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 β-lactamase 1 (*blaNDM-1*), is a concern in the treatment of multi drug-resistant agents. Here, we report the appearance of *blaNDM-1* producing *Escherichia coli* (*E. coli*) in Iran for the first time.

Case Presentation: In this study, 2 *blaNDM-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 *blaIMP* and *blaVIM* carbapenemase, whereas they can produce metallo-β-lactamases (MBL). However, genetic detection using Polymerase Chain Reaction amplification with specific primers for *blaIMP*, *blaVIM*, *blaOXA*, *blaNDM-1*, *blaOXA-10*, and *blaVIM*, genes showed that only the *blaNDM-1*-gene is amplified from the resistant isolates. Further sequencing of PCR products confirmed the presence of the *blaNDM-1*-gene in these isolates.

Conclusions: The emerging of *blaNDM-1*-producing *E. coli* is a new threat for to the health system in Iran, due to the spreading of the *blaNDM-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 β-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 carbapenemases is recently known as New Delhi metallo-β-lactamase-1 (*blaNDM-1*), which belongs to class B β-lactamases or metallo-β-lactamases (MBL) (4). For the first time, *blaNDM-1* was detected from *Klebsiella pneumonia* isolated from a urinary tract sample from a Swedish patient who had been hospitalized in India (5). Today, *blaNDM-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 *blaNDM-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 *blaNDM-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 carbapenem 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, CEP (30 μg), ciprofloxacin, CLP (5 μg), cefazolin, CEFz (30 μg), ceftriaxone, CEFx (30 μg), cefoxitin, CEFo (30 μg), TMP/SMX (25 μg), and aztreonam, AZT (30 μg). All antibiotic disks were purchased from Rosco Diagnostica Taastrup, Denmark. However, nitrofurantoin, NIT (300 μg), 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 blaTEM detection. Additionally, CDT test was used for blaSHV detection using 2 IPM (10 μg) disks and EDTA 0.5 M solution. Results showed that the strains do not produce blaTEM, whereas they can produce blaSHV enzyme.

Genotypic detection of blaKPC, blaIMP, blaVIM-4 (Verona integron-encoded metallo-β-lactamase), blaVIM-1, blaVIM-17, blaIPM-1 (Dutch imipenemase), and blaNDM-1 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 blaNDM primers and further DNA sequencing confirmed the presence of blaNDM 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 blaNDM-producing E. coli in Iran. Earlier, Shahcheraghi et al reported the first blaNDM producing K. pneumonia in Iran (15). Furthermore, recently, Fazeli et al presented the 2nd report on the detection of blaNDM gene among K. pneumonia isolates from Iran (16). Approximately, the emergence of blaNDM-producing Enterobacteriaceae is reported in all neighboring countries including: Turkey (17), Iraq (18), Pakistan (19), and Afghanistan (20). The prevalence of blaNDM-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 11.1 % in Vietnam (21). Among these blaNDM-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 blaNDM 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. blaNDM gene (19). In addition, the presence of blaNDM producing bacteria in environmental samples such as drinking-water samples could be involved in spreading of the resistant gene (23). Spreading of blaNDM gene is a global concern. Isolates harboring blaNDM gene are resistant to almost all β-lactam antibiotics, fluoroquinolones, and aminoglycosides (19). These isolates can be eradicated by aztreonam, colistin, and tige-

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**Table 1. Primer Sequences Used in This Study**

| Gene Name | Primer Sequence | Product Size | Reference |
|-----------|-----------------|--------------|-----------|
| **blaKPC** | Forward: 5′- ATG TCT GTA TGG CCG TCT-3′<br>Reverse: 5′- TTT TCAGGACCTATGCCC-3′ | 989 bp | (9) |
| **blaTEM-1** | Forward: 5′- GAAGC GTT AT GGCTATAC-3′ | 587 bp | (10) |
| **blaVIM-1** | Forward: 5′- GTG GTA GAT GC -3′<br>Reverse: 5′- ATGGTA AT CGC-3′ | 836 bp | (11) |
| **blaVIM-37** | Forward: 5′- CAA ATG GCC AGC-3′<br>Reverse: 5′- CCG TCT -3 | 390 bp | (12) |
| **blaIPM-1** | Forward: 5′- GCT GT CTC GTG GCG-3′<br>Reverse: 5′- TAC G-3 | 659 bp | (13) |
| **blaNDM** | Forward: 5′- GGG ATTTG CTA TAC G-3′<br>Reverse: 5′- GAA ATG GGC-3′ | 621 bp | (14) |
However, resistance to these antibiotics may be developed as the blaNDM-1 harboring E. coli strains in our study were also resistant to aztreonam.

In conclusion, our results show the emerging of carbapenem-resistant genes, especially blaNDM-1, is an alarm to our health system, due to the spreading of transferable blaNDM-1 gene among pathogenic bacteria, which are resistant to all available antibiotics. Therefore, early identification of blaNDM-1 harboring bacteria and prevention of their spreading must be performed in any region.

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Footnote

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References

1. Hawkey PM, Livermore DM. Carbapenem antibiotics for serious infections. BMJ. 2012;344:e3236. doi: 10.1136/bmj.e3236. [PubMed: 22654063].

2. Nordmann P, Cuzon G, Naas T. The real threat of Klebsiella pneumoniae carbapenemase-producing bacteria. Lancet Infect Dis. 2009;9(4):225–36. doi: 10.1016/S1473-3099(09)70054-4. [PubMed: 19324295].

3. Gupta N, Limbago BM, Patel JR, Kallen AJ. Carbapenem-resistant Enterobacteriaceae: epidemiology and prevention. Clin Infect Dis. 2011;53(1):60–7. doi: 10.1093/cid/cir202. [PubMed: 21653305].

4. Khan AU, Maryam L, Zarrilli R. Structure, Genetics and Worldwide Spread of New Delhi Metallo-beta-lactamase (NDM): a threat to public health. BMC Microbiol. 2017;17(1):101. doi: 10.1186/s12866-017-1012-8. [PubMed: 28449550].

5. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, et al. Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. Antimicrob Agents Chemother. 2009;53(12):5046–54. doi: 10.1128/AAC.00774-09. [PubMed: 19770275].

6. Jamal WY, Albert MJ, Rotimi VO. High Prevalence of New Delhi Metallo-beta-lactamase (NDM) Producers among Carbapenem-Resistant Enterobacteriaceae in Kuwait. PLOs One. 2016;11(3):152638. doi: 10.12998/wjcc.v4.i3.63. [PubMed: 26989670].

7. Poorabbas B, Mardaneh J, Rezaei Z, Kalani M, Pouladfar G, Alami MH, et al. Nosocomial Infections: Multicenter surveillance of antimicrobial resistance profile of Staphylococcus aureus and Gram negative rods isolated from blood and other sterile body fluids in Iran. Ir J Microbiol. 2015;7(3):127–35. [PubMed: 26668699].

8. Soltani J, Poorabbas B, Mardanesh J, Health care associated infections, antibiotic resistance and clinical outcome: A surveillance study from Sanandaj, Iran. World J Clin Cases. 2016;4(3):63–70. doi: 10.12998/wjcc.v4.i3.63. [PubMed: 28989670].

9. Marchaim D, Navon-Venezia S, Schwaber MJ, Carmeli Y. Isolation of imipenem-resistant Enterobacter species: emergence of KPC-2 carbapenemase, molecular characterization, epidemiology, and outcomes. Antimicrob Agents Chemother. 2008;52(4):1413–8. doi: 10.1128/AAC.01033-07. [PubMed: 18227979].

10. Pitout JD, Gregson DB, Poirel L, McCleare J, Le P, Church DL. Detection of Pseudomonas aeruginosa producing metallo-beta-lactamases in a large centralized laboratory. J Clin Microbiol. 2005;43(7):3129–35. doi: 10.1128/JCM.43.7.3129-3135.2005. [PubMed: 16000424].

11. Juan C, Beciro G, Gutierrez O, Alberti S, Garau M, Perez JL, et al. Characterization of the new metallo-beta-lactamase VIM-13 and its integron-borne gene from a Pseudomonas aeruginosa clinical isolate in Spain. Antimicrob Agents Chemother. 2008;52(10):3589–96. doi: 10.1128/AAC.00465-08. [PubMed: 18644957].

12. Ellington MJ, Kister J, Livermore DM, Woodford N. Multiplex PCR for rapid detection of genes encoding acquired metallo-beta-lactamas. J Antimicrob Chemother. 2007;59(2):321–2. doi: 10.1093/jac/ddl148. [PubMed: 17153500].

13. Poirel L, Walsh TR, Cuvelier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis. 2011;70(1):119–23. doi: 10.1016/j.diagmicrobio.2010.12.002. [PubMed: 21980774].

14. Nordmann P, Poirel L, Carrer A, Toleman MA, Walsh TR. How to detect NDM-1 producers. J Clin Microbiol. 2011;49(2):718–21. doi: 10.1128/JCM.01773-10. [PubMed: 21123531].
15. Shahcheraghi F, Nobari S, Rahmati Ghezelgeh F, Nasiri S, Owlia P, Nikbin VS, et al. First report of New Delhi metallo-beta-lactamase-1-producing Klebsiella pneumoniae in Iran. Microb Drug Resist. 2013;19(1):30–6. doi: 10.1089/mdr.2012.0078. [PubMed: 22984942].

16. Fazeli H, Norouzi-Barough M, Ahadi AM, Shokri D, Solgi H. Detection of New Delhi Metallo-Beta-Lactamase-1 (NDM-1) in carbapenem-resistant Klebsiella pneumoniae isolated from a university hospital in Iran. Hippokratia. 2015;19(3):205–9. [PubMed: 27418777].

17. Poirel L, Ozdamar M, Ocampo-Sosa AA, Turkoglu S, Ozer UG, Nordmann P. NDM-1-producing Klebsiella pneumoniae now in Turkey. Antimicrob Agents Chemother. 2012;56(5):2784–5. doi: 10.1128/AAC.00150-12. [PubMed: 22391536].

18. Poirel L, Fortineau N, Nordmann P. International transfer of NDM-1-producing Klebsiella pneumoniae from Iraq to France. Antimicrob Agents Chemother. 2011;55(4):1821–2. doi: 10.1128/AAC.01761-10. [PubMed: 21245442].

19. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK, a molecular, biological, and epidemiological study. Lancet Infect Dis. 2010;10(9):597–602. doi: 10.1016/S1473-3099(10)70043-2.

20. McGann P, Hang J, Clifford RJ, Yang Y, Kwak YI, Kuchner RA, et al. Complete sequence of a novel 178-kilobase plasmid carrying bla(NDM-1) in a Providencia stuartii strain isolated in Afghanistan. Antimicrob Agents Chemother. 2012;56(4):1673–9. doi: 10.1128/AAC.05604-11. [PubMed: 22290972].

21. Tran HH, Ehsani S, Shibayama K, Matsui M, Suzuki S, Nguyen MB, et al. Common isolation of New Delhi metallo-beta-lactamase-1-producing Enterobacteriaceae in a large surgical hospital in Vietnam. Eur J Clin Microbiol Infect Dis. 2015;34(5):2447–54. doi: 10.1007/s10096-015-2345-6. [PubMed: 25712442].

22. Hu H, Hu Y, Pan Y, Liang H, Wang H, Wang X. Novel plasmid and its variant harboring both a blaNDM-1 gene and type IV secretion system in clinical isolates of Acinetobacter Iwoffii. Antimicrob Agents Chemother. 2012;56(4):3698–702. doi: 10.1128/AAC.00999-11. [PubMed: 22290961].

23. Walsh TR, Weeks J, Livermore DM, Toleman MA. Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. Lancet Infect Dis. 2011;11(5):355–62. doi: 10.1016/S1473-3099(11)70059-7. [PubMed: 21478057].

24. Barantsevich EP, Churkina IV, Barantsevich NE, Pelkonen J, Schlyakhto EV, Woodford N. Emergence of Klebsiella pneumoniae producing NDM-1 carbapenemase in Saint Petersburg, Russia. Antimicrob Chemother. 2013;58(5):1204–6. doi: 10.1093/jac/dks503. [PubMed: 23315490].

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