Arch Clin Infect Dis. 2018 August; 13(4):e62029. doi: 10.5812/archcid.62029.

Published online 2017 October 31. **Case Report**

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

Shirin Eyvazi [1], Mojdeh Hakemi-Vala [2,*], Ali Hashemi [2], Fatemeh Bagheri Bejestani [3] and Negar Elahi [4]

[1] Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2] Department of Microbiology, Medical School, Shahid Beheshti University of Medical Sciences (SBMU), Tehran, Iran
[3] Department of Microbiology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran
[4] Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran

[*] *Corresponding author*: Mojdeh Hakemi-Vala, Kodakyar St, Daneshjo Blvd, Velenjak, Tehran, Iran, PO Box: 19835-151. Tel: +98-2123872556, E-mail: m.hakemi@sbmu.ac.ir

Received 2017 February 08; **Revised** 2017 July 10; **Accepted** 2017 August 12.

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 (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-β-lactamases (MBL). However, genetic detection using Polymerase Chain Reaction amplification with specific primers for bla_{KPC}, $blaI_{MP}$, 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 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 important carbapenemases is recently known as New Delhi metallo-β-lactamase-1 (bla_{NDM-1}), which belongs to class B β-lactamases or metallo-β-lactamases (MBL) (4). For the first time, $bla_{NDM-1}$was detected from *Klebsiella pneumonia* 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), 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 (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_{AmpC} detection. Additionally, CDT test was used for bla_{MBL} detection using 2 IPM (10 μg) disks and EDTA 0.5 M solution. Results showed that the strains do not produce $blaA_{mpC}$, whereas they can produce bla_{MBL} enzyme.

Genotypic detection of bla_{KPC}, bla_{IMP}, bla_{VIM-1} (Verona integron-encoded metallo-β-lactamase), bla_{VIM-1}– bla_{VIM-37}, bla_{DIM-1}(Dutch imipenemase), and bla_{NDM-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 bla_{NDM-1} primers and further DNA sequencing confirmed the presence of bla_{NDM-1}gene in these 2 *E. coli* isolates (Figure 1). *E. coli* ATCC 25922 was considered as a control in all tests.

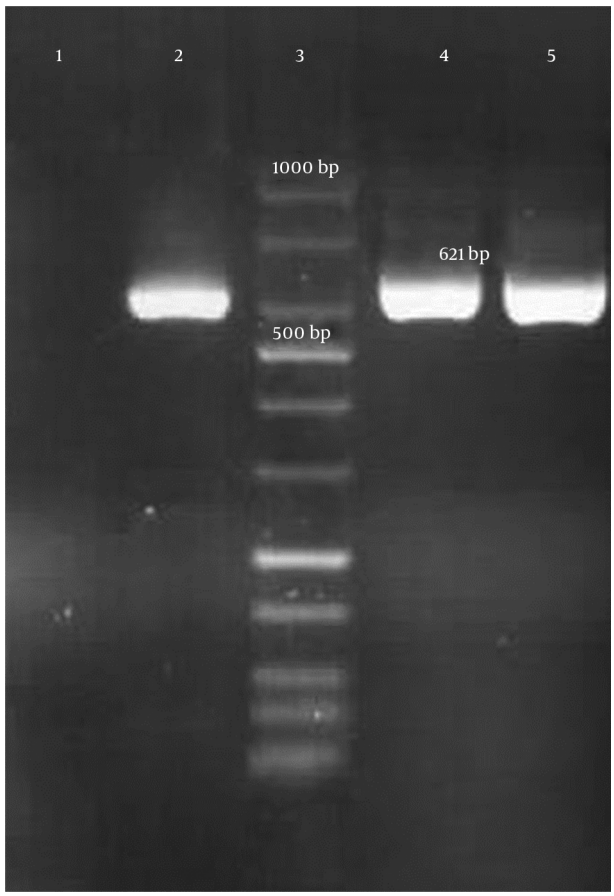
Table 1. Primer Sequences Used in This Study

Gene Name	Primer Sequence	Product Size	Reference
bla_{KPC}	Forward: 5′-ATG TCT GTA TCG CCG TCT -3′	989-bp	(9)
	Reverse: 5′-TTTTCAGAGCCTTACTGCCC-3′		
$blaI_{MP-1}$	Forward:5′--GAAGGCGTTTATGTTCATAC-3′	587-bp	(10)
	Reverse: 5′-GTACGTTTCAA-GAGTGATGC-3′		
bla_{VIM-1}	Forward: 5′-AGTGGTGAGTATCCGACAG-3′	836-bp	(11)
	Reverse: 5′-ATGAAAGTGCGTGGAGAC-3′		
$bla_{VIM-1-VIM-37}$	Forward: 5′-GATGGTGTTTGGTCGCATA-3′	390-bp	(12)
	Reverse: 5′-CGAATGCGCAGCACCAG-3′		
bla_{DIM}	Forward: 5′-GCTTGTCTTCGCTTGC-TAACG-3′	699-bp	(13)
	Reverse: 5′-CGTTCGGCTG-GATTGATTTG-3′		
bla_{NDM-1}	Forward: 5′-GGTTTGGC-GATCTGGTTTTC-3′	621-bp	(14)
	Reverse: 5′-CGGAATGGCT-CATCACGATC-3′		

3. Discussion

This is a first report on emerging of bla_{NDM-1}producing *E. coli* in Iran. Earlier, Shahcheraghi et.al reported the first bla_{NDM-1}producing *K. pneumonia* in Iran (15). Furthermore, recently, Fazeli et.al presented the 2nd report on the detection of bla_{NDM-1}gene among *K. pneumonia* isolates from Iran (16). Approximately, the emergence of bla_{NDM-1}producing Enterobacteriaceae is reported in all neighboring countries including; Turkey (17), Iraq (18), Pakistan (19), and Afghanistan (20). The prevalence of bla_{NDM-1}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). Among these bla_{NDM-1}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_{NDM-1}gene is placed near various insertion elements on transferrable plasmids (22). Medical tourisms has a considerable role in wide spreading of resistant genes e.g. bla_{NDM-1}gene (19). In addition, the presence of bla_{NDM-1}producing bacteria in environmental samples such as drinking-water samples could be involved in spreading of the resistant gene (23). Spreading of bla_{NDM-1}gene is a global concern. Isolates harboring bla_{NDM-1}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_{NDM-1} 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_{NDM-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 bla_{NDM-1}, is an alarm to our health system, due to the spreading of transferable bla_{NDM-1}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_{NDM-1}harboring bacteria and prevention of their spreading must be performed in any region.

Acknowledgments

The authors are thankful to Ms. Sattarzadeh Tabrizi M., and the other staff of the Motahari hospital for their kind cooperation.

Footnote

Financial Disclosure: No competing financial interests exist.

References

1. Hawkey PM, Livermore DM. Carbapenem antibiotics for serious infections. *BMJ*. 2012;**344**:3236. 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):228–36. doi: 10.1016/S1473-3099(09)70054-4. [PubMed: 19324295].
3. Gupta N, Limbago BM, Patel JB, 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: 28449650].
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-1 (NDM-1) Producers among Carbapenem-Resistant Enterobacteriaceae in Kuwait. *PLoS One*. 2016;**11**(3):152638. doi: 10.1371/journal.pone.0152638. [PubMed: 27031521].
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. *Iran J Microbiol*. 2015;**7**(3):127–35. [PubMed: 26668699].
8. Soltani J, Poorabbas B, Miri N, Mardaneh 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: 26989670].
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.01103-07. [PubMed: 18227191].
10. Pitout JD, Gregson DB, Poirel L, McClure JA, 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, Beceiro A, 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, Kistler J, Livermore DM, Woodford N. Multiplex PCR for rapid detection of genes encoding acquired metallo-beta-lactamases. *J Antimicrob Chemother*. 2007;**59**(2):321–2. doi: 10.1093/jac/dkl481. [PubMed: 17185300].
13. Poirel L, Walsh TR, Cuvillier 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: 21398074].
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)70143-2.
20. McGann P, Hang J, Clifford RJ, Yang Y, Kwak YI, Kuschner 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**(6):1247–54. doi: 10.1007/s10096-015-2345-6. [PubMed: 25732142].
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 lwoffii. *Antimicrob Agents Chemother*. 2012;**56**(4):1698–702. doi: 10.1128/AAC.06199-11. [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. *J Antimicrob Chemother*. 2013;**68**(5):1204–6. doi: 10.1093/jac/dks503. [PubMed: 23315490].