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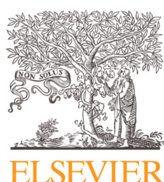

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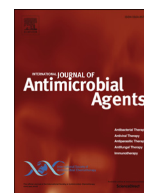

Short Communication

Activity of cefiderocol and innovative β-lactam/β-lactamase inhibitor combinations against isogenic strains of *Escherichia coli* expressing single and double β-lactamases under high and low permeability conditions

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ABSTRACT

Objectives: To analyse the impact of the most clinically relevant β-lactamases and their interplay with low outer membrane permeability on the activity of cefiderocol, ceftazidime/avibactam, aztreonam/avibactam, cefepime/enmetazobactam, cefepime/taniborbactam, cefepime/zidebactam, imipenem/relebactam, meropenem/vaborbactam, meropenem/xeruborbactam and meropenem/nacubactam against recombinant *Escherichia coli* strains.
Methods: We constructed 82 *E. coli* laboratory transformants expressing the main β-lactamases circulating in Enterobacterales (70 expressing single β-lactamase and 12 producing double carbapenemase) under high (*E. coli* TG1) and low (*E. coli* HB4) permeability conditions. Antimicrobial susceptibility testing was determined by reference broth microdilution.
Results: Aztreonam/avibactam, cefepime/zidebactam, cefiderocol, meropenem/xeruborbactam and meropenem/nacubactam were active against all *E. coli* TG1 transformants. Imipenem/relebactam, meropenem/vaborbactam, cefepime/taniborbactam and cefepime/enmetazobactam were also highly active, but unstable against most of MBL-producing transformants. Combination of β-lactamases with porin deficiency (*E. coli* HB4) did not significantly affect the activity of aztreonam/avibactam, cefepime/zidebactam, cefiderocol or meropenem/nacubactam, but limited the effectiveness of the rest of carbapenem- and cefepime-based combinations. Double-carbapenemase production resulted in the loss of activity of most of the compounds tested, an effect particularly evident for those *E. coli* HB4 transformants in which MBLs were present.

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Conclusions: Our findings highlight the promising activity that cefiderocol and new β-lactam/β-lactamase inhibitors have against recombinant *E. coli* strains expressing widespread β-lactamases, including when these are combined with low permeability or other enzymes. Aztreonam/avibactam, cefiderocol, cefepime/zidebactam and meropenem/nacubactam will help to mitigate to some extent the urgency of new compounds able to resist MBL action, although NDM enzymes represent a growing challenge against which drug development efforts are still needed.

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1. Introduction

The treatment of infections caused by ESBL and carbapenemase-producing Enterobacterales is becoming an alarming concern [1]. The approval of ceftazidime/avibactam in 2015 partly alleviated the shortage of agents available to combat these strains, but ceftazidime/avibactam resistance has been increasingly described. The most common resistance mechanism involves mutation of the KPC enzyme (e.g., L169P, D179Y), leading to structural rearrangement that affect the catalytic centre of KPC architecture and accelerate ceftazidime turnover rates [2]. In addition, ceftazidime/avibactam does not provide therapeutic cover against increasingly encountered metallo-β-lactamase (MBL)-producing strains. In this context, new antimicrobial agents are urgently needed.

Cefiderocol, imipenem/relebactam, and meropenem/vaborbactam represent recently approved options with high activity against Enterobacterales. Cefiderocol is a newly developed cephalosporin with enhanced bacterial penetration via siderophore-mediated pathways and increased stability against most β-lactamases found in Gram-negative bacteria [3]. On the other hand, imipenem/relebactam and meropenem/vaborbactam are respectively new carbapenem/diazabicyclooctane (DBO) and carbapenem/boronic acid (BOR)-derived β-lactam/β-lactamase inhibitor combinations, that exhibit promising activity against Enterobacterales producing class A or C β-lactamases, particularly KPC enzymes [4,5]. Unfortunately, resistance to these agents has already been reported [6–8] We are now witnessing the emergence of some promising β-lactam/β-lactamase inhibitors. Aztreonam/avibactam and three cefepime-based combinations (cefepime/enmetazobactam, cefepime/taniborbactam and cefepime/zidebactam) are at the late stage of development. Aztreonam/avibactam takes advantage of the particular property of aztreonam to evade hydrolysis by MBL in combination with the high potency of avibactam against serine type enzymes, thus virtually covering all types of β-lactamases [9]. Enmetazobactam includes a new tazobactam derivative with enhanced activity against ESBLs. Taniborbactam is an innovative BOR-type inhibitor which protects cefepime from hydrolysis via class A, C, D, and some class B enzymes; and zidebactam (DBO) potentiates the activity of cefepime through synergistically improved bacterial killing due to its intrinsic anti-PBP-2 activity [10]. Finally, there are early-stage inhibitors in the pipeline: nacubactam, a new DBO-type β-lactam enhancer with anti-PBP-2 activity that potentiates the efficacy of meropenem against carbapenem-resistant strains; [11] and xeruborbactam, a novel BOR derivative that has been shown to restore the activity of meropenem against all types of enzymes (including MBLs) [12].

These treatments may represent a step forward in the fight against Gram-negative infections. However, the specific activity of these agents against the main enzymes circulating in Enterobacterales has not yet been thoroughly investigated. Moreover, how additional factors that extend beyond the production of single β-lactamases may limit the effectiveness of these new agents, such as porin deficiency or simultaneous production of multiple enzymes (a phenomena commonly encountered in high-risk clones of critical priority ESKAPE targets), has not been analysed in detail. Here, we constructed an isogenic panel of *E. coli* isolates expressing single β-lactamases or double carbapenemases under low and high permeability conditions to evaluate the activity of these innovative agents, accurately determine their therapeutic niches, and anticipate their potential resistance mechanisms.

2. Material and methods

2.1. Construction of recombinant E. coli isolates expressing representative β-lactamases under low and high permeability conditions

We designed a library of up to 70 isogenic *E. coli* recombinant isolates expressing a representative array of Ambler's class A (bla_{GES-1}, bla_{GES-5}, bla_{GES-7}, bla_{GES-15}, bla_{GES-20}, $bla_{CTX-M-9}$, $bla_{CTX-M-15}$, bla_{SHV-12}, bla_{PER-1}, bla_{VEB-1}, bla_{KPC-2}, bla_{KPC-35}, $bla_{KPC-2\ N132S}$, $bla_{KPC-2\ L169A}$, bla_{KPC-3}, bla_{KPC-31}, $bla_{KPC-3\ L167R}$, $bla_{KPC-3\ D179N}$), class B (bla_{VIM-1}, bla_{VIM-2}, bla_{VIM-20}, bla_{IMP-13}, bla_{IMP-28}, bla_{NDM-1}, bla_{NDM-5}, bla_{NDM-7}, bla_{NDM-23}), class C (bla_{CMY-2}, bla_{DHA-1} and bla_{FOX-4}) and class D (bla_{OXA-2}, bla_{OXA-15}, bla_{OXA-10}, bla_{OXA-14}, bla_{OXA-48}) β-lactamases. To this end a copy of each β-lactamase was amplified from representative clinical isolates, ligated to the multicopy plasmid pUCP-24 (gentamicin resistance gene cassette) and electroporated in parallel into the *E. coli* TG1 and into the OmpC- and OmpF-deficient *E. coli* HB4 strains. Transformants were selected on LB agar plates containing 10 mg/L-gentamicin. Recombinant plasmids were characterized by MIC determination following the methodology described below.

Finally, to precisely determine the role of double carbapenemase production, bla_{VIM-1}, bla_{IMP-28}, and bla_{OXA-48} carbapenemase encoding genes were also amplified with specific primers and cloned into the multi-copy plasmid pBGS-18 (kanamycin resistance gene cassette). These recombinant pBGS-18 plasmids were then electroporated into previously constructed *E. coli* TG1 and *E. coli* HB4 transformants expressing bla_{KPC-3}, bla_{NDM-1} or bla_{OXA-48} in the pUCP-24 plasmid and selected on LB agar plates containing 10 mg/L-gentamicin and 50 mg/L-kanamycin. All of the resulting transformants were checked by PCR to confirm the presence of plasmids.

2.2. Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) of ceftazidime, ceftazidime/avibactam, aztreonam, aztreonam/avibactam, cefepime, cefepime/enmetazobactam, cefepime/taniborbactam, cefepime/zidebactam, zidebactam, cefiderocol, imipenem, imipenem/relebactam, meropenem, meropenem/vaborbactam, meropenem/nacubactam, nacubactam, and meropenem/xeruborbactam were determined for all transformants in triplicate experiments by reference broth microdilution assays. The MICs were determined using

cation-adjusted Müeller-Hinton (MH) broth in all cases, with the exception of cefiderocol, which was assessed using iron-depleted cation-adjusted MH broth prepared according to CLSI M100 guidelines [13]. Tazobactam, avibactam, and taniborbactam were tested at a fixed concentration of 4 mg/L, whereas enmetazobactam, vaborbactam and xeruborbactam were tested at 8 mg/L. Zidebactam and nacubactam were tested at a 1:1 ratio with cefepime and meropenem, respectively. EUCAST v13.1 clinical breakpoints and guidelines (http://www.eucast.org/clinical_breakpoints/) were used for reference purposes. The Enterobacterales EUCAST clinical breakpoint of the β-lactam partner was used to define susceptibility or resistance to as yet unapproved combinations. The reference strain *E. coli* ATCC 25922 was used as a control.

3. Results and discussion

3.1. Role of β-lactamases

Cumulative MIC data for the whole collection and each host strain are shown in Table S1, and detailed comparative data of *E. coli* TG1 transformants producing single β-lactamases are presented in Table 1. The addition of avibactam to ceftazidime, recovered the activity of ceftazidime against most of Amblerś class A, C and D β-lactamases, indicating the high potency of the first-in-class β-lactam/DBO combination. Concerning increases in the MIC of ceftazidime/avibactam were noted for transformants producing KPC-35 (a KPC-2 L169P mutant; MIC=8 mg/L) or KPC-31 (a KPC-3 D179Y mutant; MIC=16 mg/L). The high evolutionary potential of KPC enzymes toward ceftazidime/avibactam-resistance has been extensively reported since the combination became available for clinical use [14,15]. Although the final MIC values were still below the susceptibility breakpoint, important increases in MIC were also noted for transformants producing PER-1 (MIC=2 mg/L) or the class D extended-spectrum OXAs: OXA-14 (MIC=4 mg/L) and OXA-15 (MIC=2 mg/L), a phenomenon previously observed with isogenic strains or in patients receiving ceftazidime/avibactam treatment [16]. Fortunately, both PER and the extended-spectrum OXAs are still of minor concern in Enterobacterales since they are mainly encountered in *P. aeruginosa*. As expected, avibactam performed well against all class C variants included, but did not enhance the activity of ceftazidime against any of the MBL-producing *E. coli* TG1 strains. Aztreonam/avibactam yielded very similar MICs to those of ceftazidime/avibactam against most of class A, C and D β-lactamase transformants. The most interesting feature of these new combination is probably its outstanding activity against MBL producers, which in all cases exhibited an aztreonam/avibactam MIC of ≤0.06 mg/L. The findings clearly indicate that this combination would be an addition of upmost importance to treat infections caused by MBL-producing strains. However, aztreonam/avibactam-resistant lineages of NDM-5 producing *E. coli* have already been detected due to the presence of amino-acid insertions in PBP-3 (-YRIN-, -YRIP- or -YRIK-; involved in decreased aztreonam affinity) or the production of certain AmpC variants with increased activity toward aztreonam (e.g., CMY-42) [17,18].

Analysis of the potency of cefepime in combination with the β-lactamase inhibitors enmetazobactam, taniborbactam and zidebactam also revealed promising results. Cefepime/enmetazobactam yielded MICs in the susceptibility range against most of class A, C and D β-lactamases (MICs≤0.06-0.5 mg/L) but was not active against MBLs [19]. The combination has been shown to resist the activity of AmpCs and ESBLs when challenged against large collections of clinical isolates and is also active against some producers of KPC or OXA-48 enzymes (the latter of which is almost always accompanied by CTX-M variants) [20]. The potential use of this combination as a carbapenem-sparing regimen aimed at affording carbapenems looking for a reduction in the burden of carbapenem-resistant organism in the hospital environment is a niche that deserves exploration. Similarly, cefepime/taniborbactam also demonstrated very strong potency against all class A, C and D β-lactamases (MICs≤0.06 mg/L), but also provided good protection for cefepime against some MBL producers. The combination was active against VIM-type enzymes (MICs≤0.06-1 mg/L). However, as previously shown IMP-type enzymes were not inhibited, as indicated by the negligible effect on the MICs of cefepime for IMP-28 or IMP-13 (MICs=4–8 mg/L for both cefepime and cefepime/taniborbactam) [21]. Taniborbactam also effectively reduced the MIC of cefepime for NDM producers, although borderline susceptibility was noted for some variants (e.g., NDM-5; MIC=8 mg/L), probably explained by the high MICs for cefepime that could not be effectively decreased to below the susceptibility breakpoint. Cefepime/zidebactam exhibited the broadest range of therapeutic coverage against the whole set of *E. coli* TG1 transformants, which in all cases yielded MICs ranging from ≤0.03 to 0.25 mg/L. The potency observed for cefepime/zidebactam seems to result from the synergistically improved bacterial killing by cefepime and zidebactam, as highlighted by the high intrinsic activity of zidebactam against all the transformants regardless of the β-lactamase produced (MICs=0.06–0.5 mg/L) [22]. To date, resistance to cefepime/zidebactam has rarely been detected in clinical isolates, although specific mutations affecting PBP-2 have been detected in resistant mutants obtained *in vitro* [23]. Altogether, these results support the promising role that cefepime/taniborbactam and cefepime/zidebactam may have against carbapenemase-producing Enterobacterales, particularly those carrying MBLs.

Cefiderocol was highly active against the whole collection of *E. coli* TG1 transformants, with MICs ranging from ≤0.06 to 2 mg/L. Cefiderocol activity was significantly affected by production of SHV-12, PER-1 and NDM-like enzymes, which as previously reported increased the MICs of the cephalosporin to 1, 1 and 2 mg/L, respectively. However, in accordance with our data, increased cefiderocol MICs and clinical resistance are persistently reported for strains of Enterobacterales carrying NDM carbapenemases, thus arguing for close monitorization of its activity against MBL producers. Carbapenems were tested in combination with relebactam, in the case of imipenem, or partnered with vaborbactam, nacubactam or xeruborbactam, in the case of meropenem. Due to high baseline susceptibility to both carbapenems in the *E. coli* TG1 host, imipenem/relebactam, meropenem/vaborbactam, meropenem/xeruborbactam and meropenem/nacubactam were, as expected, active against all transformants carrying class A, C and D β-lactamases, with MICs ranging in all cases between 0.03 and 0.25 mg/L. In this regard, the highest MICs for these carbapenem/β-lactamase inhibitor combinations corresponded to the transformant carrying the OXA-48 carbapenemase, against which the addition of none of these inhibitors reduced the MIC of the β-lactam partner. Production of class B carbapenemases (and particularly NDM) was associated with greater resistance to these carbapenem combinations. Xeruborbactam or nacubactam decreased 4 two-fold dilutions the MIC of meropenem against NDM enzymes, highlighting their promising role against MBLs in the near future.

3.2. Interplay between β-lactamases and decreased outer membrane permeability

Analysis of the MIC data of the 35 OmpC/OmpF-deficient *E. coli* HB4 transformants allowed us to obtain a detailed view of how decreased β-lactam internalization could amplify levels of resistance to the agents tested (the global cumulative effect is represented in Table S1, and detailed MICs are shown in Table 2). Compared with *E. coli* TG1, clinical resistance to ceftazidime/avibactam in *E. coli* HB4 extended to PER-1 (MIC=2–16 mg/L), OXA-15 (MIC=2–

Table 1
Antibiotic susceptibility data for newly developed β-lactams and β-lactam/β-lactamase inhibitor combinations against isogenic *E. coli* TG1 transformants expressing the most relevant class A, B, C and D β-lactamases found in Enterobacterales.

Strain	Ambler class	Main Phenotype	MIC (mg/L)[a]																
			CAZ (R>4)	C/A (R>8)	ATM (R>4)	A/A (R>4)	FEP (R>4)	F/T (R>4)	F/E (R>4)	F/Z (R>4)	ZID (NA)	FDC (R>2)	IMP (R>4)	I/R (R>2)	MEM (R>8)	M/V (R>8)	M/X (R>8)	M/N (R>8)	NAC (NA)
E. coli TG1	-	Wild-type	0.125	0.125	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	≤0.03	0.06	≤0.06	0.25	0.125	≤0.06	≤0.06	≤0.06	≤0.03	2
GES-1		ESBL	4	0.125	≤0.06	≤0.06	0.125	≤0.06	≤0.06	≤0.03	0.125	≤0.06	0.125	0.125	≤0.06	≤0.06	≤0.06	≤0.03	2
GES-5		Carbapenemase	0.25	0.125	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	≤0.03	0.125	≤0.06	0.25	0.125	≤0.06	≤0.06	≤0.06	≤0.03	2
GES-7		ESBL	32	0.25	0.5	≤0.06	0.125	≤0.06	≤0.06	0.06	0.25	≤0.06	0.25	0.25	≤0.06	≤0.06	≤0.06	≤0.03	2
GES-15		ESBL	8	0.125	0.125	≤0.06	0.125	≤0.06	≤0.06	0.06	0.25	≤0.06	0.125	0.125	≤0.06	≤0.06	≤0.06	≤0.03	2
GES-20		Carbapenemase	0.25	0.125	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	≤0.03	0.25	≤0.06	0.25	0.25	≤0.06	≤0.06	≤0.06	≤0.03	2
CTX-M-9		ESBL	0.5	0.125	2	≤0.06	1	≤0.06	≤0.06	0.125	0.125	≤0.06	0.125	0.125	≤0.06	≤0.06	≤0.06	≤0.03	2
CTX-M-15		ESBL	32	0.125	32	≤0.06	32	≤0.06	≤0.06	0.125	0.125	≤0.06	0.25	0.125	≤0.06	≤0.06	≤0.06	≤0.03	2
SHV-12		ESBL	>64	0.25	>64	0.125	4	≤0.06	≤0.06	0.125	0.125	1	0.25	0.125	≤0.06	≤0.06	≤0.06	≤0.03	2
PER-1	A	ESBL	>64	2	>64	2	8	≤0.06	≤0.06	0.125	0.125	1	0.25	0.125	≤0.06	≤0.06	≤0.06	≤0.03	2
VEB-1		ESBL	>64	0.25	32	≤0.06	1	≤0.06	≤0.06	0.06	0.125	0.25	0.125	0.125	≤0.06	≤0.06	≤0.06	≤0.03	2
KPC-2		Carbapenemase	16	0.25	>64	0.125	16	≤0.06	≤0.06	0.125	0.125	≤0.06	1	0.25	0.5	≤0.06	≤0.06	0.125	2
KPC-35 (KPC-2 L169P)		ESBL	>64	8	1	≤0.06	1	≤0.06	≤0.06	0.125	0.125	≤0.06	0.25	0.125	≤0.06	≤0.06	≤0.06	≤0.03	2
KPC-2 N132S		Carbapenemase	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	≤0.03	0.125	≤0.06	0.25	0.25	0.125	≤0.06	≤0.06	0.06	2
KPC-2 L169A		ESBL	16	1	8	≤0.06	8	≤0.06	≤0.06	0.125	0.125	≤0.06	1	0.25	0.125	≤0.06	≤0.06	0.06	2
KPC-3		Carbapenemase	32	0.25	64	≤0.06	4	≤0.06	≤0.06	0.125	0.125	≤0.06	1	0.125	0.25	≤0.06	≤0.06	0.06	2
KPC-31 (KPC-3 D179Y)		ESBL	64	16	0.5	≤0.06	1	≤0.06	≤0.06	0.125	0.125	0.125	0.125	0.125	≤0.06	≤0.06	≤0.06	≤0.03	2
KPC-3 L167R		Carbapenemase	4	0.125	4	≤0.06	0.25	≤0.06	≤0.06	0.125	0.125	≤0.06	0.25	0.125	≤0.06	≤0.06	≤0.06	≤0.03	2
KPC-3 D179N		ESBL	32	8	1	0.125	1	≤0.06	≤0.06	0.125	0.125	≤0.06	0.25	≤0.06	≤0.06	≤0.06	≤0.06	≤0.03	2
VIM-1		Carbapenemase	>64	>64	≤0.06	≤0.06	32	1	32	0.125	0.125	0.125	2	2	0.5	0.5	0.5	0.5	2
VIM-2		Carbapenemase	1	1	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	≤0.03	0.06	≤0.06	0.25	0.25	≤0.06	≤0.06	≤0.06	≤0.03	2
VIM-20		Carbapenemase	2	2	≤0.06	≤0.06	0.25	≤0.06	0.25	0.125	0.125	≤0.06	0.25	0.25	≤0.06	≤0.06	≤0.06	0.06	2
IMP-13		Carbapenemase	>64	>64	≤0.06	≤0.06	8	8	4	0.125	0.125	≤0.06	0.25	0.25	0.125	0.125	0.125	0.125	2
IMP-28	B	Carbapenemase	>64	>64	≤0.06	≤0.06	8	4	8	0.125	0.125	≤0.06	0.25	0.25	≤0.06	≤0.06	≤0.06	0.06	2
NDM-1		Carbapenemase	>64	>64	≤0.06	≤0.06	32	1	16	0.125	0.125	1	2	1	2	2	0.5	0.5	2
NDM-5		Carbapenemase	>64	>64	≤0.06	≤0.06	>64	8	>64	0.125	0.25	2	32	32	16	16	1	1	2
NDM-7		Carbapenemase	>64	>64	≤0.06	≤0.06	>64	4	>64	0.125	0.125	2	16	16	8	8	1	1	2
NDM-23		Carbapenemase	>64	>64	≤0.06	≤0.06	32	1	32	0.125	0.125	2	4	2	2	2	0.5	0.5	2
CMY-2		Extended-spectrum cephamycinase	>64	0.5	16	0.25	1	≤0.06	0.5	0.25	0.5	0.125	0.5	0.25	≤0.06	≤0.06	≤0.06	≤0.03	2
DHA-1	C	Extended-spectrum cephamycinase	32	0.125	4	≤0.06	0.125	≤0.06	≤0.06	≤0.03	0.25	≤0.06	0.125	0.125	≤0.06	≤0.06	≤0.06	≤0.03	2
FOX-4		Extended-spectrum cephamycinase	>64	1	2	≤0.06	0.25	≤0.06	0.125	0.06	0.25	≤0.06	0.25	0.25	≤0.06	≤0.06	≤0.06	≤0.03	2
OXA-2		Narrow-spectrum oxacillinase	2	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	0.06	0.25	0.125	0.25	0.125	≤0.06	≤0.06	≤0.06	≤0.03	2
OXA-15		ESBL	32	2	≤0.06	≤0.06	0.25	≤0.06	≤0.06	0.125	0.125	0.25	0.25	0.125	≤0.06	≤0.06	≤0.06	≤0.03	2
OXA-10	D	Narrow-spectrum oxacillinase	0.125	≤0.06	0.5	0.125	0.25	≤0.06	≤0.06	0.125	0.125	≤0.06	0.25	0.125	≤0.06	≤0.06	≤0.06	≤0.03	2
OXA-14		ESBL	32	4	0.5	0.125	0.5	≤0.06	0.125	0.125	0.125	0.25	0.25	0.125	≤0.06	≤0.06	≤0.06	≤0.03	2
OXA-48		Carbapenemase	0.25	≤0.06	≤0.06	≤0.06	0.125	≤0.06	≤0.06	0.06	0.125	≤0.06	0.5	0.25	0.25	0.25	≤0.06	0.25	2

CAZ: ceftazidime; C/A: ceftazidime/avibactam; ATM: aztreonam; A/A: aztreonam/avibactam; FEP: cefepime; F/E: cefepime/enmetazobactam; F/T: cefepime/taniborbactam; F/Z: cefepime/zidebactam; ZID: zidebactam; FDC: cefiderocol; IMP: imipenem; IMP/REL: imipenem/relebactam; MEM: meropenem; M/V: meropenem/vaborbactam; M/X: meropenem/xeruborbactam; M/N: meropenem/nacubactam; NAC: nacubactam; NA: Not available

[a] EUCAST breakpoints indicated for Enterobacterales.

Table 2
Antibiotic susceptibility data for newly developed β-lactams and β-lactam/β-lactamase inhibitor combinations against isogenic *E. coli* HB4 transformants expressing the most relevant class A, B, C and D β-lactamases found in Enterobacterales.

Strain	Ambler class	Main Phenotype	MIC (mg/L)[a]																
			CAZ (R>4)	C/A (R>8)	ATM (R>4)	A/A (R>4)	FEP (R>4)	F/T (R>4)	F/E (R>4)	F/Z (R>4)	ZID (NA)	FDC (R>2)	IMP (R>4)	I/R (R>2)	MEM (R>8)	M/V (R>8)	M/X (R>8)	M/N (R>8)	NAC (NA)
E. coli HB4	-	OmpC/OmpF porin-deficient	1	0.5	0.25	0.125	0.5	0.5	0.5	0.25	0.25	≤0.06	0.25	0.125	≤0.06	≤0.06	≤0.06	≤0.03	2
GES-1	A	ESBL	16	1	1	0.125	2	0.5	0.5	0.25	0.5	≤0.06	0.125	0.125	0.25	0.125	≤0.06	0.125	2
GES-5		Carbapenemase	2	0.5	0.25	0.125	0.5	0.5	0.5	0.25	0.25	≤0.06	0.5	0.125	4	0.25	≤0.06	0.5	2
GES-7		ESBL	>64	1	4	0.125	4	0.5	0.5	0.25	0.5	≤0.06	0.125	0.125	0.125	≤0.06	≤0.06	0.125	2
GES-15		ESBL	>64	2	1	0.125	8	0.5	0.5	0.5	0.5	≤0.06	0.125	0.125	0.25	0.125	≤0.06	0.125	2
GES-20		Carbapenemase	2	0.5	0.25	0.125	1	0.5	0.5	0.25	0.5	≤0.06	1	0.125	2	0.125	≤0.06	0.5	2
CTX-M-9		ESBL	4	0.5	16	0.125	64	0.5	0.5	2	2	≤0.06	0.25	0.125	0.25	0.125	≤0.06	0.125	2
CTX-M-15		ESBL	>64	1	>64	0.125	>64	1	0.5	1	2	0.125	0.25	0.125	0.25	0.125	0.125	0.125	2
SHV-12		ESBL	>64	4	>64	0.5	>64	1	64	1	1	2	0.25	0.125	1	1	0.25	0.25	2
PER-1		ESBL	>64	16	>64	16	>64	1	64	1	1	2	0.25	≤0.06	0.5	0.25	0.125	0.25	2
VEB-1		ESBL	>64	2	>64	1	64	0.5	0.5	0.25	0.5	0.5	0.125	≤0.06	0.125	0.125	0.125	0.125	2
KPC-2		Carbapenemase	>64	2	>64	1	>64	1	>64	4	4	0.125	64	0.5	>64	0.5	≤0.06	2	2
KPC-35 (KPC-2 L169P)		ESBL	>64	32	8	0.25	64	2	1	1	1	1	0.25	0.125	2	0.5	0.125	1	2
KPC-2 N132S		Carbapenemase	1	0.5	0.25	0.125	0.5	0.5	0.5	0.125	0.25	≤0.06	4	4	16	0.5	0.125	1	2
KPC-2 L169A		ESBL	>64	16	32	0.25	64	2	2	2	4	0.125	4	0.25	32	0.5	0.125	1	2
KPC-3		Carbapenemase	>64	4	>64	0.125	>64	1	>64	1	1	0.25	16	0.125	>64	0.5	0.125	1	2
KPC-31 (KPC-3 D179Y)		ESBL	>64	64	4	0.25	64	2	0.5	0.5	0.5	1	0.25	0.125	1	0.5	0.125	1	2
KPC-3 L167R		Carbapenemase	32	1	32	0.125	16	0.5	2	0.25	0.5	≤0.06	4	1	4	0.25	0.125	1	2
KPC-3 D179N		ESBL	>64	16	8	0.25	8	0.5	0.25	0.125	0.125	0.25	0.25	0.125	0.125	≤0.06	≤0.06	0.125	2
VIM-1	B	Carbapenemase	>64	>64	0.25	0.125	64	8	64	0.125	0.5	0.125	2	2	2	2	2	1	2
VIM-2		Carbapenemase	8	8	0.25	0.125	1	0.5	1	0.25	0.25	≤0.06	0.25	0.25	1	1	0.5	0.5	2
VIM-20		Carbapenemase	16	16	0.25	0.125	4	0.5	4	0.125	0.25	≤0.06	1	1	2	2	0.5	1	2
IMP-13		Carbapenemase	>64	>64	0.25	0.125	32	32	32	0.125	0.25	≤0.06	1	1	8	8	8	1	2
IMP-28		Carbapenemase	>64	>64	0.25	0.125	32	32	32	0.125	0.125	≤0.06	1	1	16	16	16	2	2
NDM-1		Carbapenemase	>64	>64	0.25	0.25	>64	16	>64	0.125	0.125	2	16	16	32	8	16	1	2
NDM-5		Carbapenemase	>64	>64	0.25	0.25	>64	>64	>64	0.25	0.25	2	>64	>64	>64	>64	64	4	2
NDM-7		Carbapenemase	>64	>64	0.25	0.25	>64	>64	>64	0.25	0.25	4	>64	>64	>64	>64	64	4	2
NDM-23		Carbapenemase	>64	>64	0.25	0.25	>64	32	>64	0.125	0.25	2	8	8	64	32	8	2	2
CMY-2	C	Extended-spectrum cephamycinase	>64	2	64	1	32	0.5	8	2	4	0.25	1	0.25	1	0.25	≤0.06	0.25	2
DHA-1		Extended-spectrum cephamycinase	>64	1	32	0.25	2	0.5	2	1	4	≤0.06	0.5	0.125	1	0.25	≤0.06	0.25	2
FOX-4		Extended-spectrum cephamycinase	>64	4	8	0.25	4	0.5	4	0.5	1	≤0.06	0.25	0.125	0.5	0.5	0.125	0.25	2
OXA-2	D	Narrow-spectrum oxacillinase	16	1	0.25	0.125	1	0.5	0.5	0.25	0.5	0.25	0.5	0.5	2	2	0.125	1	2
OXA-15		ESBL	64	16	2	0.125	4	1	2	0.5	0.5	0.5	0.125	0.125	0.5	0.25	0.25	0.25	2
OXA-10		Narrow-spectrum oxacillinase	2	0.5	8	1	8	0.5	4	0.25	0.5	≤0.06	0.25	0.25	4	1	0.125	2	2
OXA-14		ESBL	>64	64	16	1	16	1	4	0.5	0.5	0.5	0.25	0.125	1	0.25	0.5	0.5	2
OXA-48		Carbapenemase	1	0.5	0.25	0.25	2	0.5	2	0.25	0.25	≤0.06	2	1	8	8	≤0.06	2	2

CAZ: ceftazidime; C/A: ceftazidime/avibactam; ATM: aztreonam; A/A: aztreonam/avibactam; FEP: cefepime; F/E: cefepime/enmetazobactam; F/T: cefepime/taniborbactam; F/Z: cefepime/zidebactam; ZID: zidebactam; FDC: cefiderocol; IMP: imipenem; I/R: imipenem/relebactam; MEM: meropenem; M/V: meropenem/vaborbactam; M/X: meropenem/xeruborbactam; M/N: meropenem/nacubactam; NAC: nacubactam; NA: Not available

[a] EUCAST breakpoints indicated for Enterobacterales.

16 mg/L) and OXA-14 (MIC=4– 64 mg/L). Moreover, some enzymes such as SHV-12, KPC-3 or FOX-4 increased resistance to levels close to the susceptibility breakpoint (MICs=4 mg/L). The effect of PER-1 in this background was also amplified for aztreonam/avibactam (MIC=16 mg/L), although in general was poorly affected by the loss of porins (MIC=0.125–1 mg/L for the rest of isolates). Cefepime was one of the substrates against which lesions in porins conferred greater defects on its activity. The combination with taniborbactam was truncated against MBLs, as the MIC increased to 8 mg/L or higher in all cases except VIM-2 and VIM-20. Cefepime/enmetazobactam showed decreased activity against several key class A targets, such as SHV-12 (MIC=64 mg/L), PER-1 (MIC=64 mg/L), KPC-2 (MIC>64 mg/L) and KPC-3 (MIC>64 mg/L), but also borderline MICs (4–8 mg/L) for several class C and class D variants. The effect of low permeability was not as evident for cefepime/zidebactam, which maintained with 100% activity, probably resulting from the mechanistic synergy triggered by PBP-3 targeting of cefepime with the enhancing effect of zidebactam.

Cefiderocol maintained rates of activity similar to those observed in *E. coli* TG1, highlighting that internalization through siderophores can bypass any permeability defect. The MIC of carbapenem combinations experimented a major increase against the HB4 collection, a fact that helped to clarify the protective effects (if any) of the different inhibitors. Imipenem/relebactam was active against most of the class A and C enzymes and class D variants with ESBL activity. While imipenem/relebactam has shown to select inactivating mutations in OmpK36 when used to combat KPC infections, it was able to reduce the MIC of imipenem by between 5 and 7 two-fold dilutions against the *E. coli* HB4 derivatives producing KPC-2 (MIC=0.5 mg/L) or KPC-3 (MIC=0.125 mg/L) [24]. Interestingly, the only class A β-lactamase able to confer clinical resistance to imipenem/relebactam was the KPC-2 N132S variant (MIC=4 mg/L). Of note, the replacement of asparagine by serine at position 132, located on the YSN triad of the KPC-2 enzyme, has recently been described *in vitro* and demonstrated to confer decreased relebactam susceptibility in terms of IC_{50}, $K_{i\ app}$ and k_2/K [25]. Vaborbactam potentiated the activity of meropenem against all class A producers, including key targets such as KPC-2 (MIC=0.5 mg/L) and KPC-3 (MIC=0.5 mg/L), but was inactive against class B (MICs=1->64 mg/L) enzymes or OXA-48 (MIC=8 mg/L). Its activity was also significantly reduced by OXA-2 (MIC=2 mg/L) and OXA-10 enzymes (MIC=1 mg/L), which significantly increase the MIC of carbapenems under conditions of low permeability [26]. Meropenem/xeruborbactam was the meropenem-based combination with highest activity serine-type enzymes, particularly active against KPC-2 (MIC=≤0.06 mg/L), KPC-3 (MIC=0.125 mg/L) and OXA-48 (MIC≤0.06 mg/L). However, the previously observed anti-MBL efficacy was not evident (MICs=0.5–64 mg/L), a fact probably explained by the presence of non-functional porins which have been shown to affect the internalization of xeruborbactam [27,28]. Finally, meropenem/nacubactam demonstrated globally higher MIC values than other compounds but its activity extended to the whole collection.

3.3. Effects of double carbapenemase production

We finally constructed double transformants to estimate the potential therapeutic role of these innovative options against the emerging threat of double carbapenemase production (Table 3). Double carbapenemase production usually resulted in the loss of ceftazidime/avibactam, imipenem/relebactam, meropenem/vaborbactam, cefepime/enmetazobactam and cefepime/taniborbactam, mostly due to production of class B enzymes (particularly noticeable in the *E. coli* HB4 strain). In this regard, probably the most challenging associations of carbapenemases were those resulting from the combination of

Table 3
Antibiotic susceptibility data for *E. coli* TG1 and *E. coli* HB4 recombinant isolates expressing double carbapenemases.

Strain	Ambler class	Main Phenotype	MIC (mg/L)[a]																
			CAZ (R>4)	C/A (R>8)	ATM (R>4)	A/A (R>4)	FEP (R>4)	F/T (R>4)	F/E (R>4)	F/Z (R>4)	ZID (NA)	FDC (R>2)	IMP (R>4)	I/R (R>2)	MEM (R>8)	M/V (R>8)	M/X (R>8)	M/N (R>8)	NAC (NA)
E. coli TG1	-	Wild-type	0.125	0.125	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	≤0.03	0.06	≤0.06	0.25	0.125	≤0.06	≤0.06	≤0.03	≤0.06	2
KPC-3 + VIM-1	A + B	Double carbapenemase	64	32	64	≤0.06	16	0.25	8	0.25	0.25	≤0.06	2	0.5	1	0.125	0.125	0.125	2
KPC-3 + IMP-28		Double carbapenemase	64	64	64	≤0.06	4	2	2	0.125	0.125	≤0.06	8	0.5	2	0.25	0.25	0.5	2
KPC-3 + OXA-48	A + D	Double carbapenemase	16	≤0.25	32	≤0.06	4	≤0.25	≤0.25	0.125	0.125	≤0.06	2	0.25	2	0.125	0.125	≤0.06	2
NDM-1 + OXA-48		Double carbapenemase	>256	>256	≤0.06	≤0.06	64	8	64	0.06	0.06	2	8	8	8	4	1	0.5	2
OXA-48 + VIM-1	B + D	Double carbapenemase	128	32	≤0.06	≤0.06	8	0.25	8	0.25	0.125	≤0.06	1	0.5	0.25	0.125	0.25	≤0.06	2
OXA-48 + IMP-28		Double carbapenemase	64	32	≤0.06	≤0.06	4	4	4	0.06	0.06	≤0.06	1	1	0.5	0.25	0.125	≤0.06	2
E. coli HB4	-	OmpC/OmpF porin-deficient	1	0.5	0.25	0.125	0.5	0.5	0.5	0.25	0.25	≤0.06	0.25	0.125	≤0.06	≤0.06	≤0.06	≤0.03	2
KPC-3 + VIM-1	A + B	Double carbapenemase	>256	128	>64	0.25	128	8	128	1	1	0.125	8	1	32	4	4	1	2
KPC-3 + IMP-28		Double carbapenemase	256	128	>64	0.25	128	16	16	0.5	1	≤0.06	8	0.5	32	8	8	1	2
KPC-3 + OXA-48	A + D	Double carbapenemase	>256	0.5	>64	0.25	256	32	64	0.5	0.5	≤0.06	8	0.5	16	8	0.5	1	2
NDM-1 + OXA-48		Double carbapenemase	>256	>256	0.25	0.25	>256	32	256	0.25	0.125	2	4	4	32	32	8	1	2
OXA-48 + VIM-1	B + D	Double carbapenemase	>256	128	0.5	0.25	128	4	128	0.25	0.25	0.125	4	2	8	8	1	1	2
OXA-48 + IMP-28		Double carbapenemase	256	128	0.5	0.25	32	32	32	0.25	0.125	≤0.06	2	1	8	8	16	1	2

CAZ: ceftazidime; C/A: ceftazidime/avibactam; ATM: aztreonam; A/A: aztreonam/avibactam; FEP: cefepime; F/E: cefepime/enmetazobactam; F/T: cefepime/taniborbactam; F/Z: cefepime/zidebactam; ZID: zidebactam; FDC: cefiderocol; IMP: imipenem; I/R: imipenem/relebactam; MEM: meropenem; M/V: meropenem/vaborbactam; M/X: meropenem/xeruborbactam; M/N: meropenem/nacubactam; NAC: nacubactam; NA: Not available

[a] EUCAST breakpoints indicated for Enterobacterales.

KPC-3+IMP-28 and NDM-1+OXA-48, which respectively reduced the activity of meropenem/xeruborbactam in *E. coli* HB4 or conferred reduced cefiderocol susceptibility. Hopefully, aztreonam/avibactam (MIC≤0.06–0.25 mg/L), cefepime/zidebactam (MIC=0.06–1 mg/L), cefiderocol (MIC≤0.06–2 mg/L) and to a lesser extent meropenem/nacubactam (MIC≤0.06–1 mg/L) will represent valuable options against isolates showing such combinations of resistance mechanisms.

4. Conclusions

The collection here evaluated only includes recombinant isolates of *E. coli* and thus their implications for other species should be precisely determined in future studies. However, our findings highlight the promising stability of these new β-lactams and β-lactam/β-lactamase inhibitor combinations against the main β-lactamases circulating in Enterobacterales, including when they are combined with low permeability or additional carbapenemases. Although some of the options evaluated here appear to mitigate the urgency of new compounds able to resist the action of MBL to some extent, drug development efforts and surveillance are needed to combat the spread of some enzymes, such as NDM, which can resist the activity of last generation inhibitors or limit the activity of cefiderocol.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2024.107150.

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