth (Bio-Rad, Marnes-la-Coquette, France) for all antibiotics or antibiotic combinations except for cefiderocol, for which iron-depleted cation-adjusted MH broth was used, according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. The results were interpreted according to the latest EUCAST breakpoints (https://www.eucast.org/clinical_breakpoints). Avibactam, relebactam, and vaborbactam were tested at fixed concentrations of 4, 4, and 8 μg/mL, respectively. The reference wild-type strain *Escherichia coli* ATCC 25922 was used for quality control.

Cloning of different minor carbapenemase genes into the broad-host range pUCp24 was performed using PCR products encompassing the entire coding sequences of all respective genes, as previously described (18). The primers used for PCR amplification of the respective genes are listed in Table S1 in the supplemental material. The recombinant plasmids were further transformed into *E. coli* strain TOP10. Selection of the respective transformants was performed on plates containing 50 μg/mL amoxicillin and 30 μg/mL gentamicin. The MICs for all obtained clones were determined as mentioned above. Cultures of *E. coli* TOP10 harboring recombinant plasmids and therefore producing the different minor carbapenemases were grown overnight at 37°C in 50 mL of brain heart infusion medium with amoxicillin (50 μg/mL). The bacterial suspension was pelleted, resuspended in 10 mL of 100-mM phosphate buffer (pH 7), disrupted by sonification (cycles of 30 s of sonication at 20 kHz and 50 s of rest for a total of 5 min) using a Vibra-Cell 75186 sonicator (Thermo Fisher Scientific), and centrifuged for 1 h at 11,000 $\times$ *g* at 4°C. The β-lactamase crude extracts were used to determine the 50% inhibitory concentrations (IC_{50}s) for avibactam, relebactam, and vaborbactam using a UV-visible Ultrospec 2100 Pro spectrophotometer (Amersham Biosciences, Buckinghamshire, UK). Various concentrations of the inhibitors were preincubated with the crude extract of the enzyme for 3 min at room temperature to determine the concentrations that would reduce the hydrolysis rate of 100 μM benzylpenicillin by 50%. The results are expressed in micromolar units.

All analyzed isolates were phenotypically resistant to both carbapenems (meropenem and imipenem), except two *Enterobacter cloacae* strains producing FRI-like enzymes that showed intermediate levels of resistance to meropenem (Table 1). All 19 isolates were susceptible to ceftazidime (MIC values, 0.25 to 4 μg/mL) and cefiderocol (MIC values, $\leq$0.125 to 0.5 μg/mL). Regarding the BL/BLI combinations, meropenem-vaborbactam showed the highest rate of activity against all tested clinical strains, followed by ceftazidime-avibactam. The MICs of ceftazidime-avibactam ranged from 0.06 to 0.5 μg/mL. Notably, all isolates exhibited low MIC levels to meropenem-vaborbactam (MIC values, $\leq$0.03 to 0.125 μg/mL), while they exhibited elevated MICs for imipenem-relebactam (MIC values, 0.25 to $>$32 μg/mL), particularly NmcA-like enzyme-producing *E. cloacae* and SME-like enzyme-producing *S. marcescens* isolates. The lower MICs observed for meropenem-vaborbactam than for ceftazidime-avibactam could be explained by the high concentration of vaborbactam (8 μg/mL) used for the MIC determination, while avibactam was tested at a lower concentration (4 μg/mL). The MICs of imipenem-relebactam for NmcA-like producers ranged from 2 to 4 μg/mL, while those producing SME-like enzymes exhibited MIC values of 2 to

TABLE 1 Genotypic and phenotypic susceptibility testing of different agents against clinical multidrug-resistant *Enterobacterales* isolates

			MIC (μg/mL) for:[a]						
Isolate	Strain	Carbapenemase	IPM	I-R	MEM	MVB	CAZ	CZA	FDC
R278	*E. cloacae*	IMI-1	>32	0.5	>32	≤0.03	1	0.25	0.25
R279	*E. cloacae*	IMI-1	>32	1	32	≤0.03	2	0.125	≤0.125
N486	*E. cloacae*	IMI-1	>32	0.5	>32	≤0.03	0.5	0.125	0.5
N1905	*E. cloacae*	IMI-1	>32	0.25	32	≤0.03	0.5	0.25	0.5
R280	*E. cloacae*	IMI-2	>32	0.5	>32	≤0.03	0.25	0.125	0.5
R281	*E. asburiae*	IMI-2	>32	1	>32	0.03	0.5	0.25	≤0.125
R282	*E. asburiae*	IMI-2	>32	1	>32	0.03	0.5	0.125	0.25
R283	*E. asburiae*	IMI-2	>32	1	32	0.03	0.25	0.25	0.25
R373	*S. marcescens*	SME-1	>32	4	>32	0.03	0.25	0.125	≤0.125
R374	*S. marcescens*	SME-1	>32	2	>32	0.06	1	0.5	≤0.125
R375	*S. marcescens*	SME-1	>32	4	>32	0.06	0.25	0.25	≤0.125
R376	*S. marcescens*	SME-1	>32	2	>32	≤0.03	0.25	0.125	≤0.125
R377	*S. marcescens*	SME-1	>32	8	>32	0.06	0.25	0.25	≤0.125
R378	*S. marcescens*	SME-2	>32	4	32	0.06	1	0.25	≤0.125
R98	*S. marcescens*	SME-2	>32	>32	>32	0.06	0.25	0.125	≤0.125
R371	*E. cloacae*	NmcA	>32	4	32	≤0.03	0.5	0.06	0.25
R372	*E. cloacae*	NmcA	>32	2	16	≤0.03	0.25	0.125	≤0.125
R2178	*E. cloacae*	FRI-1	16	0.5	8	0.125	4	0.5	≤0.125
R3133	*E. cloacae*	FRI-2	8	0.25	4	≤0.03	1	0.25	≤0.125

[a]MIC values were determined using the broth microdilution method. IPM, imipenem; I-R, imipenem/relebactam; MEM, meropenem; MVB, meropenem-vaborbactam; CAZ, ceftazidime; CZA, ceftazidime-avibactam; FDC, cefiderocol.

>32 μg/mL, being further classified as less susceptible to imipenem-relebactam. These findings are in agreement with previous findings for SME-1 and SME-4 (13, 19). Moreover, Biagi et al. reported that vaborbactam reduced the MICs of ceftazidime, imipenem, and meropenem more than did relebactam against SME-producing *S. marcescens* isolates. In addition, avibactam showed good inhibitory activity against SME-1 and SME-4 producers (19).

To clarify whether the expression of $bla_{\text{SME-like}}$, $bla_{\text{NmcA-like}}$, $bla_{\text{IMI-like}}$, and $bla_{\text{FRI-like}}$ genes might be involved in reduced susceptibility to imipenem-relebactam, cloning of these carbapenemase genes was performed. MIC determination showed that recombinant *E. coli* strains producing either SME-1 or NmcA had MIC values of imipenem-relebactam that were ≥8-fold higher than that of the recipient strain (1 versus ≤0.125 μg/mL, respectively) but still remained in the susceptibility range (Table 2). The resulting IMI-1 and FRI-1 recombinant strains showed a ≥2-fold increase in the MICs of imipenem-relebactam compared to that of the recipient counterpart (0.25 versus ≤0.125 μg/mL, respectively). Conversely, no change was observed in the MICs of meropenem-vaborbactam, ceftazidime-avibactam, and cefiderocol for recombinant strains producing SME-1, NmcA, IMI-1, and FRI-1 compared to the recipient wild-type strain (Table 2).

Since elevated MIC values were observed for imipenem-relebactam compared to meropenem-vaborbactam and ceftazidime-avibactam with several carbapenemase producers, it was therefore hypothesized that vaborbactam and avibactam have greater inhibitory activities than relebactam against the studied carbapenemases. Consequently, IC_{50} determination was performed in order to better evaluate and compare the activities of those inhibitors.

TABLE 2 Susceptibility testing of recombinant *E. coli* strains

	MIC (μg/mL) for:[a]						
Strain	IPM	I-R	MEM	MVB	CAZ	CZA	FDC
E. coli TOP10+IMI-1	>32	0.25	4–8	≤0.03	2	0.25	0.06
E. coli TOP10+SME-1	>32	1	32	≤0.03	4	0.25	0.06
E. coli TOP10+NmcA	>32	1	16–32	≤0.03	4	0.25	0.06
E. coli TOP10+FRI-1	4	0.25	0.5	≤0.03	2	0.25	0.06
E. coli TOP10	≤0.125	≤0.125	≤0.03	≤0.03	0.25	0.25	0.06

[a]MIC values were determined using the broth microdilution method. IPM, imipenem; I-R, imipenem/relebactam; MEM, meropenem; MVB, meropenem-vaborbactam; CAZ, ceftazidime; CZA, ceftazidime-avibactam; FDC, cefiderocol.

TABLE 3 IC_{50}s of different β-lactamase inhibitors against the different carbapenemases tested

Enzyme	IC_{50} (μM [mean ± SD]) for:[a]		
	Avibactam	Vaborbactam	Relebactam
NmcA	5.37 ± 0.3	4.8 ± 0.3	9.7 ± 1.2
SME-1	1.8 ± 0.5	2.2 ± 0.4	5.9 ± 1.1
IMI-1	1.9 ± 0.4	2.4 ± 0.5	6.3 ± 0.5
FRI-1	1.4 ± 0.2	7.8 ± 0.2	16.5 ± 1.5

[a]The IC_{50} represents the concentration of a drug that is required for 50% inhibition of the enzymatic activity.

Generally, avibactam and vaborbactam were the most effective inhibitors antagonizing the activity of the studied carbapenemases compared to relebactam, as shown in Table 3. This finding may be related to the higher binding affinity of these two inhibitors than that of relebactam against Ambler class A carbapenemases (20–22). Furthermore, avibactam showed greater inhibitory activity than vaborbactam against FRI-like carbapenemases (Table 3). Importantly, the potent inhibitory activity of avibactam makes ceftazidime-avibactam an interesting therapeutic alternative for treating infections caused by carbapenemase-producing *Enterobacterales* isolates. The poor inhibitory activity of relebactam against the tested carbapenemase producers was confirmed by the elevated MICs of imipenem-relebactam observed (Tables 1 and 2). Relebactam is structurally related to avibactam, showing both a conserved DBO core with differences in the side chains (22). Avibactam has a carboxyamide, while relebactam has a piperidine ring. Tooke et al. (22) investigated the structural basis for relebactam inhibition of class A serine β-lactamases (SBLs) and its structure-activity relationship compared to that of avibactam. The study showed that relebactam exhibited an inferior inhibitory effect against class A SBLs compared to that of avibactam. The X-ray crystal structures of relebactam bound to CTX-M-15, L2, KPC-2, KPC-3, and KPC-4 reveal that its C2-linked piperidine ring can sterically clash with Asn104 (CTX-M-15) or His/Trp105 (L2 and KPCs), explaining its poorer inhibition activity than that of avibactam, which has a smaller C2 carboxyamide group (22).

To the best of our knowledge, this is the first study to investigate the impact of a variety of carbapenemases, including SME-, NmcA-, IMI-, and FRI-like enzymes, on the activity of imipenem-relebactam, meropenem-vaborbactam, and ceftazidime-avibactam using an *E. coli* isogenic background and to evaluate the inhibitory activities of new β-lactamase inhibitors (avibactam, relebactam, and vaborbactam). Finally, our study supports ceftazidime-avibactam, meropenem-vaborbactam, and cefiderocol as effective therapeutic options for severe uncommon carbapenemase-producing infections, including SME-producing *S. marcescens* infections, and demonstrated that relebactam has smaller inhibitory activity than vaborbactam and avibactam against minor carbapenemases, including SME-like producers.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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