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MECHANISMS OF RESISTANCE

Impact of Acquired Broad Spectrum β-Lactamases on Susceptibility to Novel Combinations Made of β-Lactams (Aztreonam, Cefepime, Meropenem, and Imipenem) and Novel β-Lactamase Inhibitors in *Escherichia coli* and *Pseudomonas aeruginosa*

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ABSTRACT The impact of broad-spectrum β-lactamases on the susceptibility to novel β-lactamase/β-lactamase inhibitor combinations was evaluated both in *Pseudomonas aeruginosa* and *Escherichia coli* using isogenic backgrounds. Cefepime-zidebactam displayed low MICs, mainly due to the significant intrinsic antibacterial activity of zidebactam. Cefepime-taniborbactam showed excellent activity against recombinant *E. coli* strains, including metallo-β-lactamase producers, whereas aztreonam-avibactam remained the best therapeutic option against class B β-lactamase-producing *P. aeruginosa*.

KEYWORDS zidebactam, taniborbactam, enmetazobactam, relebactam, vaborbactam, nacubactam, susceptibility testing, β-lactamase, avibactam

The recently developed and commercially available β-lactam/β-lactamase inhibitor (BL/BLI) combinations ceftazidime-avibactam, meropenem-vaborbactam, and imipenem-relebactam possess a broad-spectrum activity against most multidrug-resistant Gram-negative bacteria (1, 2). In addition, new β-lactamase inhibitors have been recently developed and might be soon be available, along with the respective β-lactam partner molecule, which could lead to clinically useful BL/BLI combos. Among the newly developed β-lactamase inhibitors, there are diazabicyclooctane (DBO) molecules, namely, zidebactam and nacubactam, that efficiently inhibit most class A and class C (also class D for zidebactam) β-lactamases. Additionally, they possess an antibiotic effect on PBP2, compared to avibactam. Another class of inhibitors corresponds to the boronic acid derivatives vaborbactam and taniborbactam, both of which inhibit class A and class C β-lactamases, with taniborbactam additionally being an excellent inhibitor of the metallo-β-lactamases (MBLs) of most of the NDM- and VIM-types. Finally, another class of inhibitors corresponds to penicillin-based sulfones, such as enmetazobactam, which is a derivative of tazobactam, and it is reported to be an excellent inhibitor of class A β-lactamases. Taken together, the development of all of these new inhibitors has promoted the development and the evaluation of new BL/BLI combinations (3). Hence, novel combinations, including aztreonam-avibactam, cefepime-enmetazobactam (AAI101, penicillanic acid sulfone), cefepime-zidebactam (WCK 5107, DBO), cefepime-taniborbactam (VNRX-5133, boronate), and meropenem-nacubactam (FPI-1465, DBO) have been tested in several studies *in vitro* and are currently undergoing clinical trial evaluations (4–8).

A previous study evaluating the impact of acquired broad-spectrum β-lactamases on the susceptibility to newly developed BL/BLI reported that several ESBLs, such as PER,

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SHV, and BEL, may affect the susceptibility of last resort combination therapies, such as aztreonam-avibactam, ceftolozane-tazobactam, ceftazidime-avibactam, and cefiderocol, whereas imipenem-relebactam and meropenem-vaborbactam were mostly affected by class B and some class D β-lactamases (9).

Since little is known about the potential effect of broad-spectrum β-lactamases on these new BL/BLI, particularly with the latest combos, namely, cefepime-zidebactam, cefepime-taniborbactam, cefepime-enmetazobactam, and meropenem-nacubactam, being under consideration for clinical development, the objective of our study was to assess the impact of a large series of broad-spectrum β-lactamases on the susceptibility to these combinations, using either *E. coli* (TOP10) or *P. aeruginosa* (PAO1) strains as backgrounds.

To reach that goal, the corresponding genes of the different β-lactamases to be tested were amplified via PCR using the respective specific β-lactamase gene primers (10). The corresponding amplicons were then cloned into plasmid pUCp24, which is a shuttle vector harboring the *aacC1* gene, which encodes the gentamicin acetyltransferase-3-1 that is capable of replicating in both *E. coli* and *P. aeruginosa*. Our focus was mainly on broad-spectrum β-lactamases, namely, ESBLs and carbapenemases of different classes, with several narrow-spectrum β-lactamases being included for comparison. Hence, the different β-lactamases tested here 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-14, CTX-M-15, GES-1, BEL-1, BEL-2, SHV-1, SHV-2a, SHV-12, PER-1, PER-2, PER-6, PER-7, VEB-1), class A ESBLs with weak carbapenemase activity (CTX-M-33, GES-2, GES-5), class A carbapenemases (FRI-1, IMI-1, KPC-2, KPC-3, KPC-41, GES-6, SME-1, NMC-A), class B carbapenemases (AIM-1, DIM-1, VIM-2, VIM-1, GIM-1, NDM-1, NDM-5, NDM-7, NDM-9, IMP-1, SPM-1), class C cephalosporinase (DHA-1, CMY-2, CMY-42), a narrow-spectrum class D β-lactamase (OXA-1), and carbapenem-hydrolyzing class D β-lactamases (OXA-18, OXA-23, OXA-48, OXA-58, OXA-181, OXA-427). It is noteworthy that these enzymes were chosen as representatives, mostly of clinically relevant β-lactamases that are sources of resistance to broad-spectrum β-lactams in either *E. coli* or *P. aeruginosa*.

It is also noteworthy that some of the enzymes that were produced here in both of the latter species might mainly be found in only one of the two, such as OXA-48 and OXA-427 in Enterobacterales or OXA-2, BEL, AIM, GIM, and SPM in *P. aeruginosa*. Nevertheless, our approach did not intend to reflect the exact epidemiology but rather to better decipher the real impact of the BL/BLI combinations in different backgrounds. In total, a series of 49 *E. coli* and 48 *P. aeruginosa* recombinant strains were constructed and analyzed throughout.

The MICs were determined via broth microdilution for ceftazidime-avibactam, cefepime-zidebactam, cefepime-taniborbactam, cefepime-enmetazobactam, imipenem-relebactam, meropenem-vaborbactam, meropenem-nacubactam, and aztreonam-avibactam as well as for the corresponding β-lactams of the respective combinations alone. Ceftazidime, cefepime, and aztreonam were purchased from Sigma-Aldrich (Saint-Louis, USA), whereas imipenem and meropenem were purchased from HuiChem (Shanghai, China). All of the the inhibitors (zidebactam HY-120859, taniborbactam HY-109124, relebactam HY-16752, vaborbactam HY-19930, avibactam HY-14879, enmetazobactam HY-103095) were purchased from MedChem Express (Luzern, Switzerland). The concentrations of the following β-lactamase inhibitors were fixed at 4 μg/mL: zidebactam, taniborbactam, avibactam, nacubactam, and relebactam; however, those of vaborbactam and enmetazobactam were fixed at 8 μg/mL (10, 11). The cefepime-zidebactam and meropenem-nacubactam combinations were also evaluated at a 1:1 ratio due to the strong enhancing activities of zidebactam and nacubactam. Similarly, susceptibility testing to zidebactam and nacubactam alone was performed. The MICs were determined in triplicate via broth microdilution in Mueller-Hinton (MH) broth (Bio-Rad, Marnes-la-Coquette, France) for all of the antibiotics and antibiotic combinations listed above, according to the EUCAST guidelines (12). The results were interpreted according to the latest EUCAST breakpoints, and the susceptibility breakpoints for the novel BL/BLI combinations were defined by referring to the corresponding β-lactam breakpoints (12). The reference strain *E. coli* ATCC 25922 was used as a quality control for all of the testing. No specific CLSI/EUCAST quality controls were used

when testing the action of the different β-lactamase inhibitors. Nevertheless, our experiments included different recombinant strains that produced a wide range of β-lactamases for which the potential to be inhibited or not had been previously reported, thereby allowing for the validation of our data. Hence, KPC-2-producing *E. coli* TOP10 was used as a control when testing the meropenem-vaborbactam, meropenem-nacubactam, and imipenem-relebactam combinations, CTX-M-15-producing-*E. coli* TOP 10 was used when testing the ceftazidime-avibactam, cefepime-enmetazobactam, and aztreonam-avibactam combinations, and NDM-1-producing-*E. coli* TOP 10 was used when testing the cefepime-taniborbactam combination. For all of these combinations, low MICs were observed, and these MICs were similar to that of the reference strain *E. coli* ATCC 25922.

The results obtained for the recombinant *E. coli* strains are compiled in Table 1. Overall, the most efficient combinations were cefepime-zidebactam (at a fixed concentration of zidebactam or a 1:1 ratio), cefepime-taniborbactam, and meropenem-nacubactam (at a fixed concentration of nacubactam or a 1:1 ratio) when the MICs to zidebactam and nacubactam alone were 0.25 μg/mL and 8 μg/mL, respectively. Although low MICs were observed with cefepime-zidebactam at a fixed concentration or at a 1:1 ratio, this isogenic model of *E. coli* did not allow us to investigate the proper inhibitor activity of zidebactam, as we observed such low MICs for zidebactam alone. As expected, cefepime-taniborbactam was active against all of the ESBL and carbapenemase producers, including MBL producers, except when IMP-1 and NDM-9 were produced, and these results are in line with previous observations highlighting the lack of inhibitory activity of taniborbactam against IMP-1 and NDM-9 (13, 14). Meropenem-nacubactam was also effective against most of the recombinant strains, with the exception of the MBL producers, such as those producing AIM-1, NDM-5, VIM-1, SPM-1, and IMP-1, as expected. However, it is worth highlighting that meropenem-nacubactam significantly decreased the MICs of some of the class B β-lactamase producers (NDM-1, NDM-7, NDM-9, DIM-1, GIM-1, IMP-1), and this was likely due to the intrinsic activity of nacubactam, as previously suggested (15). The higher hydrolysis of meropenem by NDM-5 and IMP-1 probably explained why the meropenem-nacubactam (at a fixed concentration or a 1:1 ratio) combinations did not decrease the MICs below the breakpoints. Interestingly, although the production of PER- and CMY-like β-lactamases significantly contributed to the reduced susceptibility to cefepime, aztreonam-avibactam, and ceftazidime-avibactam, a complete susceptibility to cefepime-zidebactam, cefepime-taniborbactam, cefepime-enmetazobactam, and meropenem-nacubactam was observed for the corresponding producers. Likewise, although the production of the MBLs of the AIM-1, DIM-1, and GIM-1 types as well as the class A β-lactamase KPC-41 have major impacts on the susceptibility to ceftazidime-avibactam, they did not show any effect on the susceptibility to cefepime-zidebactam, cefepime-enmetazobactam, cefepime-taniborbactam, and meropenem-nacubactam. This further highlights that all of the latter combinations might represent potent therapeutic options for the treatment of infections that are caused by the corresponding isolates.

An analysis of the MICs determined using the recombinant *P. aeruginosa* strains overall mirrored what was observed for *E. coli*, but with more marked effects. As expected, given the intrinsic reduced susceptibility of *P. aeruginosa* to many β-lactams due to its lower permeability, the MIC values were overall higher than those that were obtained for *E. coli* (Table 2). In addition, the PBP2 enhancing activity of zidebactam and nacubactam at 4 μg/mL appeared less marked and more variable in *P. aeruginosa*, as was previously described at this concentration (15). In line, the MICs of zidebactam and nacubactam were found to be at 8 and >32 μg/mL, respectively. As a result, resistance to cefepime-zidebactam was observed for the *P. aeruginosa* recombinant strains producing VEB-1, KPC-like, NDM-like, VIM-like, IMP-1, and CMY-like enzymes. A reduced susceptibility was observed among SHV-1, PER-2, PER-6, PER-7, and OXA-23 producers; however, this model of isogenic *P. aeruginosa* overexpressing a wide range of β-lactamases is also limited by the major role played by the antibacterial activity of zidebactam, with its MIC alone being observed at 8 μg/mL (Table 2). In contrast, this combination remained active against producers of class D β-lactamases, as the latter enzymes do not hydrolyze cefepime at a significant level. It is noteworthy that some discrepancies were observed for the MICs between

TABLE 1 Susceptibility testing of recombinant *E. coli* TOP strains[a]

Strain (β-lactamase produced)	β-lactamase spectrum	Ambler class	Minimal inhibitory concentrations (μg/mL)																
			CAZ	CZA	FEP	FEP-TAN	FEP-ENM	MEM	MVB	MER-NAC	MER-NAC 1:1	IPM	I/R	ATM	ATM-AVI	NAC	FEP-ZID	FEP-ZID 1:1	ZID
E. coli (none)	-[b]	-	≤0.25	≤0.125	≤0.25	≤0.125	≤0.125	≤0.25	≤0.125	≤0.125	0.03	≤0.25	≤0.125	≤0.25	≤0.125	8	≤0.125	0.06	0.25
E. coli OXY-2	ESBL	A	1	0.25	2	≤0.125	0.25	≤0.25	≤0.125	≤0.125	0.03	≤0.25	≤0.125	**16**	0.25	8	≤0.125	0.25	0.25
E. coli TEM-1	Narrow	A	≤0.25	≤0.125	≤0.25	≤0.125	≤0.125	≤0.25	≤0.125	≤0.125	0.03	≤0.25	0.25	≤0.25	≤0.125	8	≤0.125	0.125	0.25
E. coli TEM-3	Narrow	A	**8**	≤0.125	≤0.25	0.25	0.25	≤0.25	≤0.125	≤0.125	0.03	≤0.25	≤0.125	1	≤0.125	8	≤0.125	0.125	0.25
E. coli CTX-M-3	ESBL	A	**8**	0.5	0.5	≤0.125	≤0.125	≤0.25	≤0.125	≤0.125	0.03	≤0.25	0.5	4	≤0.125	8	≤0.125	0.25	0.25
E. coli CTX-M-14	ESBL	A	4	0.25	2	≤0.125	≤0.125	≤0.25	≤0.125	≤0.125	0.03	≤0.25	≤0.125	4	≤0.125	8	≤0.125	0.25	0.25
E. coli CTX-M-15	ESBL	A	**256**	≤0.125	**64**	≤0.125	≤0.125	≤0.25	≤0.125	≤0.125	0.03	≤0.25	≤0.125	**>256**	0.5	8	≤0.125	0.25	0.25
E. coli CTX-M-33	Carba	A	**16**	0.25	**16**	≤0.125	≤0.125	≤0.25	≤0.125	≤0.125	0.125	0.5	≤0.125	**32**	≤0.125	8	≤0.125	0.25	0.25
E. coli GES-1	ESBL	A	**16**	0.25	≤0.25	≤0.125	≤0.125	≤0.25	≤0.125	≤0.125	0.03	≤0.25	≤0.125	0.5	≤0.125	8	≤0.125	0.125	0.25
E. coli GES-2	Carba	A	**8**	0.5	≤0.25	≤0.125	≤0.125	≤0.25	≤0.125	≤0.125	0.03	≤0.25	0.25	0.5	≤0.125	8	≤0.125	0.125	0.25
E. coli GES-5	Carba	A	1	0.25	≤0.25	≤0.125	≤0.125	≤0.25	≤0.125	≤0.125	0.06	0.5	≤0.125	≤0.25	≤0.125	8	≤0.125	0.125	0.25
E. coli GES-6	Carba	A	**64**	2	≤0.25	≤0.125	≤0.125	0.5	≤0.125	≤0.125	0.125	0.5	0.5	0.5	≤0.125	8	≤0.125	0.125	0.25
E. coli BEL-1	ESBL	A	**8**	0.25	1	≤0.125	≤0.125	≤0.25	≤0.125	≤0.125	0.03	≤0.25	≤0.125	**16**	≤0.125	8	≤0.125	0.25	0.25
E. coli BEL-2	ESBL	A	**16**	1	1	≤0.125	≤0.125	≤0.25	≤0.125	≤0.125	0.03	≤0.25	≤0.125	1	≤0.125	8	≤0.125	0.25	0.25
E. coli SHV-2a	ESBL	A	**8**	0.25	2	≤0.125	≤0.125	≤0.25	≤0.125	≤0.125	0.03	≤0.25	≤0.125	**8**	≤0.125	8	≤0.125	0.25	0.25
E. coli SHV-1	Narrow	A	**8**	0.25	≤0.25	≤0.125	≤0.125	≤0.25	≤0.125	≤0.125	0.03	≤0.25	≤0.125	0.5	≤0.125	8	≤0.125	0.125	0.25
E. coli SHV-12	ESBL	A	**>256**	0.5	**8**	≤0.125	0.25	≤0.25	≤0.125	≤0.125	0.03	≤0.25	≤0.125	**>256**	≤0.125	8	≤0.125	0.25	0.25
E. coli VEB-1	ESBL	A	**>256**	2	4	≤0.125	≤0.125	≤0.25	≤0.125	≤0.125	0.03	0.5	0.25	**128**	1	8	≤0.125	0.25	0.25
E. coli FRI-1	Carba	A	1	0.25	≤0.25	≤0.125	≤0.125	0.5	≤0.125	≤0.125	0.06	2	0.25	**128**	≤0.125	8	≤0.125	0.125	0.25
E. coli PER-1	ESBL	A	**>256**	**16**	**8**	≤0.125	≤0.125	≤0.25	≤0.125	≤0.125	0.03	≤0.25	0.5	**>256**	**8**	8	≤0.125	0.25	0.25
E. coli PER-2	ESBL	A	**128**	4	**4**	≤0.125	≤0.125	≤0.25	≤0.125	≤0.125	0.03	≤0.25	≤0.125	**128**	4	8	≤0.125	0.25	0.25
E. coli PER-6	ESBL	A	**>256**	**64**	**>256**	≤0.125	≤0.125	≤0.25	≤0.125	≤0.125	0.06	≤0.25	≤0.125	**>256**	**16**	8	≤0.125	0.25	0.25
E. coli PER-7	ESBL	A	**>256**	**32**	**>256**	≤0.125	≤0.125	≤0.25	≤0.125	≤0.125	0.06	≤0.25	≤0.125	**>256**	**8**	8	≤0.125	0.25	0.25
E. coli KPC-2	Carba	A	**32**	0.5	4	≤0.125	≤0.125	4	≤0.125	≤0.125	0.125	**32**	0.25	**128**	0.125	8	≤0.125	0.25	0.25
E. coli KPC-3	Carba	A	**128**	0.5	**8**	≤0.125	≤0.125	4	≤0.125	≤0.125	0.125	**16**	0.25	**256**	≤0.125	8	≤0.125	0.25	0.25
E. coli KPC-41	Carba	A	**256**	**16**	2	≤0.125	≤0.125	0.5	≤0.125	≤0.125	0.125	2	≤0.125	**32**	1	8	≤0.125	0.25	0.25
E. coli SME	Carba	A	**8**	0.5	0.5	≤0.125	≤0.125	**32**	≤0.125	0.25	0.25	**>256**	1	**>256**	1	8	≤0.125	0.25	0.25
E. coli IMI-1	Carba	A	2	0.25	≤0.25	≤0.125	≤0.125	**16**	≤0.125	≤0.125	0.25	**128**	0.5	**128**	0.5	8	≤0.125	0.125	0.25
E. coli NMC-A	Carba	A	2	0.5	1	≤0.125	≤0.125	**32**	0.125	4	4	**256**	1	**>256**	1	8	≤0.125	0.25	0.25
E. coli VIM-1	Carba	B	**>256**	**>128**	**32**	2	**32**	4	4	4	4	**8**	**8**	≤0.25	≤0.125	8	≤0.125	0.25	0.25
E. coli VIM-2	Carba	B	**32**	**32**	0.5	0.5	0.5	≤0.25	0.25	0.25	0.25	1	1	≤0.25	≤0.125	8	≤0.125	0.25	0.25
E. coli AIM-1	Carba	B	**16**	**16**	≤0.25	0.25	0.25	8	8	4	4	4	**4**	≤0.25	≤0.125	8	≤0.125	0.125	0.25
E. coli GIM-1	Carba	B	**32**	**32**	≤0.25	≤0.125	0.25	0.5	0.5	≤0.125	0.125	0.5	0.5	≤0.25	≤0.125	8	≤0.125	0.125	0.25
E. coli DIM-1	Carba	B	**8**	8	≤0.25	≤0.125	≤0.125	0.5	0.5	≤0.125	0.125	0.5	0.5	≤0.25	≤0.125	8	≤0.125	0.125	0.25
E. coli NDM-1	Carba	B	**>256**	**>128**	**16**	1	**16**	4	4	0.5	2	4	**4**	≤0.25	≤0.125	8	≤0.125	0.25	0.25
E. coli NDM-5	Carba	B	**>256**	**>128**	**16**	1	**16**	**16**	**16**	4	4	**8**	**8**	≤0.25	≤0.125	8	≤0.125	0.25	0.25
E. coli NDM-7	Carba	B	**>256**	**>128**	**8**	0.5	**8**	2	2	≤0.125	0.5	4	**4**	≤0.25	≤0.125	8	≤0.125	0.25	0.25
E. coli NDM-9	Carba	B	**>256**	**>128**	**16**	**16**	**16**	4	4	0.5	2	**8**	**8**	≤0.25	≤0.125	8	≤0.125	0.25	0.25
E. coli IMP-1	Carba	B	**>256**	**>128**	**16**	**16**	**16**	**32**	**32**	8	8	4	**4**	≤0.25	≤0.125	8	≤0.125	0.5	0.25
E. coli SPM-1	Carba	B	**128**	**128**	4	≤0.125	4	4	4	4	4	2	2	≤0.25	≤0.125	8	≤0.125	0.25	0.25
E. coli CMY-2	Case	C	**>256**	2	1	≤0.125	0.25	≤0.25	≤0.125	≤0.125	0.06	0.5	≤0.125	**64**	0.5	8	≤0.125	0.25	0.25
E. coli CMY-42	Case	C	**>256**	4	4	≤0.125	0.5	≤0.25	≤0.125	≤0.125	0.06	0.5	0.5	**128**	4	8	≤0.125	0.25	0.25
E. coli DHA-1	Case	C	**128**	0.25	≤0.25	≤0.125	≤0.125	≤0.25	≤0.125	≤0.125	0.03	0.25	≤0.125	**8**	≤0.125	8	≤0.125	0.125	0.25

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TABLE 1 (Continued)

Strain (β-lactamase produced)	β-lactamase spectrum	Ambler class	Minimal inhibitory concentrations (μg/mL)																
			CAZ	CZA	FEP	FEP-TAN	FEP-ENM	MEM	MVB	MER-NAC	MER-NAC 1:1	IPM	I/R	ATM	ATM-AVI	NAC	FEP-ZID	FEP-ZID 1:1	ZID
E. coli OXA-1	Narrow	D	0.5	0.25	≤0.25	≤0.125	≤0.125	≤0.25	≤0.125	≤0.125	0.03	≤0.25	≤0.125	≤0.25	≤0.125	8	≤0.125	0.125	0.25
E. coli OXA-18	Carba	D	**128**	1	1	≤0.125	≤0.125	≤0.25	≤0.125	≤0.125	0.03	≤0.25	≤0.125	**128**	0.125	8	≤0.125	0.125	0.25
E. coli OXA-23	Carba	D	0.5	0.5	≤0.25	≤0.125	≤0.125	≤0.25	≤0.125	≤0.125	0.06	0.5	0.5	0.25	0.125	8	≤0.125	0.125	0.25
E. coli OXA-48	Carba	D	0.5	0.25	≤0.25	≤0.125	≤0.125	≤0.25	≤0.125	≤0.125	0.03	0.5	0.5	≤0.25	≤0.125	8	≤0.125	0.125	0.25
E. coli OXA-58	Carba	D	0.5	≤0.125	≤0.25	≤0.125	≤0.125	≤0.25	≤0.125	≤0.125	0.06	0.5	0.5	≤0.25	≤0.125	8	≤0.125	0.125	0.25
E. coli OXA-181	Carba	D	≤0.25	≤0.125	≤0.25	≤0.125	≤0.125	≤0.25	≤0.125	≤0.125	0.06	0.5	0.5	≤0.25	≤0.125	8	≤0.125	0.125	0.25
E. coli OXA-427	Carba	D	**32**	0.5	≤0.25	≤0.125	0.25	≤0.25	≤0.125	≤0.125	0.06	0.5	0.5	4	≤0.125	8	≤0.125	0.125	0.25

[a]Narrow, narrow-spectrum; Case, Cephalosporinase; ESBL, extended-spectrum β-lactamase; Carba, Carbapenemase; CAZ, ceftazidime; CZA, ceftazidime-avibactam; FEP, cefepime; FEP-ZID, cefepime/zidebactam; FEP-TAN, cefepime/taniborbactam; FEP-ENM, cefepime/enmetazobactam; IPM, imipenem; I-R, imipenem/relebactam; MEM, meropenem; MVB, meropenem-vaborbactam; MER-NAC, meropenem/nacubactam; ATM, aztreonam; ATM-AVI, aztreonam/avibactam. The MIC values indicated in bold are those corresponding to a categorization of resistance. The shaded MIC values are those that correspond to a significantly elevated MIC value, compared to wild type *E. coli* TOP10, but are not categorized as resistant, according to EUCAST (12).

[b]-, none.

TABLE 2 Susceptibility testing of recombinant *P. aeruginosa* PAO strains[a]

Strain (β-lactamase produced)	β-lactamase spectrum	Ambler class	Minimal inhibitory concentrations (μg/mL)																
			CAZ	CZA	FEP	FEP-TAN	FEP-ENM	MEM	MVB	MER-NAC	MER-NAC 1:1	IPM	I-R	ATM	ATM-AVI	NAC	FEP-ZID	FEP-ZID 1:1	ZID
P. aeruginosa (none)	-[b]	-	1	1	1	1	1	0.5	0.25	0.25	0.25	0.5	0.25	4	2	>32	≤0.125	1	4
P. aeruginosa OXY-2	ESBL	A	4	1	**>256**	1	2	0.5	0.5	0.5	0.5	0.5	0.25	**64**	2	>32	2	2	8
P. aeruginosa TEM-1	Narrow	A	2	1	2	1	1	0.5	0.5	0.5	0.5	0.5	0.25	4	4	>32	≤0.125	2	8
P. aeruginosa TEM-3	Narrow	A	8	1	**16**	2	1	0.5	0.5	0.25	0.5	0.5	0.25	4	4	>32	≤0.125	2	8
P. aeruginosa CTX-M-3	ESBL	A	**16**	4	**256**	2	1	0.5	0.25	0.5	0.5	0.5	0.5	**>256**	2	>32	≤0.125	4	8
P. aeruginosa CTX-M-15	ESBL	A	**16**	1	**>256**	2	1	0.5	0.5	0.5	0.5	0.5	0.5	**64**	4	>32	2	4	8
P. aeruginosa CTX-M-33	Carba	A	**16**	2	**>256**	2	1	2	0.5	0.5	0.5	2	0.5	**128**	4	>32	≤0.125	4	8
P. aeruginosa GES-1	ESBL	A	**32**	1	8	1	2	0.5	0.25	0.25	0.5	0.5	0.5	4	2	>32	≤0.125	2	8
P. aeruginosa GES-2	Carba	A	8	1	8	2	2	1	0.5	0.5	1	1	0.25	**16**	4	>32	≤0.125	4	8
P. aeruginosa GES-5	Carba	A	**16**	2	2	1	2	4	1	0.5	1	2	1	4	2	>32	≤0.125	2	8
P. aeruginosa GES-6	Carba	A	**64**	2	2	1	2	8	2	2	4	4	1	4	2	>32	≤0.125	2	8
P. aeruginosa BEL-1	ESBL	A	**32**	2	**16**	2	8	0.5	0.25	0.25	0.25	0.5	0.5	**64**	2	>32	≤0.125	2	8
P. aeruginosa BEL-2	ESBL	A	**128**	4	2	1	2	0.5	0.5	0.5	0.25	0.5	0.5	**32**	4	>32	0.5	2	8
P. aeruginosa SHV-2b	ESBL	A	**128**	4	**>256**	4	**64**	0.5	0.25	0.25	0.5	1	0.25	**64**	2	>32	≤0.125	4	8
P. aeruginosa SHV-1	Narrow	A	**64**	2	**128**	2	**32**	0.5	0.25	0.25	0.5	1	0.25	8	2	>32	4	4	8
P. aeruginosa SHV-12	ESBL	A	**>256**	4	**>256**	1	**64**	0.5	0.25	0.25	0.25	1	0.5	**>256**	8	>32	0.25	4	8
P. aeruginosa VEB-1	ESBL	A	**>256**	**64**	**>256**	8	**32**	0.5	0.5	0.25	0.25	1	0.5	**>256**	16	>32	**128**	8	8
P. aeruginosa FRI-1	Carba	A	2	1	2	1	1	1	0.25	0.25	4	2	0.25	16	2	>32	0.5	2	8
P. aeruginosa PER-1	ESBL	A	**>256**	**32**	**>256**	4	**>128**	0.5	0.25	0.5	0.5	1	0.25	**>256**	16	>32	≤0.125	4	8
P. aeruginosa PER-2	ESBL	A	**128**	4	**32**	1	1	0.5	0.25	0.5	0.25	1	0.25	**>256**	8	>32	4	4	8
P. aeruginosa PER-6	ESBL	A	**>256**	**32**	**>256**	4	1	1	0.5	0.25	0.25	0.5	0.5	**>256**	**32**	>32	4	4	8
P. aeruginosa PER-7	ESBL	A	**>256**	**32**	**>256**	8	**>128**	1	0.25	0.25	0.5	1	0.5	**>256**	**32**	>32	8	8	8
P. aeruginosa KPC-2	Carba	A	**128**	4	**>256**	4	**>128**	**128**	**16**	4	4	**64**	1	**>256**	8	>32	**>128**	8	8
P. aeruginosa KPC-3	Carba	A	**>256**	8	**>256**	4	**128**	**128**	**16**	4	4	**64**	1	**>256**	8	>32	**>128**	8	8
P. aeruginosa KPC-41	Carba	A	**>256**	**>128**	**>256**	**32**	**>128**	**32**	**8**	4	4	**16**	2	**>256**	**32**	>32	**>128**	8	8
P. aeruginosa SME	Carba	A	8	1	4	1	1	**64**	2	4	4	**64**	1	**>256**	4	>32	2	2	8
P. aeruginosa NMC-A	Carba	A	4	1	4	1	1	**32**	2	2	2	**32**	1	**128**	4	>32	2	2	8
P. aeruginosa IMI-1	Carba	A	2	1	8	8	8	**16**	1	1	4	**16**	1	**128**	4	>32	≤0.125	4	8
P. aeruginosa VIM-1	Carba	B	**>256**	**>128**	**>256**	**128**	**>128**	**64**	**64**	**64**	**>32**	**32**	**32**	4	4	>32	**>128**	8	8
P. aeruginosa VIM-2	Carba	B	**128**	**128**	**128**	4	**128**	**32**	**32**	**32**	**32**	**16**	**16**	4	4	>32	**128**	8	8
P. aeruginosa AIM-1	Carba	B	**64**	**64**	**16**	**16**	**16**	**32**	**32**	**32**	**32**	**16**	**16**	4	2	>32	0.5	4	8
P. aeruginosa GIM-1	Carba	B	**64**	**64**	8	4	8	**16**	**16**	**16**	**16**	4	**4**	4	4	>32	≤0.125	4	8
P. aeruginosa DIM-1	Carba	B	**256**	**128**	2	2	2	**16**	**16**	**16**	**16**	**16**	**16**	4	4	>32	≤0.125	2	8
P. aeruginosa NDM-1	Carba	B	**>256**	**>128**	**>256**	**64**	**>128**	**64**	**64**	**64**	**>32**	**16**	**16**	4	4	>32	**>128**	8	8
P. aeruginosa NDM-5	Carba	B	**>256**	**>128**	**>256**	**64**	**>128**	**32**	**32**	**32**	**32**	**16**	**16**	4	4	>32	**>128**	8	8
P. aeruginosa NDM-7	Carba	B	**>256**	**>128**	**>256**	**64**	**>128**	**64**	**64**	**64**	**>32**	**16**	**16**	4	4	>32	**>128**	8	8
P. aeruginosa NDM-9	Carba	B	**>256**	**>128**	**>256**	**>128**	**>128**	**64**	**64**	**64**	**>32**	**16**	**16**	4	4	>32	**>128**	8	8
P. aeruginosa IMP-1	Carba	B	**>256**	**>128**	**>256**	**>128**	**>128**	**64**	**64**	**64**	**>32**	**16**	**16**	4	4	>32	**128**	8	8
P. aeruginosa SPM-1	Carba	B	**>256**	**128**	**>256**	**16**	**>128**	**32**	**32**	**32**	**32**	4	**4**	4	4	>32	≤0.125	4	8
P. aeruginosa CMY-2	Case	C	**>256**	**128**	**128**	**16**	**64**	1	0.5	0.5	1	1	1	**256**	16	>32	**32**	8	8
P. aeruginosa CMY-42	Case	C	**>256**	**64**	**128**	2	**64**	1	0.5	0.5	1	1	1	**256**	16	>32	**32**	8	8
P. aeruginosa DHA-1	Case	C	**128**	1	2	2	1	0.5	0.25	0.25	0.25	1	0.5	16	4	>32	0.5	2	8
P. aeruginosa OXA-1	Narrow	D	1	1	**32**	**32**	4	0.5	0.5	0.25	0.25	0.5	0.5	4	2	>32	2	4	8

(Continued on next page)

TABLE 2 (Continued)

Strain (β-lactamase produced)	β-lactamase spectrum	Ambler class	Minimal inhibitory concentrations (μg/mL)															
			CAZ	CZA	FEP	FEP-TAN	FEP-ENM	MEM	MVB	MER-NAC	MER-NAC 1:1	IPM	I-R	ATM	ATM-AVI	NAC	FEP-ZID	FEP-ZID 1:1	ZID
P. aeruginosa OXA-18	Carba	D	**>256**	2	2	2	2	2	2	2	2	4	**4**	**64**	4	>32	0.25	1	8
P. aeruginosa OXA-23	Carba	D	2	1	**32**	**16**	**16**	8	8	8	8	4	**4**	4	2	>32	4	4	8
P. aeruginosa OXA-48	Carba	D	1	1	1	1	1	2	2	2	2	2	2	4	2	>32	1	1	8
P. aeruginosa OXA-58	Carba	D	1	1	1	1	1	4	4	4	4	2	2	4	2	>32	≤0.125	1	8
P. aeruginosa OXA-181	Carba	D	1	1	1	1	1	1	1	1	1	1	1	4	2	>32	0.25	1	8
P. aeruginosa OXA-427	Carba	D	**>256**	**128**	**64**	4	4	4	4	4	4	2	2	**>256**	**32**	>32	≤0.125	4	8

[a]Narrow, narrow-spectrum; Case, Cephalosporinase; ESBL, extended-spectrum β-lactamase; Carba, Carbapenemase; CAZ, ceftazidime; CZA, ceftazidime-avibactam; FEP, cefepime; FEP-ZID, cefepime/zidebactam; FEP-TAN, cefepime/taniborbactam; FEP-ENM, cefepime/enmetazobactam; IPM, imipenem; I-R, imipenem/relebactam; MEM, meropenem; MVB, meropenem-vaborbactam; MER-NAC, meropenem/nacubactam; ATM, aztreonam; ATM-AVI, aztreonam/avibactam. The MIC values indicated in bold are those corresponding to a categorization of resistance. The shaded MIC values are those that correspond to a significantly elevated MIC value, compared to wild type *P. aeruginosa* PAO1, but are not categorized as resistant, according to EUCAST (12).

[b]-, none.

cefepime-zidebactam with a fixed concentration of the inhibitor at 4 μg/mL or at a 1:1 ratio, with these mainly being caused by the major role of the direct antibacterial action of zidebactam in these combinations. Moreover, resistance to cefepime-enmetazobactam was observed for many recombinant strains, including those producing β-lactamases of class A (SHV-1, SHV-2a, SHV-12, PER-1, PER-7, KPC-2, KPC-3, KPC-41), class B (NDM-type, VIM-type, SPM-1, AIM-1), class C (CMY-type), and class D (OXA-23), whereas resistance to cefepime-taniborbactam was only observed for those producing β-lactamases of class A (KPC-41), class B (VIM-1, AIM-1, NDM-type, IMP-1, SPM-1), class C (CMY-2), and class D (OXA-1, OXA-23). Finally, resistance was observed with meropenem-nacubactam for all of the class B β-lactamase producers as well for as those producing OXA-23.

Among the class A β-lactamase-producing *P. aeruginosa* strains, imipenem-relebactam, cefepime-taniborbactam, and meropenem-nacubactam appeared to be the best options, whereas aztreonam and aztreonam-avibactam remained the best options against the class B producers. It is noteworthy that although KPC-like enzymes are rarely identified in *P. aeruginosa* (16), our results indicate that only a few therapeutic options, such as cefepime-taniborbactam, meropenem-nacubactam, and imipenem-relebactam could be considered in this situation if the corresponding strain is not affected by significant permeability defects. Interestingly, OXA-427-producing *P. aeruginosa* exhibited a reduced susceptibility to most of the BL/BLI combinations.

In a recent study, Vázquez-Ucha et al. highlighted the high activities of cefepime-zidebactam, cefepime-taniborbactam, and cefepime-enmetazobactam on 400 strains of carbapenemase-producing Enterobacterales (17). Cefepime-zidebactam and cefepime-taniborbactam displayed efficient activity against 99% of the tested clinical isolates, whereas cefepime-enmetazobactam displayed lower activity (61.8%), with high MICs being found for OXA-48 and KPC producers, even though the activity of cefepime-enmetazobactam might be affected by the clonality of the *K. pneumoniae* isolates and by the fact that enmetazobactam is actually a tazobactam derivative whose main function is to inhibit ESBLs and not carbapenemases, as was recently and rightly underscored by Vázquez-Ucha et al. and Shapiro (18, 19). Another study evaluating imipenem-relebactam, cefepime-zidebactam, and cefepime-taniborbactam combinations showed promising *in vitro* activity against a collection of ceftolozane-tazobactam-resistant and ceftazidime-avibactam-resistant *P. aeruginosa* strains (20).

On the other hand, meropenem-nacubactam also exhibited excellent performance *in vitro* on a large collection of Enterobacterales and *P. aeruginosa*, including meropenem-resistant and ceftazidime-avibactam-resistant isolates, thereby highlighting the potent inhibitory and antibacterial dual-action of nacubactam (21).

To the best of our knowledge, this study is the first to investigate the relative impacts of a wide range of β-lactamases on the activity of the most recent BL/BLI combinations that are being considered for clinical development. That evaluation was made both in *E. coli* and *P. aeruginosa*, using isogenic backgrounds, which allowed for the accurate assessment of the respective involvement of different β-lactamases in the reduced susceptibility or even resistance to such new treatment options. The excellent performances of aztreonam-avibactam, meropenem-nacubactam, cefepime-zidebactam, and cefepime-taniborbactam must be underscored, even though those combinations are not yet available for clinical use. Nevertheless, due to the efficient intrinsic activities of zidebactam and nacubactam, the interpretation of the inhibitory capacities of the tested combinations should be placed in perspective. When considering commercially available combinations, good performances of ceftazidime-avibactam, imipenem-relebactam, and meropenem-vaborbactam were noticed against *E. coli*, but these combinations were less efficient against the MBL producers. Of note, the *in vitro* activities of those different combinations appeared to be more variable against the *P. aeruginosa* recombinant strains. Therefore, our results highlight that the continuous spread of class B β-lactamases (NDM, IMP, and VIM) and, more rarely, KPC-type enzymes in *P. aeruginosa* may still be considered a significant source of concern when considering treatment with newly available therapeutics.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, DOCX file, 0.02 MB.

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REFERENCES

1. Yahav D, Giske CG, Grāmatniece A, Abodakpi H, Tam VH, Leibovici L. 2020. New β-lactam-β-lactamase inhibitor combinations. Clin Microbiol Rev 34:e00115-20. https://doi.org/10.1128/CMR.00115-20.
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. Vázquez-Ucha JC, Arca-Suárez J, Bou G, Beceiro A. 2020. New carbapenemase inhibitors: clearing the way for the β-lactams. Int J Mol Sci 21:9308. https://doi.org/10.3390/ijms21239308.
4. Kaye KS, Belley A, Barth P, Lahlou O, Knechtle P, Motta P, Velicitat P. 2022. Effect of cefepime/enmetazobactam vs piperacillin/tazobactam on clinical cure and microbiological eradication in patients with complicated urinary tract infection or acute pyelonephritis: a randomized clinical trial. JAMA 328:1304–1314. https://doi.org/10.1001/jama.2022.17034.
5. ClinicalTrials.gov. 2022. Study of cefepime-zidebactam (FEP-ZID) in complicated urinary tract infection (cUTI) or acute pyelonephritis (AP). https://clinicaltrials.gov/ct2/show/NCT04979806.
6. ClinicalTrials.gov. 2022. Safety and efficacy study of cefepime/VNRX-5133 in patients with complicated urinary tract infections (CERTAIN-1). https://clinicaltrials.gov/ct2/show/NCT03840148.
7. ClinicalTrials.gov. 2022. A study to investigate the intrapulmonary lung penetration of nacubactam in healthy participants. https://clinicaltrials.gov/ct2/show/NCT03182504.
8. ClinicalTrials.gov. 2022. Efficacy, safety, and tolerability of ATM-AVI in the treatment of serious infection due to MBL-producing Gram-negative bacteria. https://clinicaltrials.gov/ct2/show/NCT03580044.
9. Poirel L, Ortiz de la Rosa JM, Sadek M, Nordmann P. 2022. 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. Antimicrob Agents Chemother 66:e0003922. https://doi.org/10.1128/aac.00039-22.
10. 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.
11. Le Terrier C, Nordmann P, Poirel L. 2022. In vitro activity of aztreonam in combination with newly developed β-lactamase inhibitors against MDR Enterobacterales and Pseudomonas aeruginosa producing metallo-β-lactamases. J Antimicrob Chemother 78:101–107. https://doi.org/10.1093/jac/dkac360.
12. EUCAST. Clinical breakpoint table v. 13.0. https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_13.0_Breakpoint_Tables.pdf.
13. Krajnc A, Brem J, Hinchliffe P, Calvopiña K, Panduwawala TD, Lang PA, Kamps JJAG, Tyrrell JM, Widlake E, Saward BG, Walsh TR, Spencer J, Schofield CJ. 2019. Bicyclic boronate VNRX-5133 inhibits metallo- and serine-β-lactamases. J Med Chem 62:8544–8556. https://doi.org/10.1021/acs.jmedchem.9b00911.
14. Le Terrier C, Gruenig V, Fournier C, Nordmann P, Poirel L. 2023. NDM-9 resistance to taniborbactam. Lancet Infect Dis https://doi.org/10.1016/S1473-3099(23)00069-5.
15. Morinaka A, Tsutsumi Y, Yamada M, Suzuki K, Watanabe T, Abe T, Furuuchi T, Inamura S, Sakamaki Y, Mitsuhashi N, Ida T, Livermore DM. 2015. OP0595, a new diazabicyclooctane: mode of action as a serine β-lactamase inhibitor, antibiotic and β-lactam 'enhancer'. J Antimicrob Chemother 70:2779–2786. https://doi.org/10.1093/jac/dkv166.
16. 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.
17. Vázquez-Ucha JC, Lasarte-Monterrubio C, Guijarro-Sánchez P, Oviaño M, Álvarez-Fraga L, Alonso-García I, Arca-Suárez J, Bou G, Beceiro A, GEMARA-SEIMC/REIPI Enterobacterales Study Group. 2022. Assessment of activity and resistance mechanisms to cefepime in combination with the novel β-lactamase inhibitors zidebactam, taniborbactam, and enmetazobactam against a multicenter collection of carbapenemase-producing Enterobacterales. Antimicrob Agents Chemother 66:e0167621. https://doi.org/10.1128/AAC.01676-21.
18. Shapiro S. 2022. Cefepime/enmetazobactam is a clinically effective combination targeting extended-spectrum β-lactamase-producing Enterobacterales. Antimicrob Agents Chemother 66:e0029822. https://doi.org/10.1128/aac.00298-22.
19. Vázquez-Ucha JC, Lasarte-Monterrubio C, Guijarro-Sánchez P, Oviaño M, Álvarez-Fraga L, Alonso-García I, Arca-Suárez J, Bou G, Beceiro A. 2022. Reply to Shapiro, "Cefepime/enmetazobactam" is a clinically effective combination targeting extended-spectrum β-lactamase-producing Enterobacterales. Antimicrob Agents Chemother 66:e0035322. https://doi.org/10.1128/aac.00353-22.
20. Lasarte-Monterrubio C, Fraile-Ribot PA, Vázquez-Ucha JC, Cabot G, Guijarro-Sánchez P, Alonso-García I, Rumbo-Feal S, Galán-Sánchez F, Beceiro A, Arca-Suárez J, Oliver A, Bou G. 2022. Activity of cefiderocol, imipenem/relebactam, cefepime/taniborbactam and cefepime/zidebactam against ceftolozane/tazobactam- and ceftazidime/avibactam-resistant Pseudomonas aeruginosa. J Antimicrob Chemother 77:2809–2815. https://doi.org/10.1093/jac/dkac241.
21. Okujava R, Garcia-Alcalde F, Haldimann A, Zampaloni C, Morrissey I, Magnet S, Kothari N, Harding I, Bradley K. 2018. Activity of meropenem/nacubactam combination against Gram-negative clinical isolates: ROSCO Global Surveillance 2017. Open Forum Infect Dis 5:S416–S416. https://doi.org/10.1093/ofid/ofy210.1190.