First Report of New Delhi Metallo- β -Lactamase-1 (NDM-1) Among *Escherichia Coli* Strains Isolated from Leukemia Patients in Iran

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Case report

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Abstract

Background: *Escherichia coli* has appeared as an important opportunistic pathogen responsible for nosocomial infections in Patients with immunodeficiency particularly in leukemia patients. New Delhi metallo-β-lactamase (NDM-1) is an enzyme that class of beta-lactam antibiotics and is used in treatment of multi-drug resistant (MDR) infections.

Case Presentation: In this study, 80 isolates of *Escherichia coli* and *Klebsiella pneumoniae* collected over the course of two years from two medical centers of Tehran, Iran. Production of carbapenemase was detected of the isolates using MHT. New Delhi metallo-beta-lactamase 1 (*bla*_{NDM-1}) genes were detected by polymerase chain reaction (PCR) amplification with specific primers 2 *bla*_{NDM-1} producing *E.coli* strains were isolated from 2 leukemia of patients, The isolates were resistant to carbapenems (imipenem, meropenem),two isolates were positive for carbapenemase production by Modified Hodge test.

Conclusions: The emerging of NDM-1 producing *E. coli* is a threat for leukemia patients in Oncology and hematology departments. We concluded that the incidence of MDR pathogens increased among patients with leukemia and is life threatening.

Background

The global increasing prevalence of NDM-1-producing *Enterobacteriaceae* is a concerning phenomenon in immunocompromised patients. Previous results indicated that *bla*_{NDM-1} gene can be carried on the IncN plasmids of different sizes along with other resistance factors. *bla*_{NDM-1} can confer resistance to almost all the β-lactams. Thus the bacteria carrying NDM-1 gene are considered resistant to all antibiotic classes except colistin and ciprofloxacin. The gene was scarcely integrated into the chromosome. The sequencing of this gene suggests a new enzyme unrelated to hitherto known as MBLs. The most similar known type is Verona integron-encoded metallo-β-lactamase (VIM-1/VIM-2) sharing a 32.4% resemblance (1).

In a study conducted in China, the presence of this gene was first reported in a patient with leukemia, especially in patients with neutropenia (2) .our study is also the first report in Iran. The purpose of this study was to investigate the existence of *NDM1* gens as a risk factor for life-threatening infection in patients with leukemia patients.

Case Presentation

In this study, 80 isolates of *Escherichia coli* and *Klebsiella pneumoniae* were collected from two medical centers of Tehran, Iran: The Hematology-Oncology Research Center, Dr. Shariati Hospital, and the Mahak Pediatric Oncology Center between 2014–2015.

The bacteria were isolated and stored in Hamadan University of Medical Sciences. All the clinical specimens were quickly sent to the laboratory and analyzed for confirmatory test. Isolates were identified using standard microbiological and biochemical procedures (3). In our previous study, antibiotic susceptibility of the isolates was tested by Kirby-Bauer disk diffusion method for imipenem, ceftazidime, ceftriaxone, cefotaxime, ciprofloxacin, levofloxacin, amikacin, ampicillin, and gentamicin (all from Mast, UK). Moreover, the combination disk method was employed to detect extended-spectrum β-lactamase (ESBL) producing isolates; while the minimum inhibitory concentrations (MICs) of selected antimicrobials were determined by the broth microdilution method. The results were interpreted according to the Clinical Laboratory Standards Institute (CLSI) guidelines (4). The carbapenem-resistant strains were investigated for carbapenemase production by modified hodge test (MHT) according to the CLSI guidelines in which, *E. coli* ATCC25922 was used as the positive control (5). Furthermore, the combined disk diffusion method was applied for *bla*_{MBL} detection using 2 IPM (10 µg) disks and

EDTA 0.5 M solution(6). For diagnosis of the NDM1, PCR was performed on DNA extracted by boiling, using NDM-F-specific primers: CAACTGGATCAAGCAGGAGA, NDM-R TCGATCCCAACGGTGATATT (Bioneer Company, Korea) (7).

The sequencing of amplicons in both directions was carried out by the Bioneer Company (Daejeon, South Korea). The data were analyzed using FinchTV software (Geospiza, USA), and the sequences were then confirmed using the NCBI web site (<http://www.ncbi.nlm.nih.gov/BLAST>). A total of 56 *E. coli* and 24 *K. pneumoniae* isolates were achieved from urine, blood, sputum, wound, and vagina. Among the 80 isolates, 52 (63%) strains were ESBL producers, followed by 5 (6.25%) metallo β -lactamase (MBL) producers. According to our antimicrobial susceptibility test on *E. coli* and *K. pneumoniae* isolates, 8 (10%) of *E. coli* isolates were imipenem-resistant. Out of 80 isolates, 8(10%) isolates were found to be carbapenems-resistant. The major MBL and carbapenems-resistant species were *Escherichia coli*. The first NDM1-containing isolate was obtained from a 26-year-old subject diagnosed with acute leukemia. The second was obtained from a 2-year-old child with acute leukemia. These two NDM-1positive *E. coli* isolates (labeled as E1–E2) were recovered from the urine and blood samples of two different hospitalized patients. All two isolates were positive for the modified Hodge test and (MBL) producers (Table 1, 2).

Table 1
Antimicrobial resistance profile of two NDM-1-producing *E. coli* isolates from two patients with leukemia.

Phenotypic tests for carbapenemase		Minimum inhibitory concentration ($\mu\text{g/mL}$) for antibiotics	
Strains	MHT	IMP	MEM
E1	+	4	8
E2	+	16	32

Table 2
Distribution of CTX-M group, *qnr* genes, *TEM* gene, IS903, IS26, and ISEcp1 elements in NDM-1 producing *E. coli* isolated from leukemia patients.

	CTXM1	CTXM2	CTXM8	CTXM9	CTXM25	ISECP1	IS26	IS903	<i>qnrA</i>	<i>qnrB</i>	<i>qnrS</i>	<i>TEM</i>
E1	+	–	–	+	–	+	+	+	+	+	–	+
E2	+	–	–	–	–	+	+	–	–	–	–	+

Discussion

The present study reports NDM-1-producing *E. coli* strain from the bloodstream and infected urinary tract of two patients. PCR results confirmed that the NDM-1-producing *E. coli* harbored *qnrA*, *qnrB*, *qnrS* and *bla*_{CTXM-1·CTX-M2·CTX-M8·CTX-M9·CTX-M25} and the insertion sequence of ISECP1, IS26, IS903. The two NDM1-producing *E. coli* isolates did not contain *qnrS* genes but they carried *bla*_{TEM} gene.

Eyvazi et. al recently reported *bla*_{NDM-1} producing *E. coli* in Iran for the first time (8). Shahcheraghi et al detected the first *bla*_{NDM-1} producing *K. pneumonia* in Iran as well (9).

In October 2011, Laurent Poirel et al. reported *bla*_{NDM-1} producing *K. pneumoniae* and *E. coli*. in a 16-year-old male patient admitted to the hematology unit of a hospital in Istanbul, turkey (10).

In a study in 2010 on a patient transferred from Iraq to France, NDM-1-producing *K. pneumoniae* was also been reported (11).

Reports have also declared the existence of *bla*_{NDM-1} producing *Enterobacteriaceae* in Pakistan and Afghanistan (12, 13). The *bla*_{NDM-1} producing bacteria could be also found in environmental samples and drinking-water (14). In our investigation, PCR confirmed that the NDM1-producing *E.coli* harbored quinolone resistance gene B (*qnrB*) and *bla*_{CTXM-9}. This result was similar to the other reports on strains carrying *bla*_{NDM-1} which also harbored other β -lactamase genes (2).

An NDM-1-producing *E.coli* strain was detected in the bloodstream of a patient in this study that exhibited high resistance to all tested β -lactam antibiotics, which can be attributed to the production of *bla*_{NDM-1} and other resistant genes.

Bahramian et al. published the first report about metallo- β -lactamase-6 (NDM-6) among *K. pneumoniae* in New Delhi. ST147 strains were also isolated from dialysis patients in Iran (15). Firoozeh et al. reported 20 (11.1%) *K. pneumoniae* isolates harboring *bla*_{NDM-1} gene (16).

We inferred that it is important to evaluate the health condition of the immunocompromised patient especially those with leukemia. Moreover, the patient receiving chemotherapy may develop bloodstream infections. Bacterial infections can result in significant morbidity and mortality due to the development of febrile neutropenia and bacteremia.

Conclusion

This is the first report on the *bla*_{NDM-1}-producing *E. coli* strains isolated from leukemia patients in Iran. NDM-1-producing *E.coli* also harbored genes encoding cefotaxime resistant (CTX-M) group, TEM, QNR, and insertion sequence of resistance enzymes. The coincidence of NDM-1 with other antibiotic-resistance genes may further limit the treatment options and makes infection control procedures more challenging among leukemia patients. Our results show the emergence of *bla*_{NDM-1} as an alarm to our health services, in particular, among the immunocompromised patient.

Declarations

Ethics approval and consent to participate:

This study was approved by the Ethics Committee of Hamadan University of Medical Sciences (CodeNo:IR.UMSHA.REC.1398.004).

Consent for publication:

all the authors agree to publish the manuscript in the European journal of medical science.

Availability of data and materials:

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests:

The authors declare that they have no competing interests

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Author's contribution:

MR, FA and SD performed microbiological and molecular tests and write the manuscript. AG, A H and HG play role in Project Administration. MRA supervised all of the stages of designing the study, conducting the research and writing the manuscript.

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