

## 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*

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### 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*<sub>NDM-1</sub>, *bla*<sub>VIM-2</sub>, and *bla*<sub>OXA-48</sub>. 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  $\beta$ -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

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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  $\beta$ -lactamases (ESBLs) and/or AmpC  $\beta$ -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  $\beta$ -lactamases: Ambler class A (*Klebsiella pneumoniae* carbapenemase [KPC], nonmetallocarbapenemase [NMC], *Serratia marcescens* enzymes [SME], imipenem-hydrolyzing  $\beta$ -lactamases [IMI], etc.), class B/metallo- $\beta$ -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  $\mu$ g), gentamicin (10  $\mu$ g) (Oxoid, UK), ofloxacin (5  $\mu$ g), azithromycin (15  $\mu$ g), amox-

icillin-clavulanic acid (30  $\mu$ g), trimethoprim-sulfamethoxazole (25  $\mu$ g) (Bioanalyse, Turkey), imipenem (10  $\mu$ g), meropenem (10  $\mu$ g), ertapenem (10  $\mu$ g), and doripenem (10  $\mu$ g) (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 5 mL 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  $\mu$ g) 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- $\mu$ g imipenem disc and a 10- $\mu$ g imipenem disc to which 10  $\mu$ L 0.5 M EDTA (Oxoid) was added to obtain the desired concentration of 750  $\mu$ g) 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  $\geq 7$  mm (14).

**Detection of carbapenemases by PCR.** PCR analysis was performed to detect *bla*<sub>IMP-2</sub>, *bla*<sub>VIM-2</sub>, *bla*<sub>NDM-1</sub>, *bla*<sub>KPC-2</sub> and *bla*<sub>OXA-48</sub>. 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'<br>R:5'-TTACTGCCCGTTGACGCCCAATCC-3' | 785                | (16) |
| NDM-1  | F:5'-GGTTTGCGATCTGGTTTTTC-3'<br>R:5'-CGGAATGGCTCATCACGATC-3'   | 621                | (17) |
| OXA-48 | F:5'-TTGGTGGCATCGATTATCGG-3'<br>R:5'-GAGCACTTCTTTTGTGATGGC-3'  | 743                | (18) |
| IMP-2  | F:5'-GGCAGTCGCCCTAAAACAAA-3'<br>R:5'-TAGTTACTTGGCTGTGATGG-3'   | 737                | (19) |
| VIM-2  | F:5'-AAAGTTATGCCGCACTCACC-3'<br>R:5'-TGCAACTTCATGTTATGCCG-3'   | 865                | (20) |

fication was performed in a 25- $\mu$ L reaction mixture containing 2  $\mu$ L of DNA template, 12  $\mu$ L of Dream Taq Green PCR Master Mix (Thermo Fisher Scientific Baltics UAB, Vilnius, Lithuania), 9  $\mu$ L of distilled water, and 1  $\mu$ L 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:  $\leq 0$ , 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 tak-

en 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 (3<sup>rd</sup> 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).

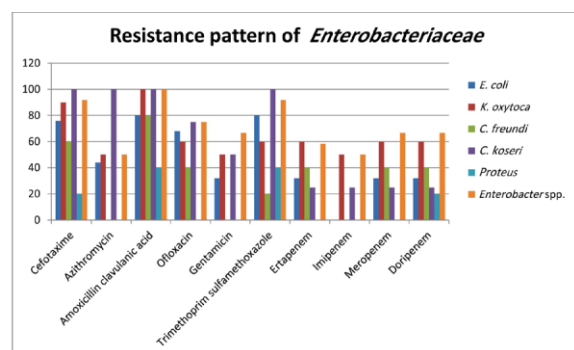
**Table 2.** Prevalence of clinical samples and isolates in different hospitals ICUs.

| Hospitals                                | No. of samples | No. of Gram-negative bacterial isolates |
|--|----------------|---|
| 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                                     |

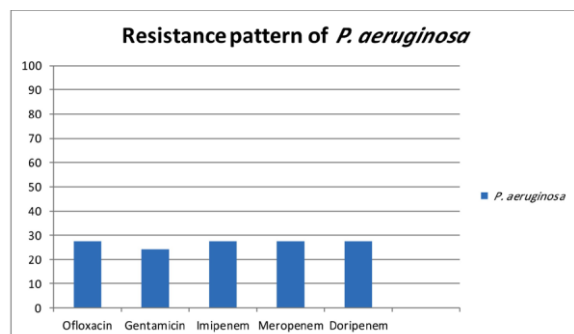
**Table 3.** Numbers and percentages (%) of CRE species in relation to patient’s age and gender.

| Age           | 0-20 | 21-40    | 41-60      | >60        | Total      |
|---------------|------|----------|------------|------------|------------|
| <b>Gender</b> |      |          |            |            |            |
| 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 carbapenemase-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).

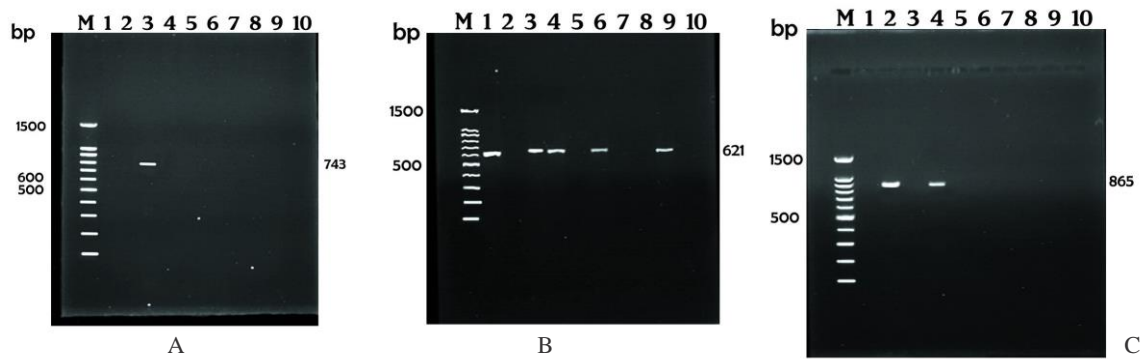
**MBL detection.** The most common MBL-producing isolate was *P. aeruginosa* (20%) followed by *E. coli* (10%), *K. oxytoca* (10%), *Enterobacter* spp. (5%) and *C. koseri* (5%) (Table 4).

**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.

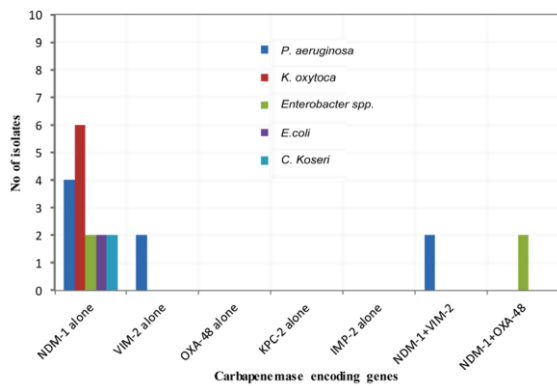
**Table 4.** Distribution of MBL and carbapenemases among CR-GNB isolates

| CR-GNB isolates (n=40)            | MHT (carbapenemases) |           | (IMP-EDTA CDT) (MBLs) |            |
|-----------------------------------|----------------------|-----------|-----------------------|------------|
|                                   | Positive             | Negative  | Positive              | Negative   |
| <i>P. aeruginosa</i> 14 (35%)     | 10 (25%)             | 4 (10%)   | 8 (20%)               | 6 (15%)    |
| <i>Enterobacter</i> spp. 12 (30%) | 8 (20%)              | 4 (10%)   | 2 (5%)                | 10 (25%)   |
| <i>K. oxytoca</i> 8 (20%)         | 4 (10%)              | 4 (10%)   | 4 (10%)               | 4 (10%)    |
| <i>E. coli</i> 4 (10%)            | 4 (10%)              | 0 (0%)    | 4 (10%)               | 0 (0%)     |
| <i>C. koseri</i> 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*<sub>OXA-48</sub>, *bla*<sub>NDM-1</sub>, and *bla*<sub>VIM-2</sub>: (A) Agarose gel electrophoresis showing PCR products of *bla*<sub>OXA-48</sub>. 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*<sub>OXA-48</sub> gene; Lanes 1, 2, and 4-9 are negative samples, (B) Agarose gel electrophoresis showing PCR products of *bla*<sub>NDM-1</sub>. 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*<sub>NDM-1</sub> gene; Lanes 2, 5, 7, and 8 are negative samples, (C) Agarose gel electrophoresis showing PCR products of *bla*<sub>VIM-2</sub>. 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*<sub>VIM-2</sub> 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 multi-drug-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*<sub>NDM-1</sub> (20, 50%), followed by *bla*<sub>VIM-2</sub> (4, 10%) and *bla*<sub>OXA-48</sub> (2, 5%) (Fig. 4). This result is in accordance with Garg et al. (42) and Tawfick et al. (43), where *bla*<sub>NDM</sub> was the most predominant carbapenemase-encoding gene. In contrast, *bla*<sub>NDM</sub> (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*<sub>VIM</sub> (28, 12.3%) as the second most predominant carbapenemase-encoding gene among isolates, while Okoche et al. (44) reported *bla*<sub>VIM</sub> (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*<sub>OXA-48</sub> as the most prevalent carbapenemase-encoding gene among isolates. In our study, none of the CR-GNB isolates harbored the *bla*<sub>KPC</sub> and *bla*<sub>IMP</sub> 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*<sub>OXA-48</sub> + *bla*<sub>NDM-1</sub> and *bla*<sub>VIM-2</sub> + *bla*<sub>NDM-1</sub>) (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.

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