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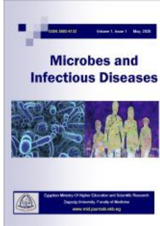

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Original article

In *vitro* activity of ceftazidime-avibactam in combination with aztreonam against carbapenem resistant *Enterobacterales* isolated from intensive care units

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ABSTRACT

Background: Carbapenem-resistant (CR) *Enterobacterales* are established causes of serious healthcare-associated infections. Development of new β-lactam/β-lactamase inhibitors was a breakthrough, but effective antibiotic treatment for metallo-β-lactamase (MBL) producers remained an unmet need. Ceftazidime-avibactam (CZA) combination with aztreonam (ATM) is an emerging option to combat these bugs. **Aim:** The aim of the study was to evaluate the *in vitro* activity of ceftazidime-avibactam alone and in combination with aztreonam on CR *Enterobacterales* isolates from intensive care units' (ICU) patients **Methods:** A total of 258 *Enterobacterales* were recovered from patients admitted to ICUs and screened for carbapenem resistance. Carbapenem-resistant isolates were subjected to antimicrobial susceptibility testing and molecular detection of the five major carbapenemase genes (bla_{KPC}, bla_{OXA-48}, bla_{NDM}, bla_{VIM}, and bla_{IMP}) using polymerase chain reaction (PCR). *In vitro* activity of ceftazidime-avibactam and aztreonam combination was evaluated using broth disk elution method. **Results:** One hundred and twenty (46.5%) of the 258 *Enterobacterales* isolates were carbapenem resistant. All were also multi-drug resistant (MDR), exhibiting resistance to most antibiotics. Metallo-β-lactamase producers were the predominant (76.7%). Susceptibility to ceftazidime-avibactam and aztreonam combination was higher in MBL producing group (59/ 92, 64.1%), all were CZA resistant, while the addition of ATM didn't demonstrate an advantage over CZA alone in MBL non producers. **Conclusion:** A high rate of CZA resistance was observed among CR *Enterobacterales* in our ICUs. The molecular mechanisms behind this resistance need to be studied. Ceftazidime-avibactam-aztreonam combination can be considered for treating MBL producers but only after susceptibility testing.

Introduction

The World Health Organization (WHO) has recognized antimicrobial resistance as one of the major global health threats. *Enterobacterales* as *Escherichia coli (E. coli)* and *Klebsiella* species are considered to be of the most important bacteria causing hospital-acquired and community-acquired infections [1].

Carbapenems were considered the last treatment option for infections caused by multidrug resistant (MDR) *Enterobacterales* due to their stability even in response to extended spectrum beta

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lactamases (ESBLs) and AmpC enzymes. However, resistance to carbapenems has emerged as a consequence of their misuse leading to reduction in their effectiveness [2].

Resistance to carbapenems is mostly mediated by carbapenemase enzymes. These enzymes include ambler class A (*Klebsiella pneumoniae* carbapenemase, KPC), class B Metallo-β-lactamases (MBL) as New Delhi MBL (NDM), and class D (Oxacillinase, OXA-48-like enzymes). Serine carbapenemases include ambler class A and D [3, 4].

An established approach for preserving the efficacy of β-lactams is reducing the activity of β-lactamases, this is achieved by using β-lactamase inhibitors to protect the simultaneously administered β-lactam agents. β-lactamase inhibitors fall in two classes according to their mechanism of action; suicide inhibitors which include the older members; clavulanic acid, sulbactam, and tazobactam, they undergo inactivation after binding to β-lactamase molecule. The newer reversible inhibitors, however, detach from the β-lactamase without modification and can inactivate other β-lactamase molecules thus sustaining prolonged action, and protect multiple β-lactam molecules. This new class includes diazabicyclooctanes and boronates [5,6].

Ceftazidime-avibactam (CZA), a β-lactam/β-lactamase inhibitor drug, was FDA approved in 2015 for the treatment of complicated intra-abdominal infections (with metronidazole) and complicated urinary tract infections, including pyelonephritis. It is also indicated for use in adult patients with hospital acquired bacterial pneumonia [7]. Ceftazidime (CAZ) is a third-generation cephalosporin that demonstrates an enhanced activity against a wide spectrum of Gram-negative bacteria (GNB), particularly *Enterobacterales* and *Pseudomonas aeruginosa*, expressing a variety of β-lactamases. However, ESBLs production and overexpression of class C β-lactamases resulted in rapid emergence of resistance to ceftazidime [8].

Avibactam, the first of the diazabicyclooctanes β-lactamase inhibitors (relebactam being the second), was added to restore the activity of ceftazidime as it has a broad-spectrum activity against class A (including KPC and ESBL), class C (AmpC), and some class D (OXA-48 like) β-lactamases [9]. So, CZA was recommended by the Infectious Diseases Society of America for the treatment of serious infections by KPC and OXA-48 like-producing carbapenem resistant *Enterobacterales* (CRE) or when the results of carbapenemase testing are either unavailable or negative [10].

Aztreonam (ATM), a monobactam, is stable in the presence of MBLs, but susceptible to be hydrolyzed by serine carbapenemases and other beta lactamases like ESBLs which are inhibited by the co-administered avibactam [8]. Therefore, ATM in combination with avibactam (aztreonam-avibactam or adding ATM to CZA is recommended for treatment of MBL-producing *Enterobacterales* [10].

Unfortunately, shortly after CZA was introduced into clinical practice, the first case of resistance to CZA in a KPC-3 producing *Klebsiella pneumoniae* (*K. pneumoniae*) was reported followed by other reports of resistance in CR isolates producing OXA enzymes, that are poor ceftazidimases, including OXA-48 like enzymes that are commonly inhibited by avibactam [11-13].

In our facility, CZA was formerly administered for treatment of severe CRE infections in the ICU, but its use was discontinued because of the poor outcomes compared to best available therapies despite the high cost. This was done without routine testing for carbapenemase production or detection of the type of carbapenemase.

This study aimed to evaluate the *in vitro* activity of ceftazidime-avibactam alone and in combination with aztreonam on CR *Enterobacterales* isolates from ICU patients considering the type of carbapenemase produced.

Material and Methods

Study design and setting

This cross-sectional study was conducted during the period from October 2023 to March 2024 in the ICUs of Zagazig University Hospitals and Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University, Egypt.

The study was approved by the Institutional Review Board of Faculty of Medicine, Zagazig University (approval #11167-17-10-2023) and was carried out in concordance with the Declaration of Helsinki. Informed written consents were provided from patients' relatives.

Bacterial isolation and identification

Two hundred and fifty-eight *Enterobacterales* isolates were obtained from clinical samples collected from ICU patients under complete aseptic conditions. Samples were cultivated on MacConkey agar and blood agar (Oxoid, UK) and incubated at 37 ºC for 48 hours in aerobic conditions. Identification was done by standard microbiological methods; colonial morphology, Gram-stained films, and biochemical tests [14]. *Enterobacterales* species were then confirmed by VITEK 2 compact system (bioMérieux, France).

Antimicrobial susceptibility testing of CR *Enterobacterales*

All *Enterobacterales* isolates were screened at first for carbapenem resistance by disk diffusion method using imipenem 10 µg, meropenem 10 µg, ertapenem 10 µg, and doripenem 10 µg disks on Mueller-Hinton agar (Oxoid, UK) incubated at 37ºC for 16-18 hours. Isolates that showed non-susceptibility to any of the four tested carbapenems were considered carbapenem resistant. Proteus isolates showing only imipenem resistance were excluded as *Proteus* species have intrinsic resistance to imipenem. Results were interpreted according to CLSI M100 guidelines [15].

Carbapenem resistant *Enterobacterales* isolates were then subjected to antibiotic susceptibility testing by disk diffusion method for the following antibiotics: amoxycillin/clavulanate (AMC, 20/10 µg), ampicillin/sulbactam (SAM, 10/10 µg), piperacillin/tazobactam (TPZ, 100/10 µg), cefotaxime (CTX, 30 µg), ceftriaxone (CRO, 30 µg), cefoxitin (FOX, 30 µg), ceftazidime (CAZ, 30 µg), cefepime (FEP, 30 µg), ceftazidime-avibactam (CZA) (30/20 µg), aztreonam (ATM, 30 µg), gentamicin (CN, 10 µg), amikacin (AK, 30 µg), ciprofloxacin (CIP, 5 µg), levofloxacin (LEV, 5 µg), trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 µg) and nitrofurantoin (F, 300 µg) for urinary isolates. All used antibiotic disks were supplied by (Oxoid, UK) except for the ceftazidime-avibactam that was supplied by (Liofilchem, Italy). Inhibition zone diameters were interpreted according to CLSI M100 [15].

Phenotypic detection of carbapenemase production

CR *Enterobacterales* isolates were initially screened for carbapenemase production using a modified carbapenem inactivation method (mCIM) and an EDTA-modified carbapenem inactivation method (eCIM) [15].

Characterization of carbapenemase genes

Characterization of carbapenemase genes was done by PCR after genomic DNA extraction using G-spinTM Genomic DNA Extraction Kit (iNtRON Biotechnology, Inc., Korea). Two multiplex PCR reactions were performed to target the five major carbapenemase genes; the 1st reaction was for genes encoding class A (bla_{KPC}) and class D carbapenemases ($bla_{\text{OXA-48}}$). The 2nd reaction was for genes encoding class B (MBL) (bla_{NDM}, bla_{VIM} and bla_{IMP}). Amplification was performed using DreamTaq Green PCR Master Mix (ThermoFisher Scientific, Germany). Primers and conditions were as described by **Poirel et al.** [16]. *K. pneumoniae* ATCC BAA-1705 and *K. pneumoniae* ATCC BAA-2146 were used as the positive controls for bla_{KPC} and bla_{NDM}, respectively.

Ceftazidime-avibactam and aztreonam combination *in vitro* activity testing

The *in vitro* activity of adding ATM to CZA was tested by the "Broth Disk Elution Method" recently endorsed by CLSI [17,18]. Briefly, four 5-mL cation-adjusted Mueller-Hinton broth (CA-MHB) tubes were prepared, to which the following were added separately; a 30-µg ATM disk, a 30/20-µg CZA disk, both disks in combination, and no disks (as growth control). Twenty-five µl of 0.5 McFarland standard inoculum were added to each tube to attain a final inoculum of around 7.5 x 10^5 CFU/ml. After overnight incubation, the tubes were assessed for turbidity (not susceptible) or no turbidity (susceptible). *K. pneumoniae* ATCC BAA-2146 and *K. pneumoniae* ATCC BAA-1705 were used as controls; they are susceptible and not-susceptible to the combination, respectively.

Statistical analysis

Statistical Package for Social Science (SPSS) software version 20.0 was used to statistically analyze the data. Categorical variables were represented in terms of numbers and percentages. Fisher exact test was used to calculate the distribution differences in categorical variables. p value < 0.05 was considered statistically significant.

Results

A total of 258 *Enterobacterales* were isolated from different clinical samples. Of the 258 *Enterobacterales,* 171 isolates were identified as *K. pneumoniae*, 58 as *E. coli*, and 29 as *Proteus mirabilis (P. mirabilis).* By disk diffusion method, carbapenem resistance was detected in 46.5%

(120/258) of the isolated *Enterobacterales,* of which 88 *K. pneumoniae*, 20 *E. coli* and 12 *P. mirabilis* isolates were carbapenem resistant. The majority of *K. pneumoniae* isolates 54 (61.4%) were recovered from endotracheal aspirates while most of the *E. coli* and *P mirabilis* isolates were isolated from urine samples; 12 (60%) and 10 (83.3%), respectively. **Table 1** displays isolates' distribution among the clinical specimens.

All 120 CR *Enterobacterales* were resistant to β-lactam/β-lactamase inhibitors, cephalosporins, aztreonam and nitrofurantoin. High rates of resistance were found to aminoglycosides, quinolones, and sulfonamides as follows; 90% of isolates were resistant to gentamicin, 85.8% were resistant to amikacin, 89.2% were resistant to levofloxacin, 87.5% were resistant to ciprofloxacin and 79.2**%** were resistant to trimethoprim-sulfamethoxazole **(Figure 1)**.

Regarding carbapenemase genes, PCR results showed that 106/120 (88.3%) of CR *Enterobacterales* isolates harbored one or more of the tested carbapenemase genes **(Table 2)**. The most frequent gene was bla_{NDM} (71.7%) followed by $bla_{\text{OXA-48}}$ (45%). Bla_{IMP} and bla_{VIM} were found in *Klebsiella* isolates only (4.5% and 2.3%), respectively. Co-existence of bla_{NDM} and $bla_{\text{OXA-48}}$ genes was observed in (38, 43.2%) and (2, 10%) of the *Klebsiella* and *E. coli* isolates, respectively. Bla_{KPC} was not detected in any of the tested isolates. None of the studied genes were detected in (14/120, 11.7%) of the isolates that also showed no carbapenemase activity by mCIM/eCIM.

Regarding the CZA susceptibility profile, 90%, 108/120 CR isolates, were CZA resistant. All MBL gene harboring isolates either alone (52 isolates) or with $bla_{\text{OXA-48}}$ (40 isolates) were resistant to CZA, of which 32 (61.5%) and 27 (67.5%) were susceptible to CZA plus ATM, respectively. Resistance rates to CZA in non MBL gene harboring isolates were 71.4% (10/14) in $bla_{\text{OXA-48}}$ harboring isolates and 35.7% (5/14) in isolates tested negative for carbapenemase genes and the same results were obtained for CZA plus ATM. A statistically significant difference between isolates with different carbapenemase genes regarding the synergistic effect of CZA-ATM combination was observed (p=0.024) **(Table 3)**.

Table 1. Distribution of CR *Enterobacterales* isolates from different clinical samples.

Sample type	***K. pneumoniae* (N=88)**	***E. coli* (N=20)**	***P. mirabilis* (N=12)**
	no %	**no %**	**no %**
Endotracheal aspirate	54 (61.4)	6 (30)	0 (0.0)
Urine	14 (15.9)	12 (60)	10 (83.3)
Blood	8 (9)	2 (10)	2 (16.7)
Cerebrospinal fluid (CSF)	2 (2.3)	0 (0.0)	0 (0.0)
Pus	6 (6.8)	0 (0.0)	0 (0.0)
Central venous catheter	2 (2.3)	0 (0.0)	0 (0.0)
Fluid aspirate	2 (2.3)	0 (0.0)	0 (0.0)

Table 2. Distribution of carbapenemase genes among CR *Enterobacterales* isolates.

Carbapenemase genes	*K. pneumoniae* (n=88)		*E. coli* (n=20)		*P. mirabilis* (n=12)		Test	*p* value
	N	%	N	%	N	%		
Class A (*bla*$_{KPC}$), n=0	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	-	-
Class B MBL, n=52	**34**	**38.6**	**14**	**70**	**4**	**33.3**		
• ***bla*$_{NDM}$**	28	31.8	14	70	4	33.3		
• ***bla*$_{VIM}$**	2	2.3	0	0.0	0	0.0	F	0.03*
• ***bla*$_{IMP}$**	4	4.5	0	0.0	0	0.0		
Class D (*bla*$_{OXA-48}$), n=14	**12**	**13.6**	**2**	**10**	**0**	**0.0**		0.51
Both *bla*$_{OXA-48}$ + *bla*$_{NDM}$, n=40	**38**	**43.2**	**2**	**10**	**0**	**0.0**		<0.001**
Carbapenemase genes negative, n=14	**4**	**4.5**	**2**	**10**	**8**	**66.7**		<0.001**

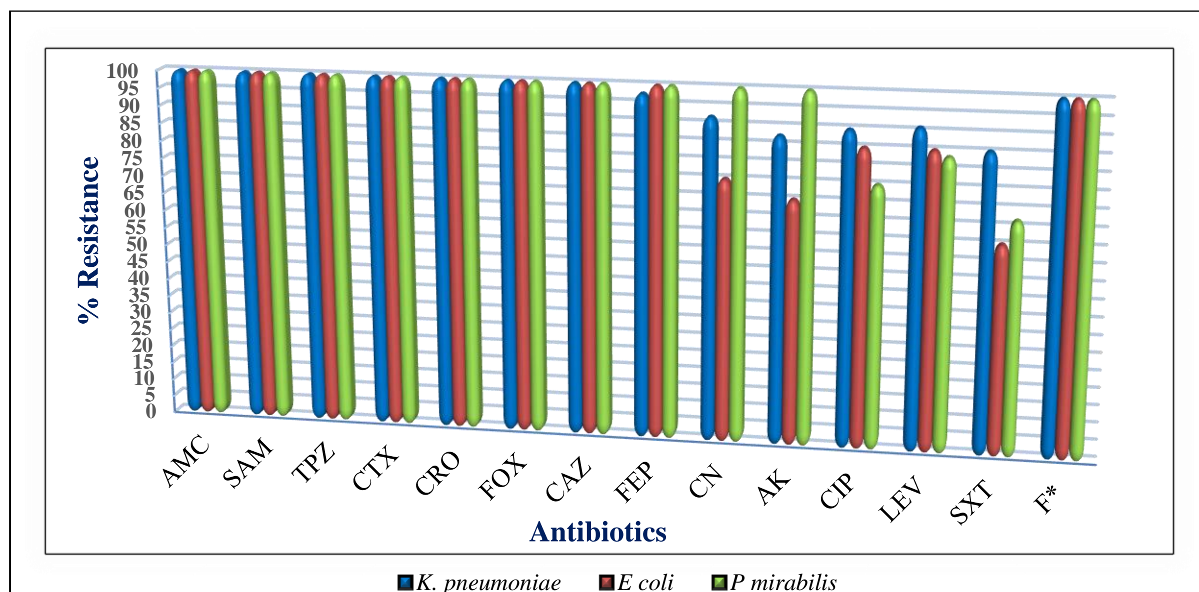
F: Fisher exact test * Statistically significant at $p < 0.05$

Table 3. Antimicrobial susceptibility pattern of ceftazidime-avibactam, aztreonam, and ceftazidime-avibactam in combination with aztreonam for all CR isolates (n=120).

Carbapenemase Gene	Isolate	CZA		ATM		CZA+ATM		F test
		S no	R no	S no	R No	S no	NS no	
MBL	*K. pneumoniae* (34) *E. coli* (14) *P. mirabilis* (4)	0 0 0	34 14 4	0 0 0	34 14 4	19 9 4	15 5 0	
	total (52)	0 (0)	52 (100)	0 (0)	52 (100)	32 (61.5)	20 (38.5)	
MBL+OXA-48	*K. pneumoniae* (38) *E. coli* (2) *P. mirabilis* (0)	0 0 0	38 2 0	0 0 0	38 2 0	25 2 0	13 0 0	
	Total (40)	0 (0)	40 (100)	0 (0)	40 (100)	27 (67.5)	13 (32.5)	*p*= 0.024
MBL producers	**n=92**	**0 (0)**	**92 (100)**	**0 (0)**	**92 (100)**	**59 (64.1)**	**33 (35.9)**	
OXA-48	*K. pneumoniae* (12) *E. coli* (2) *P. mirabilis* (0)	2 2 0	10 0 0	0 0 0	12 2 0	2 2 0	10 0 0	
	Total (14)	4 (28.6)	10 (71.4)	0 (0)	14 (100)	4 (28.6)	10 (71.4)	
Non-producers	*K. pneumoniae* (4) *E. coli* (2) *P. mirabilis* (8)	0 1 8	4 1 0	0 0 0	4 2 8	0 1 8	4 1 0	
	Total (14)	9 (64.3)	5 (35.7)	0 (0)	14 (100)	9 (64.3)	5 (35.7)	
MBL non-producers	**n=28**	**13 (46.4)**	**15 (53.6)**	**0 (0)**	**28 (100)**	**13 (46.4)**	**15 (53.6)**	
Total	**n= 120**	**12 (10)**	**108 (90)**	**0 (0)**	**120 (100)**	**72 (60)**	**48 (40)**	

Abbreviations: CZA: Ceftazidime–avibactam, ATM: Aztreonam.; S, sensitive; R, resistant; NS, not-susceptible

Figure 1. Antimicrobial resistance in CR *Enterobacterales* isolates.



Abbreviations: Amoxycillin/clavulanate (AMC); Ampicillin/sulbactam (SAM); Piperacillin/tazobactam (TPZ); Cefotaxime (CTX); Ceftriaxone (CRO); Cefoxitin (FOX); Ceftazidime (CAZ); Cefepime (FEP); Gentamicin (CN); Amikacin (AK); Ciprofloxacin (CIP); Levofloxacin (LEV); Trimethoprim-sulfamethoxazole (SXT); Nitrofurantoin (F).
*: for urinary isolates only

Discussion

Carbapenem resistant *Enterobacterales* continue to threaten healthcare systems increasing morbidity and mortality rates among vulnerable ICU patients, and the cost of health services worldwide. Safe and effective therapeutic options for these bugs are limited. Carbapenemase enzymes production is the main CR mechanism, they are heterogeneous regarding their mechanism of action and spectrum of drug substrates. Combinations of β-lactam/β-lactamase inhibitor have been developed to nullify the activity of these enzymes and to restore the activity of certain β-lactam antibiotics. Therefore, epidemiological characterization of carbapenemases and ongoing surveillance are crucial not only for the application of effective infection control measures, but also to inform ideal therapeutic choices [10]; CZA shows activity against KPC and OXA-48 producing Enterobacterales, meropenem/vaborbactam has activity against KPC-producing *Enterobacterales* but not against OXA producers. Ceftolozane–tazobactam, however, is inactive against all carbapenemase producers. Meanwhile, none of these combinations has activity against MBL-producers. Aztreonam evades MBL-mediated hydrolysis and with an added avibactam, it retains activity in presence of ESBLs, AmpC, and KPC [19]. This study was conducted to test the *in vitro* activity of ceftazidime-avibactam alone and in combination with aztreonam against CR *Enterobacterales* isolates from the ICUs.

In this study, a total of 258 *Enterobacterales* were isolated from different clinical samples and *K. pneumoniae* was the most frequent with a rate of 66.3% (171/258). Comparable findings were reported by a previous study conducted in ICUs of our facility, where *K. pneumoniae* represented (69.2%) [20]. The high frequency of *K. pneumoniae* can be attributed to its capacity to colonize different body sites of patients in ICU, as well as equipment and instruments, and the outstanding capacity to accumulate several virulence and drug resistance determinants [21].

Of the isolated *Enterobacterales,* 46.5% were carbapenem resistant which is concordant with the finding of **El-Sweify et al.** who stated a carbapenem resistance rate of 44.3% in *Enterobacerales* [22]. On the other hand, a higher rate was previously recorded in different ICUs in our facility in (81%) [23] and in Egyptian cancer patients (89.6%) [24]. Prevalence rates of carbapenem resistance in *Enterobacterales* may differ among several studies as a result of several factors including, the studied population and the carbapenem prescribing practice, in addition to the efficacy of containment measures adopted by each facility [25]. Unsurprisingly, resistance to carbapenems is increasing in Egypt; they are widely

used as empiric therapy as a result of high prevalence of ESBL producing GNB in both hospital and community settings, being safe and well-tolerated [26]. All CR *Enterobacterales* isolates in the present study were MDR. Similar results were reported by other Egyptian studies [20, 27, 28].

Our results showed that 88.3% of the isolates harbored one or more of the tested carbapenemase genes with high prevalence of MBLs (76.7%, 92/120); bla_{NDM} was the most detected MBL in 80% of *E. coli* isolates, 75% of *K. pneumoniae* isolates and 33.3% of *P. mirabilis* isolates, while bla_{IMP} and bla_{VIM} were found in *K. pneumoniae* only (4.5% and 2.3%) respectively. Bla_{OXA-48} was the only serine carbapenemase gene detected (45% 54/120) while 40 isolates (33.3%) co-harbored both bla_{NDM} and $bla_{OXA-48.}$ On the other hand, 14 (11.7%) of the isolates didn't harbor any of the tested carbapenemase genes; other mechanisms such as ESBL production together with disruption in porin expression can result in carbapenem resistance in these isolates [29]. Meanwhile, none of the tested isolated harbored bla_{kpc}.

The reported epidemiology of β-lactamases in our region and the high prevalence of ESBL and OXA-48 like carbapenemases strongly nominated ceftazidime-avibactam, among the new β-lactam/β-lactamase inhibitor combinations released in the last decade, to be a safe and effective alternative to colistin based combinations for treatment of infections caused by a considerable percentage of carbapenem resistant *Enterobacterales.* Unfortunately, our results demonstrated higher rates of MBL production either alone or combined to OXA-48 (**Table 2**). Class B (MBLs) are not inhibited by avibactam and can hydrolyze ceftazidime. Aztreonam, however, is stable to MBLs but is inactivated by ESBL and class C cephalosporinases. Thus, a combination of ceftazidime-avibactam and aztreonam can be active against MBL producers [30].

As regards susceptibility of CZA tested against CR isolates, 90 % of 120 CR isolates were CZA resistant and the resistance rate increased in MBL producers to 100%, which is near to the rates reported by older studies conducted in our facility [20, 31]. As for OXA-48 producers, 71.4% of them were resistant to CZA, despite the high susceptibility rates reported by some studies [32, 33]. Previous irrational use of the drug, that was not guided by the type of carbapenemase produced, might have driven the emergence of resistance in OXA-48 producers.

Ceftazidime-avibactam plus aztreonam *in vitro* activity on CR isolates was tested by broth disk elution method recently endorsed by CLSI with reported sensitivity and a specificity of 100% [34]. Susceptibility was detected in 72/120 isolates (60%) with higher susceptibility being observed in MBL producing group (59/ 92, 64.1%), all were CZA resistant, while in MBL non producers, 46.4% were susceptible to CZA plus ATM, all were sensitive to CZA. In other words, the addition of ATM didn't demonstrate an advantage over CZA alone in these MBL non producers. **(Table 3)**

Previous *in vitro* studies reported favorable outcomes of CZA-ATM combination; **Marshall et al.** demonstrated a synergistic bactericidal effect of the combination in 17 out of 21 MBL-producing *Enterobacterales* isolates by double disk synergy method [35]. Better results were reported by other studies where the effect of CZA combined with ATM tested by Etest strips method demonstrated high synergy rates (95-100%) [36, 37], also **Romina et al.** reported a positive synergy of 100% among double carbapenemase producers [33]. Meanwhile, real-life clinical data from a multicenter observational prospective study reported therapeutic advantage of this combination in patients with bloodstream infections due to MBL-producing *Enterobacterales* compared to other active antibiotics in terms of slower clinical failure rates at day 14, lower mortality rates at day 30, and shorter length of hospital stay [38].

However, and as usual, when new antibiotics are released, the development of resistance is inevitable; a plethora of resistance mechanisms to CZA has been described rendering the interpretation of phenotypic susceptibility results difficult. Substitutions in KPC-2, KPC3 and CTX-M β-lactamases have been widely reported, in addition to porin mutations and drug efflux that have been described as factors decreasing CZA activity [11,39-42]. OXA-48, unlike most carbapenemases, lacks significant hydrolytic activity on extended spectrum cephalosporins, including CAZ, but CAZ and CZA exposure led to single (P68A) and double (P68A, Y211S) amino acid substitutions in OXA-48 that led to increased flexibility within the OXA-48 structure, resulting in enhanced CAZ hydrolysis and resistance to avibactam hydrolytic activity that leads to CAZ inactivation [43].

Another, non β-lactamase mediated, mechanism of CZA resistance is penicillin binding protein 3 (PBP3) alteration by four amino-acid insertions after position 333, adjacent to the β-lactam drug binding site. PBP3 is the target for both ceftazidime and aztreonam, therefore these insertions can account for resistance to CZA alone and in combination with aztreonam independent of β-lactamase production [44]. In a related context, a recent study demonstrated that aztreonam/avibactam was superior to CZA plus ATM in time kill assays for NDM producing *K. pneumoniae* isolates. The authors speculated that the competitive binding of ceftazidime and aztreonam to PBP3, before ceftazidime become inactivated by NDM and lose its bactericidal effect, results in the lower combined effect of CZA plus ATM compared to aztreonam-avibactam [45].

Conclusion

Characterization of the molecular mechanisms behind the forementioned CZA resistance phenotypes is highly recommended. CZA-ATM combination can be considered for treating CR *Enterobacterale*s isolates but only for MBL producers and only after testing for *in vitro* susceptibility to the combination.

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