Infection and Drug Resistance

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Effect of Colistin, Fosfomycin and Meropenem/Vaborbactam on Carbapenem-Resistant *Enterobacterales* in Egypt: A Cross-Sectional Study

Raghdaa Shrief[1], Amira H El-Ashry[2], Rasha Mahmoud[3], Rasha El-Mahdy[2]

[1]Medical Microbiology and Immunology Department, Faculty of Medicine, Damietta University, Damietta, Egypt; [2]Medical Microbiology and Immunology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt; [3]Internal Medicine Department, Nephrology and Dialysis Unit, Mansoura University, Mansoura, Egypt

Correspondence: Rasha El-Mahdy, Medical Microbiology and Immunology, Mansoura Faculty of Medicine, Mansoura University, Mansoura, 35516, Egypt, Tel +20 10 0532 9819, Email rashaamr@mans.edu.eg

Purpose: The increasing multi-drug carbapenem resistance among *Enterobacterales* are a severe health problem limiting therapeutic options and worsen the prognosis. This study characterizes carbapenemase genes and integrons among uropathogenic carbapenem resistant *Enterobacterales* (CRE) isolates recovered from Mansoura University Hospitals and evaluates the effect of colistin, fosfomycin and meropenem-vaborbactam on these isolates.

Patients and Methods: A total of 200 *Enterobacterales* isolates were collected from patients with urinary tract infections. Antimicrobial susceptibility testing was performed by the disc diffusion method. Colistin susceptibility was tested using the broth microdilution method and fosfomycin and meropenem/vaborbactam susceptibility were tested by MIC Test Strips. Carbapenem resistant isolates were screened for carbapenemase activity phenotypically using the modified carbapenem inactivation method and EDTA-modified carbapenem inactivation method and genotypically by multiplex PCR. Integrons class 1 and 2 and *fosA* gene were assayed by PCR. Data were statistically analyzed using the Statistical Package for Social Sciences (SPSS) version 16. The Chi-square or Fisher's exact test was used to compare groups, as appropriate.

Results: Ninety-two *Enterobacterales* isolates were resistant to meropenem (46%); 52 *E. coli* and 40 *K. pneumoniae* strains. All CRE isolates were multi-drug resistant (MDR). Sensitivity of CRE isolates to colistin, fosfomycin and meropenem/vaborbactam were 67.4%, 82.6% and 58.7%, respectively. Carbapenemase genes were detected by multiplex PCR in 69.6% of CRE isolates (Carbapenemase producing *Enterobacterales* (CPE) mainly bla_{NDM} (37%). CPE isolates were significantly more resistant to meropenem/vaborbactam than non-CPE isolates; 51.6% vs 17.8%, respectively (P = 0.003) especially bla_{NDM} carrying isolates (70.6%). Class 1 integrons and *fosA* gene were detected in 91.3% and 11.9% of CRE isolates, respectively.

Conclusion: This study revealed that about half of the uropathogenic *Enterobacterales* isolates were MDR CRE. Carbapenemase gene bla_{NDM} was the main gene among CRE isolates. Meropenem/vaborbactam sensitivity was significantly higher on non-CPE than CPE isolates and limited by the predominance of bla_{NDM}.

Keywords: *Enterobacterales*, carbapenemases, carbapenem-resistant *Enterobacterales*, colistin, fosfomycin, meropenem-vaborbactam

Introduction

The increasing Carbapenem-resistant *Enterobacterales* (CRE) isolates are a severe threat worldwide due to the poor prognosis and limited therapeutic options.[1–3] The production of carbapenemases such as *Klebsiella pneumoniae* carbapenemase (KPC), Metallo- β-lactamases (MBLs) and oxacillinases (OXA) enzymes mainly cause carbapenem resistance. Moreover, the deficiency in the outer-membrane protein expression plays a minor role in carbapenem resistance.[4]

Integrons are mobile genetic elements that mediate the intracellular movement of antibiotic resistance genes. Class 1 integrons facilitate the spread of antibiotic resistance genes in *Enterobacterales*, reducing the spectrum of therapeutic options.[5]

Received: 8 August 2022
Accepted: 14 October 2022
Published: 27 October 2022

CRE strains are usually resistant to many antibiotics,[6] increasing the need for reusing old antibiotics such as colistin and fosfomycin and new drug combinations for treatment. These combinations use the old β-lactam drug with a new β-lactamase inhibitor effective on carbapenemases such as meropenem-vaborbactam (M/V) and ceftazidime-avibactam (C/A).[4,7]

Colistin (polymyxin E) is an old antibiotic effective on most *Enterobacterales* species. It binds to the lipid A, disrupting the outer cell membrane and causing leakage of cytoplasmic contents and bacterial death.[8] Colistin has been used as the last line of treatment for infections caused by CRE, yet the resistance limits its use.[7,9] Loading doses colistin and colistin methanesulfonate, an inactive prodrug of colistin, are associated with favorable outcomes in infections caused by Gram-negative pathogens only when carbapenems cannot be used and with a close monitoring of the renal functions.[10,11] The combining of the loading dose colistin and meropenem is associated with a better survival rate and can be a promising therapeutic strategy for treating carbapenems resistant infections.[12]

Fosfomycin is a broad-spectrum bactericidal antibiotic that interferes with synthesizing Gram-negative and some Gram-positive bacterial cell walls. It is the first line of treatment for uncomplicated urinary tract infections (UTIs).[13] Fosfomycin resistance is caused by many enzymes that inactivate fosfomycin, including metalloenzymes (*fosA* type);[14,15] however, there is limited data about fosfomycin resistance in Africa, including Egypt.[13,16,17]

A drug combination formed of meropenem paired with vaborbactam, a boronic acid β-lactamase inhibitor with a broad spectrum of carbapenemases inhibition, has been used to treat all types of CRE infections caused mainly by KPC-producing strains. Compared to the older drug combinations, this combination is a highly effective and safe therapy for severe infections in critically ill patients.[4,18–20]

The objective of this study was to characterize the carbapenemase genes, and integrons among the uropathogenic CRE isolates recovered from Mansoura University Hospitals (MUHs) and to evaluate the effect of colistin, fosfomycin and meropenem-vaborbactam on CRE isolates. To our knowledge, this is the first study evaluating the effectiveness of meropenem-vaborbactam on uropathogenic CRE isolates in Egypt.

Materials and Methods

This cross-sectional study assessed the efficacy of colistin, fosfomycin and meropenem-vaborbactam on the uropathogenic CRE isolates recovered from MUHs.

Bacterial Isolates

Two hundred *Enterobacterales* strains were isolated from the urine of patients with UTIs attending MUHs from September 2021 to March 2022. Each patient underwent a clinical examination after taking a medical history to diagnose the UTI.

Isolation and Identification of the *Enterobacterales* Isolates

The urine samples were collected from adult patients with UTIs under complete aseptic conditions and cultured on cystine lactose electrolyte deficient agar (CLED) (Oxoid Ltd., England) to detect different *Enterobacterales* species.

Urinary tract infection was diagnosed if the patient suffered from symptoms and signs of UTI; fever (>38.0°C), suprapubic tenderness, costovertebral angle pain or tenderness, urinary urgency, urinary frequency or dysuria and confirmed when the bacterial colony count was $\geq 10^5$ CFU/mL.

The isolates were identified by Gram staining and the standard biochemical tests; methyl red test, Voges-Proskauer test, citrate utilization test, oxidase test, Kligler iron agar, lysine iron agar and motility indole ornithine test. The isolates identification was confirmed by API 20E (BioMérieux, Marcy l'Étoile, France).

Detection of CRE

Carbapenem-resistant *Enterobacterales* (CRE) isolates are defined as any isolate that has imipenem/or meropenem MIC values of ≥ 4 μg/mL.[6] All *Enterobacterales* isolates (No = 200) were tested for meropenem susceptibility by the disc diffusion and broth microdilution methods according to the Clinical Laboratory Standards Institute (CLSI) guidelines M100 and M07, respectively. Interpretative criteria for meropenem were susceptible ≤1 mg/l, intermediate 2 mg/l and resistant ≥4 mg/l.[21,22]

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of CRE isolates was performed by the disc diffusion method for several antibiotics, including aztreonam (30 µg), amikacin (30 µg), cefuroxime (30 µg), ceftazidime (30 µg), cefoxitin (30 µg), piperacillin/tazobactam (100/10 µg), ciprofloxacin (5 µg), nitrofurantoin (300 µg) and trimethoprim/sulfamethoxazole (1.25/23.7 ug).[21]

Colistin susceptibility testing was carried out using the broth microdilution method with cation-adjusted Mueller Hinton broth (Oxoid) as a culture medium and colistin sulfate powder (Acros Organics BVBA, Geel, Belgium). The test was interpreted using EUCAST cut-offs as MIC ≤ 2 µg/mL susceptible and >2 µg/mL resistant.[23]

Fosfomycin susceptibility was tested using the disc diffusion method with fosfomycin 200 µg disc containing glucose-6-phosphate (G6P) (Liofilchem, Roseto Degli Abruzzi, Italy) on Mueller Hinton agar and interpreted using CLSI guidelines, where ≥16 mm was sensitive, 13–15 mm intermediate and ≤12 mm resistant.[21] In addition, fosfomycin sensitivity was carried out using Fosfomycin MIC Test Strip (Liofilchem) containing fosfomycin and G6P and interpreted according to breakpoints set by the CLSI; ≤64 µg/mL as susceptible and ≥256 µg/mL as resistant for *E. coli* generalized to *Enterobacterales*.[21]

Meropenem/vaborbactam susceptibility was tested by the disc diffusion method using meropenem/vaborbactam disc (20ug-10µg) (MAST Laboratories Ltd., Bootle, Merseyside, UK)[21] and the MIC Test Strip (MTS™ Meropenem-vaborbactam, Liofilchem). The susceptibility was interpreted using CLSI guidelines for *Enterobacterales* as susceptible ≥18 mm, intermediate 15–17 mm and resistant ≤14 mm using the disc diffusion method and susceptible ≤4/8 mg/l, intermediate 8/8 mg/l and resistant ≥16/8 mg/l for the MIC Test Strip method.[21]

Quality control testing was performed using *Escherichia coli* ATCC 25922 and NCTC 13353 and *Klebsiella pneumoniae* ATCC 700603 and ATCC BAA-1705 as reference strains to ensure the proper test conditions.[21]

Phenotypic and Genotypic Detection of Carbapenemase Producing *Enterobacterales*

Phenotypic detection of carbapenemase producers was performed by the modified carbapenem inactivation method (mCIM) and EDTA-modified carbapenem inactivation method (eCIM) according to CLSI guidelines to distinguish metallo-carbapenemase from serine-carbapenemase.[21]

DNA was extracted from CRE isolates using the Gene JET genomic DNA purification kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. DNA was then stored at −20°C.

All CRE isolates were screened for carbapenemase genes; bla_{IMP}, bla_{VIM}, $bla_{OXA\text{-}48}$, bla_{NDM} and bla_{KPC}, as previously described.[24] Carbapenemase genes were detected using two multiplex PCR reactions; one for bla_{IMP} and bla_{VIM} and the second for detecting $bla_{OXA\text{-}48}$, bla_{NDM} and bla_{KPC}. The thermal cycling steps involved initial denaturation of 10 min at 94°C and 36 cycles of 30s at 94°C, 40s at 52°C, and 50s at 72°C, with 5 min for the final extension. The gel electrophoresis using 2% agarose was used to detect bla_{IMP}, bla_{VIM}, $bla_{OXA\text{-}48}$, bla_{NDM} and bla_{KPC} amplicons; 232, 390, 438, 621 and 798 bp, respectively. Sequences of primers used to amplify the five carbapenemase genes are shown in Table 1.

Detection of Class 1 and 2 Integrons by Duplex PCR Among CRE Isolates

As previously reported, Integrons class 1 and 2 were detected using the genomic DNA by duplex PCR yielding 160 and 789 bp amplicons, respectively (Table 1).[25]

Detection of *fosA* Gene by PCR Among CRE Isolates

The plasmid was extracted from fosfomycin-resistant CRE isolates using Gene JET Plasmid Miniprep Kit (Thermo Scientific). The purified plasmid was used to detect the *fosA* gene by PCR[26] using the primers listed in Table 1.

Statistical Analysis

Data were statistically analyzed using the Statistical Package for Social Sciences (SPSS) version 16 (SPSS Inc, Chicago, IL, USA). Qualitative data were expressed as numbers and percentages. The Chi-square or Fisher's exact test was used to compare groups, as appropriate. Results with $p < 0.05$ were considered statistically significant.

Table 1 Primers' Sequences and Amplicons' Size Used in PCR for Carbapenemase, Integrons and *fosA* Genes Detection

Gene	Primer Sequence (5′-3′)	Amplicon (bp)	Reference
bla_{IMP}	GGAATAGAGTGGCTTAAYTCTC GGTTTAAYAAAACAACCACC	232	24
bla_{VIM}	GATGGTGTTTGGTCGCATA CGAATGCGCAGCACCAG	390	24
bla_{OXA-48}	GCGTGGTTAAGGATGAACAC CATCAAGTTCAACCCAACCG	438	24
bla_{NDM}	GGTTTGGCGATCTGGTTTTC CGGAATGGCTCATCACGATC	621	24
bla_{KPC}	CGTCTAGTTCTGCTGTCTTG CTTGTCATCCTTGTTAGGCG	798	24
Integron class 1	CAGTGGACATAAGCCTGTTC CCCGAGGCATAGACTGTA	160	25
Integron class 2	CACGGATATGCGACAAAAAGGT GTAGCAAACGAGTGACGAAATG	789	25
fosA	ATCTGTGGGTCTGCCTGTCGT ATGCCCGCATAGGGCTTCT	271	26

Note: Y = C or T.
Abbreviation: bp, base pair.

Results

The present study was conducted on 200 *Enterobacterales* strains (121 *E. coli*, 78 *Klebsiella pneumoniae*, 1 *Enterobacter cloacae*) isolated from urine of adult patients over 7 months. The patients were 61.5% female (123/200) and 38.5% male (77/200).

Enterobacterales strains were recovered from patients with UTI and confirmed by traditional biochemical reactions and API 20E. Meropenem susceptibility testing using the broth microdilution method according to CLSI guidelines (susceptible ≤1 mg/l and resistant ≥4 mg/l) revealed 92 CRE isolates (46% of *Enterobacterales* isolates); 52 *E. coli* and 40 *K. pneumoniae* strains.

Using the disc diffusion method, all the isolates were resistant to cefuroxime and ceftazidime. About 24%, 15.2% and 8.7% of CRE isolates were sensitive to amikacin, nitrofurantoin and cefoxitin, respectively and only 6.5% of the isolates were sensitive to aztreonam, ciprofloxacin and trimethoprim/sulfamethoxazole (Table 2). Out of 200 *Enterobacterales* isolates, 105 isolates (52.5%) were MDR including all the CRE isolates.

Colistin susceptibility was tested using the broth microdilution method where sensitive CRE isolates had MIC ≤ 2 µg/mL. About 67.4% of CRE isolates were sensitive to colistin (Table 2) and the colistin MIC range was 0.25–64 µg/mL.

Testing the fosfomycin susceptibility of CRE isolates using the disc diffusion method and MIC Test Strip showed that 82.6% of the isolates were sensitive to fosfomycin (Table 2), where the fosfomycin MIC range was 0.125–256 µg/mL (Figure 1).

Meropenem/vaborbactam susceptibility was evaluated by the disc diffusion method according to CLSI guidelines; 58.7% of the CRE isolates were inhibited by meropenem/vaborbactam (Table 2). Using the MIC Test Strip, the range of meropenem/vaborbactam MIC was 0.032–256 µg/mL (Figure 1).

Screening of carbapenemase genes; *bla_{IMP}*, *bla_{VIM}*, *bla_{OXA-48}*, *bla_{NDM}* and *bla_{KPC}* by two multiplex PCR revealed that 69.6% of the isolates had carbapenemase genes (Carbapenemase producing *Enterobacterales* (CPE)) mainly *bla_{NDM}* (37%) followed by *bla_{OXA-48}* and *bla_{KPC}* (13% each). Four (4.4%) and two (2.2%) CRE isolates carried *bla_{NDM}* plus *bla_{OXA-48}* and *bla_{KPC}* plus *bla_{OXA-48}*, respectively. Carbapenemase genes *bla_{IMP}* and *bla_{VIM}* were not detected in any of the CRE isolates. Twenty-eight (30.4%) CRE isolates were non-CPE isolates (Table 3). The sensitivity and specificity of

Table 2 Antimicrobial Susceptibility Pattern of the Uropathogenic CRE Strains

	CR *E. coli* (No = 52) No/%		CR *K. pneumoniae* (No = 40) No/%		CRE Isolates (No = 92) No/%	
	S	R	S	R	S	R
Aztreonam	4 (7.7)	48 (92.3)	2 (5)	38 (95)	6 (6.5)	86 (93.5)
Piperacillin/tazobactam	0 (0)	52 (100)	4 (10)	36 (90)	4 (4.3)	88 (95.7)
Amikacin	14 (27)	38 (73)	8 (20)	32 (80)	22 (23.9)	70 (76.1)
Ciprofloxacin	0 (0)	52 (100)	6 (15)	34 (85)	6 (6.5)	86 (93.5)
Nitrofurantoin	12 (23)	40 (77)	2 (5)	38 (95)	14 (15.2)	78 (84.8)
Cefuroxime	0 (0)	52 (100)	0 (0)	40 (100)	0 (0)	92 (100)
Ceftazidime	0 (0)	52 (100)	0 (0)	40 (100)	0 (0)	92 (100)
Cefoxitin	6 (11.5)	46 (77)	2 (5)	38 (95)	8 (8.7)	84 (91.3)
Trimethoprim/ sulfamethoxazole	2 (3.8)	50 (96.2)	4 (10)	36 (90)	6 (6.5)	86 (93.5)
Colistin	30 (57.7)	22 (42.3)	32 (80)	8 (20)	62 (67.4)	30 (32.6)
Fosfomycin	46 (88.5)	6 (11.5)	30 (75)	10 (25)	76 (82.6)	16 (17.4)
Meropenem/vaborbactam	30 (57.7)	22 (42.3)	24 (60)	16 (40)	54 (58.7)	38 (41.3)

Abbreviations: CR, carbapenem resistant; CRE, carbapenem-resistant *Enterobacterales*; S, sensitive, R, resistant.

the combined mCIM and eCIM for detection of carbapenemase activity among CPE isolates were 92% and 96.2%, respectively. The eCIM could not detect carbapenemase activity in 3 CPE isolates carrying both bla_{NDM} plus $bla_{OXA\text{-}48}$ (Table 4).

Carbapenemase-producing *Enterobacterales* (CPE) isolates were significantly more resistant to meropenem/vaborbactam compared to non-CPE isolates; 51.6% vs 17.8%, respectively (P = 0.003). This resistance was higher among the isolates carrying bla_{NDM} (70.6%) than other carbapenemase genes. There was no significant difference in the resistance pattern of all other tested antibiotics between the CPE and non-CPE isolates ($p \geq 0.05$, Tables 3 and 5).

Eleven out of the 16 fosfomycin-resistant CRE isolates (68.75%, 11.9% of CRE isolates) carried *fosA* gene; 66.7% (4/6) and 70% (7/10) of fosfomycin-resistant *E. coli* and *K. pneumoniae* isolates, respectively. *FosA* gene was more common among CPE than non-CPE isolates; 15.6% vs 3.6%, respectively (Table 5).

Integron class 1 was detected in 91.3% of the CRE isolates by duplex PCR, mostly among *E. coli* isolates, while integron class 2 was not detected in any of the CRE isolates. Integron class 1 was more common among non-CPE than CPE isolates; 100% vs 87.5%, respectively (Table 5).

Discussion

Carbapenem-resistant *Enterobacterales* are a global health concern associated with patients' morbidity and mortality. These strains have become an alarming issue due to limited therapeutic options that enforce the reuse of some old therapy and the development of new therapeutic alternatives.

The present study involved 200 *Enterobacterales* isolates from adult patients with UTIs attending MUHs over seven months. According to meropenem susceptibility testing, ninety-two isolates (46%) were CRE strains; *E. coli* (52, 56.5%) and *K. pneumoniae* (40, 43.5%). Similar to these results, Kotb et al also stated that 47.9% of 2306 *Enterobacterales* isolates were carbapenem-resistant, and the prevalence of CRE varied with different clinical samples.[27]

The prevalence of CRE varies in Egypt from 34.1% to 66.08%.[28–30] This variation might be due to different patients, clinical samples, laboratory techniques, antibiotic policy, and infection control measures. In Egypt, carbapenem-resistant

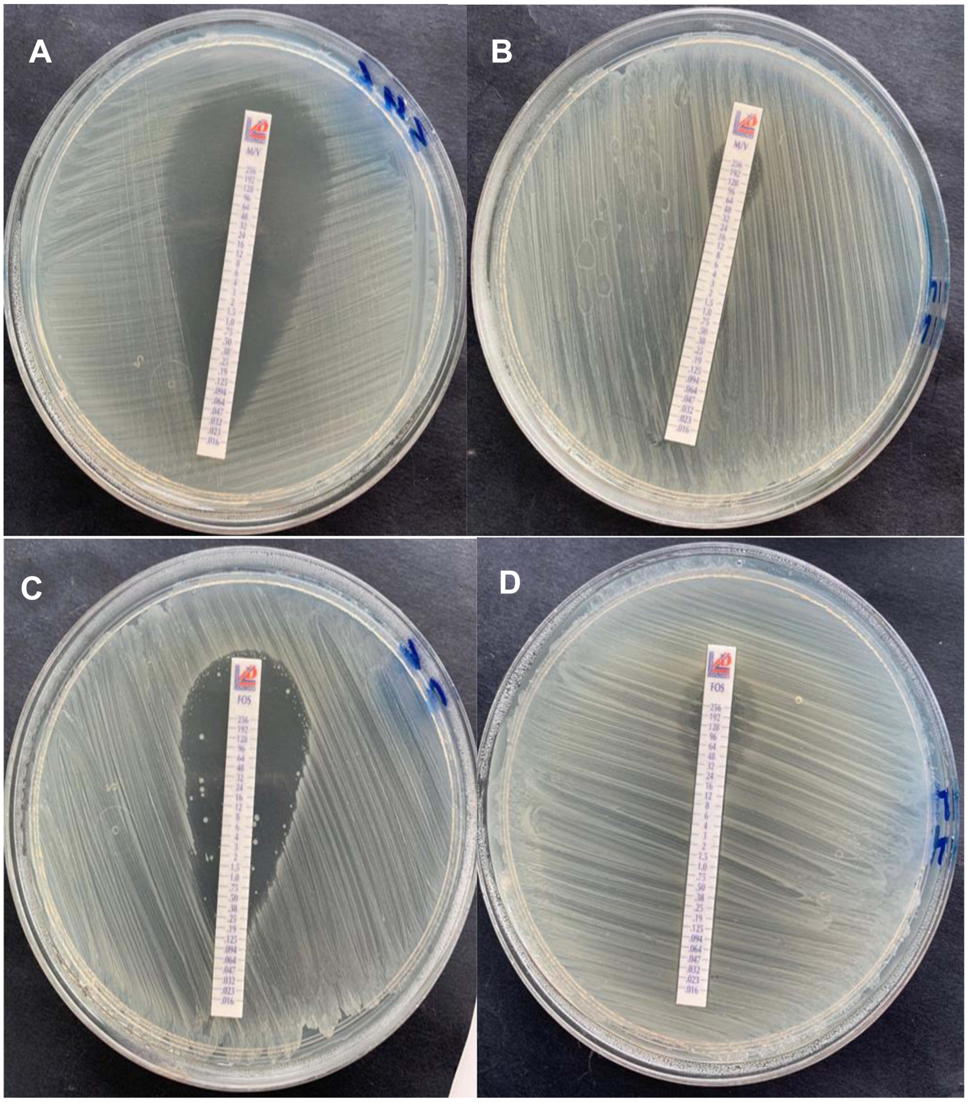

Figure 1 Meropenem/vaborbactam and fosfomycin susceptibility testing using the MIC Test Strip according to CLSI guidelines. (**A**) Meropenem/vaborbactam susceptible strain (MIC = 0.032 μg/mL), (**B**) meropenem/vaborbactam resistant strain (MIC > 256 μg/mL), (**C**) fosfomycin susceptible strain (MIC = 0.25 μg/mL) and (**D**) fosfomycin resistant strain (MIC > 256 μg/mL).

Enterobacterales are a significant health issue as half of the *Enterobacterales* isolates were resistant to carbapenem in some hospitals. This resistance level is higher than other Arab, African, Asian, European and American countries.[31,32]

Antimicrobial susceptibility testing of CRE isolates showed a high resistance pattern to most tested antibiotics with no sensitivity to cefuroxime and ceftazidime. These data are comparable to the data previously reported in India[33] and the USA.[34–36]

Of 200 *Enterobacterales* isolates, 105 isolates (52.5%) were MDR, including all CRE strains acquiring non-susceptibility to at least one agent in three or more antimicrobial categories.[37] Similar results were also reported from Ethiopia,[31] Egypt[38] and Saudi Arabia,[39] while, a higher percentage of MDR was reported in other studies[30,40–42] and a lower percentage was documented in some Arab countries[43] and USA.[44] The high prevalence of MDR in Egypt emphasizes the importance of implementing effective infection control strategies and antibiotic stewardship programs, in addition to the education of healthcare workers for early surveillance and preventive measures of MDR isolates to control their spread.

Integron class 1 was detected in 91.3% of our CRE isolates. Multi-drug resistance is strongly associated with integrons, especially class 1, which is widely distributed among resistant Gram-negative bacteria. In this study, all

Table 3 Detection of Carbapenemase and Integrons Genes by PCR and the Sensitivity of CRE Isolates to Meropenem/Vaborbactam

Bacterial Species (No)	**Non-CPE**	**Genes**					
		bla_{NDM}	bla_{OXA-48}	bla_{KPC}	bla_{NDM} and bla_{OXA-48}	bla_{KPC} and bla_{OXA-48}	**Integron Class I**
***E. coli* (52)**	16	18	12	4	2	0	50
***K. pneumoniae* (40)**	12	16	0	8	2	2	34
Total (92)	28 (30.4%)	34 (37%)	12 (13%)	12 (13%)	4 (4.4%)	2 (2.2%)	84 (91.3%)
Meropenem/vaborbactam sensitivity	23 (82.1%)	10 (29.4%)	6 (50%)	12 (100%)	2 (50%)	1 (50%)	50 (59.5%)

Abbreviation: Non-CPE, non-carbapenemase producing *Enterobacterales*.

Table 4 Performance of the Modified Carbapenem Inactivation Method (mCIM) and EDTA-Modified Carbapenem Inactivation Method (eCIM) in Screening of Carbapenemase Activity of Carbapenem Resistant Isolates

	Non-CPE	bla_{NDM}	bla_{OXA-48}	bla_{KPC}	bla_{NDM} and bla_{OXA-48}	bla_{KPC} and bla_{OXA-48}	***Total***
No of CRE strains	28	34	12	12	4	2	92
mCIM	0	34	12	12	4	2	64
eCIM	N/A	34	1	0	1	0	36

Abbreviations: CRE, carbapenem-resistant *Enterobacterales*; Non-CPE, non-carbapenemase producing *Enterobacterales*; N/A, not applicable.

Table 5 Antibiotic Resistance Pattern and the Distribution of Integron and *fosA* Genes Among Carbapenemase Producing Enterobacterales (CPE) and Non-CPE Isolates

Antibiotic	**Non-CPE Isolates (No = 28) No/%**	**CPE Isolates (No = 64) No/%**	**P value**
Aztreonam	26 (92.8%)	60 (93.7%)	1
Piperacillin/tazobactam	28 (10 0%)	60 (93.7%)	0.31
Amikacin	24 (85.7%)	46 (71.9%)	0.15
Ciprofloxacin	26 (92.8%)	60 (93.7%)	1
Nitrofurantoin	24 (85.7%)	54 (84.4%)	1
Cefuroxime	28 (100%)	64 (100%)	NA
Ceftazidime	28 (100%)	64 (100%)	NA
Cefoxitin	26 (92.8%)	58 (90.6%)	1
Trimethoprim/sulfamethoxazole	28 (100%)	58 (90.6%)	0.17
Colistin	8 (28.6%)	22 (34.4%)	0.58
Fosfomycin	2 (7.1%)	14 (21.9%)	0.13
Meropenem/vaborbactam	5 (17.8%)	33 (51.6%)	0.003*
Integrons class I	28 (100%)	56 (87.5%)	0.10
***fosA* gene**	1 (3.6%)	10 (15.6%)	0.16

Note: *P value is significant; < 0.0.
Abbreviation: CPE, carbapenemase producing *Enterobacterales*.

CRE isolates were MDR, and integron class 1 was detected among most of them, which might play a role in the spread of MDR among these isolates.[25]

In this study, 62 CRE isolates (67.4%) were sensitive to colistin adopting EUCAST cut-offs in agreement with other studies.[6,34,36] Castanheira et al,[35] Qamar et al[45] and Maraki et al[9] have recorded a higher colistin sensitivity; 83.3%, 84.1% and 87.5% among the CRE isolates, respectively. In contrast, in Turkey, a high colistin resistance (76.19%) has been reported owing to the only testing multi- and extensive drug-resistant *Enterobacterales* and the high level of antibiotic consumption.[7]

Both colistin and fosfomycin are considered a salvage treatment for multi- and extensive drug-resistant carbapenem-resistant *Enterobacterales* infections as they are associated with better prognosis.[45] In our study, fosfomycin inhibited 82.6% of the CRE isolates. The *fosA* gene was detected in 11 of 16 resistant isolates (68.75%), consistent with other studies from Egypt[16] and Pakistan.[45]

Similarly, several studies have reported the sensitivity of CRE isolates from different clinical samples to fosfomycin.[16,17,46–48] Moreover, 74% of carbapenem-resistant *Enterobacter* species were sensitive to fosfomycin, and the *fosA* gene was detected in only 42% of the resistant isolates.[49] On the contrary, a high fosfomycin resistance was detected among carbapenem-resistant *K. pneumoniae*[9,50] and extensive drug-resistant CRE isolates (67.35%).[7]

Several mechanisms are associated with fosfomycin resistance that differs with the geographic locality and the studied bacteria. Plasmid-mediated fosA enzymes are the most common mechanism of resistance in *Enterobacterales*.[49]

In the USA, the *fosA* gene was detected in 80% of carbapenem-resistant *K. pneumoniae* isolates,[51] whereas in China, the *fosA* gene has been identified in only uropathogenic *K. pneumoniae* (26.7%)[52] and has not been recognized in any fosfomycin-resistant isolates in other studies.[50,53] Lastly, Mosime et al have reported that fosA enzymes are not a common cause of resistance amongst community-acquired urinary pathogens.[54]

In the current study, 69.6% of CRE isolates were CPE, mostly bla_{NDM} producers (37%), followed by $bla_{OXA\text{-}48}$ and bla_{KPC} producers (13% each). About 4.4% and 2.2% of the CRE isolates carried bla_{NDM} plus $bla_{OXA\text{-}48}$ and bla_{KPC} plus $bla_{OXA\text{-}48}$, respectively, while 30.4% of CRE were non-CPE isolates. The sensitivity of the mCIM combined with the eCIM in the screening of carbapenemase activity was 92%.

In accordance, 75.8% of CRE isolates carried carbapenemase genes, yet, 50.9%, 10.2% and 9.5% of the isolates had bla_{KPC}, $bla_{\mathrm{OXA\text{-}48}}$ like genes and $bla_{NDM\text{-}1}$, respectively[34] consistent with other studies.[6,35] On the other hand, a lower prevalence of carbapenemase encoding genes was documented in 53.4% of CRE isolates, where bla_{KPC} was the most frequent gene (94.2%).[36]

In Egypt, carbapenem resistance genes have been recognized in 45.3% of MDR CRE isolates.[38] Another Egyptian study reported that carbapenemase gene prevalence among CRE isolates was 89.62% and similar to the current study, the most prevalent gene detected was bla_{NDM} (68.88%).[42] Furthermore, a high prevalence of bla_{NDM} has also been documented among CRE isolates in Alexandria, Egypt (67.5%)[55] and Greece (85%),[9] which might be due to its presence on conjugative plasmids that facilitate its spread between bacteria.[42]

However, some Egyptian studies have reported that $bla_{OXA\text{-}48}$ was the most common carbapenemase gene among CRE isolates[28,30] similar to the studies from Turkey[7] and Ethiopia.[40] On the other hand, Khalil et al[29] have stated that the most prevalent carbapenemase genes among CRE were bla_{KPC} in Gharbia, Egypt. This difference in the most prevalent carbapenemase gene type might be due to the different detection methods and geographic regions.[40]

In the current study, meropenem/vaborbactam inhibited 58.7% of CRE isolates, and its sensitivity was significantly higher on non-CPE than CPE isolates; 82.2% vs 48.4%, respectively, with a low sensitivity on the isolates carrying bla_{NDM} (29.4%) and a complete sensitivity on the isolates carrying bla_{KPC} (100%).

A study has stated that meropenem/vaborbactam inhibited 73.9% of CRE isolates with significant activity on bla_{KPC} producing isolates (99.5%), limited activity on $bla_{OXA\text{-}48}$ producing isolates and no activity on $bla_{NDM\text{-}1}$ producing isolates. Meropenem/vaborbactam had a lower MIC50/90 with bla_{KPC} than non bla_{KPC} producing isolates due to the large number of MBLs and OXA-48 producing isolates.[6] Meropenem-vaborbactam was 4-fold more active than meropenem alone and inhibited 84.2% of the CRE isolates.[34]

Similarly, several studies have documented meropenem-vaborbactam complete activity on bla_{KPC} producing CRE isolates regardless of the bla_{KPC} variant, limited activity on isolates producing $bla_{OXA\text{-}48}$ and no activity on MBLs producing ones.[35,36]

The low efficacy of meropenem/vaborbactam seen in our study compared to other studies might be due to the predominancy of bla_{NDM} then $bla_{OXA\text{-}48}$ and bla_{KPC} producing CRE isolates. Vaborbactam strongly inhibits bla_{KPC} and has a good outcome when combined with different carbapenems. It does not inhibit MBLs producing isolates and has limited activity on isolates producing class D oxacillinases associated with resistance to carbapenems.[34] It has also been documented that meropenem-vaborbactam was active on non-bla_{KPC} producers other than bla_{NDM} and $bla_{OXA\text{-}48.}$[56]

In the present study, meropenem/vaborbactam significantly inhibited non-CPE higher than CPE isolates. Other studies have similarly reported the whole activity of meropenem-vaborbactam on non-CPE isolates compared to other antibiotics.[35,36,57] The therapeutic options of non-carbapenem producing CRE are challenging, and further studies are still needed. The effect of meropenem/vaborbactam on the isolates varies with the bacterial species and the different resistance mechanisms.[57]

Clarifying the epidemiology of CRE isolates and their resistance mechanisms is mandatory to guide the clinicians on the appropriate therapeutic options for infections caused by these organisms to improve the clinical outcome. Surveillance of CRE, effective infection control measures and appropriate antibiotic stewardship are crucial approaches to reducing the spread of CRE. An antimicrobial restriction system could increase the appropriateness of prescribing antibiotics and decrease the expense for carbapenem.[58]

Our work emphasizes the importance of meropenem/vaborbactam therapy with the favorable clinical outcome for all physicians, pharmacists, and healthcare professionals worldwide. Meropenem/vaborbactam is a promising therapeutic option for bla_{KPC}-producing uropathogenic isolates, yet its effect on non-bla_{KPC} CRE producers (MBLs and OXA-48-like enzymes producers) is limited and needs more optimization. To the best of our knowledge, this is the first study in Egypt that highlights the effect of meropenem/vaborbactam on uropathogenic CRE isolates. However, this study had some limitations such as the short study duration and limited molecular techniques due to financial constraints. More studies are needed on different clinical samples, pathogenic bacteria and patients' groups.

Conclusions

In conclusion, this study revealed that about half of uropathogenic *Enterobacterales* were MDR CRE isolates and colistin and fosfomycin had an excellent therapeutic effect on these CRE isolates. The carbapenemase gene bla_{NDM} was the primary gene among CPE isolates, and meropenem/vaborbactam had an unsatisfactory therapeutic effect on CRE isolates due to the predominancy of bla_{NDM}.

Abbreviations

KPC, Klebsiella pneumoniae carbapenemases; OXA, oxacillinases; MDR, multi-drug resistant; UTI_S, urinary tract infections; MUHs, Mansoura University Hospitals; CLED, cystine lactose electrolyte deficient agar; CLSI, Clinical Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing; G6P, glucose-6-phosphate; MIC, minimum inhibitory concentration; CPE, carbapenemase producing *Enterobacterales*.

Data Sharing Statement

On request.

Ethics Approval and Consent to Participate

This study was approved by Mansoura Faculty of Medicine ethical committee (R22.09.1813) and a signed informed consent was obtained from each patient. This study complies with the Declaration of Helsinki.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

The article is self-funded.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Bassetti M, Peghin M, Pecori D. The management of multidrug-resistant *Enterobacteriaceae*. *Curr Opin Infect Dis*. 2016;29:583–594. doi:10.1097/QCO.0000000000000314
2. Logan LK, Weinstein RA. The epidemiology of carbapenem-resistant *Enterobacteriaceae*: the impact and evolution of a global menace. *J Infect Dis*. 2017;215:S28–36. doi:10.1093/infdis/jiw282
3. Jean -S-S, Harnod D, Hsueh P-R. Global threat of carbapenem-resistant Gram-negative bacteria. *Front Cell Infect Microbiol*. 2022;12:823684. doi:10.3389/fcimb.2022.823684
4. Novelli A, Giacomo PD, Rossolini GM, Tumbarello M. Meropenem/vaborbactam: a next generation β-lactam β-lactamase inhibitor combination. *Expert Rev Anti-Infect Ther*. 2020;18(7):643–655. doi:10.1080/14787210.2020.1756775
5. Bagheri-Nesami M, Rafiei A, Eslami G, et al. Assessment of extended-spectrum β-lactamases and integrons among *Enterobacteriaceae* in device-associated infections: multicenter study in north of Iran. *Antimicrob Resist Infect Control*. 2016;5:52. doi:10.1186/s13756-016-0143-2
6. Pfaller MA, Huband MD, Mendes RE, Flamm RK, Castanheira M. In vitro activity of meropenem/vaborbactam and characterisation of carbapenem resistance mechanisms among carbapenem-resistant *Enterobacteriaceae* from the 2015 meropenem/vaborbactam surveillance programme. *Int J Antimicrob Agents*. 2018;52:144–150.
7. Süzük Yıldız S, Kaşkatepe B, Şimşek H, Sarıgüzel FM. High rate of colistin and fosfomycin resistance among carbapenemase-producing *Enterobacteriaceae* in Turkey. *Acta Microbiol Immunol Hung*. 2019;66(1):103–112. doi:10.1556/030.65.2018.042
8. Yamamoto M, Pop-Vicas AE. Treatment for infections with carbapenem-resistant *Enterobacteriaceae*: what options do we still have? *Crit Care*. 2014;18(3):229. doi:10.1186/cc13949
9. Maraki S, Mavromanolaki VE, Moraitis P, et al. Ceftazidime-avibactam, meropenem-vaborbactam, and imipenem-relebactam in combination with aztreonam against multidrug-resistant, metallo-β-lactamase-producing *Klebsiella pneumoniae*. *Eur J Clin Microbiol Infect Dis*. 2021;40(8):1755–1759. doi:10.1007/s10096-021-04197-3
10. Katip W, Yoodee J, Uitrakul S, Oberdorfer P. Efficacy of loading dose colistin versus carbapenems for treatment of extended spectrum beta lactamase producing Enterobacteriaceae. *Sci Rep*. 2021;11(1):18. doi:10.1038/s41598-020-78098-4
11. Katip W, Uitrakul S, Oberdorfer P. Clinical efficacy and nephrotoxicity of the loading dose colistin for the treatment of carbapenem-resistant *Acinetobacter baumannii* in critically ill patients. *Pharmaceutics*. 2021;14(1):31. doi:10.3390/pharmaceutics14010031
12. Katip W, Oberdorfer P, Kasatpibal N. Effectiveness and nephrotoxicity of loading dose colistin-meropenem versus loading dose colistin-imipenem in the treatment of carbapenem-resistant *Acinetobacter baumannii* infection. *Pharmaceutics*. 2022;14(6):1266. doi:10.3390/pharmaceutics14061266
13. Aghamali M, Sedighi M, Zahedi Bialvaei A, et al. Fosfomycin: mechanisms and the increasing prevalence of resistance. *J Med Microbiol*. 2019;68(1):11–25. doi:10.1099/jmm.0.000874
14. Ohkoshi Y, Sato T, Suzuki Y, et al. Mechanism of reduced susceptibility to fosfomycin in *Escherichia coli* clinical isolates. *Biomed Res Int*. 2017;2017:5470241. doi:10.1155/2017/5470241
15. Garallah ET, Al-Jubori SS. Surveillance of *murA* and the plasmid-mediated fosfomycin resistance *fosA* gene in uropathogenic *E. coli* isolates from UTI patients. *Gene Rep*. 2020;21(7):100872. doi:10.1016/j.genrep.2020.100872
16. Mashaly G-S. Activity of fosfomycin in extended-spectrum beta-lactamases producing *Klebsiella pneumoniae* from hospital acquired urinary tract infections. *Open J Med Microbiol*. 2016;6:104–110. doi:10.4236/ojmm.2016.63014
17. Foad MF. Phenotypic detection and antimicrobial susceptibility profile of ESBL, AmpC and carbapenemase producing gram-negative isolates from outpatient clinic specimens. *Int J Curr Microbiol App Sci*. 2016;5(1):740–752. doi:10.20546/ijcmas.2016.501.075
18. Lee Y, Kim J, Trinh S. Meropenem-Vaborbactam (Vabomere™): another option for carbapenem-resistant *Enterobacteriaceae*. *PT*. 2019;44:110–113.
19. Tumbarello M, Raffaelli F, Cascio A, et al. Compassionate use of meropenem/vaborbactam for infections caused by KPC-producing *Klebsiella pneumoniae*: a multicentre study. *JAC Antimicrob Resist*. 2022;4(1):dlac022. doi:10.1093/jacamr/dlac022
20. Forteza Guillot M, Martín Cerezuela M, Ramírez P. New evidence in severe pneumonia: meropenem-vaborbactam. *Rev Esp Quimioter*. 2022;35(Suppl1):43–45. doi:10.37201/req/s01.10.2022
21. Clinical and Laboratory Standards Institute (CLSI. *Performance Standards for Antimicrobial Susceptibility Testing CLSI Supplement M100*. 31st ed. Wayne, PA: CLSI; 2021.
22. Clinical and Laboratory Standards Institute (CLSI). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. Wayne, PA: CLSI; 2018.
23. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 12.0; 2022. Available from: http://www.eucast.org. Accessed October 21, 2022.
24. Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. *Diagn Microbiol Infect Dis*. 2011;70(1):119–123. doi:10.1016/j.diagmicrobio.2010.12.002

25. Khosravi AD, Motahar M, Abbasi Montazeri E. The frequency of class1 and 2 integrons in *Pseudomonas aeruginosa* strains isolated from burn patients in a burn center of Ahvaz, Iran. *PLoS One*. 2017;12(8):e0183061. doi:10.1371/journal.pone.0183061
26. Li Y, Zheng B, Li Y, Zhu S, Xue F, Liu J. Antimicrobial susceptibility and molecular mechanisms of fosfomycin resistance in clinical *Escherichia coli* isolates in mainland China. *PLoS One*. 2015;10(8):e0135269. doi:10.1371/journal.pone.0135269
27. Kotb S, Lyman M, Ismail G, 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):1–9. doi:10.1186/s13756-019-0639-7
28. Raheel AS, Mohamed HA, Hessam WF, Abbadi SH, El Sayed AE. Detection of carbapenemase enzymes and genes among carbapenem-resistant *Enterobacteriaceae* isolates in Suez Canal University Hospitals in Ismailia, Egypt. *Microbes and Infect Dis*. 2020;1((1)):24–33.
29. Khalil HS, Wahab MAAE. Risk factors, phenotypic and genotypic characterization of carbapenem resistant *Enterobacteriaceae* in Tanta University Hospitals, Egypt. *Int J Infect Control*. 2016;12(2). doi:10.3396/ijic.v12i2.15905
30. Kamel NA, El-Tayeb WN, El-Ansary MR, Mansour MT, Aboshanab KM. Phenotypic screening and molecular characterization of carbapenemase-producing Gram-negative bacilli recovered from febrile neutropenic pediatric cancer patients in Egypt. *PLoS One*. 2018;13(8):e0202119. doi:10.1371/journal.pone.0202119
31. Bitew A, Tsige E. High prevalence of multidrug-resistant and extended-spectrum β-lactamase-producing *Enterobacteriaceae*: a cross-sectional study at Arsho advanced medical laboratory, Addis Ababa, Ethiopia. *J Trop Med*. 2020;2020:6167234.
32. El-Kholy A, El-Mahallawy HA, Elsharnouby N, Abdel Aziz M, Helmy AM, Kotb R. Landscape of multidrug-resistant gram-negative infections in Egypt: survey and literature review. *Infect Drug Resist*. 2021;14:1905–1920. doi:10.2147/IDR.S298920
33. Shanmugam P, Meenakshisundaram J, Jayaraman P. bla_{KPC} gene detection in clinical isolates of carbapenem resistant *Enterobacteriaceae* in a tertiary care hospital. *J Clin Diagn Res*. 2013;7:2736–2738. doi:10.7860/JCDR/2013/7759.3747
34. Castanheira M, Huband MD, Mendes RE, Flamm RK. Meropenem-vaborbactam tested against contemporary Gram-negative isolates collected worldwide during 2014, including carbapenem-resistant, KPC producing, multidrug-resistant and extensively drug-resistant *Enterobacteriaceae*. *Antimicrob Agents Chemother*. 2017;61:e00567–17. doi:10.1128/AAC.00567-17
35. Castanheira M, Doyle TB, Kantro V, Mendes RE, Shortridge D. Meropenem-vaborbactam activity against carbapenem resistant *Enterobacterales* isolates collected in U.S. hospitals during 2016 to 2018. *Antimicrob Agents Chemother*. 2020;64:e01951–19. doi:10.1128/AAC.01951-19
36. Carvalhaes CG, Shortridge D, Sader HS, Castanheira M. Activity of Meropenem-vaborbactam against bacterial isolates causing pneumonia in patients in U.S. hospitals during 2014 to 2018. *Antimicrob Agents Chemother*. 2020;64(3):e02177–19. doi:10.1128/AAC.02177-19
37. Magiorakos AP, Srinivasan A, Carey RB, 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):268–281. doi:10.1111/j.1469-0691.2011.03570.x
38. El-Kholy AA, Girgis SA, Shetta MAF, Abdel-Hamid DH, Elmanakhly AR. Molecular characterization of multidrug-resistant Gram-negative pathogens in three tertiary hospitals in Cairo, Egypt. *Eur J Clin Microbiol Infect Dis*. 2020;39(5):987–992. doi:10.1007/s10096-020-03812-z
39. Alkofide H, Alhammad AM, Alruwaili A, et al. Multidrug-resistant and extensively drug-resistant *Enterobacteriaceae*: prevalence, treatments, and outcomes-A retrospective cohort study. *Infect Drug Resist*. 2020;13:4653–4662.
40. Tekele SG, Teklu DS, Legese MH, et al. Multidrug-resistant and carbapenemase-producing *Enterobacteriaceae* in Addis Ababa, Ethiopia. *Biomed Res Int*. 2021;2021:9999638. doi:10.1155/2021/9999638
41. Bandyr A, Tantry B. ESBL activity, MDR, and carbapenem resistance among predominant *Enterobacterales* isolated in 2019. *Antibiotics*. 2021;10(6):744. doi:10.3390/antibiotics10060744
42. Tawfick MM, Alshareef WA, Bendary HA, Elmahalawy H, Abdulall AK. The emergence of carbapenemase bla_{NDM} genotype among carbapenem-resistant *Enterobacteriaceae* isolates from Egyptian cancer patients. *Eur J Clin Microbiol Infect Dis*. 2020;39(7):1251–1259. doi:10.1007/s10096-020-03839-2
43. Moghnieh RA, Kanafani ZA, Tabaja HZ, Sharara SL, Awad LS, Kanj SS. Epidemiology of common resistant bacterial pathogens in the countries of the Arab League. *Lancet Infect Dis*. 2018;18(12):e379–e394. doi:10.1016/S1473-3099(18)30414-6
44. Gupta V, Ye G, Olesky M, Lawrence K, Murray J, Yu K. National prevalence estimates for resistant *Enterobacteriaceae* and *Acinetobacter* species in hospitalized patients in the United States. *Int J Infect Dis*. 2019;85:203–211.
45. Qamar S, Shaheen N, Shakoor S, Farooqi J, Jabeen K, Hasan R. Frequency of colistin and fosfomycin resistance in carbapenem-resistant *Enterobacteriaceae* from a tertiary care hospital in Karachi. *Infect Drug Resist*. 2017;10:231–236.
46. Alexander BT, Marschall J, Tibbetts RJ, Neuner EA, Dunne WM, Ritchie DJ. Treatment and clinical outcomes of urinary tract infections caused by KPC producing *Enterobacteriaceae* in a retrospective cohort. *Clin Ther*. 2012;34:1314–1323.
47. Pogue JM, Marchaim D, Abreu-Lanfranco O, et al. Fosfomycin activity versus carbapenem-resistant *Enterobacteriaceae* and vancomycin-resistant *Enterococcus*, Detroit, 2008–10. *J Antibiot*. 2013;66:625–627.
48. Kaase M, Szabados F, Anders A, Gatermann SG. Fosfomycin susceptibility in carbapenem-resistant *Enterobacteriaceae* from Germany. *J Clin Microbiol*. 2014;52(6):1893–1897.
49. White BP, Stover KR, Barber KE, Galloway RC, Sullivan DC, King ST. Mechanisms of fosfomycin resistance in carbapenem-resistant *Enterobacter* sp. *Int J Antimicrob Agents*. 2017;50(5):690–692. doi:10.1016/j.ijantimicag
50. Liu P, Chen S, Wu ZY, Qi M, Li XY, Liu CX. Mechanisms of fosfomycin resistance in clinical isolates of carbapenem-resistant *Klebsiella pneumoniae*. *J Glob Antimicrob Resist*. 2020;22:238–243. doi:10.1016/j.jgar.2019.12.019
51. Elliott ZS, Barry KE, Cox HL, et al. The role of *fosA* in challenges with fosfomycin susceptibility testing of multispecies *Klebsiella pneumoniae* carbapenemase-producing clinical isolates. *J Clin Microbiol*. 2019;57(10):e00634–19. doi:10.1128/JCM.00634-19
52. Sun F, Chen S, Qiu X, et al. Antibacterial activity of fosfomycin against uropathogens. *Chemother*. 2014;60:157–161. doi:10.1159/000371734
53. Chen J, Wang D, Ding Y, Zhang L, Li X. Molecular epidemiology of plasmid-mediated fosfomycin resistance gene determinants in *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae* isolates in China. *Microb Drug Resist*. 2019;25(2):251–257. doi:10.1089/mdr.2018.0137
54. Mosime LB, Newton-Foot M, Nel P. Fosfomycin resistance in community-acquired urinary pathogens from Western Cape, South Africa. *S Afr J Infect Dis*. 2022;37(1):a321. doi:10.4102/sajid.v37i1.321
55. Iman FEG, Marwa AM, Doaa AY. Phenotypic and genotypic methods for detection of metallo beta lactamases among carbapenem resistant *Enterobacteriaceae* clinical isolates in Alexandria Main University Hospital. *Afr J Microbiol Res*. 2016;10:32–40. doi:10.5897/AJMR2015.7821

56. Kinn PM, Chen DJ, Gihring TM, et al. *In vitro* evaluation of meropenem-vaborbactam against clinical CRE isolates at a tertiary care center with low KPC-mediated carbapenem resistance. *Diagn Microbiol Infect Dis*. 2019;93(3):258–260. doi:10.1016/j.diagmicrobio.2018.09.017
57. Castanheira M, Doyle TB, Deshpande LM, Mendes RE, Sader HS. Activity of ceftazidime/avibactam, meropenem/vaborbactam and imipenem/relebactam against carbapenemase-negative carbapenem-resistant *Enterobacterales* isolates from US hospitals. *Int J Antimicrob Agents*. 2021;58(5):106439. doi:10.1016/j.ijantimicag.2021.106439
58. Wanla W, Katip W, Supakul S, Apiwatnakorn P, Khamsarn S. Effects of an antimicrobial restriction system on appropriate carbapenem use in a hospital without infectious diseases consultation. *Int J Gen Med*. 2017;10:443–449. doi:10.2147/IJGM.S145133