Infection and Drug Resistance

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ORIGINAL RESEARCH

Carbapenemase Producers Among Extensive Drug-Resistant Gram-Negative Pathogens Recovered from Febrile Neutrophilic Patients in Egypt

This article was published in the following Dove Press journal:
Infection and Drug Resistance

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Purpose: This study aimed to detect the prevalence of carbapenemase producers (CPs) among extensive drug-resistant (XDR)-carbapenemase producing Gram-negative bacteria (GNB) recovered from various clinical specimens of hospitalized neutrophilic febrile patients in two major tertiary care hospitals in Egypt.

Methods: Standard methods were used to evaluate the antimicrobial susceptibility of clinical isolates according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Phenotypic and genotypic analysis of CPs were carried out and statistically analyzed using standard methods.

Results: Three hundred and forty-two GNB were obtained from 342 clinical specimens during the period of the study, where 162 (47%) were enterobacterial isolates, including, 63 (18.4%) *Escherichia coli*, 87 (25.4%) *Klebsiella* spp., 5 (1.46%) *Enterobacter cloacae*, 5 (1.46%) *Salmonella* spp. and 2 (0.6%) *Proteus* and 180 (53%) were non-fermentative bacilli including, 129 (37.7%), *Acinetobacter baumannii*, and 51 (14.9%), *Pseudomonas* spp. Out of the 342 GNB, 188 (54.9%) isolates were multi-drug resistant (MDR). Of these, 52 (27.6%) were XDR as well as CPs as confirmed phenotypically. The MIC of imipenem against the XDR GNB against showed either low (11 isolates; 21.1%; MIC range =4–32 µg/mL) or high levels of resistance (41 isolates; 78.8%; MIC range = 64-≥1024). The most prevalent carbapenem resistance (CR) genes were *blaKPC* (63.5%) followed by *blaOXA*-48 (55.7%) and *blaVIM* (28.8%). No significant association could be observed between the MIC level and the presence of CR genes (*P* value >0.05).

Conclusion: High prevalence of MDR (54.9%) and XDR (27.6%) GNB pathogens associated with high levels of resistance to carbapenems were observed. All XDR GNB were CPs and tested positive for at least one of the CR genes. However, most of them (78.8%) showed a high level of CR (MIC range = 64-≥1024) with no significant association with the CR genes.

Keywords: carbapenem resistance, carbapenemases, MDR, XDR, Gram-negative pathogens

Introduction

The development and dissemination of XDR carbapenem-resistant (CR) Gram-negative bacteria (GNB) is considered a serious hazard to human health globally.[1–4] The development of CR is receiving remarkable attention nowadays as carbapenem antibiotics are viewed as the last line of defense against severe multidrug-resistant infections.[5–7]

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DovePress
http://doi.org/10.2147/IDR.S269971

CR could be due to decreased outer membrane permeability associated with an overproduction of AmpC β-lactamases, generation of extended-spectrum beta-lactamases (ESBLs) and overexpression of class A, B and D carbapenemase enzymes. These enzymes include *Klebsiella pneumoniae* carbapenemase (blaKPC), Verona integron encoded metallo-β-lactamase (blaVIM), New Delhi metallo-β-lactamase (blaNDM), imipenemase (blaIMP) and oxacillinases (blaOXA-48). This remains the most clinically important mechanism that drives the emergence of resistance among GNB worldwide and is involved in most of the nosocomial outbreaks recently.[8,9]

Due to the consequences of the high morbidity and mortality rates and the high chance of large-scale dissemination of CR particularly via transmissible genetic, an expeditious detection of CPs is of a crucial importance to limit this threatening public health crisis.[10,11] Also, the emergence of XDR GNB particularly those that are CPs has elevated dramatically in the last years imposing a global concern.[10,11] Moreover, the treatment options for pyogenic infection caused by XDR GNB pathogens became very limited and scarce, particularly for pediatrics.[10,11] The febrile neutrophilic patients are characterized by having leukocytosis where total leucocyte count is above 11,000 white blood cells/μL as well as fever with an oral temperature >38°C for at least 1 hour. These parameters are indicative of pyogenic infections.[11] Thus, the aim of our current study is to highlight the prevalence, phenotypic and genotypic characteristics of CPs obtained from neutrophilic febrile patients admitted to two major Tertiary Care Hospitals in Egypt.

Methods

Collection and Examination of Clinical Isolates

A total of 342 non-duplicate Gram-negative clinical isolates discharged from the Microbiology laboratories of New Kasr El Aini and El Demerdash Tertiary Care Hospitals, Cairo, Egypt were collected over a period of 6-month, between April and September 2019. The study was approved by the Faculty of Pharmacy Cairo University Ethical Committee Nr. MI (2418) in April, 2019. Both oral and written informed consent were obtained from patients or parents of the patients after clarifying them with the purpose of the study which was carried out in accordance with the guidelines outlined in the Declaration of Helsinki. Based on the hospital records, the isolates were recovered from 342 clinical specimens obtained from febrile neutrophilic patients (>11,000 white blood cells/microL with oral temperature >38°C over at least 1 hour) including, urine (n = 118), sputum (n = 112), pus (n = 42), blood (n = 17), stool (n=11), and others including catheter tips and bronchial lavage (n=42). The patients' age ranged from 1 to 65 years. There were 158 (46%) male including the age ranged 1–20 year (95; 28%), 21–40 year (20; 6%), 41–66 year (43; 12.5%) and 184 (54%) were females including the age ranged 1–20 year (112; 33%), 21–40 year (23; 7%), 41–66 year (49; 14%). Isolates were identified macroscopically, microscopically, and biochemically according to Bergey's manual of determinative bacteriology.[12] The bacterial identification was confirmed using the MicroscanR WalkAway-96 Plus auto-identification system (Beckman Coulter, Miami, FL, USA). No specific exclusion criteria were applied and all isolates were collected on routine workdays. To confirm the identification of isolates, our data were compared to the hospital's data records.

Antimicrobial Susceptibility Tests

The antibiotic susceptibility tests were performed according to Kirby-Bauer method using a panel of 13 antibiotic disks including amoxicillin/clavulanic acid (20μg/10μg), amikacin (30μg), aztreonam (30μg), cefoxitin (30μg), ceftriaxone (30μg), ciprofloxacin (5μg), levofloxacin (5μg), imipenem (10μg) meropenem (10μg), ertapenem (10μg), imipenem (10μg), sulphamethoxazole/trimethoprim (25μg), and doxycycline (30μg) were tested. Kirby-Bauer test was carried out on Mueller–Hinton agar plates one time for all tested isolates followed by measurements of the inhibition zone diameters. MDR and XDR isolates were defined based on the international standard criteria as previously reported.[13] The XDR isolates that revealed resistance pattern to any of the carbapenems tested were potentially pointed out as carbapenem-resistant and accordingly, were selected to detect their MIC against imipenem by broth microdilution method according to CLSI guidelines, 2019.[14] The broth microdilution test was done in triplicate. Enterobacteriaceae isolates that revealed MIC ≥ 4 μg/mL and non-fermentative bacilli isolates that revealed MIC≥ 8 μg/mL for imipenem were considered carbapenem-resistant isolates with great

potential for carbapenemase production according to CLSI, 2019.[14] Quality control was monitored by using *E. coli* ATCC 25922™ reference strain.

Phenotypic Detection of CPs

Modified Carbapenem Inactivation Method (mCIM)

mCIM for detection of CPs using readily available laboratory reagents was recommended by the CLSI guidelines in 2019. The test was performed in duplicates on XDR GNB isolates that were potentially CPs. A meropenem disk was briefly immersed in a suspension of tested isolate and incubated for at least 4 hours. Consequently, the disk was transferred to an inoculated plate with *E. coli* ATCC 25922™. After an overnight incubation, the tested isolates showing a zone of inhibition between 6 and 15 mm or colonies were present within 16–18 mm were considered CPs. On the other hand, isolates showing a zone of inhibition greater than or equal to 19 mm were not considered CPs.[14]

Combined Disk Test

In order to detect metallo-beta lactamases (MBLs) production, combined disk test was performed. About, 0.5 McFarland standard adjusted overnight culture was spread over the surface of Mueller-Hinton agar plate using a cotton swab. Two disks, one is imipenem and the other is imipenem/ethylenediaminetetraacetic acid (EDTA) were laid on the surface of the agar at a distance of 4–5 cm from each other using sterile forceps. After an overnight incubation, the enhancement of inhibition zone (≥7 mm) of the IPM-EDTA disk compared to imipenem disk alone was considered positive for the presence of MBLs. This test was done in duplicates to ensure the reproducibility of results.[15]

Blue-Carba Test

The Blue-carba test can detect all CPs directly from bacterial culture with 100% sensitivity and specificity. This test was carried out as previously reported by Pires et al.[16] Duplicates of the test were carried out on the promising CPs isolates to give more reproducible results.

Molecular Detection of Genes Coded for Carbapenemases

DNA of phenotypically confirmed XDR CP isolates was extracted using the Genomic DNA Purification Kit (Thermo Fisher Scientific, USA) according to the manufacturer's instructions and was used as template for PCR using the appropriate primers synthesized by Macrogen®. PCR amplification was carried out using the annealing temperatures (Ta) and appropriate primers of *blaIMP, blaKPC, blaNDM, blaOXA-48*, and *blaVIM* genes as shown in Table 1. Using agarose gel electrophoresis, the amplified PCR products were analyzed and the interpretation of the size of DNA fragments was done via comparing to a 1000 bp DNA ladder (GeneRuler 1 kb ThermoFisher Scientific, USA).

DNA Sequencing of PCR Amplicons

QIA quick PCR Purification Kit (Qiagen) was used to purify the PCR products extracted from the agarose gel, and then they were sequenced using ABI 3730xl DNA sequencer from the forward and reverse directions by the CliniLab Co, Egypt. The resulted sequences were assembled into a final consensus sequence using the Staden Package program version 3 (http://staden.sourceforge.net/). The open reading frames (ORFs) were analyzed using FramePlot 2.3.2 (http://www.nih.go.jp/~jun/cgi-bin/frameplot.pl), annotated, and submitted into the NCBI GenBank database under the accession codes MT185944, MT185945 and MT185946:

Statistical Analysis

Statistical analysis including descriptive statistics, frequency tables, and cross-tabulations was performed using Statistical Package for the Social Sciences software IBM® SPSS® version 20 (SPSS Inc., Chicago, IL, USA). In order to determine the statistical significance, analysis of the categorical variables was performed using the Chi-square test. A value of $P<0.05$ was considered statistically significant, and significance was two-sided.

Results

Identification of Recovered Isolates

A total of 342 GNB were recovered from the 342 clinical specimens throughout the period of the study. A total of 162 (47%) were enterobacterial isolates, including 63 (18.4%) *E. coli*, 87 (25.4%) *Klebsiella* spp., 5 (1.46%) *E. cloacae*, 5 (1.46%) *Salmonella* spp. and 2 (0.6%) *Proteus*. On the other hand, a total of 180 (53%) were non-fermentative bacilli including, 129 (37.7%), *Acinetobacter (A.) baumannii*, and 51 (14.9%), *Pseudomonas* spp. Antimicrobial susceptibility tests of the 342 isolates showed that 188 (54.9%) were

Table 1 Primer Sequences and Expected Sizes of PCR Products of Carbapenemase Genes

Multiplex/ MonoplexPCR	Carbapenemase Genes	Forward Primer (5′-3′)	Reverse Primer (5′-3′)	Expected PCR Product Size	Ta (°C)	Reference
A	bla_{KPC} bla_{NDM}	TGTCACTGTATCGCCGTC GGTTTGGCGATCTGGTTTTC	TATTTTTCCGAGATGGGTGAC CGGAATGGCTCATCACGATC	331 bp 621 bp	50	[17] [18]
B	bla_{VIM} bla_{OXA-48}	TCTACATGACCGCGTCTGTC GCGTGGTTAAGGATGAACAC	TGTGCTTTGACAACGTTCGC CATCAAGTTCAACCCAACCG	748 bp 438 bp	52	[17] [19]
C	bla_{IMP}	CTACCGCAGCAGAGTCTTTG	AACCAGTTTTGCCTTACCAT	587 bp	56	[17]

Notes: A and B are multiplex PCR; C is a monoplex of *imp* detection.
Abbreviation: Ta (°C), annealing temperature.

multidrug resistant (MDR), among these, 52 isolates (15.2%) were XDR-CPs.

Antimicrobial Susceptibility Testing and Data Analysis

Table 2 shows the antibiotic susceptibility patterns of the GNB isolates. Out of the 342 GNB; 188 (54.9%) isolates were MDR. The MDR GNB isolates were *A. baumannii* (86; 45.7%), *K. pneumoniae* (50; 26.6%), *E. coli* (30; 16%), *P. aeruginosa* (18; 9.6%), *E. cloacae* (3; 1.5%) and *Salmonella spp*. (1; 0.5%). Out of the 188 MDR GNB, 52 (27.6%) exhibited resistance to all of the tested antimicrobial agents; however, they remained sensitive to only one or two and therefore, were categorized as XDR GNB. Furthermore, all of XDR GNB isolates were CPs. As shown in Table 3, the broth microdilution method was used to determine the MIC of the 52 (27.6%) XDR GNB against imipenem, and based on the value of MIC, the XDR GNB were categorized into 2 categories; low-level resistance (11 isolates; 21.1%; MIC range =4–32 µg/mL) and high-level resistance (41 isolates; 78.8%; MIC range = 64-≥1024)

Phenotypic Detection of XDR GNB

As shown in Table 4, the carbapenemase enzyme producing ability of the XDR GNB (n=52) isolates was phenotypically determined using the modified carbapenem inactivation method (m CIM), blue-carba and the combined disk tests.

PCR Amplification of CPs

The extracted DNA of each of the 52 XDR GNB isolates (CPs) was used as a template for PCR amplification of *blaIMP, blaKPC, blaNDM, blaOXA-48*, and *blaVIM* carbapenemase genes.

The results of multiplex PCR revealed that *blaKPC* was amplified in 33 (63.5%) isolates (15 *A. baumannii*, 9 *Klebsiella* spp. 5 *Pseudomonas* spp, and 3 *E. coli* and 1 *E. cloacace*), followed by *blaOXA*-48 that was detected in 29 (55.7%) isolates (13 *A. baumannii*, 9 *Pseudomonas* spp., 6 *Klebsiella* spp., and 1 *E. cloacace*), followed by *blaVIM* that was observed in 15 (28.8%) isolates (7 *A. baumannii*, 5 *Klebsiella* spp., 2 *Pseudomonas* spp. and 1 *E. cloacace*). Agarose gel electrophoresis of the multiplex-PCR amplification of *blaKPC*/*blaNDM* and *blaoxa-48*/*blaVIM* genes of some of the tested XDR GNB isolates are shown in Figures 1S and 2S, respectively. On the other hand, neither *blaIMP* nor *blaNDM* was detected in any of the tested XDR isolates. Data summary of both phenotypic and genotypic findings of the XDR GNB (n=52) is tabulated in Table 5.

Statistical Analysis of the Association of MIC Level of Resistance and Carbapenemase Resistant Genes Among XDR GNB Isolates (n-52)

Statistical analysis has shown that there was no significant association between MIC level of CR (either high- or low-level resistance) and *blaKPC, blaOXA-48, blaVIM*, PCR detected CR genes (*P* value >0.05). The statistical Pearson chi-square and Likelihood Ratios are shown in Table 6.

Discussion

CR bacteria have become a major threatening crisis worldwide, which restricts the available therapeutic and treatment options.[20] Therefore, we aimed in this study to detect the prevalence of GNB carbapenemase producers, after determining their resistance profiles. The clinical isolates were recovered from various clinical specimens of infected patients attending two major

hospitals in Egypt. The patients were admitted to the hospital suffering from signs and symptoms of acute pyogenic infections including fever (oral temperature >38°C for at least 1 hour). Based on the hospital records, the complete blood count of the respective patients also showed leukocytosis white blood cells >11,000/μL. According to the hospital records, a total of 158 (46%) patients were males in the age range of 1–20 years (95; 28%), 21–40 years (20; 6%), 41–66 years (43; 12.5%). On the other hand, a total of 184 (54%) was female patients in the age range of 1–20 years (112; 33%), 21–40 years (23; 7%), and 41–66 years (49; 14%).

The fact that lower respiratory tract infectious diseases are the number one cause of deaths among infectious diseases worldwide was reflected in the predominance of sputum cultures among recovered specimens.[21]

A total of 342 GNB were recovered from the 342 clinical specimens during the period of our study, where 162 (47%) were enterobacterial isolates, including, 63 (18.4%) *E. coli*, 87 (25.4%) *Klebsiella* spp., 5 (1.46%), *E. cloacae*, 5 (1.46%) *Salmonella* spp. and 2 (0.6%) *Proteus spp*.). The remaining 180 (53%) were non-fermentative bacilli including 129 (37.7%) *A. baumannii* and 51 (14.9%) *Pseudomonas* spp.

It has been previously reported that the respiratory tract, blood, and urine tubes were the main important sources of *A. baumannii* pathogens.[22,23] In our study, 129 (37.7%) *A. baumannii* isolates were recovered and this could explain the predominance of *A. baumannii*, the most life-threatening pathogen among the recovered GNB isolates in our study. Moreover, this finding is of great medical value and challenge from the medical point of view since *A. baumannii* is one of the difficult-to-treat and nosocomial-infection-causing pathogens., this may be due to of its endless capacity to acquire antimicrobial resistance owing to the plasticity of its genome.[24] Our microbiological data were in consonance with other novel studies that confirmed that Enterobacteriaceae especially, *K. pneumoniae* and *E. coli* together with *A. baumannii*, and *P. aeruginosa* posed the highest hazards among the GNB recovered from respiratory tract infections.[25] It is disturbing to note that 44.7% (84 out of 162) of the enterobacterial isolates were considered MDR as they were resistant to three or more antimicrobial classes. Of these, 50 out of 87 (57.5%) were *Klebsiella* spp., 30 out of 63 (47.6%) were *E. coli*, 3 out of 5 (60%) were *E. cloacae* and 1 out of 5 (20%) was *Salmonella* spp. Out of the 180 nonfermentative bacilli, 86 out of 129 (66.7%) and 18 out of 51 (35.3%) were MDR from *A. baumannii*, and *Pseudomonas* spp., respectively. Accordingly, out of the 342 GNB; 188 (54.9%) isolates were MDR which is in accordance with previous studies and imposes weak infection control strategies.[26,27] Despite the fact that the antibiogram analysis of recovered GNB is raising concerns, yet, the overall sensitivity patterns of amikacin, doxycycline and tigecycline ranging from 70% to 85% rise as a promising hope.

In addition, the percentage of CR represented a cause of concern as the disk diffusion method revealed resistant patterns to at least one of the tested carbapenems (imipenem, meropenem and ertapenem) in 40.12% (65 out of 162) enterobacterial isolates and 57.78% (104 out of 180) of non-fermentative bacilli. Regarding the wide dissemination of CP GNB with devastating ramifications within clinical settings, its early disclosure is crucial for expeditious employment of infection control measures and choosing an appropriate antimicrobial therapy. CPs were preliminarily screened for the detection of MBLs using a combined disk test. In the present study, 50% (26 out of 52) of the tested isolates were positive for class B carbapenemase. This revealed that other carbapenemase types, for example, class A and class D oxacilinase (that were not inhibited by EDTA) might be included in the carbapenem-resistance pattern. This was in accordance with the study conducted by Rakhi et al that had reported 50% in the combined disk test for MBLs production.[28]

In order to comply with the latest guidelines for carbapenemase production, mCIM and blue-carba tests were further performed for these 52 CP isolates. Results showed that the former test detected 48 (92.30%), on the other hand, the latter test detected 51 (98.07%) of CP isolates. A recent study conducted by Bayraktar et al showed similar results of 92.7% and 98.1% for mCIM and blue-carba test, respectively.[29] Moreover, the blue-carba test matched the results of another recent study conducted by Cordeiro-Moura et al that reported 97.1%.[27] Accordingly, we can declare that the blue-carba test revealed a high sensitivity and specificity for the detection of carbapenemase production and hence is viewed as an auspicious method for the rapid detection of CPs among clinical settings.[30]

We then attempted to identify the carbapenemase genes prevalence, which was crucial for the suppression of such

Table 2 Antimicrobial Resistance Patterns of the Recovered GNB (N= 342)

Antimicrobial Class	Percentage Resistance (%)							
	Antimicrobial Agent	***E. coli* (n=63)**	***Klebsiella spp.* (n=87)**	***A. baumannii* (n=129)**	***Pseudomonas spp.* (n=52)**	***Salmonella spp.* (n=5)**	***Enterococcus spp.* (n=5)**	***Proteus* (n=2)**
β-lactam group	Amoxicillin/ clavulanic	82.5	94.3	nd	nd	0	100	0
	Aztreonam	41.3	65.5	nd	35.3	0	100	0
	Ceftriaxone	61.9	79.3	96.8	nd	0	60	0
	Cefoxitin	28.9	79.3	-	nd	0	100	0
	Meropenem	26.9	69	79.1	52.9	40	100	0
	Ertapenem	28.6	69	nd	nd	40	60	0
	Imipenem	38.1	79.3	97.7	54.9	60	60	0
Aminoglycosides	Amikacin	4.8	52.8	87.6	49	0	60	0
Quinolones	Ciprofloxacin	38.1	75.9	95.3	52.9	40	60	0
	Levofloxacin	38.1	72.4	83.7	54.9	nd	60	0
Sulfonamides/ diaminopyrimidines	Sulfamethoxazole/ Trimethoprim	66.7	62.1	88.4	nd	0	60	0
Tetracyclines	Doxycycline	60.3	52.8	53.5	nd	0	40	100

Abbreviation: nd, not determined.

XDR strains within clinical healthcare sets. Following phenotypic screening of CPs isolates (52 isolates), PCR was performed using the genomic DNA of each isolate and the specific primers for each CR gene. The five carbapenemase genes examined in our study were selected based on their prevalence among the GNB and for representing the three major classes of carbapenemases, including, class A serine carbapenemases (blaKPC), class B metallo-β-lactamases (blaIMP, balNDM and blaVIM) and class D serine carbapenemases (blaOXA-48).[2]

For class A carbapenemases, various genotypes of the bla_{GES} gene (coding for blaGES β-lactamase) comprise a point mutation (G493A), where serine was included instead of glycine which, therefore, displays carbapenemase activity. Reports of GES carbapenemases are rare but increasing steadily particularly because they are plasmid-mediated.[18,31,32]

The carbapenemase blaOXA-48 is characterized by high hydrolytic activity to penicillins and low activity towards carbapenems. On the other hand, it is not affected by β-lactamase inhibitors such as sulbactam and clavulanic acid and therefore of relevant medical importance.[33,34] Other blaOXA β-lactamase variants including blaOXA-23, blaOXA-24/40, and blaOXA-58 are often detected in *Acinetobacter* spp., but with low carbapenemase activity as well as lack of inhibition by β-lactamase inhibitors.[35,36]

This remains the most clinically important mechanism that drives the emergence of resistance among GNB worldwide and is involved in most of the nosocomial outbreaks recently.[8,9]

Our results showed that *blaKPC* was the most predominant CR gene (63.5%) in accordance with a novel study conducted by Li and his colleagues,[37] followed by *blaOXA*-48 23 (55.7%), then *blaVIM* 10 (28.8%). However, *blaIMP* and *blaNDM* were not detected in any isolate. On the other hand, our findings were different from another study that was conducted in Zagazig hospital in Egypt, which reported that blaOXA-23 was the most prevalent (90%) followed by, *blaNDM* (66.7%) then *blaGES* (50%) in CR *A. baumannii*, respectively.[38] However, genes coding *for blaVIM, blaGES, blaNDM* and *blaIMP* were identified in 50%, 40.9%, 27.3% and 18.2% of CR *P. aeruginosa*, respectively.[38] The difference between the respective study and our study could be attributed to other factors such as geographical and patient factors.

Interestingly, 21 (40.38%) out of 52 carbapenemase-positive isolates were found to co harbor 2 carbapenemase genes that were distributed as follows: 19 isolates co-harbored *blaKPC* and *blaOXA-48*, 1 isolate co-harbored *blaKPC* and *blaVIM* and 1 isolate co-harbored *blaOXA-48* and *blaVIM*. Moreover, 4 (7.69%) isolates co harbored 3 carbapenemase genes that were *blaKPC, blaOXA*-48 and *blaVIM*. It was remarkably noticed that the *blaKPC* was

Table 3 MIC of Imipenem Against XDR GNB (n=52)

MIC Range µg/mL		Isolate Species	No. of Isolates
High level resistance (n= 41)	512-≥1024	*E. coli* *Klebsiella* spp. *A. baumannii* *Pseudomonas spp.*	0 1 8 5
	64–256	*E. coli* *Klebsiella* spp. *A. baumannii* *Pseudomonas spp.*	2 10 10 5
Low level resistance (n= 11)	4–32	*E. coli* *Klebsiella* spp. *A. baumannii* *Pseudomonas spp.* *Enterobacter spp.*	1 3 3 3 1

the most predominant gene found in most enterobacterial strains worldwide including Egypt as reported by many studies[39–41] Although the presence of both *blaOXA*-48 and *blaVIM*-1 genes has been previously reported in Egypt in one *E. coli* isolate.[42] This is the first report confirming the high rate of coexistence of the previously mentioned carbapenemases among Gram-negative bacterial isolates in Egypt. It was not astonishing that the combined disk test was not able to identify 8 *blaKPC*, 2 *blaVIM* and 11 *blaOXA*-48 producing isolates as the latter has no available specific inhibitors. Hence, CDT was not recommended for *blaOXA*-like gene detection.[42,43] However, recent investigations are seeking for testing temocillin as a promising inhibitor for *blaOXA*-48.[44] Regarding mCIM, negative results were obtained with four isolates producing *blaKPC* and one isolate producing both *blaVIM* and *blaOXA-48*. This was due to the weak hydrolytic activity of *blaOXA*-48 to meropenem as mentioned in some studies.[45–47] Never the less, our study showed positive results with *blaOXA*-48 producing isolates that were not usually identified by phenotypic approaches and this was similar to the results reported by Gauthier et al.[48] Fortunately, as discussed in other studies and according to our findings, almost all variants of carbapenemase genes managed to be rapidly detected with nearly 100% sensitivity via the blue-carba test.[49,50]

On the other hand, according to our data 25% (n=13) of the positive phenotypically tested isolates showed negative results in all five tested primers and this could be attributed to the presence of other carbapenemase genes such as *blaSME, blaGES, blaOXA-181*, and *blaOXA-244* which were not tested in the current study. Another reason may be to the hyperproduction of ESBL and AmpC coding genes or deletion/mutation in the OmpF and OmpC coding genes.[51] Although the PCR-based method has the inherited disadvantages of being expensive and unable to detect novel genes, they remain the classical and reliable methods for better correlation of results with detailed phenotypic methods that have been used only as a primary screening for detection of carbapenemase enzymes.[52–54]

Table 4 Phenotypic Detection of Carbapenemase Producing of XDR GNB Isolates

Tested Isolate	Modified Carbapenem Inactivation Method (mCIM)[2]		Blue-Carba Test (BCT)[3]		Combined Disk Test (CDT)[4]	
	Meropenem Disk		Imipenem Powder		Imipenem/EDTA (Class B)	
	No of Cpo*/Total No Tested	%	No of Cpo*/Total No Tested	%	No of Cpo*/Total No Tested	%
E. coli	3/3	100	3/3	100	2/3	66.7
***Klebsiella* spp.**	14/14	100	14/14	100	8/14	57.1
E. cloacae	1/1	100	1/1	100	0/1	0
A. baumannii	18/21	85.7	21/21	100	12/21	57.1
Pseudomonas spp.	12/13	92.3	12/13	92.3	4/13	30.8

Abbreviations: Cpo, carbapenemase-producing organism; EDTA, ethylenediaminetetraacetic acid.

Table 5 Summarization of Phenotypic and Genotypic Characters of the XDR GNB (n=52)

Nr	Isolate No.	Isolate	MIC Imipenem µg/mL	Combined Disk Test	Modified Carbapenem Inactivation Test	Blue-Carba Test	Carbapenemase Genes
1	55A	*E. coli*	64	-	+	+	*blaKPC*
2	84S	*Klebsiella* spp.	64	-	+	+	*blaOXA-48, blaKPC*
3	36M	*A. baumannii*	8	-	+	+	*blaOXA-48*
4	99	*Pseudomonas spp.*	>1024	-	-	+	*blaKPC*
5	83	*Pseudomonas spp.*	>1024	-	+	+	*blaOXA-48, blaKPC*
6	18	*Pseudomonas spp.*	256	+	+	+	*blaVIM*
7	9G	*A. baumannii*	256	-	-	+	*blaOXA-48*
8	50	*Pseudomonas spp.*	>512	-	+	+	*blaOXA-48*
9	30M	*A. baumannii*	>1024	+	+	-	*blaVIM*
10	78S	*Pseudomonas spp.*	32	-	+	+	*blaOXA-48*
11	100	*Pseudomonas spp.*	256	-	+	+	*blaOXA-48*
12	74M	*A. baumannii*	128	+	+	+	*blaOXA-48, blaKPC*
13	109A	*A. baumannii*	128	-	+	+	*blaOXA-48*
14	2*	*E. cloacace*	16	-	+	+	*blaOXA-48, blaKPC, blaVIM*
15	6M	*A. baumannii*	32	-	+	+	*blaOXA-48, blaKPC*
16	89S	*Klebsiella* spp.	64	-	+	+	*blaOXA-48, blaKPC*
17	81S	*Klebsiella* spp.	8	-	+	+	*blaVIM*
18	63M	*A. baumannii*	512	-	+	+	*blaOXA-48, blaKPC*
19	30G	*A. baumannii*	128	-	+	+	*blaKPC, blaVIM*
20	100M	*A. baumannii*	128	-	-	+	*blaKPC*
21	55M	*A. baumannii*	>1024	+	+	+	*blaVIM*
22	25	*Klebsiella* spp.	64	-	+	+	*blaKPC*
23	34E	*E. coli*	16	-	+	+	*blaKPC*
24	106S	*Klebsiella* spp.	64	-	+	+	*blaKPC*
25	28M	*A. baumannii*	>1024	+	+	+	*blaOXA-48, blaKPC*
26	4*	*Klebsiella* spp.	256	+	+	+	*blaVIM*
27	20G	*A. baumannii*	32	+	+	+	*blaVIM*
28	11*	*Klebsiella* spp.	265	+	+	+	*blaVIM*

(*Continued*)

Table 5 (Continued).

Nr	Isolate No.	Isolate	MIC Imipenem µg/mL	Combined Disk Test	Modified Carbapenem Inactivation Test	Blue-Carba Test	Carbapenemase Genes
29	7*	*Klebsiella* spp.	128	+	+	+	*blaOXA-48, blaKPC*
30	92A	*Klebsiella* spp.	64	+	+	+	*blaOXA-48, blaKPC, blaVIM*
31	19	*Pseudomonas spp.*	512	+	+	+	*blaOXA-48*
32	7G	*A. baumannii*	256	+	-	+	*blaKPC*
33	42M	*A. baumannii*	>1024	+	+	+	*blaOXA-48, blaKPC*
34	13G	*A. baumannii*	>1024	+	-	+	*blaOXA-48, blaKPC, blaVIM*
35	77Y	*Pseudomonas spp.*	16	+	+	+	*blaOXA-48, blaVIM*
36	3M	*A. baumannii*	128	+	+	+	*blaOXA-48, blaKPC*
37	33	*Pseudomonas spp.*	512	-	+	-	*blaKPC*
38	99A	*E. coli*	64	-	+	+	*blaKPC*
39	54	*Pseudomonas spp.*	512	-	+	+	*blaOXA-48*
40	14M	*A. baumannii*	128	+	+	+	*blaOXA-48, blaKPC*
41	79A	*Klebsiella* spp.	512	+	+	+	*blaKPC*
42 43	37M 9*	*A. baumannii* *Klebsiella* spp.	512 256	+ +	+ +	+ +	*blaVIM* *blaKPC*
44	34M	*A. baumannii*	512	-	+	+	*blaKPC*
45	13M	*A. baumannii*	>1024	+	+	+	*blaOXA-48, blaKPC*
46	64M	*A. baumannii*	256	-	+	+	*blaOXA-48, blaKPC, blaVIM*
47	114S	*Klebsiella* spp.	128	+	+	+	*blaOXA-48, blaKPC*
48	111S	*Pseudomonas spp.*	256	+	+	+	*blaKPC*
49	59	*Pseudomonas spp.*	32	-	+	+	*blaOXA-48*
50	113S	*Klebsiella* spp.	32	-	+	+	*blaOXA-48, blaKPC*
51	112S	*Klebsiella* spp.	16	+	-	+	*blaVIM*
52	51	*Pseudomonas spp.*	256	-	+	+	*blaOXA-48, blaKPC*

Table 6 Association of MIC Level of Resistance and Carbapenemase Resistant Genes Among XDR GNB Isolates (n-52) and Statistically Significant Associations

MIC Resistance	Level	*blaKPC*		Total (n= 52)	Likelihood Ratio	Pearson Chi-Square
		Negative	Positive			
	N Y	6 12	5 29	11 41	0.222	0.217
		blaOXA-48				
		Negative	Positive			
	N Y	5 19	6 22	11 41	0.927	0.927
		blaVIM				
		Negative	Positive			
	N Y	8 32	3 9	11 41	0.346	0.325

Abbreviations: N, low level resistance (n= 11); Y, high level resistance (n= 41).

Conclusion

In summary, this study shows the high prevalence of carbapenemase-encoding genes among XDR GNB in Egypt. Both *blaKPC* and *blaOXA*-48 like were found to be the most predominant CR genes. All XDR GNB were CPs as confirmed by phenotypic and genotypic methods. The high prevalence of CPs in Egypt with the limited potential therapeutic options necessitates an extreme concern and immediate intervention with effective antimicrobial stewardship programs to reduce the burden of CR along with limiting the spread of this nightmare emerging threat.

Data Sharing Statement

All the data supporting the findings are included in the manuscript.

Ethical Clearance

The study was approved by the Faculty of Pharmacy Cairo University Ethical Committee Nr. MI (2418) in April, 2019. A written informed consent was obtained from wither the patients or parents of the patients after clarifying them with the purpose of the study which was carried out in accordance with the guidelines outlined in the Declaration of Helsinki.

Acknowledgments

We would like to acknowledge the microbiology laboratories of New Kasr El Aini and El Demerdash Hospitals, Cairo, Egypt for providing us with the clinical isolates. We would like to acknowledge the Department of Microbiology and immunology, of both Faculty of pharmacy, Ain Shams University (ASU) and Ahram Candian University (ACU), Cairo, Egypt for providing support and facilities whenever needed. Also, I would like to acknowledge Miss Ann Elshamy, the assistant lecturer at the Microbiology and Immunology Department, Faculty of Pharmacy, Ain Shams University for providing help and support in performing PCR and agarose gel electrophoresis.

Funding

There is no funding to report.

Disclosure

The authors declare that they have no competing interests for this work.

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