

RESEARCH

Open Access



# Ceftazidime-Avibactam plus aztreonam synergistic combination tested against carbapenem-resistant *Enterobacterales* characterized phenotypically and genotypically: a glimmer of hope

Rawan Taha<sup>1</sup>, Ola Kader<sup>1</sup>, Sherine Shawky<sup>1</sup> and Shahinda Rezk<sup>2\*</sup> 

## Abstract

**Background** Carbapenemase-producing *Enterobacterales* (CPE) show rapid global dissemination and pose a significant therapeutic challenge. This study aimed to characterize carbapenemase-producing *Klebsiella* spp. and *Escherichia coli* (*E. coli*) phenotypically and genotypically and evaluate the effect of ceftazidime/ avibactam plus aztreonam combination.

**Methods** A total of 219 *Klebsiella* species and 390 *E. coli* strains were isolated from clinical samples, in which 80 *Klebsiella* spp. and 20 *E. coli* isolates were resistant to tested carbapenems (imipenem, ertapenem, meropenem) by disk diffusion/broth dilution method and Vitek-2 compact system. MASTDISCS *Combi Carba plus* discs and real time PCR were used to determine type of carbapenemase phenotypically and genotypically, respectively. Interestingly, the synergistic effect between ceftazidime-avibactam (E-test) and aztreonam (disc) was tested against the CPE isolates.

**Results** Out of the carbapenem-resistant isolates, 76.25% *Klebsiella* spp. isolates were extensively drug-resistant (XDR) while 18.75% were pan drug-resistant (PDR), and 5% were multidrug-resistant (MDR). Regarding *E. coli*, 5% were PDR, 20% were MDR and 75% were XDR. More than one carbapenemase gene was detected in 99% of the isolates. In comparison between MAST-*Carba plus* discs and PCR results, sensitivity and specificity were (85.42–97.92%) in *Klebsiella* spp., and (69.64–100%) in *E. coli*, respectively. Moreover, a strong association was detected between both test results among *Klebsiella* spp. ( $p < 0.001$ ) and *E. coli* ( $p = 0.012$ ) isolates. Finally, ceftazidime-avibactam and aztreonam combination showed a synergistic effect in 98.8% of *Klebsiella* spp. and 95% of *E. coli*. All 16 PDR isolates showed synergy.

**Conclusion** This synergistic effect spots the light on new therapeutics for XDR and PDR CPE.

**Keywords** Carbapenem-resistant *Enterobacterales*, Carbapenemase types, Ceftazidime-Avibactam-Aztreonam, Synergy

\*Correspondence:

Shahinda Rezk  
shahinda.rezk@alexu.edu.eg

<sup>1</sup>Microbiology Department, Medical Research Institute, Alexandria University, Alexandria, Egypt.

<sup>2</sup>Lecturer of Molecular and Diagnostic Microbiology, Microbiology Department, Medical Research Institute, Alexandria University, 165 Horreya Avenue, Hadara, Alexandria, Egypt.



© The Author(s) 2023, corrected publication 2023. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

## Introduction

Antimicrobial resistance is recognized as one of the significant global health threats by the World Health Organization [1]. *E. coli* and *Klebsiella* spp. are considered to be the most important *Enterobacteriales* that commonly cause health-associated and community-associated infections [2]. Among multidrug-resistant bacteria, carbapenemase-producing *Enterobacteriales* (CPE) show rapid global dissemination and pose a significant therapeutic challenge [1, 3]. This is besides the high threat imposed by extensive drug resistant (XDR) and pan drug resistant (PDR) CPE. Multidrug-resistance is defined as resistance to at least one agent in three or more antimicrobial classes, XDR is defined as resistance to at least one agent in all but two or fewer antimicrobials, and PDR is defined as resistance to all agents in all antimicrobial classes [4].

Detection of carbapenemases rapidly is crucial to ensure the management and treatment of clinical infections [5]. These carbapenemases include Ambler class A (KPC, and GES enzymes) and class D (OXA-48-like enzymes) which are serine carbapenemases, and class B (Metallo carbapenemases); (NDM, VIM, and IMP enzymes) [6]. Phenotypic methods are commonly used in routine laboratory practice. These methods detect carbapenemase activity regardless of carbapenemase type, whereas inhibitor-based methods such as MASTDISCS *Combi Carba plus* disc (MAST-*Carba plus*; Mast Group Ltd., UK) can be used to differentiate between carbapenemase types [7]. However, PCR remains the gold standard for the detection of carbapenemase types [5].

With the advent of a new effective therapeutic option; ceftazidime-avibactam, a 3rd generation cephalosporin combined with a  $\beta$ -lactamase inhibitor, has a wide-spectrum activity against serine  $\beta$ -lactamases but is hydrolyzed by metallo  $\beta$ -lactamases [8]. In contrast, the aztreonam, monobactam, is stable in the presence of metallo  $\beta$ -lactamases but susceptible to hydrolysis by serine  $\beta$ -lactamases [3]. Thus, the combination of ceftazidime-avibactam and aztreonam is considered to have a synergistic therapeutic effect against CPE [9].

This study aims to evaluate the performance of MASTDISC *Combi Carba plus* discs for the detection of carbapenemase types and compare it to PCR results. Moreover, synergy between ceftazidime-avibactam plus aztreonam will be tested in highly resistant CPE.

## Materials and methods

### Bacterial isolates

A total of 219 *Klebsiella* species isolates and 390 *E. coli* isolates were identified by conventional biochemical methods then antibiotic susceptibility testing (AST) was performed using disc diffusion method at Microbiology Department, Medical Research Institute, Alexandria

University between June 2021 and January 2022 [10]. Imipenem (10  $\mu$ g), ertapenem (10  $\mu$ g), and meropenem (10  $\mu$ g) were used (Oxoid, Basingstoke, England).

Carbapenem-resistant isolates; 80 (36.53%) *Klebsiella* spp. and 20 (5.13%) *E. coli*; were subjected to full identification and AST to determine minimum inhibitory concentration (MIC) of carbapenems using VITEK<sup>®</sup>2 system (bioMérieux, France).

### Phenotypic detection of carbapenemase enzymes

MASTDISCS<sup>®</sup> *Combi Carba plus* disc method (MAST-*Carba plus*; Mast Group Ltd., UK) was performed according to manufacturer's instructions. The discs were placed on Muller-Hinton agar plate inoculated with 0.5 McFarland test strain. Plates were incubated at 37 °C for 18 to 24 h [7].

MAST-*Carba plus* consists of carbapenem alone (disc A) and in combination with a MBL inhibitor (disc B), a KPC inhibitor (disc C), an AmpC inhibitor (disc D), and temocillin in combination with a MBL inhibitor (disc E). The interpretation of the test is as follows: the zone of inhibition of the carbapenem disc (A) was compared to the zones of inhibition of each of the carbapenem plus inhibitor discs (B, C, and D). If disc B only shows a zone difference  $\geq 5$  mm than disc A, the organism was recorded as demonstrating M $\beta$ L activity. If disc C only shows a zone difference  $\geq 5$  mm than disc A (D-A should be  $< 5$  mm), the organism was recorded as demonstrating KPC activity. If discs C and D both show significant zone differences ( $\geq 5$  mm) compared to disc A, the organism was recorded as demonstrating AmpC activity coupled with porin loss (impermeability). If disc E shows a zone of inhibition of  $\leq 10$  mm, the organism was recorded as demonstrating OXA-48 activity [7].

### Genotypic detection of carbapenemase genes

Bacterial DNA was extracted using Thermo-Scientific Gene-JET Genomic DNA Purification Kit (Thermo-Fisher, Vilnius, Lithuania) according to manufacturer's instructions. Maxima SYBR Green qPCR Master Mix (2X) (Thermo-Fisher, Vilnius, Lithuania) was applied for detection of *bla*<sub>NDM</sub>, *bla*<sub>VIM</sub>, *bla*<sub>IMP</sub>, *bla*<sub>KPC</sub>, *bla*<sub>GES</sub> and *bla*<sub>OXA-48-like</sub> using Mx3000P<sup>™</sup> Real Time PCR thermal cycler instrument (Stratagene, USA). A total of 6  $\mu$ L of template DNA was added to the reaction mix which was composed of 10 $\mu$ L Maxima SYBR Green master mix and 0.6  $\mu$ L for each forward and reverse primers mentioned in Table 1 [11–13] and 2.8  $\mu$ L nuclease-free water. PCR started with an initial denaturation step at 95 °C for 10 min, followed by 45 cycles of DNA denaturation at 95 °C for 15 s, primer annealing at 55 °C for 30 s and primer extension at 72 °C for 30 s. Afterward, dissociation curve analysis was performed, consisting of 1 cycle

**Table 1** Primer sequences and amplicon sizes

Primer	Sequence	Gene	Expected product size, bp	References
KPC-F	CGTCTAGTTCGTGCTCTTG	<i>bla</i> <sub>KPC</sub>	232	[11]
KPC-R	CTTGTCATCCTTGTTAGGCG			
GES-F	CTATTACTGGCAGGGATCG	<i>bla</i> <sub>GES</sub>	594	[13]
GES-R	CCTCTCAATGGTGTGGGT			
NDM-F	GGTTTGGCGATCTGGTTTTC	<i>bla</i> <sub>NDM</sub>	621	[12]
NDM-R	CGGAATGGCTCATCACGATC			
IMP-F	GGAATAGAGTGGCTTAAYTC	<i>bla</i> <sub>IMP</sub>	232	[11]
IMP-R	TCGGTTTAAAYAAAACAACCACC			
VIM-F	GATGGTGTGGTGCATA	<i>bla</i> <sub>VIM</sub>	390	[11]
VIM-R	CGAATGCGCAGCACCCAG			
OXA-48-F	GCGTGGTTAAGGATGAACAC	<i>bla</i> <sub>OXA-48</sub>	438	[11]
OXA-48-R	CATCAAGTTCAACCCAACCG			

at 95 °C for 1 min, then 55 °C for 30 s, and finally 95 °C for 30 s.

#### E-test-Disc diffusion modified method

Ceftazidime-avibactam (CAZ-AVI) E-test (Liofilchem®, Italy), containing CAZ (0.016–256 µg/mL)—AVI (4 µg/mL), and aztreonam (ATM) disc (30 µg, Oxoid, Basingstoke, England) were used to determine CAZ-AVI and ATM synergy for the 100 isolates in triplicates. After adjustment to 0.5 McFarland, the tested bacterial suspension was uniformly streaked over the surface of Muller Hinton agar plates. A CAZ-AVI E-test strip was placed 15 mm from the disc such that the center of the disc was placed parallel to the sensitivity breakpoint of the CAZ-AVI E-test (8 µg/mL). Plates were incubated at 37 °C for 16 to 18 h. Two approaches were used to identify synergy, the method used depended on the pattern detected. First, the presence of an inverse-D was interpreted as synergy, and that was a qualitative approach. Second, a quantitative approach was used as the zone radius for ATM disc alone was measured, and MIC for CAZ/AVI was read using breakpoints from CLSI M100 [3, 10].

#### Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) Qualitative data were described using numbers and percentages. Significance of the obtained results was judged at the 5% level ( $p < 0.05$ ). The used tests were chi-square test, for categorical variables, to compare different groups, and Fisher's Exact in correction for chi-square when more than 20% of the cells have an expected count of less than 5.

The sensitivity was calculated from the number of MBLs-, KPC-, and OXA-48-possessing organisms that

were correctly determined. The specificity was calculated from the number of non-MBLs-, non-KPC- or non-OXA-48-possessing organisms that were correctly determined. The positive predictive value (PPV) was calculated from the probability that MBLs-, KPC-, and OXA-48-possessing organisms detected by MAST-*Carba plus* indeed do possess these enzymes. The negative predictive value (NPV) was calculated from the probability that non-MBLs-, non-KPC- or non-OXA-48-possessing organisms detected by MAST-*Carba plus* indeed do not possess these enzymes.

#### Results

A total of 219 *Klebsiella* species and 390 *E. coli* strains were isolated from clinical samples, in which 80 *Klebsiella* spp. and 20 *E. coli* were found to be carbapenem-resistant. Among the 80 carbapenem-resistant *Klebsiella* species isolates, 79 (98.75%) were identified as *Klebsiella pneumoniae* ssp *pneumoniae* while 1 *Klebsiella* species (1.25%) was identified as *Klebsiella pneumoniae* ssp *ozaeanae*. The majority of *Klebsiella* species were isolated from blood cultures 47 (58.8%) while the majority of *E. coli* were isolated from aspirate 7 (35%). Distribution of the isolates among clinical specimens is shown in Table 2.

#### Antimicrobial susceptibility testing

All 80 carbapenem-resistant *Klebsiella* species isolates were resistant to penicillins and cephalosporins group, 79 (98.75%) isolates were resistant to aztreonam. High resistance rates were detected for aminoglycosides, quinolones, and sulfonamides. In that, 77 (96.25%) isolates were resistant to tobramycin, 62 (77.5%) isolates were resistant to amikacin, 57 (71.25%) isolates were resistant to gentamycin, 65 (81.25%) isolates were resistant to ciprofloxacin, and 61 (76.25%) isolates were resistant to

**Table 2** Distribution of carbapenem-resistant *Klebsiella* species and *E. coli* among clinical specimens

Specimen	<i>Klebsiella</i> species isolates		<i>E. coli</i> isolates		Total number of isolates
	Nº	%	Nº	%	
Blood cultures	47	58.8	4	20	51
Wound swabs	13	16.3	2	10	15
Urine	7	8.8	6	30	13
Fluid aspirate	4	5	7	35	11
Sputum	6	7.5	0	0	6
Bronchoalveolar lavage (BAL)	2	2.5	0	0	2
Central venous catheter (CVC)	1	1.3	0	0	1
Pus aspirate	0	0	1	5	1
Total	80	100	20	100	100

sulfonamides. Colistin had the lowest resistance rate 19 (23.75%), as shown in Fig. 1.

All the 20 carbapenem-resistant *E. coli* isolates were resistant to the penicillin group, ceftazidime, β-lactam/β-lactamase inhibitors, and sulfonamides. Nineteen (95%) isolates were resistant to ciprofloxacin, 15 (75%) isolates were resistant to cefepime and aztreonam, and only 3 (15%) were resistant to colistin. Ten (50%) isolates were resistant to tobramycin, 9 (45%) isolates were resistant to gentamycin, and 3 (15%) isolates were resistant to amikacin, as shown in Fig. 2.

The majority of the *Klebsiella* spp. isolates were XDR (76.25%), while 18.75% were PDR, and only 5% were

MDR. On the other hand, only 5% *E. coli* showed pan drug-resistance, while the majority were either MDR (20%) or XDR (75%).

**Prevalence of carbapenemase genes**

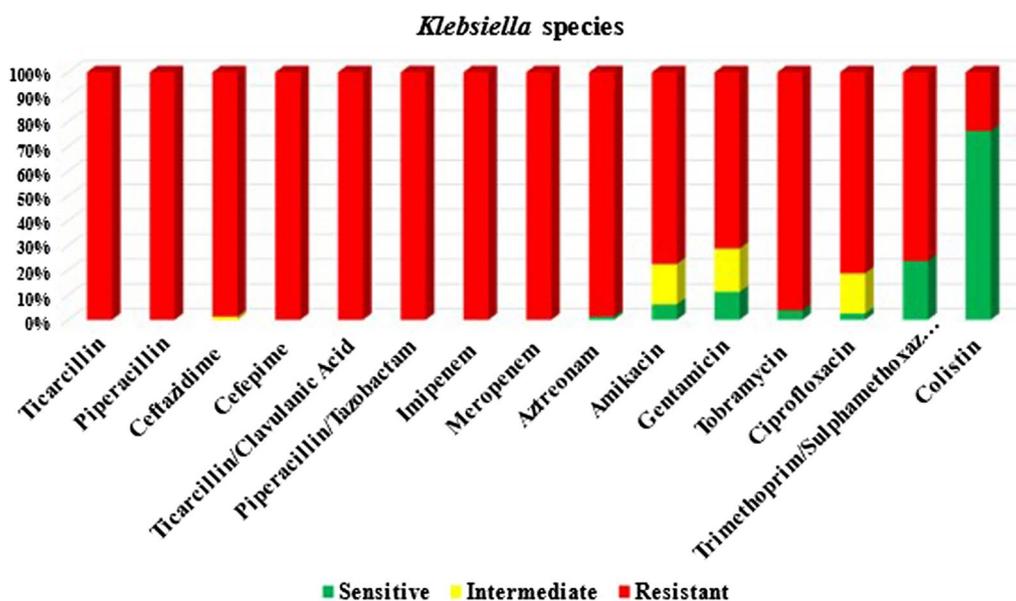
Carbapenemase production was detected in all (100%) *Klebsiella* species and *E. coli*, presented in Table 3. Among the 80 *Klebsiella* isolates, the most common gene identified was *bla<sub>NDM</sub>* (91.25%). On the other hand, *bla<sub>VIM</sub>* was detected in all *E. coli* isolates 100%. *bla<sub>IMP</sub>* wasn't detected in both genera.

**Phenotypic detection of carbapenemase enzymes by MAST-Carba plus**

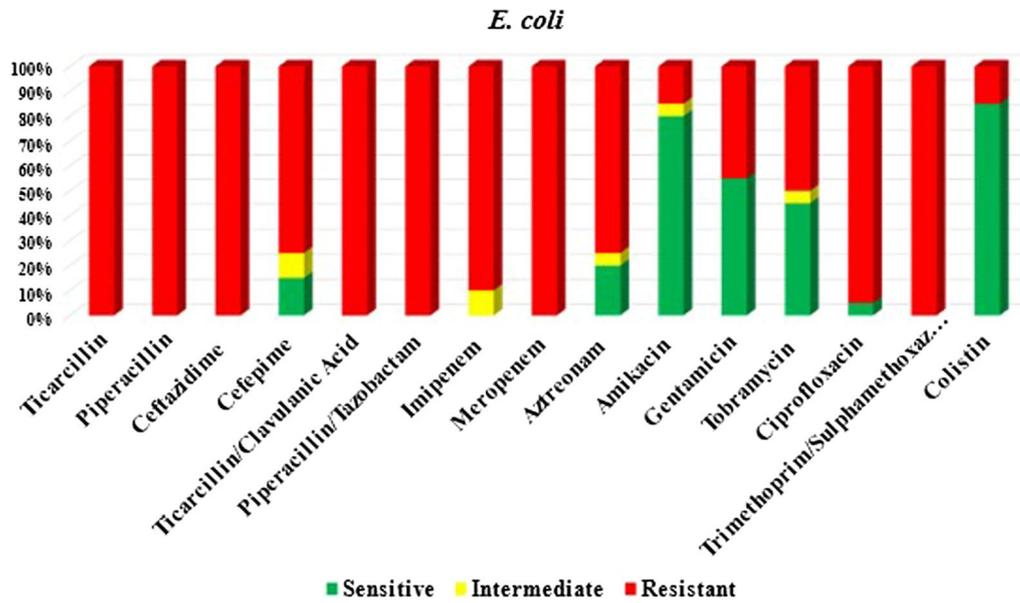
PCR results were the gold standard to evaluate the performance of MAST-Carba plus for detection of carbapenemase enzymes. Table 4 and Fig. 3 show the results of MAST-Carba plus for detection of carbapenemase enzymes.

For *Klebsiella* species isolates, the sensitivity of the MAST-Carba plus for KPC producers was 55.56%, the specificity was 98.60%, the PPV was 83.33% and the NPV was 94.60%. For MBL producers, the sensitivity was 93.51%, the specificity was 66.67%, the PPV was 98.63% and the NPV was 28.57%. For OXA-48 producers, the sensitivity was 79.31%, the specificity was 100%, the PPV was 100% and the NPV was 64.71%.

For *E. coli* isolates, the sensitivity of the MAST-Carba plus for KPC producers was 64.71%, the specificity was 100%, the PPV was 100% and the NPV was 33.33%. For MBL producers, the sensitivity was 95%,



**Fig. 1** Antimicrobial susceptibility testing of carbapenem-resistant *Klebsiella* species isolates



**Fig. 2** Antimicrobial susceptibility testing of carbapenem-resistant *E. coli* isolates

**Table 3** Genotypic detection of carbapenemase genes among carbapenem-resistant *Klebsiella* species and *E. coli* isolates

Carbapenemase genes	<i>Klebsiella</i> species (n = 80)		<i>E. coli</i> (n = 20)		$\chi^2$	P
	No	%	No	%		
<i>bla</i> <sub>KPC</sub>	9	11.25%	17	85%	45.231*	<0.001*
<i>bla</i> <sub>GES</sub>	56	70%	18	90%	3.326	0.068
<i>bla</i> <sub>NDM</sub>	73	91.25%	19	95%	0.306	<sup>FE</sup> p = 1.000
<i>bla</i> <sub>IMP</sub>	0	0.0%	0	0.0%	–	–
<i>bla</i> <sub>VIM</sub>	55	68.75%	20	100%	8.333*	0.004*
<i>bla</i> <sub>OXA-48</sub>	58	72.5%	19	95%	4.574*	0.038*

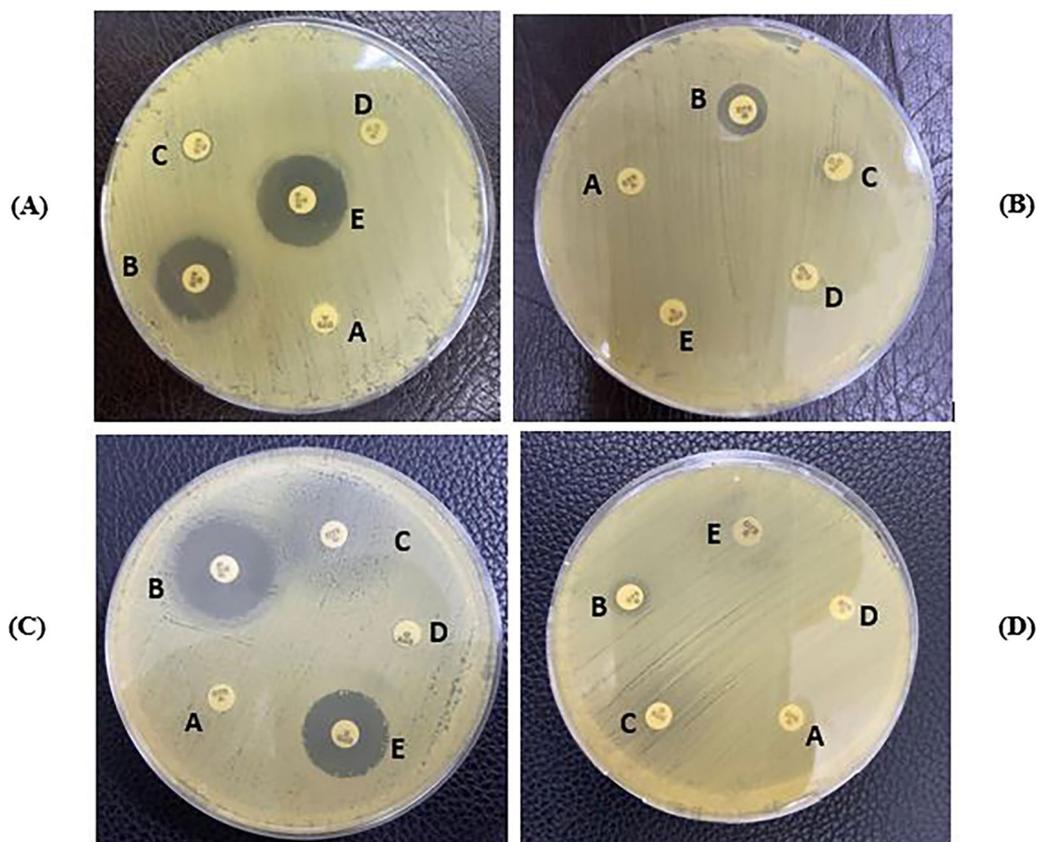
$\chi^2$ : Chi square test FE: Fisher Exact

p: p value for comparing between *Klebsiella* species and *E. coli*

\*: Statistically significant at  $p \leq 0.05$

**Table 4** Pattern of distribution of carbapenemase enzymes by using MAST-Carba plus

<i>Klebsiella</i> species (n = 80)		<i>E. coli</i> (n = 20)	
Carbapenemase enzymes	Total no. of isolates (%)	Carbapenemase enzymes	Total no. of isolates (%)
OXA-48, and Metallo $\beta$ -lactamases	38 (47.5%)	Metallo $\beta$ -lactamases, and KPC	6 (30%)
Metallo $\beta$ -lactamases	30 (37.5%)	Metallo $\beta$ -lactamases	5 (25%)
OXA-48, Metallo $\beta$ -lactamases, and KPC	4 (5%)	OXA-48, and Metallo $\beta$ -lactamases	4 (20%)
OXA-48	4 (5%)	OXA-48, Metallo $\beta$ -lactamases, and KPC	3 (15%)
None	2 (2.5%)	OXA-48, and KPC	1 (5%)
Metallo $\beta$ -lactamases, and KPC	1 (1.25%)	OXA-48, Metallo $\beta$ -lactamases, KPC, and Amp C	1 (5%)
KPC	1 (1.25%)		



**Fig. 3** Results of MAST-*Carba plus* in detection of carbapenemase-producing isolates. **A** Shows a zone of inhibition of  $\geq 5$  mm around disc B compared to that of disc A indicating M $\beta$ L activity, while discs C and D show no zones of inhibition differences compared to that of disc A indicating absence of KPC and AmpC activities and disc E shows a zone of inhibition of  $> 10$  mm indicating absence of OXA-48 activity. **B** Shows a zone of inhibition of  $\geq 5$  mm around disc B compared to that of disc A indicating M $\beta$ L activity, and disc E shows a zone of inhibition  $\leq 10$  mm indicating OXA-48 activity, while discs C and D show no zones of inhibition differences compared to that of disc A indicating absence of KPC and AmpC activities. **C** Shows a zone of inhibition of  $\geq 5$  mm around disc B compared to that of disc A indicating M $\beta$ L activity, disc C shows a reduction of growth around the disc which considered to be indicative for KPC activity while disc D shows no zone of inhibition differences compared to that of disc A indicating absence of AmpC activity, and disc E shows a zone of inhibition  $> 10$  mm indicating absence of OXA-48 activity. **D** Shows a zone of inhibition of  $\leq 10$  mm around disc E indicating OXA-48 activity, while discs B, C and D show no differences in zones of inhibition compared to that of disc A indicating absence of M $\beta$ L, KPC and AmpC activities

the specificity of test was 0.00%, the PPV was 100% and the NPV was 0.00%. For OXA-48 producers, the sensitivity was 47.37%, the specificity of test was 100%, the PPV was 100% and the NPV was 9.10%. The true positive, true negative, false positive, and false negative values of MAST-*Carba plus* in comparison to PCR results were shown in Table 5.

The highest sensitivity in the present study was for MBLs detection in *E. coli* (95%) and the lowest was for KPC detection in *Klebsiella* isolates (55.56%). There was a strong statistically significant association detected between PCR and MAST-*Carba plus* results among *Klebsiella* spp. ( $p < 0.001$ ) and *E. coli* ( $p = 0.012$ ) isolates.

#### Ceftazidime-Avibactam and aztreonam synergy testing

For *Klebsiella* spp. isolates, synergy was observed between CAZ/AVI E- test and ATM disc in 79 (98.75%) out of 80 isolates, 76 (95%) isolates were resistant to both CAZ/AVI alone and ATM alone, while 3 (3.75%) isolates were susceptible to CAZ/AVI E-test (MIC = 1.5  $\mu$ g/mL) but resistant to ATM disc. No synergy was detected in 1 (1.25%) isolate, which was susceptible to ATM disc alone (26 mm).

For *E. coli* isolates, synergy was observed between CAZ/AVI E-test and ATM disc in 19 (95%) out of the 20 isolates, 14 (70%) isolates were resistant to both CAZ/AVI alone and ATM alone, while 4 (20%) isolates were susceptible to ATM disc but resistant to CAZ/AVI E-test

**Table 5** True positive, true negative, false positive, and false negative values of MAST-*Carba plus* in comparison to PCR results

<i>Klebsiella</i> species isolates				<i>E. coli</i> isolates				
KPC		Genotypic		Total	KPC	Genotypic		Total
		Positive	Negative			Positive	Negative	
		No	No			No	No	
Phenotypic	Positive	5	1	6	Phenotypic	Positive	0	11
	Negative	4	70	74		Negative	3	9
Total		9	71	80	Total		3	20
MBL		Genotypic		Total	MBL	Genotypic		Total
		Positive	Negative			Positive	Negative	
		No	No			No	No	
Phenotypic	Positive	72	1	73	Phenotypic	Positive	0	19
	Negative	5	2	7		Negative	0	1
Total		77	3	80	Total		0	20
OXA-48		Genotypic		Total	OXA-48	Genotypic		Total
		Positive	Negative			Positive	Negative	
		No	No			No	No	
Phenotypic	Positive	46	0	46	Phenotypic	Positive	0	9
	Negative	12	22	34		Negative	1	11
Total		58	22	80	Total		1	20

and 1 (5%) isolate was susceptible to CAZ/AVI E-test (MIC=1.5 µg/mL) but resistant to ATM disc. One (5%) isolate was resistant to both ATM disc and CAZ/AVI E-test with no synergy observed between them.

All the pan drug-resistant tested isolates showed synergy between CAZ/AVI E-test and ATM disc. Different synergy patterns are shown in Fig. 4.

## Discussion

Carbapenems are often considered the last-line antibiotics used for the treatment of MDR *Enterobacterales* infections since they are stable even in response to ESBLs and AmpC enzymes. However, carbapenem resistance is progressively reported among *Enterobacterales* in recent years. Therefore, the rapid spread of CPE is considered to be a serious global health problem, and the early detection of CPE is crucial for targeted antimicrobial therapy and for timely and effective infection control [5].

This study detected a highly statistically significant difference between the prevalence of carbapenem-resistance among *Klebsiella* spp. isolates (80/219, 36.53%) and *E. coli* isolates (20/390, 5.13%), ( $p < 0.001$ ). Several recent studies have reported similar results, in which carbapenem resistance was more prevalent in *Klebsiella* spp. than *E. coli* [8, 14–19].

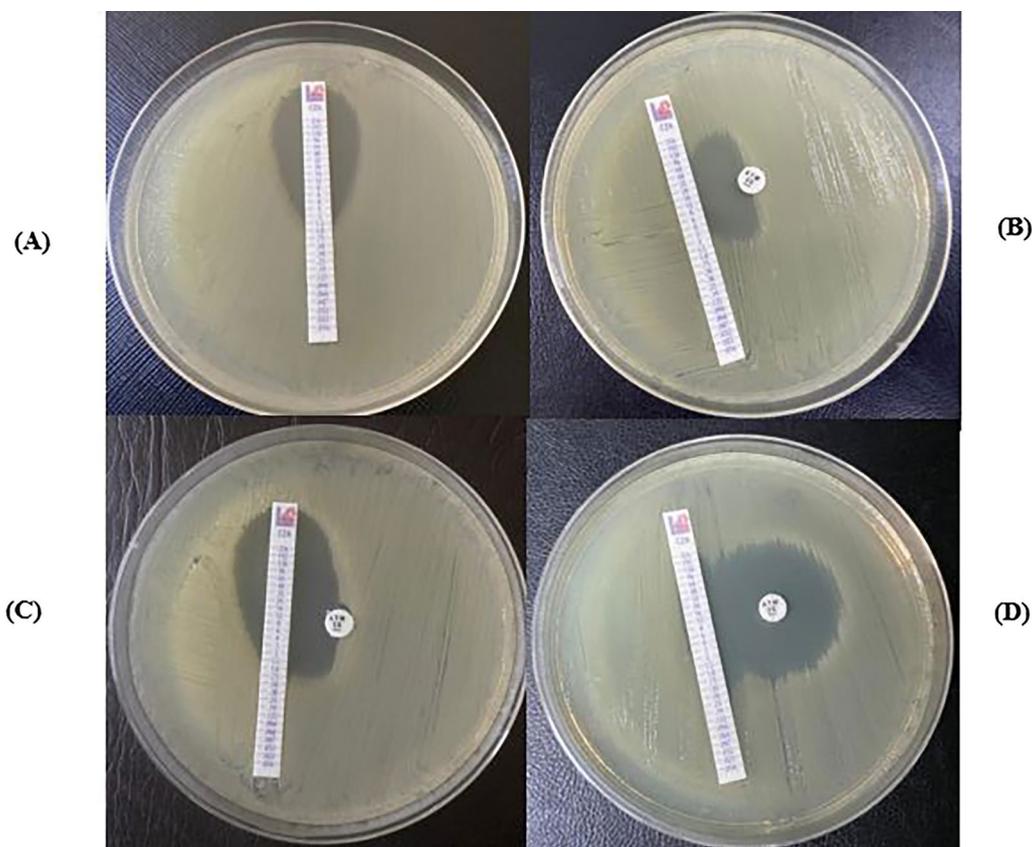
The prevalence of  $\beta$ -lactam resistant *Klebsiella* spp. and *E. coli* isolates is increasing gradually. This may be due to misuse of antibiotics and repeated exposure to  $\beta$ -lactam drugs such as penicillin, third and fourth-generation cephalosporins, and carbapenems [20].

Extensively and pan drug-resistant isolates are increasingly being detected globally. The development of pan drug-resistant isolates is worrisome because they are practically not affected by any antibiotic, therefore higher rates of morbidity and mortality are expected. Regarding carbapenem-resistant *Klebsiella* spp. isolated in this study, 76.25% were XDR, 18.75% were PDR, and only 5.00% were MDR, while among *E. coli* isolates, 75% were XDR, 20% were MDR, and 5% showed PDR. An alarming elevated prevalence of MDR, XDR, and PDR isolates was also revealed by many up-to-date studies [20–23].

The carbapenemase genes are mainly located on mobile genetic elements, facilitating their spread among different bacteria [13]. The prevalence of carbapenemase genes is highly affected by geographic region and has been associated with serious epidemics that represent a major therapeutic challenge [24].

In light of this, rapid detection of CPE producers might be the best way to prevent their spread. PCR is always used as the gold standard technique for the detection of carbapenemase genes [11]. In the current study and in another Egyptian study [25], *bla*<sub>NDM</sub> gene followed by *bla*<sub>OXA-48</sub> gene showed the highest prevalence in the *Klebsiella* isolates, while *bla*<sub>IMP</sub> gene was not detected among all isolates.

Furthermore, other studies in different geographical regions reported different gene distributions in *Klebsiella* spp. isolates. In a European survey, Grundmann et al. [14] reported that the most frequently detected carbapenemases were KPC enzymes. Consistent results



**Fig. 4** Pattern of susceptibility between CAZ-AVI E-test and ATM disc. **A** Shows MIC reading to CAZ/AVI alone (MIC = 1.5 µg/mL, reported as 2 µg/mL) **B** Shows inverse D-zone demonstrating the synergy between CAZ/AVI E-test and ATM disc **C** Shows susceptibility to CAZ/AVI E-test alone and synergy between CAZ/AVI E-test and ATM disc **D** Shows susceptibility to ATM disc with 30 mm zone diameter and synergy between CAZ/AVI E-test and ATM disc

were also declared by Han et al. [26] in China. Furthermore, in Iraq; Haji et al. [20] reported *bla*<sub>VIM</sub> gene to be the most prevalent gene detected and no *bla*<sub>KPC</sub> gene was found. Regarding Saudi Arabia, Alhazmi et al. [27] found that *bla*<sub>OXA-48</sub> gene was the most common gene detected, and consistent with our results all of their isolates were negative for the *bla*<sub>TMP</sub> gene.

In the present study, *bla*<sub>VIM</sub> gene was detected all carbapenem-resistant *E. coli* isolates, whereas *bla*<sub>TMP</sub> gene was not detected at all. Variations in the distribution of the carbapenemase genes was also recognized among the *E. coli* isolates in various countries [14, 20, 26, 28–33].

More than one carbapenemase gene was detected among 79/80 *Klebsiella* isolates and in all *E. coli* isolates. These findings were similarly stated by other researchers [18, 20, 30, 31, 34–36]. Hereby, the high prevalence of more than one carbapenemase gene in *Enterobacteriales* isolates may illustrate the hastily propagating resistance. Consequently, determination of the mobile carbapenemase genes became an urge to contain this elevating

resistance and to apply strict infection control measures in order to save lives.

In developing countries, many laboratories have no accessible PCR instruments due to its high cost. Accordingly, this study aimed to evaluate MAST-*Carba plus* in relation to PCR as a gold standard for the detection of carbapenemase-producing *Klebsiella* species and *E. coli* isolates. A statistically significant association was found between MAST-*Carba plus* and PCR results regarding detection of carbapenemase enzymes among carbapenem-resistant *Klebsiella* species ( $p < 0.001$ ), and among carbapenem-resistant *E. coli* isolates ( $p = 0.012$ ). We agree with Doyle et al. [37] who stated that if a clinical laboratory can't afford to perform molecular tests, MAST-*Carba plus* can be used to confirm the presence of carbapenemase.

Resistance to safe and broad-spectrum drugs such as carbapenems often poses a challenge in antibiotic therapy, especially for MBLs producing-bacteria. Due to the very high level of resistance and high prevalence of carbapenemase genes, one of our main objectives was to

evaluate a new synergetic combination between CAZ/AVI and ATM that would treat carbapenem-resistant *Enterobacteriales*. Promising results were revealed. Synergy was observed between CAZ/AVI E-test and ATM disc in 79 (98.75%) out of the 80 carbapenem-resistant *Klebsiella* species isolates, and in 19 (95%) out of the 20 carbapenem-resistant *E. coli* isolates. A glimmer of hope emerged when all the 16 PDR showed a synergistic effect.

Few recent studies had also demonstrated the synergy between CAZ/AVI and ATM in MDR, XDR and PDR isolates by using different testing techniques. [3, 38–42].

In the present study, among the 100 carbapenem-resistant *Klebsiella* species and *E. coli* isolates, 90 (90%) were resistant to both CAZ/AVI E-test alone and ATM disc alone but showed synergy between CAZ/AVI and ATM, these isolates produce both metallo  $\beta$ -lactamases and serine  $\beta$ -lactamases. Similarly, Jayol et al. [43] who used E-test strips method reported that the combination of CAZ/AVI and ATM was synergistic for all their 63 isolates, and stated that this combination is effective against MBL-producing *Klebsiella pneumoniae* and particularly against isolates producing more than one carbapenemase genes. Therefore, the combination of CAZ/AVI and ATM is considered an effective therapeutic option particularly against *Klebsiella* species and *E. coli* isolates producing more than one carbapenemase gene of metallo  $\beta$ -lactamases and serine  $\beta$ -lactamases.

However, in vivo efficacy and safety of this regimen have to be evaluated. To the best of our knowledge, there was only one recent study conducted by Falcone et al. [9] to clinically compare the effect of CAZ/AVI and ATM combination to other active antibiotics on the outcome of patients with bloodstream infections due to MBL-producing *Enterobacteriales* and showed that the treatment with CAZ/AVI and ATM was linked with lesser clinical failure at day 14, lower mortality at day 30, and shorter length of hospitalization.

## Conclusion

MAST-*Carba plus* is recommended to be used as a phenotypic screening method for detecting the carbapenemase enzymes in laboratories with no molecular resources. The enhanced activity observed with the combination of CAZ/AVI and ATM, compared to either agent alone, is promising and requires us to carefully consider the combination of CAZ/AVI and ATM as a new therapeutic option to treat infections caused by highly resistant *Enterobacteriales* strains. Nevertheless, we are in need for more in vivo studies.

## Author contributions

Dr RT: practical phenotypic and genotypic methods and draft manuscript preparation. Prof. Dr OK: conceptualization of the research and interpretation

of the results. Prof. Dr SS: collection of data and interpretation of the results. Dr SR: practical work, collection of data, and preparation of draft manuscript and final manuscript revision. All authors reviewed the results and approved the final version of the manuscript.

## Funding

Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB).

## Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

## Declarations

### Ethics approval and consent to participate

The study took the approval from the Ethics Committee of the Medical Research Institute, Alexandria University (IORG#: IORG0008812). An informed written consent was obtained from participating patients. It was conducted in accordance to Helsinki Declaration for studies on human subjects. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

### Consent for publication

Written informed consent was obtained from the patients for publication of this research article. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

### Competing interests

The authors declare that they have no competing interests.

Received: 18 January 2023 Accepted: 12 March 2023

Published: 21 March 2023

## References

- Sattler J, Brunke A, Hamprecht A. Systematic comparison of three commercially available combination disc tests and the zinc-supplemented carbapenem inactivation method (zCIM) for carbapenemase detection in *Enterobacteriales* isolates. *J Clin Microbiol*. 2021;59(9): e0314020.
- van Duin D, Doi Y. The global epidemiology of carbapenemase-producing *Enterobacteriaceae*. *Virulence*. 2017;8(4):460–9.
- Rawson TM, Brzeska-Trafny I, Maxfield R, Almeida M, Gilchrist M, Gonzalo X, et al. A practical laboratory method to determine ceftazidime-avibactam-aztreonam synergy in patients with New Delhi metallo-beta-lactamase (NDM)-producing *Enterobacteriales* infection. *J Global Antimicrobial Resist*. 2022;29:558–62.
- 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(3):268–81.
- Hu W, Li M, Lu W, Guo S, Li J. Evaluation of MASTDISCS combi Carba plus for the identification of metallo- $\beta$ -lactamases, KPC and OXA-48 carbapenemase genes in *Enterobacteriaceae* clinical isolates. *Lett Appl Microbiol*. 2020;70(1):42–7.
- Saito R, Koyano S, Dorin M, Higurashi Y, Misawa Y, Nagano N, et al. Evaluation of a simple phenotypic method for the detection of carbapenemase-producing *Enterobacteriaceae*. *J Microbiol Meth*. 2015;108:45–8.
- Ohsaki Y, Kubo R, Hobson J, Davies M, Osaka S, Hayashi W, et al. MAST-DISCS combi Carba plus, a simple method for discriminating carbapenemase-producing *Enterobacteriaceae*, including OXA-48-type producers. *Microbiol Immunol*. 2018;62(1):60–5.
- Zou C, Wei J, Shan B, Chen X, Wang D, Niu S. In vitro activity of ceftazidime-avibactam and aztreonam-avibactam against carbapenem-resistant *Enterobacteriaceae* isolates collected from three secondary hospitals in Southwest China between 2018 and 2019. *Infect Drug Resist*. 2020;13:3563–8.
- 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- $\beta$ -lactamase-producing *Enterobacteriales*. *Clinical Infect Dis*. 2021;72(11):1871–8.
10. Clinical and Laboratory Standards Institute [CLSI]. Performance standards for antimicrobial susceptibility testing, approved standard M100-S30. 31st ed. Wayne, PA.: CLSI; 2021.
  11. Nordmann P, Naas T, Poirel L. Global spread of Carbapenemase-producing *Enterobacteriaceae*. *Emerg Infect Dis*. 2011;17(10):1791–8.
  12. Poirel L, Walsh TR, Cuivillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. *Diagn Microbiol Infect Dis*. 2011;70(1):119–23.
  13. Monteiro J, Widen RH, Pignatari AC, Kubasek C, Silbert S. Rapid detection of carbapenemase genes by multiplex real-time PCR. *J Antimicrob Chemother*. 2012;67(4):906–9.
  14. Grundmann H, Glasner C, Albiger B, Aanensen DM, Tomlinson CT, Andrasević AT, et al. Occurrence of carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* in the European survey of carbapenemase-producing *Enterobacteriaceae* (EuSCAPE): a prospective, multinational study. *Lancet Infect Dis*. 2017;17(2):153–63.
  15. Kotb S, Lyman M, Ismail G, Abd El Fattah M, Girgis SA, Etman A, et al. Epidemiology of Carbapenem-resistant *Enterobacteriaceae* in Egyptian intensive care units using National Healthcare-associated Infections Surveillance Data, 2011–2017. *Antimicrob Resist Infect Control*. 2020;9(1):2.
  16. Sader HS, Carvalhaes CG, Arends SJR, Castanheira M, Mendes RE. Aztreonam/avibactam activity against clinical isolates of *Enterobacteriales* collected in Europe, Asia and Latin America in 2019. *J Antimicrob Chemother*. 2020;76(3):659–66.
  17. Hegazy EE, Bahey MG, Abo Hagar AM, Elkholy AA, Mohamed EA. Carbapenem-Resistant gram-negative *Bacilli* causing ventilator associated *Pneumonia*: Study of MASTDISCS Combi Carba Plus for detection of Carbapenemase producing *Enterobacteriales*. *Infect Drug Resist*. 2022;15:6331–42.
  18. Vamsi SK, Moorthy RS, Hemilamma MN, Chandra Reddy RB, Chandera-kant DJ, Sirikonda S. Phenotypic and genotypic detection of carbapenemase production among gram negative bacteria isolated from hospital acquired infections. *Saudi Med J*. 2022;43(3):236–43.
  19. Tian X, Zheng X, Sun Y, Fang R, Zhang S, Zhang X, et al. Molecular mechanisms and epidemiology of carbapenem-resistant *Escherichia coli* Isolated from Chinese patients during 2002–2017. *Infect Drug Resist*. 2020;13:501–12.
  20. Haji SH, Aka STH, Ali FA. Prevalence and characterisation of carbapenemase encoding genes in multidrug-resistant Gram-negative bacilli. *PLoS ONE*. 2021;16(11): e0259005.
  21. Al-Baz AA, Maarouf A, Marei A, Abdallah AL. Prevalence and antibiotic resistance profiles of carbapenem-resistant *Klebsiella Pneumoniae* isolated from tertiary care hospital Egypt. *Egyptian J Hos Med*. 2022;88(1):2883–90.
  22. He G, Huang J, Huang S, Sun J, Zhou Y, Tan H, et al. Risk factors affecting clinical outcome in patients with carbapenem-resistant *K pneumoniae*: a retrospective study. *Med Sci Monitor*. 2020;26:e925693.
  23. El-Badawy MF, El-Far SW, Althobaiti SS, Abou-Elazm FI, Shohayeb MM. The First Egyptian report showing the co-existence of bla (NDM-25), bla (OXA-23), bla (OXA-181), and bla (GES-1) among carbapenem-resistant *K. pneumoniae* clinical isolates genotyped by BOX-PCR. *Infect Drug Resist*. 2020;13:1237–50.
  24. Cui X, Zhang H, Du H. Carbapenemases in *Enterobacteriaceae*: detection and antimicrobial therapy. *Front Microbiol*. 2019;10:1823.
  25. Tawfik MM, Alshareef WA, Bendary HA, Elmahlawy H, Abdullal AK. The emergence of carbapenemase bla<sub>NDM</sub> genotype among carbapenem-resistant *Enterobacteriaceae* isolates from Egyptian cancer patients. *Eur J Clin Microbiol Infect Dis*. 2020;39(7):1251–9.
  26. Han R, Shi Q, Wu S, Yin D, Peng M, Dong D, et al. Dissemination of carbapenemases (KPC, NDM, OXA-48, IMP, and VIM) among carbapenem-resistant *Enterobacteriaceae* isolated from adult and children patients in China. *Front Cell Infect Microbiol*. 2020;10:314.
  27. Alhazmi W, Al-Jabri A, Al-Zahrani I. The molecular characterization of nosocomial carbapenem-resistant *Klebsiella pneumoniae* Co-Harboring bla<sub>NDM</sub> and bla<sub>OXA-48</sub> in Jeddah. *Microbiol Res*. 2022;13(4):753–64.
  28. Makhariya RR, El-Kholy I, Hetta HF, Abdelaziz MH, Hagagy FI, Ahmed AA, et al. Antibiogram and genetic characterization of carbapenem-resistant gram-negative pathogens incriminated in healthcare-associated infections. *Infect Resist*. 2020;13:3991–4002.
  29. Naim H, Rizvi M, Gupta R, Azam M, Taneja N, Shukla I, et al. Drug resistance and molecular epidemiology of carbapenem resistant gram-negative *Bacilli* isolates. *J Global Infect Dis*. 2018;10(3):133–9.
  30. Abdelaziz NA. Phenotype-genotype correlations among carbapenem-resistant *Enterobacteriales* recovered from four Egyptian hospitals with the report of SPM carbapenemase. *Antimicrob Resist Infect Control*. 2022;11(1):13.
  31. Govindaswamy A, Bajpai V, Khurana S, Batra P, Mathur P, Malhotra R. Prevalence and characterization of carbapenemase-producing *Escherichia coli* from a tertiary care hospital in India. *J Global Infect Dis*. 2019;11(3):123–4.
  32. Ortega A, Sáez D, Bautista V, Fernández-Romero S, Lara N, Aracil B, et al. Carbapenemase-producing *Escherichia coli* is becoming more prevalent in Spain mainly because of the polyclonal dissemination of OXA-48. *J Antimicrob Chemother*. 2016;71(8):2131–8.
  33. Jomehzadeh N, Jahangirimehr F, Chegeni SA. Virulence-associated genes analysis of carbapenemase-producing *Escherichia coli* isolates. *PLoS ONE*. 2022;17(5): e0266787.
  34. Ragheb SM, Tawfik MM, El-Kholy AA, Abdullal AK. Phenotypic and genotypic features of *Klebsiella pneumoniae* harboring carbapenemases in Egypt: OXA-48-like carbapenemases as an investigated model. *Antibiotics*. 2020. <https://doi.org/10.3390/antibiotics9120852>.
  35. Çalik Ş, Kansak N, Aksaray S. Phenotypic detection of carbapenemase production in carbapenem-resistant isolates with the rapid carbapenemase detection method (rCDM). *J Microbiol Methods*. 2022;200: 106536.
  36. Gatyia Al-Mayahie SM, Al-Guranie DRdT, Hussein AA, Bachai ZA. Prevalence of common carbapenemase genes and multidrug resistance among uropathogenic *Escherichia coli* phylogroup B2 isolates from outpatients in Wasit Province/Iraq. *PLoS ONE*. 2022;17(1):e0262984.
  37. Doyle D, Peirano G, Lascols C, Lloyd T, Church DL, Pitout JD. Laboratory detection of *Enterobacteriaceae* that produce carbapenemases. *J Clin Microbiol*. 2012;50(12):3877–80.
  38. Pragasam AK, Veeraraghavan B, Shankar BA, Bakthavatchalam YD, Mathuram A, George B, et al. Will ceftazidime/avibactam plus aztreonam be effective for NDM and OXA-48-Like producing organisms: lessons learnt from In vitro study. *Indian J Med Microbiol*. 2019;37(1):34–41.
  39. Wenzler E, Deraedt MF, Harrington AT, Danizger LH. Synergistic activity of ceftazidime-avibactam and aztreonam against serine and metallo- $\beta$ -lactamase-producing gram-negative pathogens. *Diagn Microbiol Infect Dis*. 2017;88(4):352–4.
  40. Marshall S, Hujer AM, Rojas LJ, Papp-Wallace KM, Humphries RM, Spellberg B, et al. Can Ceftazidime-avibactam and aztreonam overcome  $\beta$ -lactam resistance conferred by metallo- $\beta$ -lactamases in *Enterobacteriaceae*? *Antimicrobial Agents Chemother*. 2017. <https://doi.org/10.1128/AAC.02243-16>.
  41. 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(11): e0084621.
  42. Sreenivasan P, Sharma B, Kaur S, Rana S, Biswal M, Ray P, et al. In-vitro susceptibility testing methods for the combination of ceftazidime-avibactam with aztreonam in metallo-beta-lactamase producing organisms: role of combination drugs in antibiotic resistance era. *J Antibiot*. 2022;75(8):454–62.
  43. Jayol A, Nordmann P, Poirel L, Dubois V. Ceftazidime/avibactam alone or in combination with aztreonam against colistin-resistant and carbapenemase-producing *Klebsiella pneumoniae*. *J Antimicrob Chemother*. 2017;73(2):542–4.

## Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.