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Short Communication

In vitro effectiveness of ceftazidime-avibactam in combination with aztreonam on carbapenemase-producing Enterobacterales

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ABSTRACT

Objective: This work aimed to describe the *in vitro* performance of the combined activity of ceftazidime-avibactam (CZA) plus aztreonam (ATM) against carbapenemase-producing Enterobacterales (CPE).
Methods: We studied 44 CPE clinical isolates: NDM-1 (31), KPC-2 (5), KPC-3 (3), VIM-2 (2), NDM-1+KPC-2 (2), and OXA-48 (1). The efficacy of CZA in combination with were determined by two methods: (i) Kirby-Bauer's double disk synergy test and; (ii) Determination of the minimum inhibitory concentration to CZA by E-test, in either Mueller-Hinton agar alone or, supplemented with ATM 4 mg/L. Additionally, the Fractional inhibitory concentration index (FICI) was determined; values of ≤ 0.5 were interpreted as synergistic, while FICI > 0.5 were considered indifferent.
Results: All isolates were carbapenem-resistant, 14 were resistant to CZA and ATM, 15 were only CZA resistant, 12 were only ATM resistant, and three were susceptible to both. 34/44 isolates presented positive double disk synergy tests between CZA and ATM regardless of their susceptibility profile, the isolates with negative synergy tests were susceptible to at least one of the agents. On the other hand, the 21 isolates selected to compare the MIC to CZA alone and CZA plus 4 mg/L ATM of exhibited FICI values between 0.016 and 0.125, indicating a synergistic effect.
Conclusions: This method is available to clinical laboratories and would provide valuable information to guide the treatment of infections with CZA and ATM. In this sense, the use of CZA together with ATM is a potentially suitable combination for the treatment of carbapenemase-producing microorganisms.

1. Introduction

Carbapenemase-producing Enterobacterales (CPE) are a high-priority problem for the development of new antimicrobials [1]. Although there are geographic differences in individual prevalence, the major carbapenemases that threaten human health are KPC-2 (within class A), NDM-1 (within class B), and OXA-48 (within class D) [2].

According to Magiorakos et al. [3], multidrug-resistant microorganisms (MDR) are defined as non-susceptibility to at least one agent in three or more antimicrobial categories. Given that penicillins, penicillins plus β-lactamase inhibitors, cephamycins, narrow-spectrum cephalosporins, extended-spectrum cephalosporins and carbapenems are separated categories, the mere presence of any carbapenemase defines the microorganism as MDR. Furthermore, CPEs are frequently associated with resistance determinants to several antibiotic families, such as quinolones, aminoglycosides, other β-lactams, and even colistin, which makes infection caused by these bacteria challenging to treat [4].

A few years ago, several antibiotics of limited use returned to be employed in this context such as fosfomycin, colistin, and tigecycline, or have been drastically increased, like in the case of aminoglycosides. However, some of them present difficulties for their susceptibility testing in the clinical laboratory; and others,

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such as colistin, present worse PK/PD parameters and more adverse effects compared to β-lactams [5,6].

In addition, it is possible to find either Enterobacterales, *Pseudomonas* spp. or *Acinetobacter* spp. isolates co-expressing two carbapenemases, usually KPC and NDM, which limits the antimicrobial treatment of these pathogens and are challenging to detect given the convergence of resistance mechanisms. In Uruguay, the prevalence of double carbapenemase-producing Enterobacterales was 0.93% during the period 2017-2019, meanwhile during 2021 it increased to 5.6%. This could have been because of the effects of the COVID-19 pandemic, which lead to an increased number of hospitalized inpatients, extended hospital stays, higher consumption of broad-spectrum antibiotics, and hospital-acquired infections, among others [7].

Recently, new β-lactamase inhibitors that are active against carbapenemases, and that can restore the efficacy of the antibiotics used as partners are gaining relevance. Among these, avibactam emerged as one of the best options because of the broad spectrum of enzymes it inhibits and the greater effectiveness of inhibition because of its molecular mechanism and recycling capability. Avibactam is a β-lactamase inhibitor not derived from β-lactams, with activity against class A (extended-spectrum β-lactamase and serine-carbapenemases), class C, and some class D (including OXA-48) β-lactamases, but not against class B (Metallo-β-lactamases, MBL) [8].

Given that the monobactam aztreonam has activity against MBL, its association with avibactam appears promising to treat infections caused by MBL-producing bacteria, which often also produce class A β-lactamases that hydrolyse aztreonam. However, until the combination aztreonam-avibactam is available, the association ceftazidime-avibactam plus aztreonam is a promising option for the treatment of infections caused by MBL-producing microorganisms [9].

In this report, we describe the in vitro performance of the combined activity of ceftazidime-avibactam plus aztreonam against 44 carbapenemase-producing Enterobacterales, including NDM-1, VIM-2, KPC-2, KPC-3, OXA-48 and both KPC-2/NDM-1.

2. Materials and Methods

2.1. Bacterial strains, antibiotic susceptibility testing and carbapenemase resistance screening

Forty-four non-repeated clinical isolates of carbapenemase-producing Enterobacterales, belonging to the *Enterobacteriaceae* (n = 37) and *Morganellaceae* (n = 7) families, were studied [10–13]. Some isolates were previously reported and others were characterized for the present work. *E. coli* ATCC 25922 was used as a control strain.

Bacterial identification was performed using a VITEK 2 Compact System (bioMérieux, Marcy-l'Étoile, France). Antibiotic susceptibility testing was performed using Sensititre™ (Thermo Fisher Scientific) for all available antibiotics, except for aztreonam which was performed by agar dilution, and ceftazidime-avibactam (CZA) by disk diffusion test and a sub-set (see below) by E-test according to the manufacturer instructions. All susceptibility results were interpreted according to CLSI 2022 guidelines [14].

To detect carbapenemases we used the imipenem–EDTA/SMA (ethylenediaminetetraacetic acid 372 mg/sodium mercaptoacetic acid 900 mg) double disk [15] and Rosco Diagnostica Neo-Sensitabs KPC and MBL confirmation kit. Testing was performed following the manufacturers' instructions.

2.2. Resistance mechanisms characterization

Molecular confirmation of carbapenemase genes (bla_{KPC}, bla_{VIM}, bla_{IMP}, bla_{NDM}, bla_{GES} and bla_{OXA-48}) and extended-spectrum β-lactamase genes ($bla_{CTX-M-group}$, bla_{PER-1}, bla_{PER-2}, bla_{TEM} and bla_{SHV}), were assessed by PCR and sequencing as previously described [11].

DNA extraction, preparation of libraries, and short read genome sequencing using Illumina MiSeq-I with Nextera XT libraries were performed for a subset of isolates as previously described [16].

2.3. Antibiotic susceptibility to aztreonam plus ceftazidime-avibactam

The effectiveness of CZA in combination with aztreonam (ATM) was determined by two methods:

1) Kirby-Bauer's diffusion double-disk synergy test, placing CZA and ATM disks at a distance of 15-25 mm. Enlargement of the inhibition zone of either of the antibiotics in the area between both disks was interpreted as a positive synergy.
2) Minimum inhibitory concentration (MIC) determination to CZA by E-test, both in conventional Mueller-Hinton and supplemented with aztreonam 4 mg/L was performed on a subset of isolates, see below [16].

To determine the possible synergistic effect between ceftazidime and ATM, the MIC of both agents and CZA was determined on *E. coli* ATCC 25922. Then it was compared with the susceptibility to ceftazidime and CZA in the presence of ATM as described above but at a final concentration of one dilution below the MIC.

The effectiveness of the combination of CZA plus ATM was defined when the microorganism was susceptible to either of both antibiotics or when either of the two methods evaluated evidenced a positive synergy. The synergistic effect in the quantitative method was determined by using an adaptation of the fractional inhibitory concentration index (FICI). FICI was defined as $FICI = \frac{A}{A'} + \frac{B}{B'}$, where A represents the MIC of ATM in combination with CZA (which was fixed at 4 mg/L), A' is the MIC to ATM alone, B equals the MIC of CZA in combination with ATM and B' the MIC value to CZA alone. A FICI $\leq$ 0.5 was interpreted as synergistic, while a FICI $>$ 0.5 was considered indifferent [17].

3. Results

3.1. Strains and antibiotic susceptibility

We studied 44 non-redundant carbapenemase-producing Enterobacterales: *Klebsiella pneumoniae* (n=20), *Enterobacter cloacae* (n=7), *Citrobacter freundii* (n=5), *E. coli* (n=3), *Proteus mirabilis* (n=4), *Providencia rettgeri* (n=2) and *Morganella morganii* (n=1), *Klebsiella aerogenes* (n=1) and *Klebsiella oxytoca* (n=1). Regarding the carbapenemase production, 31 isolates were bla_{NDM-1} producers, 5 bla_{KPC-2}, 3 bla_{KPC-3}, 2 bla_{VIM-2}, 2 bla_{NDM-1} + bla_{KPC-2} and 1 bla_{OXA-48} (See Table 1).

All 44 isolates were carbapenem-resistant, 14 were resistant to ceftazidime-avibactam (CZA) and aztreonam (ATM), 15 were CZA resistant but ATM susceptible, 12 ATM resistant but CZA susceptible, and 3 were susceptible to both.

3.2. Antibiotic susceptibility to aztreonam plus ceftazidime-avibactam

3.2.1. Metallo-carbapenemase producers

Among the 33 MBL producers (31 harbouring bla_{NDM-1} and 2 bla_{VIM-2}), the double disk synergy test between CZA and ATM resulted positive in 23/33 isolates regardless of their susceptibility

Table 1
Features and susceptibility results from the 44 clinical isolates studied and the control strain *E. coli* ATCC 25922.

Strain	Species	Source	Carba-penemase	ESBL / AmpC	MIC MEM[a]	MIC IPM[a]	CZA zone diameter[b]	CZA-ATM ddst	MIC ATM[a]	MIC CZA[a]	MIC CZA[a] with ATM 4 mg/L	FICI
HI003	*K. pneumoniae*	Urine	NDM-1	CTX-M-15	≥16 (R)	16 (R)	16	Positive	≥ 256 (R)	> 256 (R)	< 0.016	0.016
HI004	*K. pneumoniae*	Blood	NDM-1	CTX-M-15,OXA-1, OXA-9, OXA-10, TEM-1b, SHV-1	≥16 (R)	≥16 (R)	20	Positive	128 (R)	0.5 (S)	< 0.016	0.063
HI005	*E. cloacae*	Blood	NDM-1	AmpC	≥16 (R)	≥16 (R)	16	Positive	128 (R)	> 256 (R)	< 0.016	0.031
HI006	*K. pneumoniae*	Urine	NDM-1	CTX-M-15	8 (R)	2 (I)	20	Positive	128 (R)	> 256 (R)	< 0.016	0.031
HI007	*K. pneumoniae*	Urine	NDM-1	CTX-M-15	≥16 (R)	≥16 (R)	20	Positive	128 (R)	> 256 (R)	< 0.016	0.031
HI008	*K. pneumoniae*	Blood	NDM-1	CTX-M-15, OXA-1, TEM-1	≥16 (R)	≥16 (R)	14	Positive	64 (R)	> 256 (R)	< 0.016	0.063
HI009	*E. cloacae*	Blood	NDM-1	AmpC	≥16 (R)	≥16 (R)	16	Positive	64 (R)	> 256 (R)	< 0.016	0.063
HI010	*C. freundii*	Blood	NDM-1	AmpC	≥16 (R)	≥16 (R)	20	Positive	32 (R)	> 256 (R)	< 0.016	0.125
HI011	*K. pneumoniae*	Blood	NDM-1	CTX-M-15, SHV-1, OXA-1, TEM-1b	≥16 (R)	≥16 (R)	18	Positive	128 (R)	> 256 (R)	< 0.016	0.031
HI012	*E. cloacae*	Blood	NDM-1	CTX-M-15	≥16 (R)	≥16 (R)	17	Positive	64 (R)	> 256 (R)	< 0.016	0.063
HI013	*K. pneumoniae*	Peritoneal fluid	NDM-1	CTX-M-15	≥16 (R)	≥16 (R)	14	Positive	≥ 256 (R)	> 256 (R)	< 0.016	0.016
HI023	*K. aerogenes*	Peritoneal fluid	NDM-1	CTX-M-15, OXA-1, OXA-9, OXA-10	≥16 (R)	8 (R)	18	Positive	128 (R)	R [c]		
HI024	*K. pneumoniae*	Urine	NDM-1	-	≥16 (R)	≥16 (R)	17	Positive	0.25 (S)	R [c]		
HI025	*E. cloacae*	Blood	NDM-1	CTX-M-15	≥16 (R)	≥16 (R)	14	Positive	64 (R)	R [c]		
HI026	*P. rettgeri*	Urine	NDM-1	TEM-1B, OXA-9, OXA-10	≥16 (R)	≥16 (R)	10	Positive	≤ 0.06 (S)	R [c]		
HI027	*E. coli*	Broncho- alveolar lavage	NDM-1	-	≥16 (R)	≥16 (R)	20	Negative	≤ 0.06 (S)	> 256 (R)		
HI028	*M. morganii*	Skin	NDM-1	-	4 (R)	≥16 (R)	20	Negative	≤ 0.06 (S)	> 256 (R)		
HI029	*P. rettgeri*	Blood	NDM-1	-	≥16 (R)	≥16 (R)	10	Negative	≤ 0.06 (S)	R [c]		
HI030	*K. pneumoniae*	Blood	NDM-1	-	≥16 (R)	≥16 (R)	18	Positive	1 (S)	R [c]		
HI031	*C. freundii*	Urine	NDM-1	TEM-1b, CMY-48, SCO-1, OXA-9, OXA-10	≥16 (R)	≥16 (R)	16	Negative	0.5 (S)	R [c]		
HI032	*P. mirabilis*	Urine	NDM-1	CTX-M-2, OXA-2	≥16 (R)	≥16 (R)	17	Positive	0.25 (S)	R [c]		
HI033	*C. freundii*	Bone	NDM-1	-	≥16 (R)	≥16 (R)	14	Negative	0.25 (S)	R [c]		
HI034	*E. coli*	Peritoneal fluid	NDM-1	-	≥16 (R)	≥16 (R)	20	Negative	0.25 (S)	> 256 (R)		
HI035	*C. freundii*	Skin	NDM-1	-	≥16 (R)	≥16 (R)	15	Negative	0.125 (S)	R [c]		
HI036	*E. coli*	Respiratory secretion	NDM-1	-	≥16 (R)	≥16 (R)	18	Positive	0.125 (S)	R [c]		
HI037	*E. cloacae*	Bone	NDM-1	-	≥16 (R)	≥16 (R)	16	Positive	0.125 (S)	R [c]		
HI038	*P. mirabilis*	Urine	NDM-1	-	≥16 (R)	≥16 (R)	17	Negative	≤ 0.06 (S)	R [c]		
HI039	*P. mirabilis*	Blood	NDM-1	-	16 (R)	≥16 (R)	24	Negative	≤ 0.06 (S)	S [c]		
HI040	*K. pneumoniae*	Urine	NDM-1	CTX-M-15	16 (R)	≥16 (R)	23	Positive	128 (R)	S [c]		
HI041	*K. oxytoca*	Blood	NDM-1	OXY-1	≥16 (R)	≥16 (R)	14	Positive	0.25 (S)	R [c]		
HI042	*P. mirabilis*	Surgical wound	NDM-1	TEM-1b, OXA-9, OXA-10	≥16 (R)	≥16 (R)	32	Negative	≤ 0.06 (S)	2 (S)		
HI001	*E. hormaechei*	Blood	VIM-2	CTX-M-15, TEM-1b, ACT-14, OXA-1	≥16 (R)	≥16 (R)	22	Positive	64 (R)	4 (S)	< 0.016	0.0665
HI022	*E. cloacae*	Blood	VIM-2	CTX-M-9, TEM-1b, ACT-15	≥16 (R)	≥16 (R)	20	Positive	4 (S)	4 (S)		
HI015	*K. pneumoniae*	Urine	KPC-3	SHV-12	≥16 (R)	8 (R)	26	Positive	128 (R)	0.38 (S)	< 0.016	0.073
HI014	*K. pneumoniae*	Catheter tip	KPC-3	-	≥16 (R)	≥16 (R)	24	Positive	≥ 256 (R)	1 (S)	< 0.016	0.032
HI016	*K. pneumoniae*	Urine	KPC-3	-	≥16 (R)	≥16 (R)	24	Positive	64 (R)	0.75 (S)	< 0.016	0.083
HI019	*K. pneumoniae*	Blood	KPC-2	-	≥16 (R)	≥16 (R)	23	Positive	≥ 256 (R)	1 (S)	< 0.016	0.032
HI020	*C. freundii*	Urine	KPC-2	-	≥16 (R)	≥16 (R)	30	Positive	128 (R)	1 (S)	< 0.016	0.047
HI021	*K. pneumoniae*	Urine	KPC-2	-	≥16 (R)	≥16 (R)	28	Positive	32 (R)	0.5 (S)	< 0.016	0.157
HI043	*K. pneumoniae*	Respiratory secretion	KPC-2	CTX-M-15	≥16 (R)	≥16 (R)	26	Positive	128 (R)	S [c]		
HI044	*K. pneumoniae*	Catheter tip	KPC-2	-	≥16 (R)	≥16 (R)	24	Positive	≥ 256 (R)	S [c]		
HI002	*K. pneumoniae*	Urine	OXA-48	CTX-M-15, TEM-1b, SHV-1	≥16 (R)	≥16 (R)	26	Positive	≥ 256 (R)	0.25 (S)	< 0.016	0.08
HI017	*K. pneumoniae*	Rectal swab	KPC-2 +NDM-1	CTX-M-15	≥16 (R)	≥16 (R)	17	Positive	≥ 256 (R)	> 256 (R)	< 0.016	0.016
HI018	*K. pneumoniae*	N/A	KPC-2 +NDM-1	-	≥16 (R)	≥16 (R)	15	Positive	≥ 256 (R)	> 256 (R)	< 0.016	0.016
ATCC 25922	*E. coli*	ATCC	-	-	0.12	0.03		Negative	0.06	0.06	< 0.016 (ATM 0.03 mg/L)	

Abbreviations: ATM, aztreonam; CZA, ceftazidime-avibactam; ddst, double disk synergy test; ESBL, extended-spectrum β-lactamase; FICI, fractional inhibitory concentration index; I, intermediate; IPM, imipenem; MEM, meropenem; MIC, minimum inhibitory concentration; R, resistant; S, susceptible.

[a] Minimum inhibitory concentration expressed in mg/L. Interpretation according to CLSI breakpoints is indicated in brackets.

[b] Disk diffusion zone diameter expressed in mm.

[c] Susceptibility was determined by disk diffusion. Strains highlighted in bold were studied using whole genome sequencing.

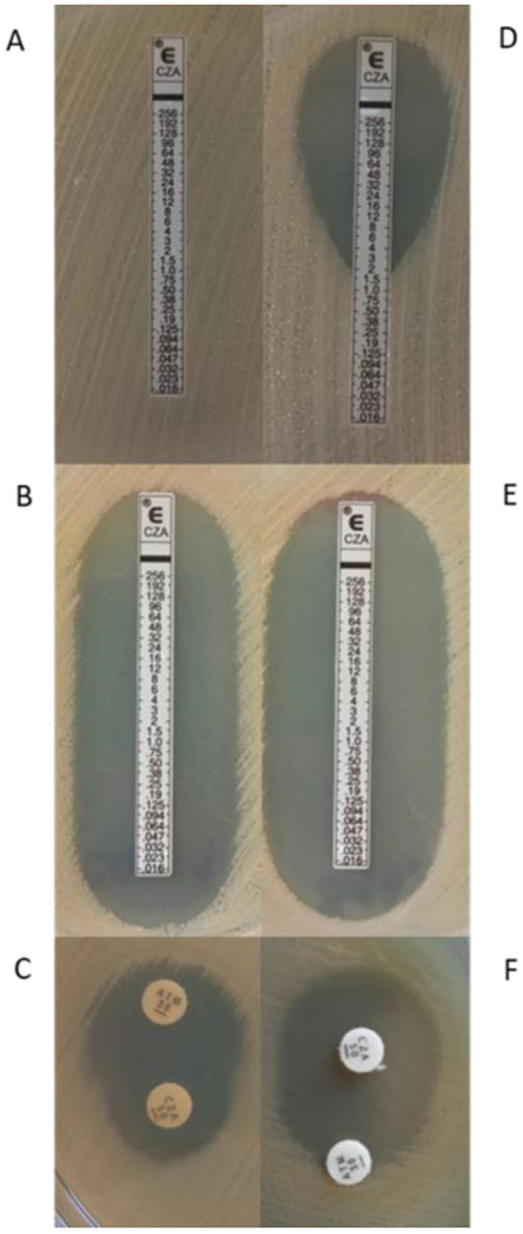

Fig. 1. Examples of synergistic effects between CZA and ATM; Panels A, B and C, Isolate HI008 (*K.pneumoniae* NDM-1 + CTX-M-15); panels D, E and F, Isolate HI016 (*K. pneumoniae* KPC-3). Panel A and D, CZA alone; panels B and E, CZA plus ATM (4 mg/L); panels C and F, double disk synergy test between CZA and ATM disks.

profile (Table 1; Fig. 1C). The ten remaining isolates, with negative synergy test, were susceptible to ATM, and two to CZA (Table 1).

Fifteen MBL-producing isolates were resistant to ATM, in twelve of them the presence of $bla_{CTX\text{-}M\text{-}15}$ was confirmed either by PCR or whole-genome sequencing. The three remaining isolates exhibited a phenotypic profile compatible with the expression of a derepressed AmpC.

Regarding the CZA susceptibility profile, 27/33 MBL-producing isolates were resistant to this antibiotic. Of note, nine of the 33 isolates displayed 20 to 22 mm inhibition zones to CZA, hence it was necessary to confirm their susceptibility using the MIC value obtained by E-test. Consequently, three isolates were categorized as susceptible (MIC 0.5 and 4 mg/L), and the remaining six as resistant (MIC > 256 mg/L) (Table 1).

Twelve ATM-resistant isolates were selected to determine the MIC to CZA in the presence of 4 mg/L of ATM. Among them, 10/12 were resistant to CZA with MIC > 256 mg/L, whereas the remaining two were susceptible with CZA MICs of 0.5 and 4 mg/L, respectively. However, when tested in Mueller-Hinton supplemented with ATM, all isolates exhibited a CZA MIC < 0.016 mg/L. FICI values ranged between 0.016 and 0.125, demonstrating a synergistic effect of CZA plus ATM (FICI < 0.5) (Table 1; Fig. 1A-B).

3.2.2. Serine-carbapenemase producers

Serine-carbapenemase producers included a total of nine isolates harbouring $bla_{KPC\text{-}2}$ (n=5), $bla_{KPC\text{-}3}$ (n=3) and $bla_{OXA\text{-}48}$ (n=1). All nine were susceptible to CZA and resistant to ATM and exhibited a positive double disk synergy test between CZA and ATM (Table 1; Fig. 1F). The OXA-48-producing isolate also produced CTX-M-15, accounting for the observed resistance to ATM.

The CZA MIC in the presence of ATM was determined in seven isolates with CZA MICs ranging from 0.25 to 1 mg/L. In Mueller-Hinton supplemented with 4 mg/L ATM, all seven exhibited CZA MICs < 0.016 mg/L. FICI values were < 0.5 (0.032 to 0.157) demonstrating a synergistic effect of CZA plus ATM (Table 1; Fig. 1D-E).

3.2.3. Double-carbapenemase producers

Two *K. pneumoniae* harbouring both $bla_{NDM\text{-}1}$ and $bla_{KPC\text{-}2}$ were studied. The two isolates were resistant to ATM and CZA (MICs ≥ 256 and > 256 mg/L respectively) and exhibited a positive double disk synergy test to both antibiotics. When determined in Mueller-Hinton supplemented with 4 mg/L, the CZA MICs dropped to < 0.016 mg/L and FICI values were 0.016, evidencing a synergistic effect between both antibiotics (Table 1).

3.3. E. coli *ATCC 25922 results*

E. coli strain ATCC 25922 presented MIC values to ATM, ceftazidime (CAZ) and CZA of 0.06, 0.125 and 0.064 mg/L, respectively. On the other hand, CAZ and CZA MIC values dropped to 0.032 and < 0.016 mg/L in the presence of ATM at 0.03 mg/L, which is equivalent to a 4-fold MIC decrease in both cases.

4. Discussion and Conclusions

Our results are in accordance with works from other authors that reported the combined use of either ATM plus avibactam or ATM plus CZA as effective in treating MBL-producing bacterial infections [9,18,19]. However, the literature is more limited regarding the study of effectiveness of CZA plus ATM against serine-carbapenemase involving infections [20].

In this sense, we found that the combination of CZA plus ATM resulted in a synergistic effect, not only among MBL-producing bacteria but also among either OXA-48, KPC-2, KPC-3, and even double carbapenemase producers. The combination of CZA plus ATM resulted in effectiveness in the 44 isolates, either by a positive synergy (n=34) or by susceptibility to at least one of the drugs. FICI values obtained for all 21 analysed isolates were below 0.5, reaching values as low as 0.016 in KPC-2 and NDM-1 double carbapenemase producers.

Even though either KPC-2, KPC-3 and OXA-48 producers are usually susceptible to CZA, its use in combination with ATM could result in a reduction of the amount of antibiotic consumed during the treatment, or by ensuring favourable clinical and/or microbiological results, preventing relapses.

On the other hand, six MBL-producing isolates, including two VIM-2 and four NDM-1, were susceptible to CZA. Even though these results were not the expected according to the inhibitory profile of avibactam, there had been previous reports of a limited proportion of metallo-carbapenemase-producing Enterobacterales and *Pseudomonas aeruginosa* susceptible to CZA [16,21]. This CZA susceptibility may be explained by the presence of weak

promoters that affect the gene expression as has been previously described [22]. This is interesting since in the presence of a multidrug-resistant microorganism, the production of a metallo-carbapenemase does not exclude the use of CZA, which could become a useful therapeutic option.

Interestingly, we observed a 4-fold MIC decrease of both CAZ and CZA when combined with 0.03 mg/L of ATM (½ MIC) for the control strain *E. coli* ATCC 25922, which is not a β-lactamase producer. Recently, Khan et al. reported a negative synergistic effect of ATM plus CZA for this strain, however, concentrations above the MICs were evaluated [19].

In this regard, assays using lower concentrations of ATM, such as ¼ MIC (0.015 mg/L), yielded non-reproducible results (data not shown), probably due to issues concerning the dilution of the antibiotic in a solid culture media at low concentrations. Because of this, we cannot affirm there is a synergistic effect between ATM and either CAZ or CZA, given that according to the FICI definition, at least a 4-fold decrease of MICs should be observed for both agents to define a synergy. However, the observed decrease of both CAZ and CZA MICs in the presence of ATM should be highlighted.

In our work, the performance of the double-disk synergy test between CZA and ATM displayed positive results in 34 isolates, which included several carbapenemase producing-isolates, even in those in which the MIC for both antibiotics was > 256 mg/L. The remaining ten isolates with negative synergy tests were susceptible to at least one of the antibiotics. This method is available to clinical laboratories and would provide valuable information to guide the treatment of infections with CZA and ATM, even in microorganisms classified as extensive drug-resistant or pandrug-resistant. Alternative drugs such as tigecycline, colistin and aminoglycosides are associated with worse pK/pD parameters and more adverse effects than β-lactams, making the latter, and particularly the combination ATM plus CZA, a better treatment option even for carbapenemase-producing strains [5,6].

Among the strengths of our work are the great diversity of Enterobacterales species analysed as well as the diversity of β-lactamases involved, including two double-carbapenemase-producing isolates. Among the main weaknesses is the lack of in vivo studies that support the in vitro results. However, the results obtained are more encouraging than other available therapeutic options. The availability of CZA is still restricted in many low-income countries such as Latin American countries. In Uruguay, the use of CZA is restricted to special requests for compassionate use, therefore collecting clinical information related to the response to these treatments is difficult. The availability of data generated in such countries could help improve access to these drugs.

Competing interests: None declared.

Ethical approval: Not required.

References

[1] Tacconelli E, Magrini N, Kahlmeter G, Singh N. Global Priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. Lancet Infect Dis 2018;18:318–27. doi:10.1016/S1473-3099(17)30753-3.

[2] Bush K. Past and present perspectives on β-lactamases. Antimicrob Agents Chemother 2018;62:e01076 18. doi:10.1128/AAC.01076-18.

[3] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18:268–81. doi:10.1111/j.1469-0691.2011.03570.x.

[4] Bush K. Carbapenemases: Partners in crime. J Glob Antimicrob Resist 2013(1):7–16. doi:10.1016/j.jgar.2013.01.005.

[5] Paul M, Carrara E, Retamar P, Tängdén T, Bitterman R, Bonomo RA, et al. European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines for the treatment of infections caused by multidrug-resistant Gram-negative bacilli (endorsed by European society of intensive care medicine). Clin Microbiol Infect 2022;28:521–47. doi:10.1016/j.cmi.2021.11.025.

[6] Aslan AT, Akova M. The Role of Colistin in the era of new β-Lactam/β-Lactamase inhibitor combinations. Antibiotics 2022;11:277. doi:10.3390/antibiotics11020277.

[7] Romero Thomas G, Corso A, Pasterán F, Shal J, Sosa A, Pillonetto M, et al. Increased detection of carbapenemase-producing Enterobacterales bacteria in Latin America and the Caribbean during the COVID-19 pandemic. Emerg Infect Dis 2022;28:E1–8. doi:10.3201/eid2811.220415.

[8] Bush K, Bradford PA. Interplay between β-lactamases and new β-lactamase inhibitors. Nature Rev Microbiol 2019;17:295–306. doi:10.1038/s41579-019-0159-8.

[9] Falcone M, Daikos GL, Tiseo G, Bassoulis D, Giordano C, Galfo V, et al. Efficacy of ceftazidime-avibactam plus aztreonam in patients with bloodstream infections caused by metallo-β-lactamase-producing Enterobacterales. Clin Infect Dis 2021;72:1871–8. doi:10.1093/cid/ciaa586.

[10] Seija V, Medina Presentado JC, Bado I, Papa Ezdra R, Batista N, Gutierrez C, et al. Sepsis caused by New Delhi metallo-β-lactamase (bla_{NDM-1}) and *qnrD*-producing *Morganella morganii*, treated successfully with fosfomycin and meropenem: case report and literature review. Int J Infect Dis 2015;30:e20–6. doi:10.1016/j.ijid.2014.09.010.

[11] Papa-Ezdra R, Caiata L, Palacio R, Outeda M, Cabezas L, Bálsamo A, et al. Prevalence and molecular characterisation of carbapenemase-producing Enterobacterales in an outbreak-free setting in a single hospital in Uruguay. J Glob Antimicrob Res 2021;24:58–62. doi:10.1016/j.jgar.2020.11.006.

[12] Garcia-Fulgueiras V, Zapata Y, Papa-Ezdra R, Avila P, Caiata L, Seija V, et al. First characterization of *K. pneumoniae* ST11 clinical isolates harbouring bla_{KPC-3} in Latin America. Rev Argent Microbiol 2020;52:211–16. doi:10.1016/j.ram.2019.10.003.

[13] Araújo Pírez L, Ávila P, García Fulgueiras V, Papa Ezdra R, Mota M, López M, et al. First report of plasmidic OXA-48 in *Klebsiella pneumoniae* In Uruguay Poster presentation at ASM Microbe; 2020.

[14] Performance CLSI. Standards for Antimicrobial Susceptibility Testing. CLSI supplement M100. 32nd ed. Clinical and Laboratory Standards Institute; 2022.

[15] Nordmann P, Poirel L, Carrër A, Toleman MA. Walsh TR How to detect NDM-1 producers. J Clin Microbiol 2011;49:718–21. doi:10.1128/JCM.01773-10.

[16] Papa-Ezdra R, Cordeiro NF, Outeda M, Garcia-Fulgueiras V, Araújo L, Seija V, et al. Novel Resistance regions carrying Tn*aphA6*, bla_{VIM-2}, and bla_{PER-1}, embedded in an IS*Pa40*-derived transposon from two multi-resistant *Pseudomonas aeruginosa* clinical isolates. Antibiotics 2023;12:304. doi:10.3390/antibiotics12020304.

[17] Garcia LS, Isenberg HD, editors. Clinical Microbiology Procedures Handbook, 2. 3rd ed. ASM Press; 2010.

[18] Vázquez-Ucha JC, Alonso-Garcia I, Guijarro-Sánchez P, Lasarte-Monterrubio C, Álvarez-Fraga L, Cendón-Esteve A, et al. Activity of aztreonam in combination with novel β-lactamase inhibitors against metallo-β-lactamase-producing Enterobacterales from Spain. Int J Antimicrob Agents 2023;61:106738. doi:10.1016/j.ijantimicag.2023.106738.

[19] Khan A, Erickson SG, Pettaway C, Arias CA, Miller WR, Bhatti MM. Evaluation of susceptibility testing methods for aztreonam and ceftazidime-avibactam combination therapy on extensively drug-resistant gram-negative organisms. Antimicrob Agents Chemother 2021;65:e0084621. doi:10.1128/AAC.00846-21.

[20] Mikhail S, Singh NB, Kebriaei R, Rice SA, Stamper KC, Castanheira M, et al. Evaluation of the synergy of ceftazidime-avibactam in combination with meropenem, amikacin, aztreonam, colistin, or fosfomycin against well-characterized multidrug-resistant *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2019;63(8):e00779-19. doi:10.1128/AAC.00779-19.

[21] Karlowsky JA, Kazmierczak KM, Valente MLN de F, Luengas EL, Baudrit M, Quintana A, et al. In vitro activity of ceftazidime-avibactam against Enterobacterales and *Pseudomonas aeruginosa* isolates collected in Latin America as part of the ATLAS global surveillance program, 2017-2019. Braz J Infect Dis 2021;25:101647. doi:10.1016/J.BJID.2021.101647.

[22] Papagiannitsis CC, Tzouvelekis LS, Miriagou V. Relative strengths of the class 1 integron promoter hybrid 2 and the combinations of strong and hybrid 1 with an active p2 promoter. Antimicrob Agents Chemother 2009;53:277–80. doi:10.1128/AAC.00912-08.