



## Emergence of NDM-1-Producing *Escherichia coli* in Iran

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### Abstract

**Introduction:** Carbapenems are a broad-spectrum class of beta-lactam antibiotics, which are used in treatment of multi-drug resistant infections. Unfortunately, global emerging and spreading of carbapenemase, especially New Delhi metallo  $\beta$  lactamase 1 ( $bla_{NDM-1}$ ), is a concern in the treatment of multi drug-resistant agents. Here, we report the appearance of  $bla_{NDM-1}$ -producing *Escherichia coli* (*E. coli*) in Iran for the first time.

**Case Presentation:** In this study, 2  $bla_{NDM-1}$ -producing *E. coli* strains were isolated from 2 burn wounds of patients in the Motahari hospital, Tehran. The isolates were resistant to carbapenems (imipenem, meropenem and ertapenem) and other common antibiotics except nitrofurantoin. Combined Disk Test showed that the isolates could not produce  $bla_{AmpC}$  and  $bla_{KPC}$  carbapenemase, whereas they can produce metallo- $\beta$ -lactamases (MBL). However, genetic detection using Polymerase Chain Reaction amplification with specific primers for  $bla_{KPC}$ ,  $bla_{IMP}$ ,  $bla_{VIM-1}$ ,  $bla_{VIM-1} - bla_{VIM-37}$ , and  $bla_{NDM-1}$  genes showed that only the  $bla_{NDM-1}$  gene is amplified from the resistant isolates. Further sequencing of PCR products confirmed the presence of the  $bla_{NDM-1}$  gene in these isolates.

**Conclusions:** The emerging of  $bla_{NDM-1}$ -producing *E. coli* is a new threat for to the health system in Iran, due to the spreading of the  $bla_{NDM-1}$  gene among pathogenic bacteria, which resulted in the emergence of multi drug resistant photogenes. Therefore, early identification of these isolates is mandatory.

**Keywords:** Beta-Lactamase NDM-1, Iran, *Escherichia coli*

### 1. Introduction

Carbapenems, including imipenem (IMP), meropenem (MEM), and ertapenem (ERP) are the last resort for extended-spectrum  $\beta$ -lactamases (ESBLs) producing bacteria (1). Unfortunately, today, the emerging of Carbapenemase-Producing Enterobacteriaceae (CPE) or Carbapenem-Resistant Enterobacteriaceae (CRE) is a concern in the treatment of multidrug-resistant Gram-negative infections (2). CRE infections are associated with a high mortality rate (3).

One of the most important carbapenemases is recently known as New Delhi metallo- $\beta$ -lactamase-1 ( $bla_{NDM-1}$ ), which belongs to class B  $\beta$ -lactamases or metallo- $\beta$ -lactamases (MBL) (4). For the first time,  $bla_{NDM-1}$  was detected from *Klebsiella pneumoniae* isolated from a urinary tract sample from a Swedish patient who had been hospitalized in India (5). Today,  $bla_{NDM-1}$  was detected in other bacterial species including *Escherichia coli* (*E. coli*) (6). The prevalence of carbapenem-resistant *E. coli* strains isolated from Iran is very low. In 2 recent studies, the susceptibility of *E. coli* isolates to imipenem was reported as 99.3% (7) and 100% (8). However, the appearance of carbapenemases

gene, especially  $bla_{NDM-1}$ , can be a serious risk factor in increasing carbapenem resistance rates among the *E. coli* strains or other bacteria.

Here, we report emergence of  $bla_{NDM-1}$ -producing *E. coli* strains in Iran for the first time.

### 2. Case Presentation

In September 2015 and June 2016, 2 swab samples from burn wound infections of a 64 year old woman as well as a 54-yr-old woman, who had been hospitalized in Motahari hospital (Iran University of medical sciences, Tehran, Iran) for a third-degree burning, were taken. The 2 patients used silver sulfadiazine, trimethoprim sulfamethoxazole, TMP/SMX, and ceftazidime as medication. The swabs were transferred to transport medium and sent to the microbiology lab of Shahid Beheshti University of Medical Science for isolation of bacterial strains. Standard identification methods including phenotypic survey on MacConkey Agar (Merck, Germany), TSI (Merck, Germany), IMViC, motility, urea (Merck, Germany) mediums, and also oxidase test, confirmed the identity of isolates as *E. coli* strains.

Antibiotic susceptibility test of the isolates by disk diffusion method for carbapenems including imipenem, IMP (10 µg), meropenem, MEM (10 µg), and ertapenem, ERP (10 µg) showed that the isolates are resistant to the antibiotics. The minimum inhibitory concentrations (MICs) of imipenem and meropenem (Jaber Ebne Hayyan Pharmaceutical Company, Iran) were determined as 8 and 64 µg/mL, respectively, by the microdilution method. Furthermore, the isolates were resistant to several common antibiotics including, piperacillin, PIP (100 µg), amoxicillin clavulanate, AMC (20/10-µg), gentamicin, GEN (10 µg), amikacin, AMN (30 µg), cefepime, CEFp (30 µg), ciprofloxacin, CIP (5 µg), cefazolin, CEFz (30 µg), ceftriaxone, CEFx (30 µg), ceftazidime, CEFt (30 µg), TMP/SMX (25 µg), and aztreonam, AZT (30 µg). All antibiotic disks were purchased from Rosco Diagnostica Taastrup, Denmark. However, nitrofurantoin, NIT (300µg), is the only antibiotic, which the carbapenem resistant isolates are sensitive to it.

Phenotypic detection of β-lactamases was done by Combined Disk Test (CDT) using ceftazidime (CAZ; 30 µg), ceftazidime/clavulanic acid (CAZ30/CLAV10), and boronic acid (BA; 250 µg) disks for *bla<sub>AmpC</sub>* detection. Additionally, CDT test was used for *bla<sub>MBL</sub>* detection using 2 IPM (10 µg) disks and EDTA 0.5 M solution. Results showed that the strains do not produce *bla<sub>AmpC</sub>*, whereas they can produce *bla<sub>MBL</sub>* enzyme.

Genotypic detection of *bla<sub>KPC</sub>*, *bla<sub>IMP</sub>*, *bla<sub>VIM-1</sub>* (Verona integron-encoded metallo-β-lactamase), *bla<sub>VIM-1</sub>-bla<sub>VIM-37</sub>*, *bla<sub>DIM-1</sub>* (Dutch imipenemase), and *bla<sub>NDM-1</sub>* genes was done by polymerase chain reaction (PCR) amplification method and further sequencing of PCR products. All primer sequences are shown in Table 1. The presence of the mentioned genes in carbapenem-resistant isolates were not confirmed by PCR. However, a 621-bp band was amplified by *bla<sub>NDM-1</sub>* primers and further DNA sequencing confirmed the presence of *bla<sub>NDM-1</sub>* gene in these 2 *E. coli* isolates (Figure 1). *E. coli* ATCC 25922 was considered as a control in all tests.

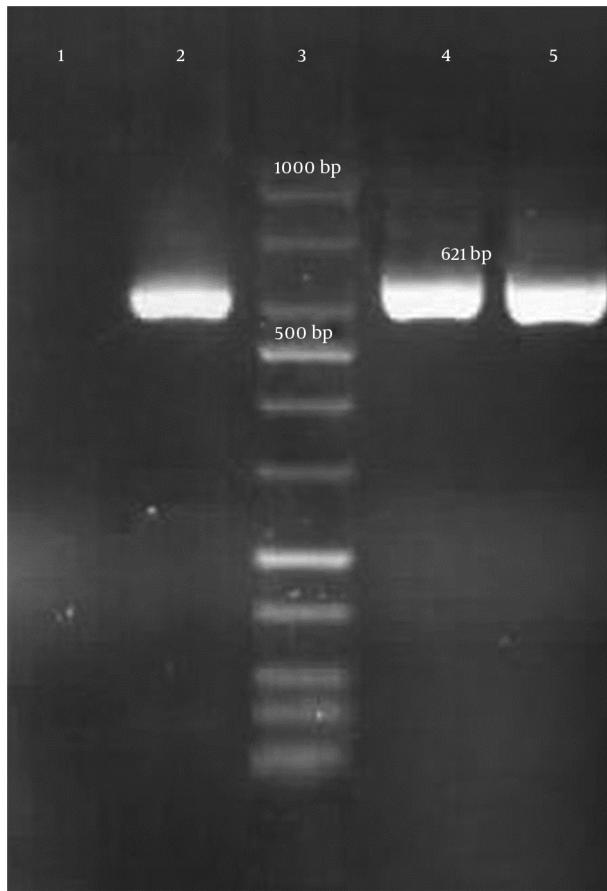
### 3. Discussion

This is a first report on emerging of *bla<sub>NDM-1</sub>* producing *E. coli* in Iran. Earlier, Shahcheraghi et.al reported the first *bla<sub>NDM-1</sub>* producing *K. pneumonia* in Iran (15). Furthermore, recently, Fazeli et.al presented the 2nd report on the detection of *bla<sub>NDM-1</sub>* gene among *K. pneumonia* isolates from Iran (16). Approximately, the emergence of *bla<sub>NDM-1</sub>* producing Enterobacteriaceae is reported in all neighboring countries including; Turkey (17), Iraq (18), Pakistan (19), and Afghanistan (20). The prevalence of *bla<sub>NDM-1</sub>* producing Enterobacteriaceae was reported from different countries as follows: 2.7 % in Kuwait (6), 1.2 % in India, Pakistan, and the United Kingdom (19), as well as 1.1 % in Vietnam (21).

Table 1. Primer Sequences Used in This Study

Gene Name	Primer Sequence	Product Size	Reference
<i>bla<sub>KPC</sub></i>	Forward: 5'-ATG TCT GTA TCG CCG TCT -3' Reverse: 5'-TTTTCAGAGCCTTACTGCC-3'	989-bp	(9)
<i>bla<sub>IMP-1</sub></i>	Forward: 5'-GAAGGCGTTTATGTCATAC-3' Reverse: 5'-GTACGTTTCAA-GAGTIGATGC-3'	587-bp	(10)
<i>bla<sub>VIM-1</sub></i>	Forward: 5'-AGTGGTGAGTATCCGACAG-3' Reverse: 5'-ATGAAAGTCCGTTGAGAC-3'	836-bp	(11)
<i>bla<sub>VIM-1-VIM-37</sub></i>	Forward: 5'-GATGGTGTGTCGCATA-3' Reverse: 5'-CGAATGCCAGCACCAG-3'	390-bp	(12)
<i>bla<sub>DIM</sub></i>	Forward: 5'-GCTTGCTTCGCTTGC-TAACC-3' Reverse: 5'-CGTTCGGCTG-GAATTGATTG-3'	699-bp	(13)
<i>bla<sub>NDM-1</sub></i>	Forward: 5'-GGTTTGGC-GATCTGTTTTC-3' Reverse: 5'-CGGAATGGCT-CATCAGGATC-3'	621-bp	(14)

Among these *bla<sub>NDM-1</sub>* producing Enterobacteriaceae, the prevalence of *E. coli* was very significant: 19 % in Kuwait (6), 20 % in India, Pakistan, and the United Kingdom (19), as well as 12.26 % in Vietnam (21). The *bla<sub>NDM-1</sub>* gene is placed near various insertion elements on transferrable plasmids (22). Medical tourism has a considerable role in wide spreading of resistant genes e.g. *bla<sub>NDM-1</sub>* gene (19). In addition, the presence of *bla<sub>NDM-1</sub>* producing bacteria in environmental samples such as drinking-water samples could be involved in spreading of the resistant gene (23). Spreading of *bla<sub>NDM-1</sub>* gene is a global concern. Isolates harboring *bla<sub>NDM-1</sub>* gene are resistant to almost all β-lactam antibiotics, fluoroquinolones, and aminoglycosides (19). These isolates can be eradicated by aztreonam, colistin, and tige-

**Figure 1.** PCR Result of *bla<sub>NDM-1</sub>* Gene

1: negative control, 2: positive control, 3: marker 50 bp 4: strain 1 and 5: strain 2.

cycline (24). However, resistance to these antibiotics may be developed as the *bla<sub>NDM-1</sub>* harboring *E. coli* strains in our study were also resistant to aztreonam.

In conclusion, our results show the emerging of carbapenem-resistant genes, especially *bla<sub>NDM-1</sub>*, is an alarm to our health system, due to the spreading of transferable *bla<sub>NDM-1</sub>* gene among pathogenic bacteria, which resulted in the emergence of multi drug resistant photogenes, which are resistant to all available antibiotics. Therefore, early identification of *bla<sub>NDM-1</sub>* harboring bacteria and prevention of their spreading must be performed in any region.

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### Footnote

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