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ORIGINAL ARTICLE

The emergence of carbapenemase *bla*$_{NDM}$ genotype among carbapenem-resistant *Enterobacteriaceae* isolates from Egyptian cancer patients

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Abstract

Carbapenem resistance among *Enterobacteriaceae* is a major concern that is increasingly reported worldwide. The objective of this study is to determine the incidence of carbapenem resistance as well as to investigate for carbapenemase-encoding genes among *Enterobacteriaceae* clinical isolates from cancer patients at different cancer institutes in Egypt. This determination was a cross-sectional study with a total of 135 clinical isolates collected over a period of 1 year. All isolates were sub-cultured on ChromID agar and subjected to phenotypic and molecular detection of carbapenemases. Most of the *Enterobacteriaceae* isolates were MDR with high resistance rates against tested antimicrobials. Overall, the results of PCR assays revealed that 89.62% (121/135) of isolates harbored one or more of the carbapenemase-encoding genes, while phenotypic assays revealed the production of carbapenemases in 68.88% (93/135) of isolates. BlastN analysis against the non-redundant genome sequences available in the GenBank database revealed that the *bla*$_{NDM-1}$ gene was the most prevalent genotype of carbapenemases in 93/135 (68.88%), followed by *bla*$_{OXA-48}$ 44/135 (32.59%), *bla*$_{OXA-23}$ 42/135 (31.11%), and *bla*$_{KPC-2}$ 2/135 (1.48%). *Klebsiella pneumoniae* isolates harbored the highest number of carbapenemase-encoding genes 34/121 (28.09%). The high prevalence of carbapenemases and/or their encoding genes among MDR *Enterobacteriaceae* bacteria in Egypt is alarming, thus, the management of serious infections caused by *Enterobacteriaceae*, particularly in cancer patients will be challenging to clinicians. Carbapenemase *bla*$_{NDM}$ genotype is emerging in cancer healthcare settings in Egypt, which could be the cause of the current increase in carbapenemase-producing *Enterobacteriaceae*.

Keywords Carbapenemases · *bla*$_{NDM}$ · *Enterobacteriaceae* · Cancer patients · Resistance · MDR

Introduction

Enterobacteriaceae members are Gram-negative bacteria, which are mainly inhabitants of the gut flora [1, 2]. Most members of this family cause such human infections as gastrointestinal infections, septicemia, pneumonia, meningitis, peritonitis, and urinary tract infections [2, 3]. In countries with low resources such as Egypt, cases of carbapenem-resistant enterobacterial infections, for instance, carbapenem-resistant *Klebsiella pneumoniae* (CRKP) infections and/or

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occurrence of carbapenemase-producing *K. pneumoniae* is increasing and present a serious burden which is a threat to public health [4]. These organisms easily acquire and transfer drug-resistant encoding genes through the mobile genetic elements, plasmids, and transposons. The acquisition of these determinants leads to the production of β-lactamases of which extended-spectrum β-lactamases (ESBLs) are the most common [3]. ESBLs in *Enterobacteriaceae* are reported to coexist with resistance determinants of other antimicrobial classes and as such these organisms become multi-drug resistant (MDR), hence limiting treatment options of infectious diseases. Carbapenem drugs have been used as a last resort to treat infections caused by MDR Gram-negative bacteria [5, 6]. However, there has been a global emergence of carbapenem-resistant bacteria [2], thought to be because of the extensive use and/or misuse without proper diagnosis of infection or self-medication by patients [7, 8]. As a result, there is selective pressure on microorganisms, which in turn enhances antimicrobial resistance. Infections caused by bacteria resistant to carbapenems often fail to respond to conventional treatment and are said to kill up to 50% of patients with bloodstream infections [2, 9]. Resistance to carbapenems is mostly mediated by the production of carbapenemases, which are β-lactamase enzymes with a capacity to hydrolyze not only the carbapenems but also all the other β-lactam agents [10]. The most common carbapenemases include veronica integron metallo-β-lactamases types (VIM), imipenemase (IMP) types, *K. pneumoniae* carbapenemase (KPC), oxacillinase-48 (OXA-48), and New Delhi metallo-β-lactamase-1 (NDM-1) encoded by carbapenem resistance, determining genes bla_{VIM}, bla_{IMP}, bla_{KPC}, $bla_{\text{OXA-48}}$, and bla_{NDM}, respectively [2]. In most sub-Saharan Africa, there is limited data on the prevalence and distribution of carbapenem resistance among *Enterobacteriaceae* [2, 11]. The aim of the current study was to study the molecular epidemiology of different types of carbapenemases among carbapenemase-producing *Enterobacteriaceae* isolates from cancer patients in Egypt.

Materials and methods

Study design and bacterial isolates

This study was a cross-sectional laboratory-based study that included 135 *Enterobacteriaceae* isolates from diverse biological specimens, collected from patients admitted to Cancer Institutes in Cairo, during the period from October 2016 to September 2017. The specimens were cultured on MacConkey's agar plates, and then sub-cultured on ChromID CARBA SMART media (bioMérieux, USA) to help in primary screening for carbapenemase-producing isolates. The bacterial species were identified based on cultural characteristics on a selective culture media, such as UriSelect (Bio-Rad Laboratories, France) and biochemical testing. Identification was furthermore confirmed using VITEK 2 automated system (bioMe'rieux, France).

Antimicrobial susceptibility testing and determination of MIC

The antimicrobial susceptibility profiles of the included *Enterobacteriaceae* isolates were determined against diverse antimicrobial classes using Kirby Bauer disk diffusion in order to study the correlation between the production of carbapenemases and antimicrobial resistance patterns according to other reports [5, 12]. The test antimicrobials included amoxicillin-clavulanate (AMC, 30 μg/disc), piperacillin-tazobactam (TPZ, 10 μg/disc), cefoxitin (FOX, 30 μg/disc), cefepime (FEP, 30 μg/disc), ceftazidime (CAZ, 30 μg/disc), cefotaxime (CTX, 30 μg/disc), cefoperazone-sulbactam (CFS, 50/50 μg/disc), cefazoline (CZ, 30 μg/disc), ceftriaxone (CRO, 30 μg/disc), temocillin 30 μg/disc, meropenem (MEM, 10 μg/disc), imipenem (IMP, 10 μg/disc), ertapenem (ETP, 10 μg/disc), and colistin (CT, 10 μg/disc). Minimum inhibitory concentration (MIC) of each meropenem, imipenem, and ertapenem was determined by the agar diffusion method against the test isolates. Results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines [13, 14]. MIC_{50} and MIC_{90} were also calculated [14]. *Escherichia coli* ATCC 25922 was used as a quality control strain.

Phenotypic confirmation of carbapenemase production among *Enterobacteriaceae* isolates

The confirmation phenotypic assays for carbapenemase production were performed as described in previously published studies [2, 11, 14–18]. These assays were the modified Hodge test (MHT) and the carbapenemase inhibition tests including both boronic acid-based combined disc test (CDT) and double disc synergy test (DDST) and EDTA-based combined disc test (CDT) and double disc synergy test (DDST). The Carba Nordmann Poirel test (Carba NP test) was performed according to the manufacturer's instructions. High-level temocillin resistance was proposed as a surrogate marker for OXA-48 production with a zone diameter cut-off of < 11 mm with temocillin 30 μg/disc [19–23].

PCR-based detection of carbapenemase-encoding genes and identification of carbapenemase type

Plasmid DNA was extracted from test isolates using QIAprep Spin Miniprep kit (QIAGEN, USA) according to the manufacturer instructions. Uniplex PCR-based detection of carbapenemase-encoding genes bla_{NDM}, bla_{VIM} bla_{KPC}, bla_{IMP}, $bla_{\text{oxa-48}}$, and $bla_{\text{OXA-23}}$ was performed. The PCR

oligonucleotide primers used in this study, synthesized by Vivantis Technologies Sdn Bhd (Malaysia), are listed in Table 1. Amplicons were analyzed using 1.5% TAE-agarose gel electrophoresis and documented using a bioimager. To identify the carbapenemase type, the PCR amplicons were sequenced at Clinilab, Cairo, Egypt and/or Macrogen, South Korea, and the generated sequences were analyzed for the carbapenemase type using the nucleotide BlastN search tool available at the NCBI website against non-redundant genomes available in the GenBank database.

Results

Bacterial species identified among *Enterobacteriaceae* isolates

Based on the procedures of identifying bacterial species, the 135 *Enterobacteriaceae* isolates recovered from diverse clinical specimens comprised 79 isolates (58.5%) *K. pneumoniae*, 51 isolates (37.8%) *E. coli*, four isolates (2.9%) *Enterobacter* spp., and one isolate (0.74%) *K. oxytoca*. The highest percentage of these isolates (60%) were recovered from blood specimens (Table 2).

Antimicrobial susceptibilities and phenotypic detection of carbapenemase production

Overall, the antimicrobial susceptibility studies indicated that the *Enterobacteriaceae* isolates included in this study showed high resistance rates to all tested antimicrobials. High frequencies of MDR isolates were recorded among *K. pneumoniae* isolates and *E. coli* isolates with frequencies of 98.73% and 96.07%, respectively. Resistance rates to carbapenem drugs varied among the isolates of different *Enterobacteriaceae* species included in this study. *K. pneumoniae* isolates showed higher resistance rates to each meropenem, imipenem, and ertapenem compared with *E. coli* isolates and *Enterobacter* spp. isolates; with a higher resistance rate was to ertapenem among *K. pneumonia* and *E. coli* isolates (Table 3).

Phenotypic screening for carbapenemase-producing isolates showed that a total of 98 out of 135 isolates (72.6%) were suspected of carbapenemase production, which were intermediate or resistant to ertapenem (ETP) and/or meropenem (MEM) and/or imipenem (IPM) according to CLSI breakpoints. Overall, phenotypic confirmation assays indicated that 93 of 135 (69%) isolates were positive for the production of one or more carbapenemases. Carbapenemase activity was detected in 36/135 (26.7%) by the modified Hodge test, in 14/135 (10.37%) of isolates by boronic acid-based inhibition tests and in 81/135 (57.7%) of isolates by EDTA-based inhibition tests. Regarding the detection of OXA-48 using temocillin resistance, 16 out of 98 (16.3%) temocillin-resistant isolates were suspected to be OXA-48 type producers. These isolates showed negative results for other phenotypic tests. On the other hand, it was recorded that 24/135 (18%) isolates were susceptible to meropenem, imipenem, and ertapenem with inhibition zone diameters ≥25 mm. Although these isolates recorded negative results in phenotypic detection, they showed positive results under PCR experiments.

Distribution of carbapenemase-encoding genes among carbapenemase-producing isolates

Based on the PCR-based assays, a total of 121 out of 135 (89.62%) of the *Enterobacteriaceae* isolates showed amplification of one or more of the carbapenemase-encoding genes (Table 4). Overall, the most prevalent carbapenemase encoding gene among isolates was bla_{NDM} 93/135 (68.88%), followed by bla_{OXA-48} 44/135 (32.59%), bla_{OXA-23} 42/135 (31.11%) and bla_{KPC} 2/135 (1.48%). Among bla_{NDM} positive isolates, 48/79 (60.75%) and 41/51 (80.39%) of *K. pneumoniae* isolates and *E. coli* isolates, respectively, harbored bla_{NDM} gene. The gene of bla_{OXA-48} was found in 34/79 (43.03%) of *K. pneumoniae* isolates and 10/51

Table 1 PCR primer sets for amplification of carbapenem-resistance encoding genes

Gene	Primer sequence (5′–3′)	Amplicon size	Reference
bla_{KPC}	Forward: ATGTCACTGTATCGCCGTCT Reverse: TTTTCAGAGCCTTACTGCCC	893	[24]
bla_{NDM}	Forward: GCGAAAGTCAGGCTGTGTTG Reverse: CATTAGCCGCTGCATTGATG	445	[25]
bla_{IMP}	Forward: GGCGGAATAGAGTGGCTTAATTCTC Reverse: CGTACGGTTTAACAAAACAACCACC	250	[25]
bla_{VIM}	Forward: AGTGGTGAGTATCCGACAG Reverse: ATGAAAGTGCGTGGAGAC	261	[26]
bla_{OXA-48}	Forward: GCTTGATCGCCCTCGATT Reverse: GATTTGCTCCGTGGCCGAAA	238	[27]
bla_{OXA-23}	Forward: ACACAATACATATCAACTTCGC Reverse: AGTGTGTTTAGAATGGTGATC	813	[24]

Table 2 Bacterial species identified from various clinical specimens in this study

Type of specimen	Bacterial species					
	K. pneumoniae (79 isolates)	*E. coli* (51 isolates)	*Enterobacter* spp. (4 isolates)	*K. oxytoca* (1 isolate)	Total	%
Blood	43	35	2	1	81	60
Pus swabs	13	12	1	0	26	19.25
Wound swabs	7	4	1	0	12	8.88
Oropharyngeal swabs	6	0	0	0	6	4.44
Sputum	4	0	0	0	4	2.96
CVP	2	0	0	0	2	1.48
Nephrostomy	2	0	0	0	2	1.48
Ear swabs	2	0	0	0	2	1.48

CVP, central venous catheter

(19.60%) of *E. coli* isolates while bla_{KPC} was detected only in one isolate of each *K. pneumoniae* and *Klebsiella oxytoca*. Based on *Enterobacteriaceae* species, *K. pneumoniae* had the highest number of these genes (60%, $n = 108$), followed by *E. coli* (37.01%, $n = 67$), *Enterobacter* spp. (2.22%, $n = 4$), and *K. oxytoca* (1.01%, $n = 1$). The DNA sequencing of PCR products and BlastN searches revealed that the bla_{NDM-1} gene was the most prevalent genotype of carbapenemases in this study. In addition, the other genotypes were bla_{KPC-2} and bla_{VIM-1}. The bla_{IMP} and bla_{VIM} carbapenemase genes were not detected in the set of isolates included in this study.

Discussion

Antimicrobial resistance is increasing worldwide, particularly in developing countries, because of greater access to antibiotic drugs. This increase in microbial drug resistance is caused mainly by the use of antimicrobials in humans and other animals [28, 29]. The emergence of carbapenem-producing *Enterobacteriaceae* bacteria causes an increasing global concern, which leads to limited therapeutic options and threatens public health. Therefore, continuous surveillance and epidemiological investigation of carbapenemases are of great importance to control infections, particularly among the immunocompromised such as cancer patients. In the present study, we report the occurrence of carbapenemase-producing *Enterobacteriaceae* isolated from cancer patients in different cancer institutes in Egypt. Following identification, it was found that 79 and 51% of the recovered isolates were *K. pneumoniae* and *E. coli*, respectively. A previous report [30] documented that *K. pneumonaie* and *E. coli* were the most important infectious agents causing health-care severe-associated infections in hospitals and other health-care facilities. These infections included those acquired by immunocompromised patients who have cancer. Millions of patients are affected by such infections worldwide each year, leading to significant mortality and financial losses for health systems [31].

In the current study, there was an emergence of multi-drug resistance among *Enterobacteriaceae* isolates. Our investigation showed around 99% of *K. pneumoniae* isolates and 96% of *E. coli* were MDR as these isolates were resistant to at least one antimicrobial agent in three or more different antimicrobial classes. High resistance to ertapenem was recorded among the isolates of *K. pneumoniae* and *E. coli* with frequencies, 76 and 55%, respectively. Ertapenem seems to be the right candidate for detecting most of the carbapenemase producers, as MICs of ertapenem are usually higher than those of other carbapenems. However, the detection of carbapenemase producers based only on the MIC values of ertapenem lacks specificity [1]. In the current study, 81% of carbapenem-resistant *Enterobacteriaceae* (CRE) were resistant to ertapenem with high levels of MICs. Our findings were higher than those reported in the literature as mentioned that 64.3% of CRE isolates were resistant to ertapenem [32]. Additionally, Baran and Aksu [33] found that 56% of isolates were ertapenem resistant. The rate of resistance in cancer patients included in this study was high, while these patients are at high risk. They are subjected to multiple procedures ranging from catheterization to biopsy to major surgical procedures. In addition, they receive chemotherapy and radiotherapy along with multiple courses of antibiotics, rendering them at high risk of developing an infection by more virulent pathogens. Other studies isolated also hypervirulent *K. pneumoniae* (hvKp) and *E. coli* from human cancer [34].

Detection of CRE is a significant subject, as carbapenemases are usually associated with many other resistance determinants, giving rise to multidrug resistance and even pan-drug resistance [1]. Carbapenemase production in

Table 3 Antimicrobial susceptibilities of *Enterobacteriaceae* isolates

Antimicrobial agent	No. of Bacterial isolates (%)				
	K. pneumoniae (79) No. (%)[a] of isolates	*E. coli* (51)	*Enterobacter* spp. (4)	*K. oxytoca* (1)	Total number (135) No. (%)[b] of isolates
AMC					
S	3 (3.79%)	2 (3.92%)	0 (0%)	0 (0%)	5 (3.70%)
I	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
R	76 (96.20%)	49 (96.07%)	4 (100%)	1 (100%)	130 (96.29%)
TZP					
S	10 (12.65%)	8 (15.68%)	3 (75%)	0 (0%)	21 (15.55%)
I	4 (5.06%)	2 (3.92%)	0 (0%)	0 (0%)	6 (4.44%)
R	65 (82.27%)	41 (80.39%)	1 (25%)	1 (100%)	107 (79.25%)
FOX					
S	1 (1.26%)	1 (1.96%)	0 (0%)	0 (0%)	2 (1.48%)
I	2 (2.53%)	1 (1.96%)	0 (0%)	1 (100%)	4 (2.96%)
R	76 (96.20%)	49 (96.07%)	4 (100%)	0 (0%)	129 (95.55%)
FEP					
S	1 (1.26%)	2 (3.92%)	3 (75%)	0 (0%)	6 (4.44%)
I	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
R	78 (98.73%)	49 (96.07%)	1 (25%)	1 (100%)	129 (95.55%)
CZ					
S	0 (0%)	2 (3.92%)	0 (0%)	0 (0%)	2 (1.48%)
I	1 (1.26%)	0 (0%)	0 (0%)	0 (0%)	1 (0.74%)
R	78 (98.73%)	49 (96.07%)	4 (100%)	1 (100%)	132 (97.77%)
CRO					
S	0 (0%)	1 (1.96%)	0 (0%)	0 (0%)	1 (0.74%)
I	0 (0%)	0 (0%)	1 (25%)	0 (0%)	1 (0.74%)
R	79 (100%)	50 (98%)	3 (75%)	1 (100%)	133 (98.51%)
CAZ					
S	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
I	8 (10.12%)	2 (3.92%)	0 (0%)	0 (0%)	10 (7.4%)
R	71 (89.87%)	49 (96.07%)	4 (100%)	1 (100%)	125 (92.59%)
CTX					
S	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
I	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
R	79 (100%)	51 (100%)	4 (100%)	1 (100%)	135 (100%)
CFS					
S	12 (15.18%)	12 (23.52%)	1 (25%)	0 (0%)	25 (18.51%)
I	5 (6.32%)	7(13.72%)	2 (50%)	0 (0%)	14 (10.37%)
R	62 (78.48%)	32(62.74%)	1 (25%)	1 (100%)	96 (71.11%)
MEM					
S	21 (26.58%)	19 (37.25%)	3 (75%)	0 (0%)	43 (31.85%)
I	7 (8.86%)	6 (11.76%)	0 (0%)	0 (0%)	13 (9.62%)
R	51 (64.55%)	26 (50.98%)	1 (25%)	1 (100%)	79 (58.51%)
IMP					
S	18 (22.78%)	20 (39.21%)	3 (75%)	0 (0%)	41 (30.37%)
I	10 (12.65%)	7 (13.72%)	0(0%)	0(0%)	17 (12.59%)
R	51 (64.55%)	24 (47.05%)	1 (25%)	1 (100%)	77 (57.03%)
ETP					
S	15 (18.98%)	20 (39.21%)	2 (50%)	0 (0%)	37 (27.40%)
I	4 (5.06%)	3 (5.88%)	1 (25%)	0 (0%)	8 (5.92%)
R	60 (75.94%)	28 (54.94%)	1 (25%)	1 (100%)	90 (66.66%)
MDR[c]	78 (98.73%)	49 (96.07%)	3 (75%)	1 (100%)	131 (97.03%)

AMC, amoxicillin-clavulanate; *CZ*, cefazolin; *CAZ*, ceftazidime; *FOX*, cefoxitin; *CTX*, cefotaxime; *CRO*, ceftriaxone; *FEP*, cefepime; *CFS*, cefoperazone-sulbactam; *ETP*, ertapenem; *IMP*, imipenem; *MEM*, meropenem; *TZP*, piperacillin-tazobactam

[a] Percent related to the total number of isolates of each species

[b] Percent related to the total number of isolates in this study

[c] Multi-drug-resistant isolates

Enterobacteriaceae may be detected either phenotypically or genotypically [35]. Several tests have been developed for the phenotypic detection of carbapenemase production such as the modified Hodge test, both boronic acid-based inhibition tests and EDTA-based inhibition tests, Carba NP test, and detection of OXA-48 depending upon high resistance to

Table 4 Frequencies of carbapenemase-encoding genes among *Enterobacteriaceae* isolates

Bacterial species	Number of isolates (%)[a] harboring carbapenemase genes			
	bla_{NDM}	bla_{KPC}	bla_{OXA-23}	bla_{OXA-48}
K. pneumoniae (79)	48 (60.75%)	1 (1.26%)	25 (31.64%)	34 (43.03%)
E. coli (51)	41 (80.39%)	0 (0%)	16 (31.37%)	10 (19.60%)
Enterobacter spp. (4)	3 (75%)	0 (0%)	1(25%)	0 (0%)
K. oxytoca (1)	1(100%)	1 (100%)	0 (0%)	0 (0%)
Total No. (%)[b]	93 (68.88%)	2 (1.48%)	42 (31.11%)	44 (32.59%)

[a] Percent related to the total number of isolates of each species

[b] Percent related to the total number of isolates in this study (135 isolates)

temocillin [19, 36]. In this study, although the results of inhibitor-based phenotypic assays almost consistent with the PCR-based identification, some results were deceptive. This observation has been frequently noticed in other earlier studies [37] and it is explained by the higher sensitivity and specificity of genotypic detection methods, compared with conventional inhibitor-based phenotypic detection tests. Although, 100% of the isolates which showed negative results in the inhibitors-based phenotypic tests harbored at least one of the carbapenem encoding genes and significantly giving positive results when retested by the Carba NP detection test kit [38]. Accordingly, we recommend using the Carba-NP test kit for optimal and rapid carbapenemase activity detection in clinical laboratory settings when molecular detection facilities are not available.

The overall detection rate of carbapenemases by the non-specific modified Hodge test in the present study was 32.4% among the 98 isolates that were suspected of carbapenemase production, which was consistent with the study of Kumar and Mehra [39], who reported a similar record. However, this test is unable to differentiate between different classes of carbapenemases and lacks sensitivity and specificity [40]. This means, not all carbapenemase-producing isolates of *Enterobacteriaceae* are MHT positive, and MHT-positive results may be encountered in isolates with carbapenem resistance mechanisms other than carbapenemase production [14]. The synergy combined disc tests used in our investigation for the characterization of MBLs were dependent on specific inhibition of the enzymes by a chelating agent such as EDTA in EDTA-based combined disc synergy tests. The tests revealed 70% of CRE that agreed with the findings in [41]. Detection of OXA-48-type producers remains a big challenge for current microbiological diagnostics [42]. Until date, there are no compounds capable of inhibiting class D carbapenemases. Although, interestingly, temocillin is used as a suggestive marker to screen for OXA-48-like determinants that confer high-level resistance [19]. A previous study reported that isolates negative for KPC and MBL must be further examined for other carbapenem resistance mechanisms, most importantly, OXA-48 production [23]. The current study showed that 16.3% of the 98 isolates that were suspected of carbapenemase production isolates were resistant to temocillin with an inhibition zone diameter of less than 11 mm.

Phenotypic assays are used to identify carbapenemase activity, while PCR-based molecular assays have been developed to identify carbapenemase encoding genes [2, 11]. In addition, molecular techniques remain the reference standard for the precise genotypic identification of carbapenemases. Using the PCR technique performed directly on colonies, carbapenemase genes can be detected within 4–6 h, with excellent sensitivity and specificity [13, 23]. Furthermore, some carbapenem-sensitive isolates indicated by the phenotypic detection could have positive carbapenemase genes in PCR [43]. In this study, we detected 28 out of 135 (20.7%) carbapenem-susceptible enterobacterial isolates ($\geq$ 25 mm) that also recorded negative phenotypic while showed amplified carbapenemase encoding genes in the PCR assays. According to this finding, it might be interpreted as silent genes indicating that susceptibility testing and phenotypic detection are not enough for preliminary screening of carbapenemases [44].

In East Africa, a few studies have been done in Kenya and Tanzania. A surveillance study in Kenya reported the isolation of NDM-1 producing *K. pneumoniae* from urine samples [45], while in Tanzania, a study reported the prevalence of 35.24% carbapenemase-encoding genes among MDR Gram-negative bacteria [5]. Isolation of carbapenemase producers among ESBL-producing isolates was also reported in South Africa [46, 47]. This study demonstrated that the rate of bla_{NDM}-carrying isolates was 68.88% among *Enterobacteriaceae* isolates. Similarly, a high prevalence of NDM of 67.5% was also documented in a previous study from Egypt [48], this might be due to the presence of highly mobile conjugative plasmids encoding for NDM enzymes, which facilitate horizontal inter- and intra-species transfer between bacteria rather than clonal spread [33]. Furthermore, bla_{NDM} was the most predominant genotype detected in the present study among the carbapenemase positive *E. coli* and *K. pneumoniae* (80 and 61%, respectively). Moreover, these isolates showed the co-existence of bla_{NDM} with other oxacillinase genes, including

bla_{OXA-23} and bla_{OXA-48}. It was previously described in many recent reports that the NDM genotype was commonly found in *E. coli* and *K. peumoniae* isolates. Its coexistence with other resistance determinants such as OXA, SHV, and VIM was also documented [49–51]. However, we did not detect bla_{IMP} and bla_{VIM} among the test isolates as reported in a similar study [52]. The DNA sequencing of amplified genes and BlastN analysis of obtained sequences against the non-redundant genome sequences available in the GenBank database revealed that the bla_{NDM-1} gene was the most prevalent genotype of carbapenemases in this study. In addition, the other genotypes were bla_{KPC-2} and bla_{VIM-1}. The bla_{IMP} and bla_{VIM} carbapenemase genes were not detected in the set of isolates included in this study.

In conclusion, carbapenems have been considered to be an important choice to fight against MDR *Enterobacteriaceae* bacterial infections. Therefore, the emergence of resistance to carbapenems represents a challenge to clinicians and threatens public health. This study revealed a high percentage of resistance to carbapenem drugs among *Enterobacteriaceae* bacteria isolated from cancer patients; particularly, the increased prevalence of NDM-1 genotype among isolates included in this study. Notably, the co-production of two carbapenemase enzymes by single bacterial isolate could lead to a high level of resistance to the carbapenems. Therefore, it is recommended to do continuous surveillance for carbapenemase determinants on all recovered isolates, weather carbapenem-susceptible, or resistant. Restricted prescriptions of carbapenems should be followed to reduce the emergence of such resistance. Additionally, besides the proper management of infection control in the cancer institutes is a must to decrease the incidence of infections among cancer patients.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was approved by the Ethical Committee of Egyptian Hospitals.

Informed consent No written informed consent was necessary for this type of study.

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