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EMERGENCE OF NDM-1 AMONG CARBAPENEM-RESISTANT *KLEBSIELLA PNEUMONIAE* IN IRAQI HOSPITALS

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Carbapenems are the last drugs of choice apart from colistin against serious infections caused by Gram-negative bacteria. However, there are increasing number of reports indicating prevailing emergence of metallo-β-lactamase (MBL)-producing clinical isolates worldwide and among them New Delhi MBL (NDM) is the most prevalent one. This study reports NDM-1 for the first time among *Klebsiella pneumoniae* from hospitalized patients in Baghdad, Iraq. Fifty-five clinical isolates of *K. pneumoniae* resistant to carbapenem were investigated from burned wounds, sputum, and blood samples. The susceptibility to different antibiotics was tested by VITEK-2 system. All strains were multidrug-resistant and they showed nine different antimicrobial-resistant patterns (A-I) and the most effective antibiotic on these strains was levofloxacin (85.45%). The phenotypic detection of carbapenemases by MAST-DISCS D70C revealed 29 (52.73%) strains were MBL-producing, out of 55 were carbapenem-resistant *K. pneumoniae* strains. The $bla_{\text{NDM-1}}$ and other MBL genes were detected by conventional PCR and the result showed 37 (67.27%) strains positive for $bla_{\text{NDM-1}}$ gene and only 5 (9.1%) strains harbored bla_{IMP} gene, while all strains were negative for bla_{VIM}, bla_{SIM}, bla_{GIM}, and bla_{SPM} genes. Our results showed the coexistence of both $bla_{\text{NDM-1}}$ and bla_{IMP} genes in three strains of *K. pneumoniae*, while indicated widespread NDM-1 in Baghdad, Iraq. Hence, it is necessary to follow proper infection control practices and physicians should be aware of the patients with such risk factors.

Keywords: *K. pneumoniae*, carbapenemases, carbapenem-resistance, $bla_{\text{NDM-1}}$ gene, MBL genes

Introduction

Klebsiella pneumoniae is a member of the family *Enterobacteriaceae* that causes severe infections [1], particularly respiratory tract infections, blood stream

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infections, and urinary tract infections. During the past decades, it became an important cause of nosocomial infections [2]. *K. pneumoniae* is considered to be the second most common cause of nosocomial Gram-negative pathogen after *Escherichia coli* [3]. It has emerged as one of the most antibiotic-resistant pathogen responsible for outbreaks in the health-care systems [1]. The growing increase in the rates of antimicrobial resistance is a major cause for concern in *Enterobacteriaceae* family, particularly *E. coli* and *K. pneumoniae* [4]. Broad-spectrum carbapenems are often considered as last therapeutic choices for treatment of infections due to multidrug-resistant Gram-negative bacteria [5, 6]. The emergence of carbapenem-resistant *Enterobacteriaceae* is increasingly notified worldwide and is becoming an important topic in health-care systems [7]. In *K. pneumoniae*, resistance to carbapenems is mainly related to the production of carbapenem-hydrolyzing β-lactamase [8]. *Enterobacteriaceae*-producing New Delhi metallo-β-lactamase (NDM) presents a recognized threat to the health-care system. The NDM-1 gene can spread rapidly and has been found in various bacterial species in health-care systems and also in the environment [9]. The aim of this study was to determine the presence of $bla_{\text{NDM-1}}$ and other metallo-β-lactamase (MBL) genes including IMP, VIM, SIM, GIM, and SPM genes among carbapenem-resistant *K. pneumoniae* isolated from hospitalized patients in two hospitals in Baghdad, Iraq.

Methods

Bacterial strains and susceptibility testing

Fifty-five carbapenem-resistant *K. pneumoniae* clinical strains were isolated from burned wounds, sputum, and blood samples of hospitalized patients in hospitals in Baghdad Medical city (The Burn Specialist Hospital, The Martyr Ghazi Al-Hariri Hospital, and Baghdad Teaching Hospital). These strains were isolated through a period extended from March 2014 to November 2015. Identification of *K. pneumoniae* strains was performed by conventional and automated (VITEK-2 system, bioMérieux, France) methods using ID-GNB cards according to the manufacturer's instructions.

Antibiotic susceptibility testing was performed by VITEK-2 system (bioMérieux, France) for the following antibiotics: imipenem, ertapenem, nitrofurantoin, ampicillin, cefazolin, amoxicillin/clavulanic acid, ampicillin/sulbactam, ceftriaxone, ceftazidime, piperacillin/tazobactam, cefepime, ciprofloxacin, levofloxacin, gentamicin, tobramycin, and trimethoprim/sulfamethoxazole using AST cards according to the manufacturer's instructions.

Phenotypic detection of carbapenemases

Carbapenemases were phenotypically investigated by MASTDISCS D70C carbapenemase detection disc set (Mast Group Ltd., UK). Each bacterial suspension was adjusted to a turbidity equivalent to 0.5 McFarland standard and then used to inoculate Mueller–Hinton agar plates and the plates were incubated overnight.

In this method, the inhibition zone diameters of disc B (carbapenem + MBL inhibitor), disc C (carbapenem + KPC inhibitor), and disc D (carbapenem + AmpC inhibitor) are compared with inhibition zone diameter of disc A (carbapenem without inhibitor). The inhibition zone diameters around the discs were measured and the results were estimated according to manufacturer's instructions. *E. coli* ATCC 25922 was used as the carbapenem-susceptible strain and it was obtained from Teaching Laboratories/Medical city, Baghdad.

Molecular detection of carbapenemases by PCR assay

Genomic bacterial DNA was extracted from all 36 carbapenem-resistant *K. pneumoniae* strains using a commercial purification system Presto Mini gDNA Bacteria Kit (Geneaid, Thailand). Primers used in this study (Alpha DNA, Canada) were provided in lyophilized form then dissolved in sterile deionized distilled water (Table I).

A simplex PCR amplification was carried out for detection of $bla_{\text{NDM-1}}$ in all carbapenem-resistant *K. pneumoniae* strains on a thermal cycler instrument (Agilent Sure Cycler 8800, Santa Clara, CA, USA) using the primers NDM-1

Table I. The sequences of primers used in this study

Primers	Sequence (5′ → 3′)	Product size (bp)	Reference
NDM-1-F	GGTGCATGCCCGGTGAAATC	661	[10]
NDM-1-R	ATGCTGGCCTTGGGGAACG		
Pre-NDM-1-F	CACCTCATGTTTGAATTCGCC	983	[11]
Pre-NDM-1-R	CTCTGTCACATCGAAATCGC		
IMP-F	GGAATAGAGTGGCTTAAYTCTC	188	[12]
IMP-R	CCAAACYACTASGTTATCT		
VIM-F	GATGGTGTTTGGTCGCATA	390	
VIM-R	CGAATGCGCAGCACCAG		
SIM-F	TACAAGGGATTCGGCATCG	570	
SIM-R	TAATGGCCTGTTCCCATGTG		
GIM-F	TCGACACACCTTGGTCTGAA	477	
GIM-R	AACTTCCAACTTTGCCATGC		
SPM-F	AAAATCTGGGTACGCAAACG	271	
SPM-R	ACATTATCCGCTGGAACAGG		

(661 bp) for the amplification of internal gene and Pre-NDM-1 (983 bp) for the amplification of entire gene sequence. The following program was separately used for each primer (NDM-1 and Pre-NDM-1): initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 45 s, annealing at 52 °C for 45 s, and extension at 72 °C for 60 s, then final extension at 72 °C for 8 min [10]. The amplification reaction was separately prepared for each primer (NDM-1 and Pre-NDM-1) with a final volume 25 µl of 12.5 2× Master mix (Promega, USA), 1 µl of each primer (forward and reverse), 4 µl of template DNA, and 6.5 µl nuclease-free water. *E. coli* ATCC 25922 used as negative control.

Real-time PCR for detection of bla$_{NDM-1}$ *gene*

Real-time PCR confirmed the detection of *bla*$_{\text{NDM-1}}$ gene. Real-time PCR was carried out for carbapenem-resistant *K. pneumoniae* strains by using Bio-Rad, USA. The amplification reaction was prepared in this study with a final volume 20 µl of Go Taq qPCR Master mix (Promega, USA), 1 µl of each forward and reverse primer (Pre-NDM-1 primer), 4 µl of template DNA, and 4 µl nuclease-free water. The same program of conventional PCR for detection of *bla*$_{\text{NDM-1}}$ gene was used. The standard curve was generated by performing three serial dilutions for the *bla*$_{\text{NDM-1}}$ gene.

Multiplex PCR for other MBL genes

Multiplex PCR amplification was carried out for the detection of *bla*$_{\text{IMP}}$, *bla*$_{\text{VIM}}$, *bla*$_{\text{SIM}}$, *bla*$_{\text{GIM}}$, and *bla*$_{\text{SPM}}$ genes in all carbapenem-resistant *K. pneumoniae* strains on a thermal cycler instrument (Agilent Sure Cycler 8800) using the following cycling conditions: 94 °C for 5 min as an initial denaturation step, followed by 36 cycles of 94 °C for 30 s, 52 °C for 40 s, and 72 °C for 50 s, final elongation step at 72 °C for 5 min. The reaction of PCR consisted of 2× of 25 Master mix (Promega, USA), 1 µl of each forward and reverse primers (*bla*$_{\text{IMP}}$, *bla*$_{\text{VIM}}$, *bla*$_{\text{SIM}}$, *bla*$_{\text{GIM}}$, and *bla*$_{\text{SPM}}$), 5 µl of template DNA, and PCR grade water to a final volume 50 µl. *E. coli* ATCC 25922 was used as negative control [12]. The products of PCR were electrophoresed for 60 min and visualized with the aid of RedSafe staining (iNtRON, Korea) and UV transilluminator documentation system [13].

Sequencing of PCR products and phylogenetic analysis

Sequencing of *bla*$_{\text{NDM-1}}$ (983 bp) amplicons was carried out by Macrogen DNA Sequencing (Seoul, Korea), and the sequence of each amplicon was

compared with the sequences in the GenBank nucleotide database/BLAST. The phylogenetic data were obtained by alignment and phylogenetic analysis of the sequences. Phylogenetic relationships were analyzed by MEGA6 program.

Results

Bacterial strains

During the period of March 2014 to November 2015, altogether 55 carbapenem-resistant *K. pneumoniae* strains were isolated, among them 23 (41.82%) were isolated from blood specimens, 18 (32.73%) were isolated from sputum specimens, and 14 (25.45%) were isolated from burned wound specimens (Table II).

Antibiotic susceptibility testing

The antibiotic susceptibility test revealed that all 55 carbapenem-resistant *K. pneumoniae* clinical strains were multidrug-resistant and they were resistant to most antibiotics under test and it showed an elevated resistance to numerous classes of β-lactam and non-β-lactam antibiotics. On the other hand, these strains showed high sensitivity rate to levofloxacin 47 (85.45%), followed by 36 (65.45%) to trimethoprim/sulfamethoxazole and 33 (60%) to ciprofloxacin as shown in Table III.

Also, the result showed nine different antimicrobial-resistant patterns among the 55 carbapenem-resistant *K. pneumoniae* strains under the study numbered from A to I, as summarized in Table IV (the strains that showing intermediate levels of susceptibility were considered as resistant).

Phenotypic detection of carbapenemases

The phenotypic detection of carbapenemases by MASTDISCS D70C was performed according to manufacturer's instructions in which the diameters of inhibition zones around the discs were measured and the bacterial strain recorded as MBL producer if disc B only showed a zone difference ≥5 mm than disc A (the discs D–A and the discs C–A should be <4 mm). The results of this test revealed among the 55 carbapenem-resistant *K. pneumoniae* strains, 29 (52.73%) were identified as MBL-producing as shown in Table II and Figure 1.

Table II. Phenotypic and genotypic detection of metallo-β-lactamase production by MASTDISCS D70C and PCR assay

Strain no.	Source of strain	Phenotypic detection (MBL)	PCR assay					
			bla$_{NDM-1}$	*bla*$_{IMP}$	*bla*$_{VIM}$	*bla*$_{SIM}$	*bla*$_{GIM}$	*bla*$_{SPM}$
K1	Sputum	Positive	Positive	Negative	Negative	Negative	Negative	Negative
K2	Blood	Negative	Negative	Negative	Negative	Negative	Negative	Negative
K3	Burn	Negative	Negative	Negative	Negative	Negative	Negative	Negative
K4	Blood	Negative	Negative	Negative	Negative	Negative	Negative	Negative
K5	Sputum	Negative	Negative	Negative	Negative	Negative	Negative	Negative
K6	Burn	Positive	Positive	Negative	Negative	Negative	Negative	Negative
K7	Sputum	Positive	Positive	Negative	Negative	Negative	Negative	Negative
K8	Burn	Positive	Positive	Negative	Negative	Negative	Negative	Negative
K9	Burn	Negative	Positive	Negative	Negative	Negative	Negative	Negative
K10	Blood	Negative	Negative	Negative	Negative	Negative	Negative	Negative
K11	Sputum	Positive	Positive	Negative	Negative	Negative	Negative	Negative
K12	Burn	Positive	Negative	Positive	Negative	Negative	Negative	Negative
K13	Burn	Positive	Positive	Negative	Negative	Negative	Negative	Negative
K14	Blood	Negative	Positive	Negative	Negative	Negative	Negative	Negative
K15	Burn	Negative	Negative	Negative	Negative	Negative	Negative	Negative
K16	Sputum	Negative	Negative	Negative	Negative	Negative	Negative	Negative
K17	Sputum	Positive	Positive	Negative	Negative	Negative	Negative	Negative
K18	Blood	Positive	Positive	Negative	Negative	Negative	Negative	Negative
K19	Blood	Negative	Positive	Negative	Negative	Negative	Negative	Negative
K20	Burn	Negative	Positive	Negative	Negative	Negative	Negative	Negative
K21	Sputum	Positive	Positive	Negative	Negative	Negative	Negative	Negative
K22	Blood	Negative	Positive	Negative	Negative	Negative	Negative	Negative
K23	Blood	Positive	Negative	Positive	Negative	Negative	Negative	Negative
K24	Sputum	Negative	Positive	Negative	Negative	Negative	Negative	Negative
K25	Blood	Negative	Positive	Negative	Negative	Negative	Negative	Negative
K26	Blood	Negative	Negative	Negative	Negative	Negative	Negative	Negative
K27	Blood	Positive	Positive	Negative	Negative	Negative	Negative	Negative
K28	Burn	Positive	Positive	Negative	Negative	Negative	Negative	Negative
K29	Sputum	Negative	Positive	Negative	Negative	Negative	Negative	Negative

K30	Sputum	Positive	Positive	Negative	Negative	Negative	Negative
K31	Burn	Positive	Positive	Negative	Negative	Negative	Negative
K32	Blood	Positive	Positive	Negative	Negative	Negative	Negative
K33	Sputum	Positive	Positive	Negative	Negative	Negative	Negative
K34	Burn	Positive	Positive	Negative	Negative	Negative	Negative
K35	Blood	Negative	Negative	Negative	Negative	Negative	Negative
K36	Blood	Positive	Positive	Positive	Negative	Negative	Negative
K37	Blood	Negative	Positive	Negative	Negative	Negative	Negative
K38	Burn	Positive	Positive	Positive	Negative	Negative	Negative
K39	Sputum	Negative	Positive	Negative	Negative	Negative	Negative
K40	Burn	Positive	Positive	Negative	Negative	Negative	Negative
K41	Sputum	Positive	Positive	Negative	Negative	Negative	Negative
K42	Sputum	Positive	Negative	Negative	Negative	Negative	Negative
K43	Blood	Negative	Negative	Negative	Negative	Negative	Negative
K44	Sputum	Negative	Negative	Negative	Negative	Negative	Negative
K45	Burn	Negative	Negative	Negative	Negative	Negative	Negative
K46	Blood	Negative	Negative	Negative	Negative	Negative	Negative
K47	Blood	Positive	Positive	Negative	Negative	Negative	Negative
K48	Blood	Negative	Negative	Negative	Negative	Negative	Negative
K49	Sputum	Negative	Positive	Negative	Negative	Negative	Negative
K50	Blood	Positive	Negative	Negative	Negative	Negative	Negative
K51	Blood	Positive	Positive	Positive	Negative	Negative	Negative
K52	Sputum	Positive	Positive	Negative	Negative	Negative	Negative
K53	Sputum	Negative	Positive	Negative	Negative	Negative	Negative
K54	Blood	Positive	Positive	Negative	Negative	Negative	Negative
K55	Blood	Positive	Positive	Negative	Negative	Negative	Negative

Note: PCR: polymerase chain reaction; MBL: metallo-β-lactamase.

Table III. The results of antibiotic susceptibility test of 55 *K. pneumoniae* clinical strains

Antibiotics	N (S%)	N (I%)	N (R%)
Ampicillin	0 (0)	0 (0)	55 (100)
Amoxicillin/clavulanic acid	0 (0)	0 (0)	55 (100)
Ampicillin/sulbactam	0 (0)	0 (0)	55 (100)
Piperacillin/tazobactam	0 (0)	0 (0)	55 (100)
Cefazolin	0 (0)	0 (0)	55 (100)
Ceftazidime	0 (0)	0 (0)	55 (100)
Ceftriaxone	0 (0)	0 (0)	55 (100)
Cefepime	0 (0)	0 (0)	55 (100)
Imipenem	0 (0)	0 (0)	55 (100)
Ertapenem	0 (0)	0 (0)	55 (100)
Gentamicin	0 (0)	0 (0)	55 (100)
Tobramycin	1 (1.82)	0 (0)	54 (98.18)
Ciprofloxacin	33 (60)	16 (29.1)	6 (10.9)
Levofloxacin	47 (85.45)	1 (1.82)	7 (12.73)
Nitrofurantoin	8 (14.55)	17 (30.91)	30 (54.54)
Trimethoprim/sulfamethoxazole	36 (65.45)	0 (0)	19 (34.55)

Note: N: number of strains; R: resistant; I: intermediate; S: sensitive.

Table IV. Antimicrobial-resistant patterns of 55 *K. pneumoniae* clinical strains

Pattern	Description	Number of strains	Percentage
A	Resistant to all tested antibiotics	4	7.27
B	Resistant to all tested antibiotics except levofloxacin	10	18.18
C	Resistant to all tested antibiotics except nitrofurantoin and trimethoprim/sulfamethoxazole	2	3.64
D	Resistant to all tested antibiotics except trimethoprim/ sulfamethoxazole	6	10.91
E	Resistant to all tested antibiotics except levofloxacin, ciprofloxacin, nitrofurantoin, and trimethoprim/sulfamethoxazole	2	3.64
F	Resistant to all tested antibiotics except tobramycin	1	1.82
G	Resistant to all tested antibiotics except levofloxacin and nitrofurantoin	4	7.27
H	Resistant to all tested antibiotics except levofloxacin and ciprofloxacin	5	9.1
I	Resistant to all tested antibiotics except levofloxacin, ciprofloxacin, and trimethoprim/sulfamethoxazole	26	47.27

Molecular detection of bla_{NDM-1}

The sequencing of $bla_{\text{NDM-1}}$ amplicons (983 bp) was carried out and aligning of the $bla_{\text{NDM-1}}$ amplicon sequences with the reference strains in GenBank confirmed the correct identification of $bla_{\text{NDM-1}}$ gene among carbapenem-resistant

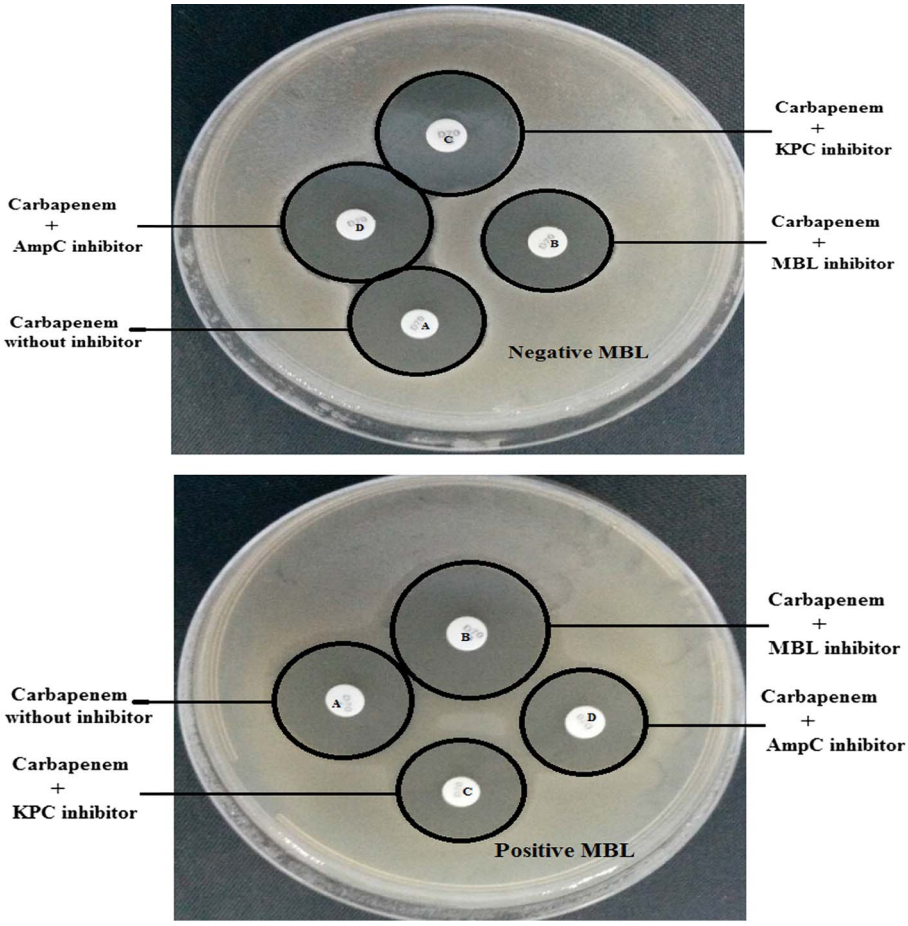

Figure 1. Phenotypic detection of metallo-β-lactamase production by MASTDISCS D70C (positive and negative MBL)

K. pneumoniae. The results showed the presence of a $bla_{\text{NDM-1}}$ gene (661 and 983 bp) in 37 (67.27%) carbapenem-resistant *K. pneumoniae* strains. On the other hand, 18 (32.73%) strains of carbapenem-resistant *K. pneumoniae* did not harbor $bla_{\text{NDM-1}}$ gene as shown in Table II and Figures 2 and 3. The result showed the highest percentage of strains harboring $bla_{\text{NDM-1}}$ gene isolated from sputum specimens followed by strains isolated from burned wounds and the lowest percentage was from strains isolated from blood as shown in Table II. Out of 18 carbapenem-resistant *K. pneumoniae* strains isolated from sputum, 14 (77.77%) were harboring $bla_{\text{NDM-1}}$ gene and out of 14 strains isolated from burned wounds 10 (71.43%) were

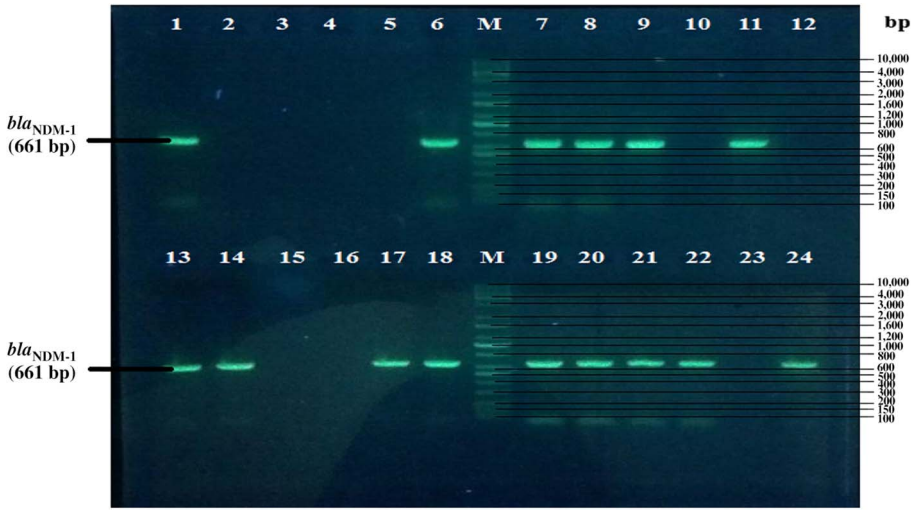

Figure 2. PCR amplification fragments for the detection of *bla*$_{\text{NDM-1}}$ gene (661 bp) among carbapenem-resistant *Klebsiella pneumoniae* ssp. pneumoniae strains. Lanes 1–24: *Klebsiella pneumoniae* ssp. *pneumoniae* strains; Lane M: 100-bp DNA ladder; Lane C: negative control. Amplicons were electrophoresed on agarose gel (1%) at 5 V/cm for 1 h, stained with RedSafe (iNtRON, Korea), and visualized using an UV transilluminator documentation system

harboring *bla*$_{\text{NDM-1}}$ gene, whereas out of 23 strains isolated from blood 13 (56.52%) were harboring *bla*$_{\text{NDM-1}}$ gene (Table II).

Detection of bla$_{IMP}$, bla$_{VIM}$, bla$_{SIM}$, bla$_{GIM}$, *and* bla$_{SPM}$ *genes*

The results of *bla*$_{\text{IMP}}$, *bla*$_{\text{VIM}}$, *bla*$_{\text{SIM}}$, *bla*$_{\text{GIM}}$, and *bla*$_{\text{SPM}}$ genes distribution of among carbapenem-resistant *K. pneumoniae* strains showed that only five strains were positive for *bla*$_{\text{IMP}}$ gene 5 (9.1%), and none of the strains harbored *bla*$_{\text{VIM}}$, *bla*$_{\text{SIM}}$, *bla*$_{\text{GIM}}$, and *bla*$_{\text{SPM}}$ genes (Figures 3 and 4, Table II).

Real-time PCR for expression and detection of bla$_{NDM-1}$ *gene*

Real-time PCR experiments for the *bla*$_{\text{NDM-1}}$ gene were performed among 55 carbapenem-resistant *K. pneumoniae* showed different expressions in different strains (Figure 5).

Phylogenetic tree

Phylogenetic tree based on the nucleotide sequences of the *bla*$_{\text{NDM-1}}$ gene was shown in Figure 6. The data for the phylogenetic analysis were obtained from

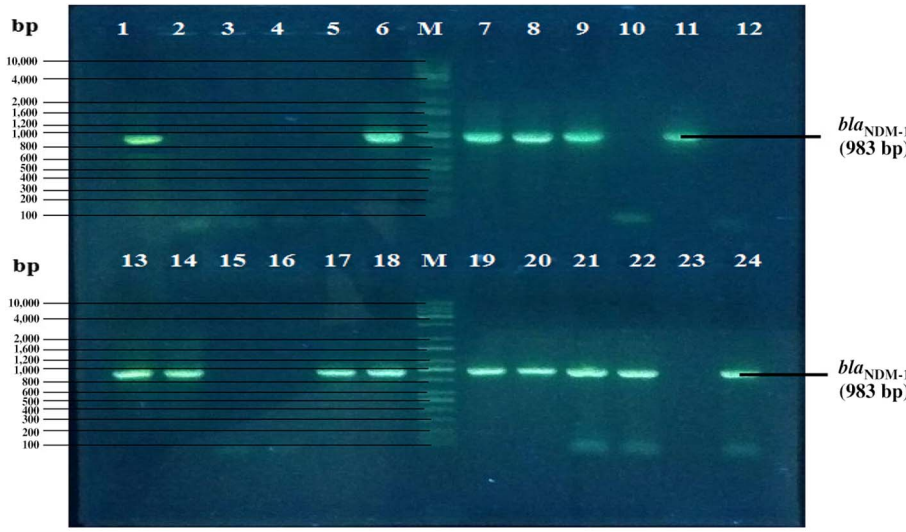

Figure 3. PCR amplification of the *bla*$_{\text{NDM-1}}$ gene (983 bp) in carbapenem-resistant *Klebsiella pneumoniae* ssp. *pneumoniae* strains. Lane M: 100-bp DNA ladder; Lanes 1–24: *Klebsiella pneumoniae* ssp. *pneumoniae* strains; Lane C: negative control. Amplicons were electrophoresed on agarose gel (1%) at 5 V/cm for 1 h, stained with RedSafe (iNtRON, Korea), and visualized using an UV transilluminator documentation system

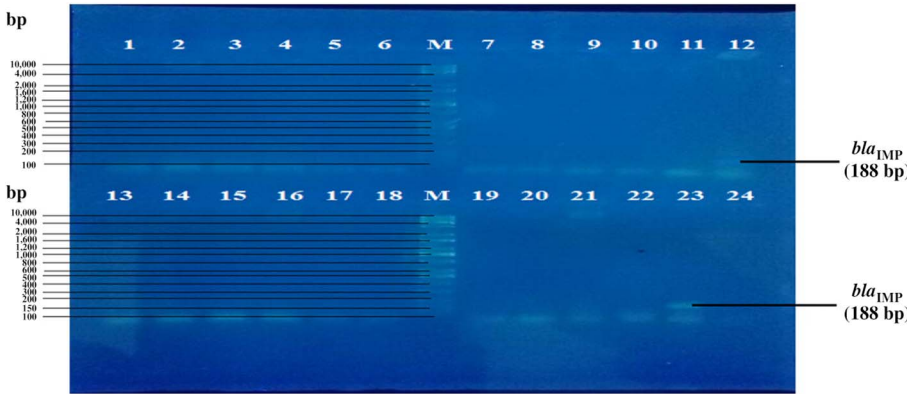

Figure 4. Multiplex PCR amplification of other MBL genes in carbapenem-resistant *Klebsiella pneumoniae* ssp. *pneumoniae* strains. Lane M: 100-bp DNA ladder; Lanes 1–55: *Klebsiella pneumoniae* ssp. *pneumoniae* strains; Lane C: negative control. Amplicons were electrophoresed on agarose gel (1%) at 5 V/cm for 1 h, stained with RedSafe (iNtRON, Korea), and visualized using an UV transilluminator documentation system

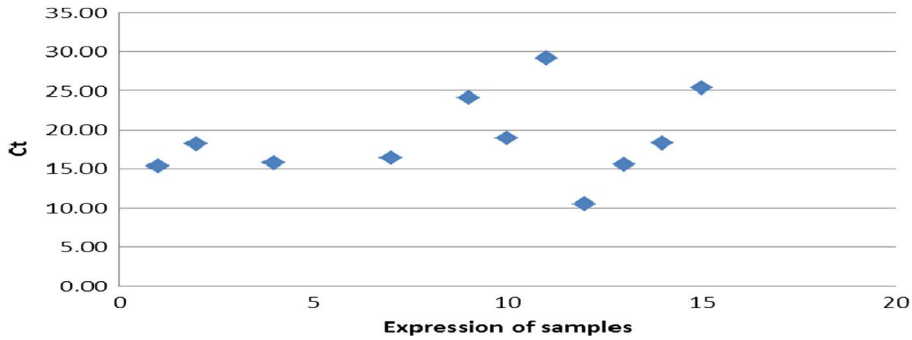

Figure 5. The expression of samples ($bla_{\text{NDM-1}}$ gene)

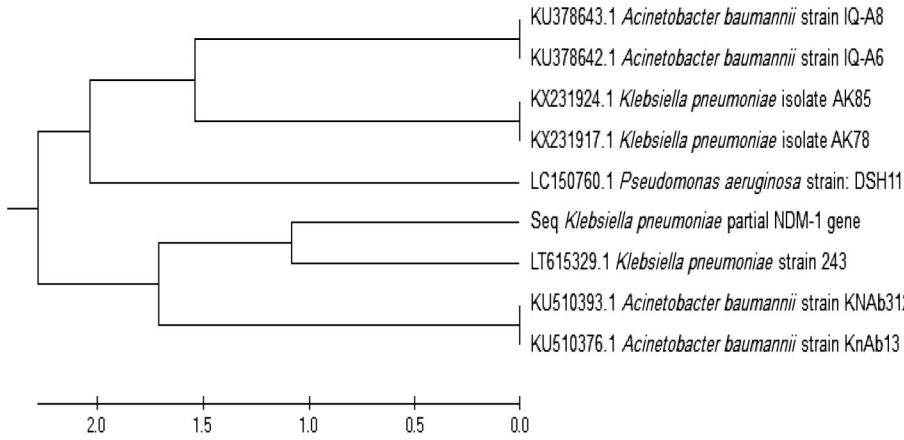

Figure 6. Phylogenetic tree based on the nucleotide sequences of the $bla_{\text{NDM-1}}$ gene

sequences in the GenBank nucleotide sequence database. Table II shows the accession numbers and the percentage of nucleotide identity and similarity of the $bla_{\text{NDM-1}}$ gene for *K. pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* sequences in the GenBank.

Discussion

Carbapenems are the drugs of choice against serious infections caused by Gram-negative bacteria, but several studies have reported the prevalence of MBL-producing clinical strains worldwide. Among MBL genes, NDM-1 has

emerged and it confers resistance to all β-lactam antibiotics and being reported in *K. pneumoniae*, *E. coli*, and *A. baumannii* as the main hosts. NDM-1 gene from India and Pakistan has been specified as reservoirs of NDM producers [14, 15]. In Iraq, hardly any information regarding the NDM-1-producing *K. pneumoniae* is available. This study reports for the first time the presence of NDM-1 among *K. pneumoniae* in Baghdad, Iraq. Antibiotic susceptibility test results showed higher resistant rates for most of the antibiotics except levofloxacin. All the strains were multidrug-resistant showing nine different antimicrobial-resistant patterns and sensitive to levofloxacin (85.45%).

The phenotypic detection of carbapenemases by MASTDISCS D70C revealed 29 (52.73%) strains were MBL-producing, out of 55 were carbapenem-resistant *K. pneumoniae* strains. This study evidenced the silent spread of NDM-1-producing (67.27%) *K. pneumoniae* strains from hospital settings in Baghdad, Iraq, which is significantly more than expected in any city of Iraq. Till date, more than six different $bla_{\text{NDM-1}}$ allotypes are known [16]. Since NDM-1 is carried on a plasmid or on chromosomes, the rapid emergence of $bla_{\text{NDM-1}}$ has been directly related to a transferable plasmid which has spread in many countries [16]. This is the first report on the prevalence of NDM-1 genes in Iraqi hospitals among *K. pneumoniae* isolates.

Several countries have reported the alarming spread of carbapenem-resistant *E. coli* and *K. pneumoniae*, but NDM-1 has been reported only from Oman, a neighboring country [17]. Initially, NDM-1 was reported in *K. pneumoniae* and *E. coli* recovered from a Swedish patient transported from India [18]. Since then it has been disseminated widely in over 40 countries [19]. All these reports from different countries have indicated a probable source of NDM-1 producers from the Indian subcontinent, with both hospital and community acquisition and has spread to Austria, Australia, Belgium, Canada, Denmark, France, Germany, Kenya, the Netherlands, Norway, the Sultanate of Oman, and the United States [17, 20–22]. Following the initial identification of the $bla_{\text{NDM-1}}$ gene in clinical isolates from Egypt due to unknown sources, it can be concluded that the NDM-producing strains have already emerged and spread in the Middle East as in Iraq and Oman. Recently, a case of NDM-producing *K. pneumoniae* has been described in France from an Iraqi patient [23]. A study performed by Pesesky et al. [24] indicated the rapid spread of carbapenem resistance between strains. This study also underlines the spread of the $bla_{\text{NDM-1}}$ gene worldwide, as exemplified by the report of NDM-1-producing *E. coli*, *Enterobacter cloacae*, and *K. pneumoniae* in the United States [25], NDM-1-producing *E. coli* from Australia [26], and the dissemination of NDM-1 gene among *K. pneumoniae*

isolates in Africa [27]. Bastian et al. [16] reported the first case of NDM-1-producing *K. pneumoniae* in Caribbean islands. Zheng et al. [28] reported plasmid encoding $bla_{\text{NDM-1}}$ from Enterobacteriaceae strains in several regions of China including Shanghai, Beijing, Shandong province, and Hong Kong.

A recent study from Turkey tested 77 isolates of *K. pneumoniae* and found that 74 isolates (89.16%) produced OXA-48 carbapenemase, whereas nine isolates (10.84%) produced both OXA-48 and NDM-1 by both phenotypic tests included CarbaNP test and CIM test [29]. Genotypic characterization of ESBL and carbapenemase genes by the Check-MDR CT102 was fully in agreement with phenotypic testing in the detection of 8 MBL in study by Somily et al. [30].

Different genes are involved in carbapenem resistance among *Enterobacteriaceae*, which may vary from country to country. In this study, $bla_{\text{NDM-1}}$ and bla_{IMP} genes were detected by conventional PCR and the result showed 37 (67.27%) strains harbored $bla_{\text{NDM-1}}$ gene, but only 5 (9.1%) strains harbored bla_{IMP} gene. Also, the results showed the coexistence of both $bla_{\text{NDM-1}}$ and bla_{IMP} genes in three strains of *K. pneumonia* under the study. Our report is in contrary with other reports from Turkey, Greece, Saudi Arabia, and Israel where bla_{VIM} genes were reported rather than bla_{IMP} genes [31–35]. On the other hand, no strain under the study carried bla_{VIM}, bla_{SIM}, bla_{GIM}, and bla_{SPM} genes and thus we may conclude that $bla_{\text{NDM-1}}$ gene was responsible for the spread of resistance to carbapenems in these strains.

This study revealed that most carbapenem-resistant *K. pneumoniae* strains carried the $bla_{\text{NDM-1}}$ gene 37 (67.27%) out of 55 strain and this is a high percentage compared with the countries of the world, which may attribute to the reason that many Iraqi patients transport for treatment in India and conduct various surgical procedures, thus leading to the gene transfer to Iraqi hospitals. Moreover, the treatment of patients infected with carbapenem-resistant *Enterobacteriaceae* is more challenging due to their high-level resistance as they very often carry on the same transposon the genes responsible for resistance to multiple antibiotics and also limited treatment options.

In conclusion, Iraq is also facing an alarming threat with the emergence of the imported NDM-1 gene in *Enterobacteriaceae*. Hence, it is necessary to follow proper infection control practices and physicians should be aware of the patients with such risk factors. A multidisciplinary approach to limit the spread of such organisms is essential followed by prevention, detection, proper antimicrobial stewardship, and adequate infection control measures should help in limiting the spread of these organisms.

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Conflict of Interest

The author has nothing to disclose.

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