ORIGINAL ARTICLE

Dissemination of NDM-1 and OXA-48 Co-producing Carbapenem-resistant Enterobacterales at Two Tertiary Hospitals in Egypt

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ABSTRACT

*Background. The global incidence of carbapenem-resistant Enterobacterales (CRE) is increasing, posing a major treatment challenge and serious infection control concern. **Objective.** We aimed to characterize carbapenemases both genotypically and phenotypically among CRE isolated from two tertiary hospitals in Egypt, and to detect the genetic relatedness among studied isolates. **Methodology.** A total of 107 non-duplicate CRE strains isolated from different clinical specimens of hospitalized patients were assessed for presence of carbapenemases genes using multiplex PCR. One-hundred isolates were also assessed for the presence of carbapenemases using MASTDISCS Combi Carba Plus kit. Molecular typing of the isolates was done by Enterobacterial Repetitive Intergenic Consensus region PCR (ERIC-PCR). **Results.** Klebsiella pneumoniae (K. pneumoniae) represented most of the studied isolates (n=85; 79.4%). The most prevalent carbapenemase gene among our isolates was blaNDM-1 (n=103; 96.2%) followed by blaOXA-48 (n=68; 63.5%). Among them, 64 isolates (59.8%) carried more than one gene as blaNDM-1 and blaOXA-48 genes. blaKPC, blaVIM and blaIMP were not detected in the isolates. The sensitivity of the Carba Plus kit for the detection of MBL was much better than OXA-48 (82.29% and 9.52%, respectively), however, the negative predictive value was poor for the detection of both (19.05% and 39.36%, respectively). The constructed dendrogram for each genus from each hospital revealed significant genetic diversity among the investigated isolates. **Conclusions.** The study highlights the wide dissemination of diverse clones of CRE isolates that co-harbor blaOXA-48 and blaNDM-1 genes among our hospitals as well as low accuracy of detection using phenotypic techniques like Carba plus kit.*

INTRODUCTION

Antimicrobial resistance (AMR) is now recognized as an urgent threat to global health, accounting for more than 35,000 fatalities each year in the US alone [1]. Carbapenem-resistant *Enterobacterales* (CRE) are among the most worrisome multidrug-resistant (MDR) pathogens. The increased prevalence of resistance to carbapenems adds complexity to the treatment of critically ill patients, as they frequently rely on carbapenems as a final line of treatment against extended-spectrum β-lactamases (ESBL) and AmpC-producing bacteria[2]. According to the WHO alert report, CRE isolates are now included within the high-risk MDR category, emphasizing the need for efficient treatment alternatives [3].

Resistance to carbapenems can be acquired through different mechanisms, including the production of different carbapenemases, structural alterations in porins that restrict drug access to their targets, and

overexpression of efflux pumps [4]. Carbapenemase-producing *Enterobacterales* (CPE) are of special concern among CRE because their genes can easily spread, and they are often concurrently resistant to several non-ß-lactam antibiotics [5]. Carbapenemases are categorized into three out of four classes of ß-lactamases, Ambler classes A, B, and D. Class A predominantly consists of KPC enzymes, class B comprises NDM, IMP, and VIM enzymes, and OXA-48-like enzymes make up class D carbapenemases [6].

Infections caused by CPE are linked to high fatality rates and are frequently managed using antibiotics, which have insufficient efficiency data and significant toxicity like polymyxins [7]. To address these therapeutic challenges, new combinations of ß-lactam-ß-lactamase inhibitors (BL/BLI) have been developed. These advanced combinations involve cephalosporins or carbapenems paired with BLI^ . Considering the variations in inhibition profiles among different BLI, optimizing the use of these agents would require precise

detection of carbapenemases, and assessing the susceptibility of various carbapenemase producers to these drugs [9]. Phenotypic techniques like Carba NP and the modified carbapenem inactivation method (mCIM) endorsed by CLSI guidelines, are widely employed in routine laboratory procedures [10]. Nevertheless, these methods identify carbapenemase activity independent of the presence of specific carbapenemase genes [11]. While molecular methods are considered the gold standard for identifying carbapenemase genes, but they are not widely available, as only a limited number of reference laboratories offer them [12]. Therefore, inhibitor-based phenotypic tests like MASTDISCS combi Carba plus disc system (Mast Group Ltd., UK), have been developed to discern the presence of carbapenemase gene utilizing faropenem combined with carbapenemase inhibitors to detect organisms producing MBLs, KPC, and OXA-48 enzymes [13].

Genes encoding carbapenemases can be easily transferred within the healthcare setting through mobile genetic elements[13], therefore, when conducting epidemiological research, identifying the source of infection is crucial for enhancing infection control and monitoring the local spread of resistant bacterial strains [14]. Molecular typing techniques serve as potent tools to ascertain genetic relatedness among pathogenic species and gain insights into their transmission[15]. One such method, repetitive element sequence-based polymerase chain reaction (PCR), explores repetitive nucleotide sequences in the bacterial genome, grouping bacterial strains accordingly [16]. The Enterobacterial repetitive intergenic consensus (ERIC)-PCR technique offers a swift, dependable, and affordable means of molecular typing for the Enterobacterales family, facilitating the distinction of their genetic diversity [15].

Understanding antibiotic resistance contributes to the decision-making of antimicrobial stewardship initiatives, infection control committees, and public health authorities regarding strategies for handling these organisms. The worldwide spread of different antibiotic-resistant genes in CRE, along with insufficient data in the Middle East area, enticed us to examine the occurrence of carbapenemase genes in clinical strains isolated from two tertiary hospitals in Cairo and Alexandria. Our objectives also included assessing the effectiveness of the MASTDISCS Combi Carba plus for identifying carbapenemases phenotypically and investigating the genetic relatedness among the studied isolates.

METHODOLOGY

The present study has been approved by the Research Ethics Committee, Faculty of Medicine, Cairo University (N172-2023). All procedures were conducted following the ethical consideration standards of the 1964 Declaration of Helsinki.

Sample collection, bacterial identification, and detection of carbapenem resistance:

In this cross-sectional study, we included 107 non-duplicate CRE strains isolated from isolated from different clinical specimens of hospitalized patients at two tertiary hospitals in Cairo (n=74) and Alexandria (n=33) during the period from September 2021 to December 2023. Isolates were identified by standard biochemical reactions [17]. Resistance to carbapenems was detected by testing the susceptibility of the isolates to meropenem (10μg), imipenem (10μg), and ertapenem(10μg) antibiotic discs, Mast, UK, by disc diffusion method according to standardized microbiological procedures. Isolates were defined as CRE if they were intermediate or resistant to one or more carbapenems according to the CLSI guidelines [18].

Molecular detection of carbapenemases:

DNA was extracted using a straightforward boiling technique. In actuality, 400 µl of 1× TE buffer (10 mM Tris, 1 mM EDTA) was used to suspend 2-3 colonies of isolates grown over night by vortexing. For ten minutes, the suspension was heated to 95°C in a boiling bath. Centrifugation was then used to remove the cell debris for 10 minutes at ×12000 RPM. For PCR reactions, one microliter of supernatant served as the template DNA [19]. Multiplex PCR amplification of *blaOXA-48, blaNDM-1, blaVIM, blaKPC,* and *blaIMP* genes was done using previously published primers [20] listed in Table S1. A total volume of 25 µL of amplification was used, containing 12.5 µL of Go Taq®G2 Hot Start Green Master Mix 2x (Promega, USA), 1 µL of each gene's forward and reverse primers (10 mM), and 2.5 µL of DNA template. The cycling conditions were as follows: initial denaturation at 95°C for 2 minutes, 35 cycles of denaturation at 95 °C for 1 minute, annealing at 60 °C for 1 minute, and extension at 72°C for 2 minutes, and a final extension at 72°C for 10 minutes. A negative control was included in each run. Amplicons were visualized and imaged under UV light after being separated by electrophoresis on a 1.5% agarose gel containing 4 µL/100 mL ethidium bromide at 90V.

Phenotypic detection of carbapenemases.

Table S1: List of primers used in the study.

I. Multiplex PCR for detection of carbapenemases:				
Primer Name	**Sequence (5′–3′)**	**5'–3' Product length**	**Target**	**Reference**
OXA- 48-F **OXA-48-R**	TTG GTG GCA TCG ATT ATC GG GAG CAC TTC TTT TGT GAT GGC	744 bp	*blaOXA-48*	
NDM-1-F **NDM-1-R**	GTA GTG CTC AGT GTC GGC A GGG CAG TCG CTT CCA ACG GT	475 bp	*blaNDM-1*	
VIM -F **VIM -R**	SGR TRS RTG GRCR CATAS CRCS TCS SCR GRA CCR SAG CRC ACR	360 bp	*blaVIM*	
KPC -F **KPC -R**	SAC CRC STC GCR GSA CCS RT SCC RSC ASG CCS GRT RTC S	275 bp	*blaKPC*	
IMP-F **IMP-R**	GRA ASA GAR TRG CST AST CSA CRT TST CTR/T RAG TGS	181 bp	*blaIMP*	
	R = G or T, S = A or T			
II. ERIC-PCR:				
ERIC 1	5'TGTAAGCTCCTGGGGATTCAC3	Variable		
ERIC 2	5'AAGTAAGTGACTGGGGTGAGCG 3'			

One hundred isolates were tested for the presence of different carbapenemases and OXA-48 enzymes using MAST DISCS Combi Carba plus (*Enterobacterales*), Mast Group Ltd., UK. kit according to manufacturer instructions and the results were compared with the gold standard PCR method. The test contains 5 discs including Penem (A) and 3 different combinations of penems with MβL, KPC, and Amp C inhibitors (B-D), in addition to Temocillin + MβL inhibitor (E). The zone of inhibition of penem disc was compared to zones of inhibition of each penem plus inhibitor discs to detect MβL, KPC, and Amp C enzymes. If no synergy is obtained between previous discs and disc E showed a zone of inhibition of ≤10 mm the organism was recorded as having OXA-48 activity.

Molecular typing of clinical isolates by Enterobacterial Repetitive Intergenic Consensus region PCR (ERIC-PCR)

Genetic relatedness of isolates included in the study (n=106) was studied by ERIC-PCR. Single *E. coli* strain among Alexandria isolates was not subjected to further analysis since it represents a singleton. Analysis of DNA polymorphism was done after amplification of the conserved sequences from the Enterobacterial Repetitive Intergenic Consensus region by ERIC-PCR using previously published primers [21], listed in (table S1). Amplification was conducted in a 25 µL total reaction volume, comprising 12.5 µL of Go Taq®G2 Hot Start Green Master Mix 2x (Promega, USA), 1 µL each of forward and reverse primers (10 mM), 2 µL of DNA template, and 8.5 µL of nuclease-free water, following a protocol previously described. [22] The cycling parameters were as follows: an initial denaturation at 95°C for 2 minutes, followed by 30 cycles of 94°C for 1 minute, 50°C for 1 minute, and 72°C for 1.5 minutes, with a final elongation at 72°C for 7 minutes.

Amplification products were subjected for electrophoresis (1.5% agarose containing 4 µL/100 mL ethidium bromide at 90 V and were visualized and imaged under UV light [22].

Interpretation of ERIC PCR patterns and constructing a phylogenetic tree, Total lab TL20 ID v2009, (c) Nonlinear Dynamics Ltd. March was used. Dendrograms were constructed through fragments detection, fragments length calculation, binary matrix, design, and finally, phylogenetic tree construction. Separate dendrograms were constructed for each genus isolated from each hospital.

Statistical analysis:

Data analysis were done using the Statistical Package for Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA). Data were summed up (%) using frequency (count) and relative frequency. Calculations were made for common diagnostic indices such sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), and diagnostic efficacy [23]. The Chi-square (2) test was applied to categorical data comparison. Furthermore, an exact test was utilized when the anticipated frequency was lower than 5 [24]. A p-value of less than or equal to 0.05 was used to indicate statistical significance.

RESULTS

Most of the studied isolates were *K. pneumoniae* (n=85, 79.4%) followed by Enterobacter and *E. coli* (n=11, 10.3% for each). Regarding species distribution in the two hospitals, *K. pneumoniae* was dominant in both Cairo (n=64, 86.5%) and Alexandria (n=21, 63.3%) followed by *E. coli* in Cairo (n=10, 13.5%) and

Enterobacter cloacae in Alexandria (n=11, 33.3%). Only one *E. coli* strain (3%) was among the studied isolates from Alexandria.

All isolates included in the study (n=107) were assessed for the presence of genes encoding different carbapenemases using multiplex PCR (Fig. 1A). Table 1 shows the distribution of different carbapenemases genes among Cairo and Alexandria isolates. Most isolates (n=64; 59.8%) carried both *blaNDM-1* and *blaOXA-48* genes, 39 isolates (36.4%) had *blaNDM-1*, while only 4 isolates (3.7%) had *blaOXA-48* gene.

bla_{KPC}, bla_{VIM}, and bla_{IMP} genes were not detected in any of the studied isolates. There was no statistically significant difference in gene distribution in isolates between Cairo and Alexandria hospitals (p-value=0.059). The concomitant presence of NDM and OXA-48 genes was the dominant genotype among both *K. pneumoniae* and Enterobacter isolates, while the sole presence of NDM was the dominant genotype among *E. coli* isolates, with a statistically significant difference (p=0.05).
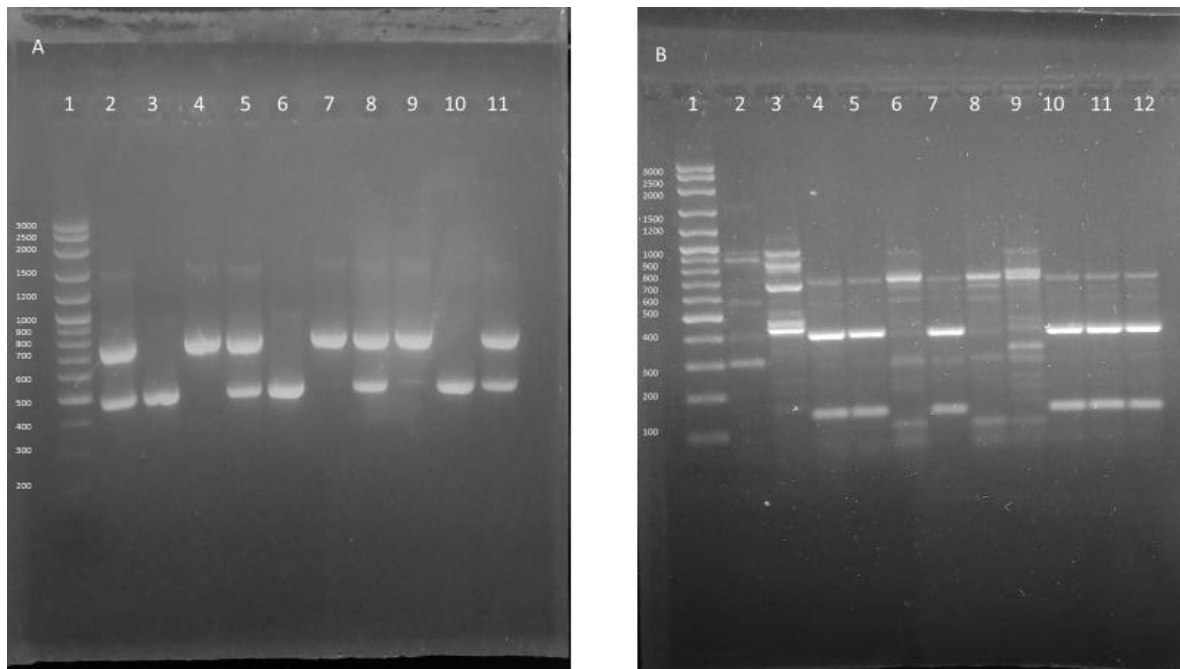

Fig.1 (A): Multiplex PCR amplification of Carbapenemases genes. **Lane 1:** DNA ladder, **Lane 2,5,8,11: Samples positive for** *blaNDM-1and blaOXA-48,* **Lane 3,6,10:** Samples positive for *blaNDM-1,* **Lane 4,7,9**: Samples positive for *blaOXA*-48 at 744bp **(B): ERIC-PCR. Lane 1:** DNA ladder, **Lanes 2-12:** Different ERIC-PCR patterns of the tested samples.

Table 1: Distribution of different carbapenemases genes among Cairo and Alexandria isolates

	Cairo isolates			*Alexandria isolates*			**Total**	**P value I**	**P value II**
	blaNDM & bla blaOXA	*blaNDM*	*blaOXA*	*blaNDM & bla blaOXA*	*blaNDM*	*blaOXA*			
K. pneumoniae	36	26	2	17	4	0	85	*0.05	**0.059
E. coli	3	6	1	0	1	0	11		
Enterobacter				8	2	1	11		
Total	39 (52.7%)	32 (43.2%)	3 (4.1%)	25 (75.8%)	7 (21.2%)	1 (3%)	107		
*: Difference in gene distribution between different species.									
**: Difference in gene distribution between Cairo and Alexandria hospitals.									

One hundred isolates were assessed for the presence of different carbapenemases namely, MBL, KPC, and Amp C in addition to OXA-48, enzymes using MASTDISCS Combi Carba plus (*Enterobacterales*). Most of the isolates (n=80; 80%) harbored MBL, 6 isolates (6%) showed OXA-48 activity, and only one isolate showed KPC activity. In the remaining 13 isolates (13%) mechanism of carbapenem resistance could not be identified (11 isolates showed synergy with 2 of the combination discs (B-C), while 2 isolates showed no synergy with any of the combination discs and did not fit criteria for OXA-48 activity).

Table 2 displays the accuracy metrics of the MASTDISCS Combi Carba plus. The sensitivity of the kit in identifying MBL surpassed that of OXA-48 (82.29% compared to 9.52%, respectively). Conversely, the specificity was higher for detecting OXA-48 than MBL (100% compared to 80%, respectively). However, the negative predictive value (NPV) was low for both (39.36% and 19.05%, respectively).

Table 2: Accuracy indices of MASTDISCS Combi Carba plus

	MBL detection	OXA-48 detection
Sensitivity	82.29%	9.52%
Specificity	80%	100.00%
Positive Predictive Value	98.75%	100.00%
Negative Predictive Value	19.05%	39.36%
Accuracy	82.18%	43%

For the interpretation of ERIC-PCR patterns and constructing a phylogenetic tree, Total lab TL20 ID v2009, (c) Nonlinear Dynamics Ltd. March was used. Separate dendrograms were constructed for each genus isolated from each hospital. The constructed dendrogram for each genus from each hospital revealed significant genetic diversity among the investigated isolates. Cairo *K. pneumoniae* isolates were separated into 6 clusters. The 1st cluster was separated at 42% of genetic similarity and included 9 strains. The 2nd and 3rd clusters were both separated at 30 % of genetic similarity and both included 8 strains. At 28 % of genetic similarity, the 4th cluster was separated and included 19 strains. The 5th and 6th clusters were separated at 18% and 8% of genetic similarity, respectively, and included 11 and 9 strains, respectively (fig. 2A).

Alexandria *K. pneumoniae* isolates were separated into 4 clusters. The 1st cluster was separated at 47% of genetic similarity and included 3 strains. At 40% of genetic similarity, the 2nd cluster was separated and included 4 strains. The 3rd and 4th clusters were separated at 27% and 10% genetic similarity, respectively, and included 7 and 4 strains, respectively. Two singletons could not group with any of the above clusters (fig. 2B).

Cairo *E. coli* isolates were separated into 2 clusters at 8% of genetic similarity. At 14% of genetic similarity, the 1st cluster was separated into 2 subclusters including 2 and 3 strains, respectively. Moreover, at 14% of genetic similarity also, the 2nd cluster was separated into 2 subclusters including 3 and 2 stains, respectively (fig. 2C).

Alexandria Enterobacter isolates were separated into 3 clusters. The 1st cluster was separated at 50 % of genetic similarity and included 2 strains. The 2nd cluster was separated at 18% of genetic similarity and included 5 strains. The 3rd cluster was separated at 10 % of genetic similarity and included 4 strains (fig. 2D).

Regarding the distribution of carbapenemases genes among ERIC-PCR clusters (table S2), the dominant genotype among most clusters was the concomitant presence of MBL and *OXA-48* genes except for one Klebsiella and one *E. coli* cluster in Cairo isolates where NDM was the dominant genotype. In addition, the presence of NDM alone was equal to the concomitant presence of NDM and OXA-48 genes in one *K. pneumoniae* and one *Enterobacter spp*. clusters.

Table S2: Distribution of ERIC PCR clusters according to MBL and OXA-48 gene detection

| Clusters	No. (Isolates included)	*bla-NDM* No. (%)	*bla- OXA-48* No. (%)	*bla-NDM & bla- OXA-48* No. (%)
Cairo *Klebsiella pneumoniae* isolates				
Cluster 1	8 (C 64,59,63,50,6,3,1,2)	4 (50%)	0 (0%)	4 (50%)
Cluster 2	8 (C54,19,48,45,11,29,9,7)	3 (37.5%)	0 (0%)	5 (62.5%)
Cluster 3	19 (C 62, 47, 57, 39, 55, 17, 43, 41, 42, 44, 61, 53, 60, 58, 51, 49, 28, 40 ,32)	7 (36.8%)	1 (5.3%)	11 (57.9%)
Cluster 4	9 (C23, 14, 22, 20, 21, 16, 18, 15, 13)	4 (44.4%)	0 (0%	6 (55.6%)
Cluster 5	11 (C52, 46, 33, 30, 8 ,4, 12, 5, 10, 38, 36)	6 (54.5%)	0 (0%)	5 (45.5%)
Cluster 6	9 (C34, 26, 37, 24, 56, 35, 25, 27, 31)	2 (22.2%)	1 (11.1%)	6 (66.7%)
Alex *klebsiella pneumoniae* isolates				
Cluster 1	5 (A23, 13, 8 ,31,18)	2 (40%)	0 (0%)	3 (60%)
Cluster 2	7 (A32, 28, 29, 27, 25, 24, 22)	1 (14.3%)	0 (0%)	6 (85.7%)
Cluster 3	4 (A34, 30, 26 ,9)	0 (0%)	0 (0%)	4 (100%)
Cluster 4	3 (A47, 41,42)	0 (0%)	0 (0%)	3 (100%)
Cairo *E. coli* isolates				
Cluster 1	5 C72,74,73,65,67	4 (80%)	1 (20%)	0 (0%)
Cluster 2	5 (C 71,69,68,66,70)	2 (40%)	0 (0%)	3 (60%)
Alex *Enterobacter* isolates				
Cluster 1	4 (A40, 19, 35 ,33)	0 (0%)	0 (0%	4 (100%)
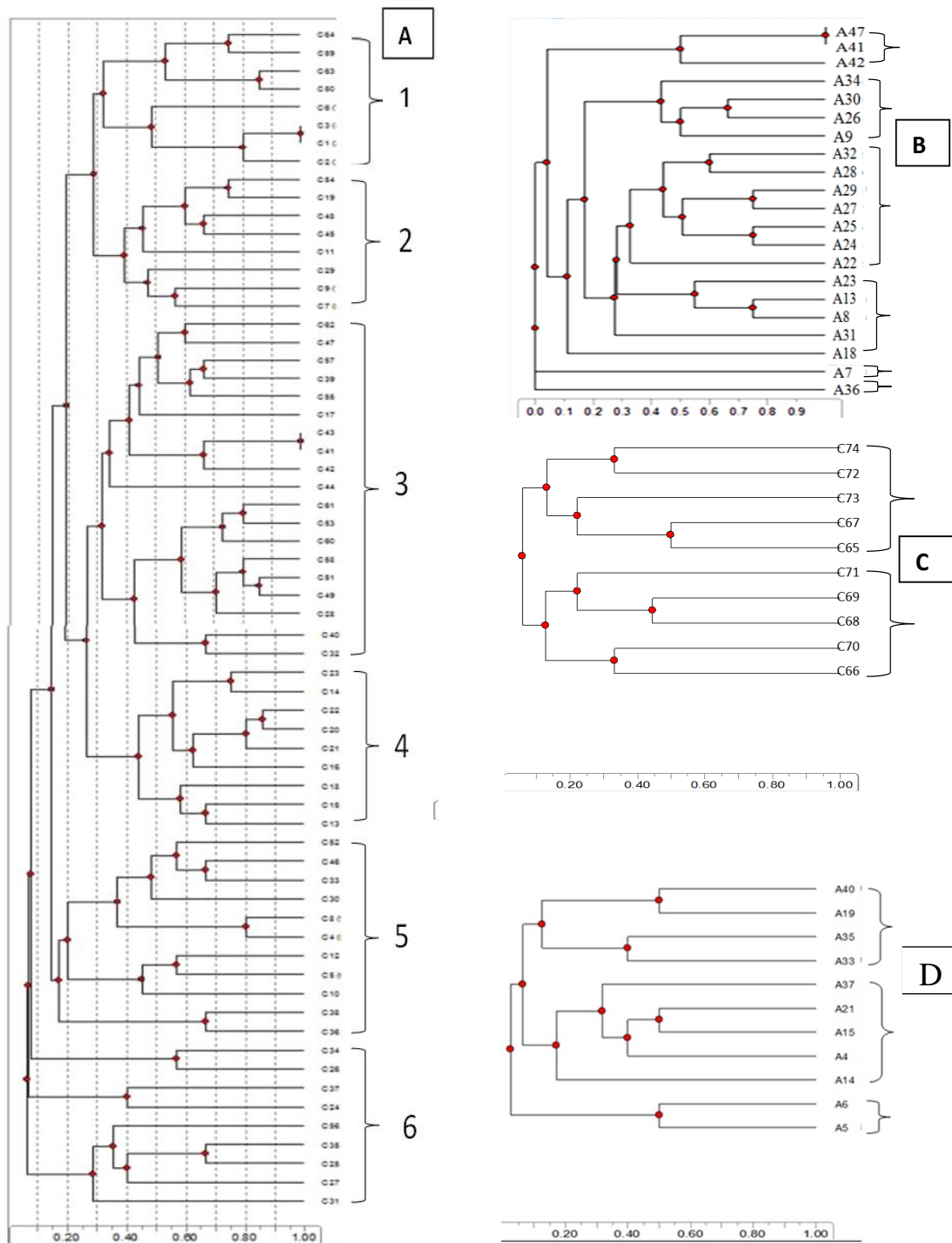
Fig. 2: Phylogenetic trees of tested isolates based on ERIC-PCR patterns. A: Phylogenetic tree of Cairo *K. pneumoniae* isolates., **B:** Phylogenetic tree of Alexandria *K. pneumoniae* isolates; **C:** Phylogenetic tree of Cairo *E. coli* isolates.**; D:** Phylogenetic tree of Alexandria *Enterobacter* isolates

DISCUSSION

The WHO has endorsed a global action plan to address the AMR crisis with five primary objectives [25]. One of these objectives is to enhance knowledge and evidence base through surveillance and research. Surveillance data is crucial in monitoring factors driving AMR and tracking trends across different sectors [26]. Hence, the objective of our current investigation was to detect and analyze carbapenemases in clinical isolates of CRE utilizing both molecular and phenotypic methods. We examined 107 non-repeated CRE strains sourced from various clinical samples obtained from two tertiary hospitals in Cairo and Alexandria. Among our isolates, the predominant species was *K. pneumoniae* (79.4%), followed by Enterobacter and *E. coli* (10.3% each). Similar findings were documented in prior studies conducted in Egypt, where *K. pneumoniae* ranked as the most prevalent species among their analyzed isolates, followed by *E. coli* [6,14].

All isolates included in our study were assessed for the presence of genes encoding different carbapenemases using multiplex PCR. Among studied isolates, *blaNDM-1* was the most prevalent (96.2%) followed by *blaOXA-48* (63.5%), however, bla_{KPC}, bla_{VIM}, and bla_{IMP} genes were not identified in any of the studied isolates. Similar findings were reported in recent studies from different institutions in Egypt, where Shawky *et al*. found that 75% of their isolates carried *blaNDM*, followed by *blaOXA-48* (59%) [27]. Additionally, El Defrawy *et al*. reported that *blaNDM* was prevalent (84%), followed by blaOXA-48 (6%), with only 2% of isolates carrying *blaKPC* [6]. Moreover, within cancer institutions in Egypt, *blaNDM* emerged as the dominant genotype (68.88%), succeeded by blaOXA-48 (32.59%) [28]. Although NDM has been identified in Egypt since 2013, outbreaks of this strain have been infrequent [28]. Nonetheless, in our study, the emergence of NDM as the predominant carbapenemase gene is noteworthy and suggests a shifting trend. NDM is recognized as a global health threat due to its widespread dissemination and association with recent hospital outbreaks [29]. On the other hand, few studies in Egypt [30–32] revealed that *blaOXA-48* gene showed the highest prevalence followed by *blaNDM-1*. The OXA-48 gene first emerged in *K. pneumoniae* in Turkey [33]. From there, it quickly spread throughout the Middle East and eventually the rest of the world. Moreover, these strains have also been linked to several nosocomial outbreaks [34]. OXA-48 has been identified as a principal carbapenemase in *Enterobacterales* from several Gulf nations, including Saudi Arabia [35–37]. Geographical location has a significant impact on the distribution of carbapenemase genes [38]. Grundmann *et al*. [39] observed that KPC enzymes were the most frequently encountered carbapenemases in a survey

conducted throughout Europe. In China, Han *et al*. likewise claimed consistent results with KPC [40]. By the same token, numerous studies have shown the prevalence of KPC-producing strains among CRE isolates across diverse regions in the United States [41].

Interestingly, most of our isolates (59.8%) coharbored a combination of two carbapenemases genes namely *blaNDM-1* and *blaOXA-48*. The initial identification of a *K. pneumoniae* strain simultaneously expressing NDM-1 and OXA-48 occurred in Morocco in 2013 [42]. Subsequently, numerous studies have reported the simultaneous presence of *blaNDM-1* and *blaOXA-48* carbapenemases in isolates of *K. pneumoniae* and *E. coli* [43–45]. In Egypt, the concomitant presence of two or more carbapenemases genes of different combinations was previously reported by few studies [14,46,47], however, the high frequency of simultaneous presence of NDM-1 and OXA-48 (48%) was not reported in Egypt until 2021 [48]. Nonetheless, this substantial dissemination of NDM-1 and OXA-48 combination among Egyptian hospitals detected in our study has not been reported before. This could be explained by the fact that these two genes are highly prevalent in Egypt and the countries nearby. In addition, because they are carried on mobile genetic elements, they can be easily transported between hospital settings raising risks in terms of public health [47]. The co-existence of these carbapenemase genes poses a serious therapeutic problem for medical professionals, considering the limited range of available treatments and the possibility of worldwide spread via cross-border transmission [43].

Although PCR is consistently regarded as the most dependable technique for detecting carbapenemase gene [49], it is quite expensive and many laboratories in developing nations including Egypt do not have access to it [50]. Our study aimed to evaluate MAST-Carba Combi Plus kit for the detection of different carbapenemases genes. Regarding the identification of MBL/NDM-1 among CRE isolates, the sensitivity of the kit for detection of MBL was much better than OXA-48 (82.29% and 9.52%, respectively), while the PPV of the kit for detection of OXA-48 and MBL was satisfactory (100%, 98.75% respectively). On the other hand, the specificity of the kit for the detection of OXA-48 was better than MBL (100% and 80%, respectively), while the NPV of the kit was very poor in detection of both NDM and OXA-48 with slightly better NPV in detection of OXA-48 than MBL (39.36% and 19.05%, respectively). Since decreases in the prevalence, increases the NPV, the difference in NPV between NDM and OXA-48 could be explained by the low prevalence of OXA-48 among our studied isolates [51]. Our results partially match previous

studies which reported high sensitivity (68%-100%) and specificity (66%- 97%) for MBL producers and higher specificity for detection of OXA-48 producers compared to MBL[50,52,53]. The discrepancy in our results regarding the sensitivity of the kit in the detection of OXA-48 producers can be explained by the fact that most of our isolates co-harbored both OXA-48 and NDM-1 genes. Although Taha *et al*. [50] reported the successful detection of combined OXA-48 and MBL producers by MAST-Carba plus, the authors interpreted the test based on their own modified criterion (inhibition zone of temocillin disc only) ignoring the other criterion as advised by the manufacturer (absence of synergy between discs A, B, C, and D). Interpreting the test this way needs further validation by the manufacturer as it could lead to false positive OXA-48 results among MBL-only producers as previously reported [13]. Further assessment is required to examine the potential co-production of different carbapenemase genes, such as MBLs alongside KPC or OXA-48, to enhance the accuracy and reliability of the analysis [52]. Finally, our results showed one false positive KPC isolate detected by MAST-Carba plus and not by PCR, this could be explained by low specificity of the test in detecting KPC which was reported previously by other investigators [54].

The spread of resistance genes, particularly those encoding carbapenemases, underscores the need for typing methods to investigate characteristics and clonality of isolates carrying various antimicrobial determinants [55]. In our study, we employed ERIC-PCR to evaluate the genetic similarity among our CPE isolates. The constructed dendrogram for each genus from each hospital unveiled notable genetic variation among the examined isolates. Notably, the prevalent genotype in most clusters involved the simultaneous presence of MBL and OXA-48 genes. The dissemination of dual carbapenemase producers within most of our bacterial clusters raises concerns for public health in our region, as these isolates display resistance to carbapenems through different mechanisms. Like our investigation, recent studies conducted in Egypt highlighted the prevalence of various clones of CRE using various molecular typing techniques like ERIC-PCR, PFGE, and BOX PCR [14,56].

CONCLUSION

In conclusion, Our study findings indicate that NDM carbapenemase is the most commonly detected among CRE isolates in our hospital settings, followed by *blaOXA-48*. The rise of isolates carrying both *blaNDM-1* and *blaOXA-48* genes among CPE is concerning, as it poses a major public health threat. Accurate detection of dual carbapenemase producers is difficult with phenotypic methods like MAST-Carba plus, which need further validation by the manufacturer. Our findings emphasize the importance of continuous screening for CRE strains, especially in cases where both *blaNDM-1* and *blaOXA-48* genes are co-produced, it is crucial to swiftly identify and prevent their dissemination. Novel treatment alternatives, efficient antimicrobial stewardship, and robust infection control measures are essential to mitigate the dissemination of these strains.

The current research is constrained by its small sample size and the focus on samples from hospitals in Egypt's central cities, limiting the generalizability of the findings. Moreover, the restricted variety of carbapenemases found in our samples poses challenges in accurately assessing the efficacy of MAST-Carba plus. To address these limitations, it is advised to undertake similar studies with a larger and more diverse sample, encompassing various locations across Egypt. Additionally, to enhance the evaluation of MAST-Carba plus, it is suggested to incorporate isolates carrying different types of carbapenemases.

Author Contributions:
Conceptualization: ElFeky D.S., Awad A.M. **Methodology and validation:** ElFeky D.S., Awad A.M. and Baddour M.M. **Writing original draft and figure preparation:** ElFeky D.S., Hamed R.M. and Mowafy H.L. **Writing review and editing:** ElFeky D.S., Awad A.M., Baddour M.M., Mowafy H.L., Hamed R.M. **Supervision:** ElFeky D.S., Awad A.M, Baddour M.M.
All authors reviewed the manuscript.

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