Molecular Characterization of Carbapenemase-Producing Gram-negative Bacteria Isolated from Clinical Specimens in Baghdad, Iraq

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Abstract

The emergence and spread of carbapenem-resistant Gram-negative bacteria is a worldwide emerging public health threat responsible for large number of nosocomial infections. Metallo-β-lactamases including IMP, VIM, and NDM as well as carbapenem hydrolyzing class D β-lactamase (OXA-48 like) are the predominant types that confer resistance to Carbapenem group of antibiotics. The aim of this study was to identify the carbapenemase encoding genes among Gram negative bacteria isolates. 42 isolates were identified depending on routine morphological tests followed by species identification using the VITEK 2 system. The 16S rDNA gene sequence was used for confirmation of the detection of Enterobacteriaceae and Pseudomonas aeruginosa. Antimicrobial susceptibility testing was performed using VITEK 2 system. For phenotypic detection of carbapenemase activity, modified carbapenem inactivation method (mCIM) was performed. The carbapenemases encoding genes (bla_oXA-48, bla_vIM, bla_NDM, bla_rex, bla_mop, bla_vES, bla_ups, bla_tin, bla_vIM, bla_uIM) were amplified by PCR and the amplified products were sequenced. Forty two Gram-negative bacteria isolates including 25 of P. aeruginosa (59.5%) and 17 of Enterobacteriaceae family (40.4%) were identified. According to PCR-based method results, carbapenemase gene bla_oXA-48 was detected in 31 (73.8%) of isolates, bla_vIM in 23 (54.7%) and bla_NDM in 2 (4.76%) of isolates. Twelve (28.5%) of isolates harbored a combination of bla_oXA-48 and bla_vIM (2.4%) coexistence, bla_oXA-48 andbla_NDM gene and (2.4%) of isolates harbored a bla_oXA-48, bla_vIM and bla_NDM genes. No other carbapenemase genes were identified. Based on the present study, it was concluded that the high prevalence was in bla_oXA-48 gene, followed by bla_vIM gene among carbapenemase-producing Gram-negative bacteria isolates.

Keywords: Enterobacteriaceae Pseudomonas aeruginosa, Carbapenemases.
INTRODUCTION

Gram-negative bacteria that produce Carbapenemase have been related to increased mortality and critical nosocomial outbreaks that represent the main challenge in both therapeutic and infection control.

The concern of carbapenemase-producing Gram-negative bacteria that emerged currently is due to it is often related to the occurrence of multiple drug resistant isolates for which few choices of antimicrobials stay available.

Carbapenems are β-lactam antibiotics that used most frequently as last resource antibiotics for treating of multidrug-resistant Gram negative bacilli-causing infections, since they have the wide spectrum of bactericidal action and their stability against most of the β-lactamases, including ESBLs.

The increase of carbapenem resistance in these microorganisms is a major concern globally. The most common mechanism of resistance is the production of carbapenem-hydrolysing enzymes, carbapenemases that hydrolyse most β-lactams.

These enzymes encoding by multiple genes of resistance, which is associated with different mobile genetic determinants, thus conferring resistance to various classes of antimicrobials, such as aminoglycosides, fluoroquinolones, tetracyclines, trimethoprim, sulphonamides, and phenicols.

The major public health threat is with transmissible carbapenemases, which can increase the rate of mortality and limit the choice of appropriate antibiotic therapy. The transmissible enzymes can be acquired unpredictably by important nosocomial pathogens such as Pseudomonas aeruginosa, Acinetobacter baumannii and members of the family Enterobacteriaceae.

The most serious form of carbapenem resistance is mediated by carbapenem-hydrolyzing β-lactamases, including metallo-β-lactamases (MBLs), such as imipenemase (IMP), Verona imipenemase (VIM), New Delhi metallo-β-lactamase (NDM), Ambler class A Klebsiella pneumoniae carbapenemase (KPC), and class D oxacillinase-48 (OXA-48).

Because of the lack of implementing standardized protocols for detection of carbapenemase-producing isolates in many countries that probable to be the major reservoirs, the actual prevalence of these producers is still unknown. It is estimated that we are on the rim of a global epidemic with carbapenemase-producing isolates, which in the hospital environment is likely to be caused mostly by all types of nosocomial carbapenemase-producers (e.g. KPC, IMP, VIM, NDM and OXA-48). In Iraq, we showed that a significant increase in carbapenem-resistant bacteria in the last two decades, especially after the 2003 War, maybe is due to the Iraq’s openness to the world and the entry of foreign workers, especially from the endemic area from the Indian subcontinent like Bangladesh and other countries.

For all of the mentioned above, as well as, few report investigated the molecular basis of resistance to carbapenems among Gram-negative bacteria, this study aimed to identify the genes which is responsible for encoding carbapenemase enzymes in these organisms.

MATERIALS AND METHODS

Bacterial isolates

A total of 42 different Gram-negative isolates were collected from various patient specimens from different hospitals in Baghdad city, Iraq during a period between October 2017 and February 2018. The isolates were identified initially depending on morphological characteristics as described previously, followed by identification by using VITEK® 2 compact system (bioMeriux, France). Genotypic method was used to confirm the identification of isolates at species level using 16S rDNA gene sequences.

Antimicrobial susceptibility test

Antimicrobial susceptibility test was performed with the VITEK 2 system (bioMe’rieux, France) using AST 69 card as described by the manufacturer. The tested antibiotics were: Ampicillin, Amoxicillin/Clavulanic Acid, Ampicillin / Sulbactam, Cefazolin, Ceftazidime, Ceftriaxone, Cefepime, Ciprofloxacin, Levofoxacin, Imipenem, Amikacin, Gentamicin, Trimethoprim/ Sulframethoxazole and Nitro-furantoin.

Phenotypic detection of carbapenemase production

The modified carbapenem inactivation
method (mCIM) was performed according to CLSI guidelines. In briefly, using sterile inoculating loop, 1μl of test organism was suspended in 2ml of tryptic soy broth, the bacterial suspension was homogenized by vortex. Then, a 10-μg meropenem disk was immersed into the suspension. Subsequently, the culture was incubated for 4 hours at 35°C, prepared 0.5 McFarland suspension of E. coli ATCC® 25922 (a carbapenem-susceptible strain) that was inoculated on Mueller-Hinton agar (MHA) plates streaked as cell lawn.

After the incubation, the disk was removed using a 10-μl inoculating loop; the loop was dragged along the edge of the tube during removal to remove excess liquid, and the disk was placed onto the inoculated MHA plate, which was then incubated in for 18-24 hours at 35°C. Following the incubation, diameter of the inhibition zone around the disc was measured, a zone diameter of 6-10 mm or presence of colonies within a 16–18 mm zone was considered to be a positive result, 16–18 mm an indeterminate result, and 19 mm a negative result.

**Genotypic identification of carbapenemase-encoding genes**

**DNA isolation of bacteria**

Pure culture of bacterial isolates were grown overnight in liquid nutrient broth medium (NB) for the isolation of genomic DNA using the Genomic DNA purification kit (Promega, USA) according to the manufacturers protocol.

All isolates were subjected to molecular screening to detect carbapenemase-encoding genes by using PCR amplification technique. In this study, multiplex PCR was used to detect carbapenemase encoding genes from clinical isolates and the PCR products were sequenced.

**Multiplex PCR**

Eleven pairs of primers (Alpha DNA, 12.5μl of Go Taq Green Master Mix, 1μl each of the following primers: IMP, SPM, VIM, NDM, BIC, KPC or OXA and the volume was completed to 25μl with nuclease free water. While the reaction mixture of 25μl for each of the following primers: AIM, SIM, GIM or DIM composed from 2μl of template DNA, 12.5μl of Go Taq Green Master Mix, 1μl of each primers and 1.5μl of dimethyl sulfoxide (DMSO) and the volume was completed to 25μl with nuclease free water. Cycle conditions were as followed: 10 min at 94°C and 36 cycles of amplification consisting of 30 sec at 94°C, 40 sec at 52°C and 50 sec at 72°C, with 5 min at 72°C for the final extension.

The amplified products were electrophoresed in 2% agarose gel in 1x TBE buffer containing red safe dye at 100 V for 50 minutes. Then, the PCR products were visualized under UV light by UV transilluminator. The E. coli ATCC 25922 strain was used as negative control.

**Sequencing of PCR products**

The amplified PCR products were sequenced at Macrogen DNA sequencing Company (Seoul, Korea). DNA sequences were analyzed and compared with standard strain using BLAST (Basic Local Alignment Search Tool) in National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/BLAST).

**Genebank accession numbers**

The 16S rDNA gene, bla_vim, 2 gene, bla_oxa-48 gene and bla_oxa-48 gene sequences from this study were deposited in Genbank database under accession numbers MK182251 to MK182258, MK156197 to MK156202 and MK159338 to MK159352.

**RESULTS**

Forty two clinical isolates of Gram-negative bacteria (Pseudomonas aeruginosa and members of Enterobacteriaceae family) were isolated from 32 patients including: 40 (95.2%) isolates from burns and 2 (4.76%) isolates from urine infection were collected. From 42 isolates, 25(59%) were P. aeruginosa and 17(40%) were Enterobacteriaceae (six of Klebsiella pneumoniae, seven of E. coli and four of Enterobacter cloacae) (Table 2).

The patterns of antimicrobial resistance were as followed: (100%) to ampicillin and cefazolin and Ceftriaxone, (95.2%) to ampicillin/sublactam and imipenem, (92.8%) to amoxicillin/clavulanic acid and trimethoprim/sulfamethoxazol, (85.71%) to cefepime, (80.95%) to...
ceftazidime and gentamicin, (76.1%) to amikacin, (69.04%) to ciprofloxacin, (66.6%) to levofloxacin and (64.28%) to nitrofurantoin (Fig. 1).

For mCIM, all isolates showed positive results (25 isolates of Pseudomonas aeruginosa and 17 isolates of Enterobacteriaceae family).

![Antibiogram pattern of isolates towards antimicrobials used in this study. (R): Resistant, (I): Intermediate and (S): Susceptible.](image)

**Fig. 1.** The Antibiogram pattern of isolates towards antimicrobials used in this study. (R): Resistant, (I): Intermediate and (S): Susceptible.

| Table 1. The primers used in this study |
|-------------------------------|-----------------|-----------------|--------|
| Gene | Primer | Sequence (5' - 3') | Product size (bp) | Reference |
|-------------------|-------------|------------------|-----------------|-----------|
| PASS PA-SS-F | GGGGGATCTTCGGACCTCA | 956 | 13 |
| PA-SS-R | TCCTTAGAGTGGCCACCCCG | | |
| 16SrRNA 27 F | AGAGTTTGATCTGCTGCTACG | 930 | 14 |
| 1492 R | CTTGTGCGGGCCCCCGTCCTTC | | |
| blaIMP IMP-F | GGAATAGAGTGGCTAATCTC | 232 | 12 |
| IMP-R | GGTTTAAYAAAAACACCAACC | | |
| blaSPM SPM-F | AAAACTTGGGTACGCAAACG | 271 | |
| SPM-R | ACATTATCCCGCTTGAAACAGG | | |
| blaAIM AIM-F | CTGAGGGTGCTACGGAAACAC | 322 | |
| AIM-R | GTTCGCCCACCTGGAATTG | | |
| blaVM VIM-F | GATGCTGTTTGGCTGCCATA | 390 | |
| VIM-R | CGAATGCGCAGCACCAG | | |
| blaOKA OXA-F | GGCTGTTAAGGATGAACAC | 438 | |
| OXA-R | CATCAAGTTCAAACCCAG | | |
| blaGIM GIM-F | TCGACACACCTGGTCTGAA | 477 | |
| GIM-R | AACTCACAACCTGGCCATGC | | |
| blaBIC BIC-F | TATGCGACCTCTCTTAAAGGC | 537 | |
| BIC-R | TCATTGGCGGTACG | | |
| blaSIM SIM-F | TAAAAGGATTGCCATCG | 570 | |
| SIM-R | TAATGGCCCTGTCCACTGTG | | |
| blaNDM NDM-F | GTTTTGCGCATCCTGTTCCTTTC | 621 | |
| NDM-R | CGGAATGCGCATCACGATC | | |
| blaDIM DIM-F | GCTTGCCTCTGCTCTGAAAC | 699 | |
| DIM-R | CGTTGCCGTGGAAATGTG | | |
| blaKPC KPC-Fm | CTGCTAGTTCTGCCTGCTTG | 798 | |
| KPC-Rm | CTGTCATCTCTGTAGCCG | | |
| Pre NDM pre-NDM-F | CACCTCATTGGTCTAGCTG | 984 | 15 |
| pre-NDM-R | CTTGTCACATCGAC | | |
indicating the production of carbapenemase by these strains. Molecular tests confirmed that at least one carbapenemase gene in all isolates that were phenotypically carbapenemase positive, with the exception of one *Pseudomonas aeruginosa* isolate was carbapenemase-positive by phenotypic test despite being negative for the detection of carbapenemase encoding genes.

Multiplex PCR-based methods were conducted to detect the carbapenem-resistant

| Isolates No. | Bacterial species            | Genes            | Isolation source |
|--------------|------------------------------|------------------|------------------|
| SN1          | *Escherichia coli*           | blaVIM           | Burn             |
| SN2          | *Enterobacter cloacae*       | blaVIM           | Burn             |
| SN3          | *Pseudomonas aeruginosa*     | blaVIM           | Burn             |
| SN4          | *Escherichia coli*           | blaVIM           | Burn             |
| SN5          | *Pseudomonas aeruginosa*     | blaVIM\& blaOXA-48 | Burn         |
| SN6          | *Pseudomonas aeruginosa*     | blaVIM\& blaOXA-48 | Burn         |
| SN7          | *Pseudomonas aeruginosa*     | blaVIM\& blaNDM\& blaOXA-48 | Burn         |
| SN8          | *Pseudomonas aeruginosa*     | blaVIM\& blaOXA-48 | Burn         |
| SN9          | *Pseudomonas aeruginosa*     | blaVIM           | Burn             |
| SN10         | *Pseudomonas aeruginosa*     | blaNDM\& blaOXA-48 | Burn         |
| SN11         | *Pseudomonas aeruginosa*     | blaVIM\& blaOXA-48 | Burn         |
| SN12         | *Pseudomonas aeruginosa*     | blaOXA-48        | Burn             |
| SN13         | *Pseudomonas aeruginosa*     | blaVIM           | Burn             |
| SN14         | *Pseudomonas aeruginosa*     | blaVIM           | Burn             |
| SN15         | *Pseudomonas aeruginosa*     | blaVIM\& blaOXA-48 | Burn         |
| SN16         | *Escherichia coli*           | blaVIM           | Burn             |
| SN17         | *Pseudomonas aeruginosa*     | blaOXA-48        | Burn             |
| SN18         | *Enterobacter cloacae*       | blaVIM\& blaOXA-48 | Burn         |
| SN19         | *Pseudomonas aeruginosa*     | blaOXA-48        | Burn             |
| SN20         | *Pseudomonas aeruginosa*     | blaOXA-48        | Burn             |
| SN21         | *Pseudomonas aeruginosa*     | blaVIM           | Burn             |
| SN22         | *Pseudomonas aeruginosa*     | None             | Burn             |
| SN23         | *Pseudomonas aeruginosa*     | blaOXA-48        | Burn             |
| SN24         | *Pseudomonas aeruginosa*     | blaOXA-48        | Burn             |
| SN25         | *Pseudomonas aeruginosa*     | blaOXA-48        | Burn             |
| SN26         | *Escherichia coli*           | blaOXA-48        | Burn             |
| SN27         | *Escherichia coli*           | blaVIM\& blaOXA-48 | Burn         |
| SN28         | *Klebsiella pneumoniae*      | blaOXA-48        | Burn             |
| SN29         | *Pseudomonas aeruginosa*     | blaOXA-48        | Burn             |
| SN30         | *Escherichia coli*           | blaVIM           | urine            |
| SN31         | *Klebsiella pneumoniae*      | blaOXA-48        | Burn             |
| SN32         | *Enterobacter cloacae*       | blaOXA-48        | Burn             |
| SN33         | *Pseudomonas aeruginosa*     | blaVIM\& blaOXA-48 | Burn         |
| SN34         | *Enterobacter cloacae*       | blaOXA-48        | Burn             |
| SN35         | *Klebsiella pneumoniae*      | blaVIM\& blaOXA-48 | Burn         |
| SN36         | *Klebsiella pneumoniae*      | blaOXA-48        | Burn             |
| SN37         | *Pseudomonas aeruginosa*     | blaOXA-48        | Burn             |
| SN38         | *Klebsiella pneumoniae*      | blaOXA-48        | Burn             |
| SN39         | *Pseudomonas aeruginosa*     | blaVIM\& blaOXA-48 | Burn         |
| SN40         | *Klebsiella pneumoniae*      | blaVIM\& blaOXA-48 | Burn         |
| SN41         | *Escherichia coli*           | blaOXA-48        | Burn             |
| SN41         | *Pseudomonas aeruginosa*     | blaVIM\& blaOXA-48 | Burn         |
genes. The most prevalent carbapenemase gene \( bla_{OXA-48} \) was found in 31(73.8%) of the 42 isolates, followed by \( bla_{VIM} \) gene that was detected in 23 (54.7%) and \( bla_{NDM} \) gene in 2(4.76%). The results showed the presence of \( bla_{OXA-48} \) gene were found in 19(45.2%) of \( P.\ aeruginosa \), 6 (14.2%) of \( K.\ pneumoniae \), 3 (7.1%) of \( E.\ coli \) isolates and in 3 (7.1%) of \( E.\ cloacae; \) \( bla_{VIM} \) gene in 14 (33.3%) of \( P.\ aeruginosa \) isolates, 5 (11.9%) of \( E.\ coli \) isolates and 2 (4.7%) of \( E.\ cloacae \) isolates and presence of \( bla_{NDM} \) gene in 2 (4.76%) of \( P.\ aeruginosa \) (Table 1). On the other hand, \( bla_{IMP}, bla_{SIM}, bla_{KPC}, bla_{BIC}, bla_{AIM}, bla_{SIM}, bla_{GIM} \) and \( bla_{TEM} \) genes were not detected in all isolates. Interestingly, twelve isolates (28.5%) harbored a combination of \( bla_{OXA-48} \) and \( bla_{VIM} \), one isolate (2.4%) co-harboring three carbapenemase-encoding genes \( bla_{OXA-48} \) and \( bla_{NDM} \) gene and 1 (2.4%) harboring \( bla_{OXA-48}, bla_{VIM} \) and \( bla_{NDM} \) genes.

**DISCUSSION**

Carbapenemase-producing bacteria have become a major problem worldwide, which has emerged due to the increased dependence on carbapenems as a last resource to treat bacteria with multidrug-resistant\(^6\).

Carbapenemases represent the stringent threat for global human health and stand as one of the most challenging issues facing infectious disease containment in the subsequent years\(^7\).

Notwithstanding the small number of isolates, the author found the dominant OXA-48 carbapenemases among studied isolates. The most current and concern development is the rapid rise in emerging and dissemination of OXA-48, particularly in \( K.\ pneumoniae \). In 2001, the OXA-48-producing Enterobacteriaceae was first identified in Turkey, then later reported in various countries including the Middle East, North Africa, and Europe\(^8\).

In a local study carried by Abdulla et al. (2016), they reported that the \( bla_{OXA-48} \) genes were detected in 25%, of the \( E.\ coli \) isolates and 21.4% in \( K.\ pneumoniae \) isolates\(^9\). Several studies in regional countries reported the predominance of \( bla_{OXA-48} \) among Gram negative bacteria, as it was 49% in Arabian Gulf \(^{10}\), 53.3% in the UAE\(^\text{21}\), 88% in Lebanon\(^\text{22}\), 49.2% in Egypt \(^\text{16}\) and 86% in Turkey \(^\text{8}\), on the other hand, a study by Mohamed et al., reported decrease in the rate (22.4%) of this gene in \( P.\ aeruginosa \) isolates\(^{23}\).

The increasing incidence of \( bla_{OXA-48} \) has been described in varying worldwide countries, in France, \( bla_{OXA-48} \) gene represented 76.8% of carbapenem producing \( K.\ pneumoniae \), 81.1% of carbapenem- producing \( E.\ coli \), 75.8% of carbapenem-producing \( Enterobacter \) spp.\(^{24}\), in Romania, 80% of \( K.\ pneumoniae \) isolates harbored a \( bla_{OXA-48} \) gene\(^{25}\), while in Canada, Matasige et al., reported that \( bla_{OXA-48} \) found in 31.3% of isolates \(^{26}\).

In regard to existence of the \( bla_{VIM} \) gene, the results also showed increasing in prevalence rate, as it was 54.7% of carbapenem-resistant isolates have possessed this gene.

A local study done by Al-Jubori et al. (2016) showed that the prevalence rate of \( bla_{VIM} \) gene was 25% in \( A.\ baumannii \)\(^{17}\), while another study done by Hammadi et al. (2015), reported that all \( E.\ coli \) isolates did not carry \( bla_{VIM} \) gene\(^{28}\).

The VIM types are the most frequent among class B carbapenemases which have been detected in all continents\(^{29}\). VIM enzymes were firstly reported in isolates of \( P.\ aeruginosa \), and then emerged in \( Enterobacteriaceae \) as well. Subsequently in a number of regional countries, a study carried out in Saudi Arabia describing that \( P.\ aeruginosa \) strain harboring the \( bla_{VIM-2} \) gene from a Saudi patient hospitalized in France\(^{30}\).

In Iran, Rajabnia et al. (2015), reported that the \( bla_{VIM-1} \) gene are presence in 30% of \( K.\ pneumoniae \) isolates \(^{31}\), while in Turkey, Haciseyitoglu et al. (2017) found that the percentage rate of this gene was low when it reached only 10% in \( E.\ cloacae \)\(^\text{32}\).

Hammami et al. (2011) revealed that the percentage of \( bla_{VIM-2} \) gene was 67% in Tunisia\(^{33}\).

In Romania, Mereuta et al. (2013), showed that 48% of \( P.\ aeruginosa \) isolates carried \( bla_{VIM-2} \) gene\(^{34}\), while in Korea, Hong et al. showed that 69% of isolates harbored the \( bla_{VIM-2} \) gene\(^{35}\), on the other hand, Touati et al. (2013) mentioned that the percentage rate was 82% of the studied isolates\(^{36}\).

Since the detection of NDM-1 is firstly reported in India, there has been a global rise in the dissemination of NDM-1 carrying organisms. At first, the existence of NDM-1 was predominantly
reported in *Enterobacteriaceae*, but reports occurring recently pointed out to its spread in *Acinetobacter* spp. and *Pseudomonas* spp. as well.

The author found that *bla*$_{NDM}$ gene is presence in two isolates (4.7%) of *P. aeruginosa* among carbapenem resistant isolates.

In local studies, Al-shara *et al.* (2014) reported that out of 36 carbapenem resistant *P. aeruginosa* isolates, only 5.6% of isolates harbored *bla*$_{NDM}$ gene, another study by AL-Harmoosh (2015) showed that the prevalence rate of *bla*$_{NDM}$-1 gene was 20%$^{39}$, while in recent study by Hussein (2017), revealed that *bla*$_{NDM}$-1 gene was 40% in *E. coli* isolates$^{45}$, however Hammoudi *et al.* showed that the percentage of the prevalence of *bla*$_{NDM}$-1 gene was 100% in isolates$^{41}$.

Bacterial isolates that produce NDM-1 enzyme may express numerous other unrelated resistance genes, such as OXA-48 type and VIM type that encode other carbapenemases, AmpC, extended-spectrum beta-lactamases, and other classes of antimicrobials$^{42}$.

The prevalence of NDM-1 producing isolates were reported from different countries including the Gulf Corporation Council (GCC) which investigated in a total of 200 isolates collected from 16 hospitals in Saudi Arabia, Kuwait, Oman and the United Arab Emirates. Overall, NDM-1 was the most common encountered carbapenemase gene 46.5%$^{43}$, 47.6% in Egypt$^{16}$, 29.5% in Turkey$^{8}$ and 7.8% in Tunisia$^{44}$.

In Bangladesh, Khatun *et al.* (2015), reported the rate 73.7% of *bla*$_{NDM}$-1$^{45}$, in Romania a study done by Dortet *et al.* (2015), revealed that 15 carbapenemase producing *Enterobacteriaceae*, were recovered from patients hospitalized between 2011 and 2013, 60% harbored a *bla*$_{NDM}$-1 gene$^{35}$, 100% in Russia$^{46}$, as well as Dort *et al.* (2013) reported that the percentage of *bla*$_{NDM}$-1 gene was 7.9% in France$^{24}$.

The global emergence of double- and multi-carbapenemase-producing hospital-acquired Gram-negative pathogens, are mostly *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*.$^{47}$

In Kuwait, Jamal *et al.* reported that two of the *bla*$_{NDM}$-1-producing isolates co-harbored *bla*$_{OXA}$-48 carbapenemase$^{48}$, 22.1% in Dubai$^{21}$, 4.5% in Turkey$^{22}$ and 92.1% in Tunis$^{44}$, while in India, kaziet *et al.* (2014) revealed that 3.6% of the isolates possessed dual carbapenemase *bla*$_{NDM}$ and *bla*$_{VIM}$ genes.$^{37}$

A *K. pneumoniae* co-producing NDM-1 and OXA-232 (an OXA-48 variant) was imported to the USA from India$^{49}$, and another was found also in a French hospital$^{50}$, where its cross-transmission was documented$^{47}$.

The ratio of carbapenemase producing isolates differs by geographic region, type of infection, specimen source, and selective pressure due to antibiotics. This difference also associated with variation among the different patients studied and the different rates of antibiotic used in different hospitals$^{51}$.

The diversity of carbapenemases depends on the country; may be affected by historical and cultural relationships.$^{17}$ In our country, the wars, medical tourism, and Cross border transfer of patients particularly incoming workers might play a significant role in emerging and dissemination of different variants of carbapenemase encoding genes. There is an urgent need to find guidelines and appropriate procedures of infection control in order to deny such infections among patients.

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**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

**AUTHORS’ CONTRIBUTION**

SH designed the experiments and analyzed the data. NH performed the experiments and wrote the manuscript. SH read and approved the manuscript.

**FUNDING**

None.

**DATA AVAILABILITY**

The datasets of 16S rDNA gene, *bla*$_{VIM}$-2 gene, *bla*$_{OXA}$-48 gene and *bla*$_{NDM}$ gene
sequences from this study were deposited in NCBI database under accession numbers MK182251 to MK182258, MK156197 to MK156202 and MK159338 to MK159352. All relevant data are available from the authors upon request.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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