IJM Iranian Journal of Microbiology

Volume 14 Number 6 (December 2022) 832-840

ORIGINAL ARTICLE

Phenotypic and genotypic detection of resistance mechanisms in carbapenem-resistant gram-negative bacteria isolated from Egyptian ICU patients with first emergence of NDM-1 producing *Klebsiella oxytoca*

Reem Hossam Fawzy, Gamal Fadl Mahmoud Gad, Heba Ahmed Mohamed*

Department of Microbiology and Immunology, Faculty of Pharmacy, Minia University, Minia, Egypt

Received: August 2022**, Accepted:** October 2022

ABSTRACT

Background and Objectives: Carbapenems are considered the last resort to treat several infections, particularly in intensive care units (ICUs). However, increasing carbapenem resistance is problematic because it leads to high morbidity and mortality rates. This study aimed to determine the rate of carbapenem resistance among Gram-negative bacteria collected from patients in ICUs and to identify their resistance mechanisms using phenotypic and genotypic methods.
Materials and Methods: Antimicrobial susceptibility testing was carried out using the disc diffusion method among 180 Gram-negative bacterial isolates. Productions of carbapenemases, metallo-beta-lactamases (MBLs) and the harboring of carbapenemase-encoding genes, were detected in 40 selected carbapenem-resistant Gram-negative bacteria (CR-GNB).
Results: Of 40 selected CR-GNB isolates, 28 (70%), and 20 (50%) isolates were phenotypically positive for carbapenemase, and MBL production, respectively. Furthermore, 22 (55%) showed amplification of one or more of the carbapenemase-encoding genes, including $bla_{\text{NDM-1}}$, $bla_{\text{VIM-2}}$, and $bla_{\text{OXA-48}}$. This study described the first emergence of NDM-1 producing *Klebsiella oxytoca* in Egyptian ICUs.
Conclusion: High incidence of CR-GNB detected in the ICUs in our study area may be attributed to the overuse of antibiotics, including carbapenems, and improper application of infection control measures. These findings confirm the need for the application of a strict antibiotic stewardship program.

Keywords: Antimicrobial drug resistance; Carbapenems; Gram-negative bacteria; Intensive care units; *Klebsiella oxytoca*

INTRODUCTION

Carbapenems are β-lactam antibiotics that inhibit bacterial cell wall synthesis through the inactivation of transpeptidase enzymes (1). They are often reserved for treating severe infections, especially those caused by highly resistant bacteria. Recently, the occurrence of carbapenem-resistant Gram-negative bacteria (CR-GNB) in intensive care units (ICUs) has increased dramatically (2). The World Health Organization priority list of antibiotic-resistant bacteria ranks carbapenem-resistant *Enterobacteriaceae* (CRE), *Acinetobacter baumannii* and *Pseudomonas aeruginosa* within the critical priority level (3). Evidence suggests high morbidity and mortality rates in patients infected by carbapenem-resistant pathogens compared with patients infected by carbapenem-susceptible pathogens (4). Several studies reported that

*Corresponding author: Heba Ahmed Mohamed, Ph.D, Department of Microbiology and Immunology, Faculty of Pharmacy, Minia University, Minia, Egypt. Tel: +201020296850 Fax: +20862369075 Email: Heba.ahmed@mu.edu.eg

Copyright © 2022 The Authors. Published by Tehran University of Medical Sciences.

This work is licensed under a Creative Commons Attribution-Non Commercial 4.0 International license (https://creativecommons.org/licenses/by-nc/4.0/). Noncommercial uses of the work are permitted, provided the original work is properly cited.

the prevalence of carbapenem resistance is increasing in the world, especially in Egypt (5-7).

The main mechanisms of carbapenem resistance include: (i) enzymatic hydrolysis of carbapenems by carbapenemases, (ii) production of extended-spectrum β-lactamases (ESBLs) and/or AmpC β-lactamases, which possess weak carbapenemase activity, combined with bacterial cell membrane alterations or efflux pump upregulation, and (iii) modification of penicillin-binding proteins (8, 9).

A large variety of carbapenemases have been identified as belonging to three classes of β-lactamases: Ambler class A (*Klebsiella pneumoniae* carbapenemase [KPC], nonmetallocarbapenemase [NMC], *Serratia marcescens* enzymes [SME], imipenem-hydrolyzing β-lactamases [IMI], etc.), class B/metallo-β-lactamases (MBLs) (New Delhi MBL [NDM], imipenemase [IMP], Verona integron-encoded MBL [VIM], etc.), and class D (oxacillinase [OXA] and *Pseudomonas*-specific enzymes [PSE]) (10). These classes are of great clinical importance among nosocomial pathogens. Class B carbapenemases/MBLs have been reported as responsible for carbapenem resistance among *Enterobacteriaceae* (11).

This study aimed to determine the prevalence and distribution of carbapenem resistance patterns in different ICUs in Egypt and to identify carbapenemases production as a resistance mechanism using phenotypic methods and genotypic detection of carbapenemase-encoding genes by PCR.

MATERIALS AND METHODS

Sample collection. A total of 400 clinical samples were collected from the ICUs of four major hospitals in Minia governorate, Egypt, including El-Minia University Hospital, El-Minia Health Insurance Hospital, El-Minia Gynecology and Obstetrics Hospital, and El-Minia Nephrology and Urology University Hospital, between November 2018 and October 2019. Samples collected included 318 urine (79.5%), 76 blood (19%), and 6 sputum (1.5%). Of them, 180 Gram-negative bacterial isolates were recovered and identified by conventional methods.

Antimicrobial susceptibility testing. All isolates underwent antimicrobial susceptibility testing using cefotaxime (30 μg), gentamicin (10 μg) (Oxoid, UK), ofloxacin (5 μg), azithromycin (15 μg), amoxicillin-clavulanic acid (30 μg), trimethoprim-sulfamethoxazole (25 μg) (Bioanalyse, Turkey), imipenem (10 μg), meropenem (10 μg), ertapenem (10 μg), and doripenem (10 μg) (Lilofilchem, Italy), according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (12) by the disc diffusion method using Mueller–Hinton agar (MHA) (Oxoid). *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as quality control strains.

40 selected CR-GNB isolates (*P. aeruginosa* (n=14, 35%), *Enterobacter* spp. (n=12, 30%), *Klebsiella oxytoca* (n=8, 20%), *E. coli* (n=4, 10%) and *Citrobacter koseri* (n=2, 5%)) for use in further investigations.

Phenotypic detection of carbapenemases by modified Hodge test (MHT). Briefly, MHT was performed by adjusting a suspension of *E. coli* ATCC 25922 to 0.5 McFarland turbidity standard in 5mL of sterile broth or saline. Then, 4.5 mL of sterile broth or saline was added to 0.5 mL of this suspension then it was streaked as a lawn onto a MHA plate. A disc of ertapenem (10 μg) was placed at the center of the plate. The test isolate was streaked in a straight line from the disc's edge to the plate's edge. One disc of ertapenem was used to test four isolates per plate. The plate was incubated overnight in ambient air at 37°C. The appearance of inhibition zones in a cloverleaf-like pattern indicated carbapenemase production and was considered as MHT-positive result (13).

Detection of MBLs by disc diffusion method or imipenem-EDTA combined disc test (IMP-EDTA CDT). Briefly, the isolates were streaked onto MHA plates. Then, two discs (a 10-μg imipenem disc and a 10-μg imipenem disc to which 10 μL 0.5 M EDTA (Oxoid) was added to obtain the desired concentration of 750 μg) were placed on the plate and incubated at 35°C for 16-18 hours. The tested isolates were considered MBL producers when the difference between the inhibition zones of the imipenem-EDTA disc and that of imipenem disc alone without EDTA was ≥7 mm (14).

Detection of carbapenemases by PCR. PCR analysis was performed to detect bla_{IMP-2}, bla_{VIM-2}, bla_{NDM-1}, bla_{KPC-2} and bla_{OXA-48}. The primers are listed in Table 1. Briefly, after DNA extraction using boiling method (15), PCR was performed using T-Personal Thermal Cycler (Biometra, Germany). PCR ampli-

Table 1. Primers sequences, amplicon size, annealing temp., and PCR conditions

Target	Sequence (5′ → 3')	Amplicon size (bp)	Ref.
KPC-2	F:5′-TCGCTAAACTCGAACAGG-3' R:5′-TTACTGCCCGTTGACGCCCAATCC-3'	785	(16)
NDM-1	F:5′-GGTTTGGCGATCTGGTTTTC-3' R:5′-CGGAATGGCTCATCACGATC-3'	621	(17)
OXA-48	F:5′-TTGGTGGCATCGATTATCGG-3' R:5′-GAGCACTTCTTTTGTGATGGC-3'	743	(18)
IMP-2	F:5′-GGCAGTCGCCCTAAAACAAA-3' R:5′-TAGTTACTTGGCTGTGATGG-3'	737	(19)
VIM-2	F:5′-AAAGTTATGCCGCACTCACC-3' R:5′-TGCAACTTCATGTTATGCCG-3'	865	(20)

fication was performed in a 25-µL reaction mixture containing 2 µL of DNA template, 12 µL of Dream Taq Green PCR Master Mix (Thermo Fisher Scientific Baltics UAB, Vilnius, Lithuania), 9 µL of distilled water, and 1 µL each of 20 pmol forward and reverse primers. The resultant amplicons were analyzed by electrophoresis in a 1.5% Top Vision agarose gel (Thermo Fisher Scientific Baltics UAB) stained with ethidium bromide (Sigma-Aldrich, USA) to identify the specific amplified product by comparing its size against a Gene Ruler 100 bp DNA Ladder (Thermo Fisher Scientific Baltics UAB).

Statistical analysis. Data were statistically analyzed using SPSS 20.0 statistical software (IBM Corp.,Armonk, NY, USA). Categorized data were expressed as numbers and percentages. The level of agreement between phenotypic and genotypic methods was estimated using the Cohen kappa (K), which was interpreted as follows: ≤ 0, no agreement; 0.01-0.20, none to slight agreement; 0.21-0.40, fair agreement; 0.41-0.60, moderate agreement; 0.61-0.80, substantial agreement; and 0.81–1.00, almost perfect agreement (21). A p-value < 0.05 was considered significant. The sensitivity, specificity, positive and negative predictive values, and accuracy of the phenotypic methods were evaluated using the PCR results as the gold standard (22).

RESULTS

400 clinical samples were collected from ICUs of four hospitals in El-minia governorate as listed in (Table 2). Out of the 400 samples, 214 (53.5%) were taken from male patients while 186 (46.5%) were taken from female ones. Age over 40 years is considered a high risk factor for CRE infection (Table 3). We recovered 180 Gram-negative bacterial isolates as follows: *P. aeruginosa* (n = 58, 32%), *E. coli* (n = 50, 28%), *Enterobacter* spp. (n = 24, 13%), *K. oxytoca* (n = 20, 11%), *Proteus* (n = 10, 6%), *Citrobacter freundii* (n = 10, 6%) and *C. koseri* (n = 8, 4%).

Antimicrobial susceptibility. Figs. 1 and 2 summarize the resistance rates of the isolates. *Enterobacteriaceae* showed the highest resistance to amoxicillin-clavulanic acid (85.2%), followed by cefotaxime (3rd generation cephalosporins) (77%) and sulfamethoxazole-trimethoprim (72.1%) and the lowest rate of resistance to ertapenem (39.3%) and gentamicin (37.7%), with gentamicin being the most effective antimicrobial agent. Among the carbapenem antibiotics, meropenem and ertapenem were the most effective (Fig. 1).

Table 2. Prevalence of clinical samples and isolates in different hospitals ICUs.

Hospitals	No. of samples	No. of Gram-negative bacterialisolates
El-Minia university hospital	232	106
El-Minia health insurance hospital	88	38
El-Minia women and obstetric hospital	52	24
El-Minia nephrology and urology hospital	28	12
Total	400	180

Table 3. Numbers and percantages (%) of CRE species in relation to patient's age and gender.

Age / Gender	0-20	21-40	41-60	>60	Total
Female	0	0	2 (7.7%)	8 (30.8%)	10 (38.5%)
Male	0	2 (7.7%)	10 (38.5%)	4 (15.4%)	16 (61.5%)
Total CRE	0	2 (7.7%)	12 (46.2%)	12 (46.2%)	26 (100%)

CRE: carbapenem-resistant *Enterobacteriaceae*

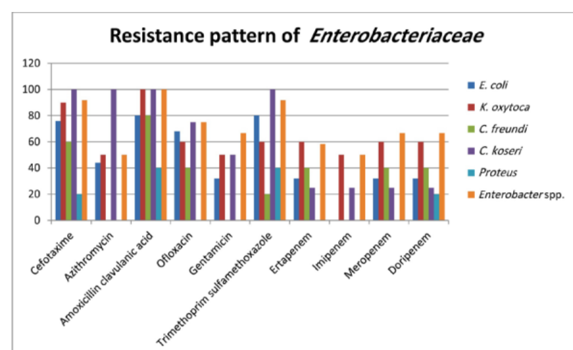

Fig. 1. Antimicrobial resistance rate (%) among *Enterobacteriaceae* isolates.

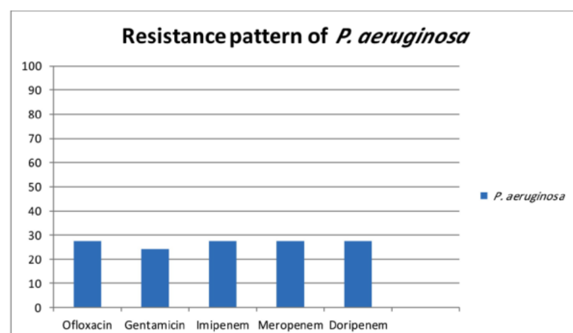

Fig. 2. Antimicrobial resistance rate (%) among *P. aeruginosa* isolates.

P. aeruginosa showed the least resistance (24.1%) to gentamicin, making it the most effective antimicrobial agent. The resistance rate was 27.6% for both ofloxacin and the carbapenems (imipenem, meropenem, and doripenem) (Fig. 2).

Resistance mechanisms. We selected 40 CR-GNB isolates for detection of resistance mechanisms including MBL, carbapenemases production as well as detection of cabapenemase-encoding genes. All selected CR-GNB were isolated from urine.

Detection of carbapenemase production. The most common carbapenemase-producing isolate was *P. aeruginosa* (25%), followed by *Enterobacter* spp. (20%), *E. coli* (10%), *K. oxytoca* (10%), and *C. koseri* (5%) (Table 4).

MBL detection. The most common MBL-producing isolate was *P. aeruginosa* (20%) followed by *E.coli* (10%), *K. oxytoca* (10%), *Enterobacter* spp. (5%) and *C. koseri* (5%) (Table 4).

PCR-based detection of carbapenemase-encoding genes. Out of the 40 Gram-negative isolates, 22 were identified as carbapenemases harboring CR-GNB (Fig. 3). Of these 22 CR-GNB, four (18.2%) harbored more than one carbapenemase gene, including 2 *Enterobacter* spp., which harbored bla_{OXA48} + bla_{NDM-1} genes and 2 *P. aeruginosa*, which harbored bla_{VIM-2} + bla_{NDM-1} genes. Isolates carrying bla_{NDM-1} alone, bla_{VIM-2} alone, bla_{OXA-48} + bla_{NDM-1}, and bla_{VIM-2} + bla_{NDM-1} constituted (16, 72.7%), (2, 9.1%), (2, 9.1%), and (2, 9.1%), respectively (Fig. 4), noting that this is the first time to detect NDM-1 gene in *K. oxytoca* from Egyptian ICUs.

Table 4. Distribution of MBL and carbapenemases among CR-GNB isolates

CR-GNB isolates (n=40)	MHT (carbapenemases)		(IMP-EDTA CDT) (MBLs)	
	Positive	Negative	Positive	Negative
P. aeruginosa 14 (35%)	10 (25%)	4 (10%)	8 (20%)	6 (15%)
Enterobacter spp. 12 (30%)	8 (20%)	4 (10%)	2 (5%)	10 (25%)
K. oxytoca 8 (20%)	4 (10%)	4 (10%)	4 (10%)	4 (10%)
E. coli 4 (10%)	4 (10%)	0 (0%)	4 (10%)	0 (0%)
C. koseri 2 (5%)	2 (5%)	0 (0%)	2 (5%)	0 (0%)
Total (CR-GNB) (n=40)	28 (70%)	12 (30%)	20 (50%)	20 (50%)
Total (CRE) (n=26)	18 (69.2%)	8 (30.8%)	12 (46.2%)	14 (53.8%)

CR-GNB: carbapenem-resistant Gram-negative bacteria, CRE: carbapenem-resistant *Enterobacteriaceae*, MHT: modified Hodge test, IMP-EDTA CDT: imipenem-EDTA combined disc test, MBLs: metallo-beta-lactamases.

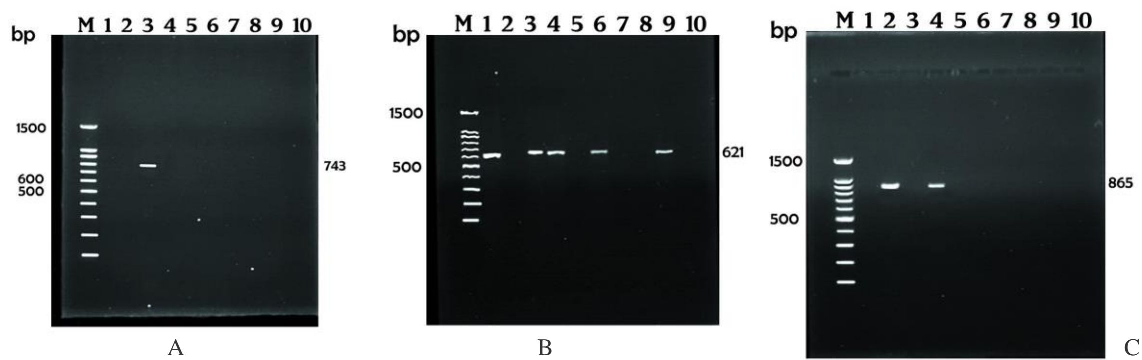

Fig. 3. Agarose gel electrophoresis showing PCR products of $bla_{\text{OXA-48}}$, $bla_{\text{NDM-1}}$, and $bla_{\text{VIM-2}}$: (A) Agarose gel electrophoresis showing PCR products of $bla_{\text{OXA-48}}$. Lane M is a DNA ladder 100 bp; Lane 10 is a negative control; Lane 3 is a typical band size of 743 bp corresponding to the molecular size of $bla_{\text{OXA-48}}$ gene; Lanes 1, 2, and 4-9 are negative samples, (B) Agarose gel electrophoresis showing PCR products of $bla_{\text{NDM-1}}$. Lane M is a DNA ladder 100 bp; Lane 10 is a negative control; Lane 1, 3, 4, 6, and 9 are typical bands size of 621 bp corresponding to the molecular size of $bla_{\text{NDM-1}}$ gene; Lanes 2, 5, 7, and 8 are negative samples, (C) Agarose gel electrophoresis showing PCR products of $bla_{\text{VIM-2}}$. Lane M is a DNA ladder 100 bp; Lane 10 is a negative control; Lane 2, and Lane 4 are typical bands size of 865 bp corresponding to the molecular size of $bla_{\text{VIM-2}}$ gene; Lanes 1, 3, and 5-9 are negative samples.

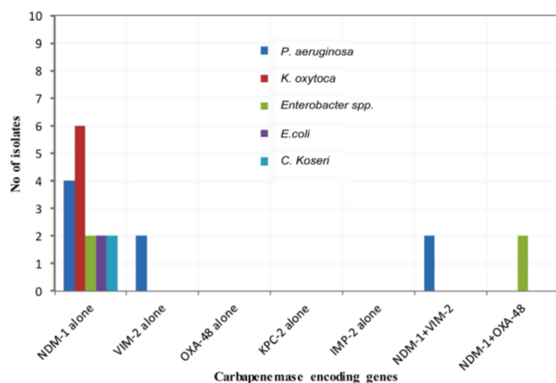

Fig. 4. The distribution of carbapenemase-encoding genes in the study isolates.

Phenotypic and genotypic correlation. Of the 40 isolates that underwent PCR testing for carbapenemase-encoding genes, 22 were PCR-positive, and 18 were PCR-negative. All 22 PCR-positive isolates were MHT-positive (K = 0.271, P = 0.071), with the exception of four (two each of *K. oxytoca* and *Enterobacter* spp.), while eight isolates (4 *Enterobacter* spp., 2 *P. aeruginosa*, and 2 *K. oxytoca*) were CDT-negative (K = 0.3, P = 0.057). Of the 18 PCR-negative isolates, 10 were MHT-positive. Phenotypic and genotypic results were not statistically significant.

Considering PCR as the gold standard test, the IMP-EDTA CDT showed higher specificity and accuracy compared with the MHT. However, the MHT was more sensitive (Table 5).

DISCUSSION

Carbapenems were used as the drug of choice and the last resort for treating infections due to multidrug-resistant Gram-negative bacilli acquired in ICUs. However, the increasing resistance to carbapenems, mainly among Gram-negative bacteria, is a concerning issue as it leads to treatment failure and high morbidity and mortality rates (23). Thus, this study focused on determining carbapenem resistance rates and mechanisms in Egypt. The most common Gram-negative bacteria isolated from the ICUs in our study was *P. aeruginosa* (32.2%). This result agreed with studies conducted in tertiary-care hospital ICUs done by Moolchandani et al. (24) and Uc-Cachón et al. (25), in which *Pseudomonas* spp. were the most common isolates (19.09% and 30.41%, respectively). Another study performed in Egyptian ICU also correlated with our results (26).

In our study, the isolated Gram-negative bacteria exhibited a high resistance rate to different antimicrobial agents (Figs. 1 and 2). Of concern, some showed resistance to all tested agents, reflecting a worrisome situation in ICUs. The overall resistance of Gram-negative bacteria was 40% for imipenem, which was higher than previous studies recorded in ICUs in Egypt. A Previous study done in ICU in Egypt in 2010 (27) detected no imipenem resistance. Later studies done in ICUs in Egypt in 2013 (28), 2014 (29) and 2018 (30) in which imipenem re-

Table 5. Sensitivity, specificity, TP, TN, FP, FN, NPV, and PPV in MHT versus IMP.EDTA test

	TP	TN	FP	FN	Sensitivity	Specificity	PPV	NPV	Accuracy
IMP-EDTA CDT	14	12	6	8	63.6%	66.7%	70%	60%	65%
MHT	18	8	10	4	81.8%	44.4%	64.3%	66.7%	60%

TP: True positive, TN: True negative, FP: False positive, FN: False-negative, NPV: Negative predictive value, PPV: Positive predictive value, IMP-EDTA CDT: Imipenem-EDTA Combined disc test, MHT: modified Hodge test.

sistance represented 16%, 23.4%, and 25%, respectively. These findings confirm the increasing rate of carbapenem resistance in Egypt. This may occurred as a result of the overuse of carbapenems in Egypt in the last ten years.

The *Enterobacteriaceae* isolates in our study showed the least resistance to ertapenem and meropenem among the carbapenems. In contrast, Xie et al. reported that *Enterobacteriaceae* showed the highest resistance rate to ertapenem (31). The resistance rate of the *P. aeruginosa* isolates in our study to carbapenems was the same for imipenem, meropenem, and doripenem (27.6%) (Fig. 2). However, a higher imipenem-resistance rate (82%) was detected by Siwakoti et al. (32) in an ICU in Nepal.

In the present study, 28 (70%) Gram-negative and 18 (69.2%) *Enterobacteriaceae* isolates were identified as carbapenemase producers by MHT. This agrees with Dirar et al. (33), who reported carbapenemase production by MHT in 67.3% and 74.5%, respectively. However, our results were lower than that of Begum and Shamsuzzaman (80%) (34) and higher than those of Rao et al. (51.42%) (35).

In this study, the rate of carbapenemase producers by MHT was highest for *P. aeruginosa* (25%), followed by *Enterobacter* spp. (20%) (Table 4). Amjad et al. (36) reported that the rate of carbapenemase production was highest in *E. coli* (38%), followed by *P. aeruginosa* (30%), while Rao et al. (35) found that *Klebsiella* spp. (14%), *P. aeruginosa* (14%), and *E. coli* (14%) represented the majority of carbapenemase-producing isolates.

In our study 20 of the 40 (50%) isolates were MBL producers (Table 4) , which agreed with Gautam et al. (37), who reported that the prevalence of MBL producers among isolates was 50.6%. However, a lower percentage of MBL prevalence was reported by Gupta et al. (38) (21.4%). Furthermore, a higher percentage of MBL producers (80%) was reported by Namaei et al. (39). In our study, the highest rate of MBL producers was observed in *P. aeruginosa* (Table 4). The same result was obtained by Gupta et al. (38).

Regarding PCR testing, 55% (22/40) of the isolates in the current study were PCR-positive for one or more of the carbapenemase-encoding genes. Similar results were reported by Elbadawi et al. (40), who detected carbapenemase genes in 58.7% of isolates. Among the 22 PCR-positive isolates for the carbapenemase-encoding genes in our study, *P. aeruginosa* (8, 36.4%) showed the highest rate of harbouring carbapenemase-encoding genes, followed by *K. oxytoca* (6, 27.2%), *Enterobacter* spp. (4, 18.2%), *E. coli* (2, 9.1%), and *C. koseri* (2, 9.1%). Codjoe et al. (41) reported the highest rate of harbouring carbapenemase-encoding genes in isolates of *P. aeruginosa* and *Acinetobacter* spp.

Among the 40 isolates, the most prevalent carbapenemase-encoding gene was $bla_{\text{NDM-1}}$ (20, 50%), followed by $bla_{\text{VIM-2}}$ (4, 10%) and $bla_{\text{OXA-48}}$ (2, 5%) (Fig. 4). This result is in accordance with Garg et al. (42) and Tawfick et al. (43), where bla_{NDM} was the most predominant carbapenemase-encoding gene. In contrast, bla_{NDM} (5, 2.6%) was recorded by Okoche et al. (44) as the least prevalent carbapenemase-encoding gene among isolates. Mushi et al. (45) reported bla_{VIM} (28, 12.3%) as the second most predominant carbapenemase-encoding gene among isolates, while Okoche et al. (44) reported bla_{VIM} (21, 10.7%) as the most prevalent carbapenemase-encoding gene among isolates. In contrast to our current study, El-Mahallawy et al. (46) reported $bla_{\text{OXA-48}}$ as the most prevalent carbapenemase-encoding gene among isolates. In our study, none of the CR-GNB isolates harbored the bla_{KPC} and bla_{IMP} genes, which correlated with the findings of Baran and Aksu (47).

In the present study, of the 22 bacterial isolates harboring one or more of the carbapenemase-encoding genes, 18 (81.8%) carried a single gene, and 4 (18.2%) carried more than one carbapenemase gene ($bla_{\text{OXA-48}}$ + $bla_{\text{NDM-1}}$ and $bla_{\text{VIM-2}}$ + $bla_{\text{NDM-1}}$) (Fig. 4). These results agreed with Mushi et al. (45) and

Kazi et al. (10), who reported that multiple carbapenemase-encoding genes were harbored by 18.91% and 18.75% of isolates, respectively. In our study, the multi-carbapenemases CR-GNB co-producers show near-complete resistance to the tested antimicrobials and, thus, limitation in treatment options.

In our study, carbapenemase screening and detection were performed using both phenotypic and genotypic tests. Differences between the phenotypic and genotypic results were not statistically significant. Considering PCR as the gold standard test, the MHT was more sensitive than the CDT (Table 5). This agreed with a study that reported MHT and CDT sensitivity as 65.62% and 55.22%, respectively (48). The IMP-EDTA CDT had higher specificity (66.7%) and accuracy (65%) compared with the MHT (Table 5). These results were lower than those of Galani et al. (49). Despite phenotypic tests being cheap, it has some disadvantages including difficulty in interpretation, differences in sensitivity or specificity depending on the tested isolates, and time-consuming. Thus, genotypic tests are reliable and used to overcome these disadvantages.

CONCLUSION

Our results indicate a high prevalence of CR-GNB in ICUs in Egypt, particularly *P. aeruginosa*, which was the most prevalent Gram-negative bacteria. This may be attributed to the overuse and misuse of antibiotics, including carbapenems, and the improper application of infection control measures in certain Egyptian hospitals and ICUs. First detection of NDM-1-producing *K. oxytoca* among ICUs in Egypt is alarming and highlights the importance of the application of an antibiotic stewardship program to reduce the dissemination of carbapenem-resistant isolates.

REFERENCES

1. Sauvage E, Kerff F, Terrak M, Ayala JA, Charlier P. The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis. *FEMS Microbiol Rev* 2008; 32: 234-258.
2. Akova M, Daikos GL, Tzouvelekis L, Carmeli Y. Interventional strategies and current clinical experience with carbapenemase-producing Gram-negative bacteria. *Clin Microbiol Infect* 2012; 18: 439-448.
3. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect Dis* 2018; 18: 318-327.
4. Cai B, Echols R, Magee G, Arjona Ferreira JC, Morgan G, Ariyasu M, et al. Prevalence of carbapenem-resistant Gram-negative infections in the United States predominated by *Acinetobacter baumannii* and *Pseudomonas aeruginosa. Open Forum Infect Dis* 2017; 4: ofx176.
5. Khalifa HO, Soliman AM, Ahmed AM, Shimamoto T, Hara T, Ikeda M, et al. High carbapenem resistance in clinical Gram-negative pathogens isolated in Egypt. *Microb Drug Resist* 2017; 23: 838-844.
6. 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: 2.
7. Jean S-S, Harnod D, Hsueh P-R. Global threat of carbapenem-resistant Gram-negative bacteria. *Front Cell Infect Microbiol* 2022; 12: 823684.
8. Messaoudi A, Mansour W, Jaidane N, Chaouch C, Boujaâfar N, Bouallègue O. Epidemiology of resistance and phenotypic characterization of carbapenem resistance mechanisms in *Klebsiella pneumoniae* isolates at Sahloul University Hospital-Sousse, Tunisia. *Afr Health Sci* 2019; 19: 2008-2020.
9. Anderson REV, Boerlin P. Carbapenemase-producing *Enterobacteriaceae* in animals and methodologies for their detection. *Can J Vet Res* 2020; 84: 3-17.
10. Kazi M, Drego L, Nikam C, Ajbani K, Soman R, Shetty A, et al. Molecular characterization of carbapenem-resistant *Enterobacteriaceae* at a tertiary care laboratory in Mumbai. *Eur J Clin Microbiol Infect Dis* 2015; 34: 467-472.
11. Queenan AM, Bush K. Carbapenemases: the versatile beta-lactamases. *Clin Microbiol Rev* 2007; 20: 440-458.
12. Weinstein MP, Lewis JS, 2nd. The clinical and laboratory standards institute subcommittee on antimicrobial susceptibility testing: background, organization, functions, and processes. *J Clin Microbiol* 2020; 58(3): e01864-19 .
13. Lee K, Chong Y, Shin HB, Kim YA, Yong D, Yum JH. Modified Hodge and EDTA-disk synergy tests to screen metallo-beta-lactamase-producing strains of *Pseudomonas* and *Acinetobacter* species. *Clin Microbiol Infect* 2001; 7: 88-91.
14. Thapa P, Bhandari D, Shrestha D, Parajuli H, Chaudhary P, Amatya J, et al. A hospital based surveillance of

metallo-beta-lactamase producing Gram negative bacteria in Nepal by imipenem-EDTA disk method. *BMC Res Notes* 2017; 10: 322.

15. Abdulall AK, Tawfick MM, El Manakhly AR, El Kholy A. Carbapenem-resistant Gram-negative bacteria associated with catheter-related bloodstream infections in three intensive care units in Egypt. *Eur J Clin Microbiol Infect Dis* 2018; 37: 1647-1652.
16. Biberg CA, Rodrigues ACS, Carmo SFD, Chaves CEV, Gales AC, Chang MR. KPC-2-producing *Klebsiella pneumoniae* in a hospital in the Midwest region of Brazil. *Braz J Microbiol* 2015; 46: 501-504.
17. Farajzadeh Sheikh A, Rostami S, Jolodar A, Tabatabaiefar MA, Khorvash F, Saki A, et al. Detection of metallo-beta lactamases among carbapenem-resistant *Pseudomonas aeruginosa. Jundishapur J Microbiol* 2014; 7(11): e12289.
18. Aktaş Z, Kayacan ÇB, Schneider I, Can B, Midilli K, Bauernfeind A. Carbapenem-hydrolyzing oxacillinase, OXA-48, persists in *Klebsiella pneumoniae* in Istanbul, Turkey. *Chemotherapy* 2008; 54: 101-106.
19. Khurana S, Mathur P, Kapil A, Valsan C, Behera B. Molecular epidemiology of beta-lactamase producing nosocomial Gram-negative pathogens from North and South Indian hospitals. *J Med Microbiol* 2017; 66: 999-1004.
20. Yan JJ, Hsueh PR, Ko WC, Luh KT, Tsai SH, Wu HM, et al. Metallo-beta-lactamases in clinical Pseudomonas isolates in Taiwan and identification of VIM-3, a novel variant of the VIM-2 enzyme. *Antimicrob Agents Chemother* 2001;45: 2224-2228.
21. McHugh ML. Interrater reliability: the kappa statistic. *Biochem Med (Zagreb)* 2012; 22: 276-282.
22. Ilstrup DM. Statistical methods in microbiology. *Clin Microbiol Rev* 1990; 3: 219-226.
23. Katchanov J, Asar L, Klupp E-M, Both A, Rothe C, König C, et al. Carbapenem-resistant Gram-negative pathogens in a German university medical center: Prevalence, clinical implications and the role of novel β-lactam/β-lactamase inhibitor combinations. *PLoS One* 2018; 13(4): e0195757.
24. Moolchandani K, Sastry AS, Deepashree R, Sistla S, Harish BN, Mandal J. Antimicrobial resistance surveillance among intensive care units of a tertiary care hospital in Southern India. *J Clin Diagn Res* 2017; 11(2): DC01-DC07.
25. Uc-Cachón AH, Gracida-Osorno C, Luna-Chi IG, Jiménez-Guillermo JG, Molina-Salinas GM. High prevalence of antimicrobial resistance among Gram-negative isolated Bacilli in intensive care units at a tertiary-care Hospital in Yucatán Mexico. *Medicina (Kaunas)* 2019; 55: 588.
26. Hasanin A, Eladawy A, Mohamed H, Salah Y, Lotfy A, Mostafa H, et al. Prevalence of extensively drug-resistant Gram negative bacilli in surgical intensive care in Egypt. *Pan Afr Med J* 2014; 19: 177.
27. Talaat M, Hafez S, Saied T, Elfeky R, El-Shoubary W, Pimentel G. Surveillance of catheter-associated urinary tract infection in 4 intensive care units at Alexandria university hospitals in Egypt. *Am J Infect Control* 2010; 38 :222-228.
28. Fahmey SS. Early-onset sepsis in a neonatal intensive care unit in Beni Suef, Egypt: bacterial isolates and antibiotic resistance pattern. *Korean J Pediatr* 2013; 56: 332-337.
29. Kishk RM, Mandour MF, Farghaly RM, Ibrahim A, Nemr NA. Pattern of blood stream infections within neonatal intensive care unit, Suez Canal University Hospital, Ismailia, Egypt. *Int J Microbiol* 2014; 2014: 276873.
30. Seliem WA, Sultan AM. Etiology of early onset neonatal sepsis in neonatal intensive care unit–Mansoura, Egypt. *J Neonatal Perinatal Med* 2018; 11: 323-330.
31. Xie S, Fu S, Li M, Guo Z, Zhu X, Ren J, et al. Microbiological characteristics of carbapenem-resistant *Enterobacteriaceae* clinical isolates collected from county hospitals. *Infect Drug Resist* 2020; 13: 1163 -1169.
32. Siwakoti S, Subedi A, Sharma A, Baral R, Bhattarai NR, Khanal B. Incidence and outcomes of multidrug-resistant Gram-negative bacteria infections in intensive care unit from Nepal-a prospective cohort study. *Antimicrob Resist Infect Control* 2018; 7: 114.
33. Dirar M, Bilal N, Ibrahim ME, Hamid M. Resistance Patterns and Phenotypic Detection of β-lactamase Enzymes among *Enterobacteriaceae* isolates from referral hospitals in Khartoum State, Sudan. *Cureus* 2020; 12(3): e7260.
34. Begum N, Shamsuzzaman SM. Emergence of carbapenemase-producing urinary isolates at a tertiary care hospital in Dhaka, Bangladesh. *Ci Ji Yi Xue Za Zhi* 2016; 28: 94-98.
35. Rao MR, Chandrashaker P, Mahale RP, Shivappa SG, Gowda RS, Chitharagi VB. Detection of carbapenemase production in *Enterobacteriaceae* and P*seudomonas* species by carbapenemase Nordmann–Poirel test. *J Lab Physicians* 2019; 11: 107-110.
36. Amjad A, Mirza IA, Abbasi S, Farwa U, Malik N, Zia F. Modified Hodge test: A simple and effective test for detection of carbapenemase production. *Iran J Microbiol* 2011; 3: 189-193.
37. Gautam S, Bhattarai NR, Rai K, Poudyal A, Khanal B. Detection of bla NDM-1 encoding imepenemase among the imipenem-resistant Gram-negative bacilli isolated from various clinical samples at a tertiary care hospital of eastern Nepal: a descriptive cross-sectional study. *Int J Microbiol* 2020; 2020: 8861204.
38. Gupta R, Malik A, Rizvi M, Ahmed M. Presence of metallo-beta-lactamases (MBL), extended-spectrum

beta-lactamase (ESBL) & AmpC positive non-fermenting Gram-negative bacilli among Intensive Care Unit patients with special reference to molecular detection of $bla_{CTX\text{-}M}$ & bla_{AmpC} genes. *Indian J Med Res* 2016; 144: 271-275.
39. Namaei MH, Yousefi M, Askari P, Roshanravan B, Hashemi A, Rezaei Y. High prevalence of multi-drug-resistant non-fermentative Gram-negative bacilli harboring bla_{IMP}-1and $bla_{VIM\text{-}1}$ metallo-beta-lactamase genes in Birjand, south-east Iran. *Iran J Microbiol* 2021; 13: 470-479.
40. Elbadawi HS, Elhag KM, Mahgoub E, Altayb HN, Ntoumi F, Elton L, et al. Detection and characterization of carbapenem resistant Gram-negative bacilli isolates recovered from hospitalized patients at Soba University Hospital, Sudan. *BMC Microbiol* 2021; 21: 136.
41. Codjoe FS, Donkor ES, Smith TJ, Miller K. Phenotypic and genotypic characterization of carbapenem-resistant Gram-negative bacilli pathogens from hospitals in Ghana. *Microb Drug Resist* 2019; 25: 1449-1457.
42. Garg A, Garg J, Kumar S, Bhattacharya A, Agarwal S, Upadhyay GC. Molecular epidemiology & therapeutic options of carbapenem-resistant Gram-negative bacteria. *Indian J Med Res* 2019; 149: 285-289.
43. 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: 1251-1259.
44. Okoche D, Asiimwe BB, Katabazi FA, Kato L, Najjuka CF. Prevalence and characterization of carbapenem-resistant *Enterobacteriaceae* isolated from Mulago National Referral Hospital, Uganda. *PLoS One* 2015; 10(8):e0135745.
45. Mushi MF, Mshana SE, Imirzalioglu C, Bwanga F. Carbapenemase Genes among multidrug resistant Gram negative clinical isolates from a tertiary hospital in Mwanza, Tanzania. *Biomed Res Int* 2014; 2014: 303104.
46. ElMahallawy HA, Zafer MM, Amin MA, Ragab MM, Al-Agamy MH. Spread of carbapenem resistant *Enterobacteriaceae* at tertiary care cancer hospital in Egypt. *Infect Dis (Lond)* 2018; 50: 560-564.
47. Baran I, Aksu N. Phenotypic and genotypic characteristics of carbapenem-resistant *Enterobacteriaceae* in a tertiary-level reference hospital in Turkey. *Ann Clin Microbiol Antimicrob* 2016; 15: 20.
48. Kamel NA, Tohamy ST, Yahia IS, Aboshanab KM. Insights on the performance of phenotypic tests versus genotypic tests for the detection of carbapenemase-producing Gram-negative bacilli in resource-limited settings. *BMC Microbiol* 2022; 22: 248.
49. Galani I, Rekatsina PD, Hatzaki D, Plachouras D, Souli M, Giamarellou H. Evaluation of different laboratory tests for the detection of metallo-beta-lactamase production in *Enterobacteriaceae*. *J Antimicrob Chemother* 2008; 61: 548-553.