

# The occurrence of antibiotic resistance, ESBLs, MBL and NDM-1 in Uropathogenic *Escherichia coli* in central part of Iran

Farshad Kakian<sup>1</sup>, Javad Fathi<sup>1</sup>, Fatemeh Alvandi<sup>2</sup>, Mahnaz Moumivand<sup>2</sup>, Abbas Rabei Gholami<sup>3</sup>, Abolfazl Gholipour<sup>4</sup>, Mohamad hossein Rezaei<sup>5</sup>, Behnam Zamanzad<sup>6,\*</sup>

<sup>1</sup>Department of Bacteriology and Virology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

<sup>2</sup>Department Microbiology, Hamadan University of Medical Sciences, Hamadan, Iran

<sup>3</sup>Cellular and Molecular Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran

<sup>4</sup>Cellular and Molecular Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran

<sup>5</sup>Cellular and Molecular Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran

<sup>6</sup>Department of Microbiology and Immunology, School of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran

## Abstract

Extended spectrum  $\beta$ -lactamases (ESBLs) are enzymes that capable of destroying the antibiotics of  $\beta$ -lactam, and cephalosporin, and Metallo- $\beta$ -lactamase enzymes (MBL) can also deactivate all  $\beta$ -lactams and carbapenems. This study aimed to determine ESBLs and MBLs enzymes and the frequency of NDM-1 gene. In this study, 200 *Escherichia coli* isolates of women with urinary tract infection were collected (100 inpatients and 100 outpatients). Minimum inhibitory concentration (MIC) for ceftazidime and meropenem was determined by E-test. A phenotypic confirmation test was used to detect ESBL enzymes. MBLs production was performed with modified Hodge test (MHT) and EDTA disk synergy (EDS) test. PCR was used for detecting the presence of NDM-1 gene. From 200 isolates, 93 isolates produce ESBL enzymes. Overall, 97 isolates were resistant to ceftazidime, and 38 isolates resistant to meropenem. The results of the MHT and EDS positive tests were 41 and 16 isolates, respectively. NDM-1 was not found in any of the patients. The prevalence of *E. coli* isolates producing both ESBLs and MBLs enzymes, is a serious threat to clinical infections. Accordingly, for the control and treatment of these strains, rapid and accurate identification can be greatly helpful.

**Keywords:** *Escherichia coli*, MIC, ESBL, NDM-1

## 1. Introduction

One of the most important infections that affect parts of the urinary tract is urinary tract infection (UTI). Bacteria are the main reason for this infection. The most common bacterial cause for UTI is *Escherichia coli*. UTIs report about 150 million cases each year and are more common in women than men [1-3]. Up to 10% of women have a UTI in a given year,

and half of them have at minimum one infection at some point in their lifetime [4]. There is a rising worry for multidrug-resistant bacteria that produce extended-spectrum  $\beta$ -lactamases (ESBLs). *E. coli* becomes progressively resistant to expanded-spectrum cephalosporins by the production of ESBLs [5,6]. Class D  $\beta$ -lactamases or oxacillinases (OXA) are commonly distributed between clinically relate Gram-

### \* Corresponding author:

Dr. Behnam Zamanzad, MD, Ph.D  
Department of Microbiology and Immunology, School of Medicine,  
Shahrekord University of Medical Sciences, Shahrekord, Iran  
Tel/Fax: +98 38 33346732; +98 38 3334911  
Email: bzamanzad22@gmail.com  
<http://orcid.org/0000-0001-8348-7827>

© The Author(s) 2020

Received: November, 5, 2020  
Accepted: November, 30, 2020



negative bacteria [7,8]. Carbapenem antibiotics are now used to treat infections caused by  $\beta$ -lactam resistant bacteria [2,9]. This antibiotic is used to treat infections caused by penicillin or cephalosporin-resistant Gram-negative bacilli because this antibiotic, in addition to a broad spectrum of action, has acceptable stability against most  $\beta$ -lactamase [2,10,11]. Resistance to carbapenem is mainly created with Metallo- $\beta$ -lactamases (MBL) such as NDM (New Delhi Metallo- $\beta$ -lactamase), a class B type of  $\beta$ -lactamases that know bivalent metal ions [12,13]. Recently, a new MBL named NDM-1 appeared in *E. coli* [14,15]. This gene causes resistance to penicillins, cephalosporins, and carbapenems [16]. This study aimed to determine ESBL, and MBL production ability and determination of the frequency of NDM gene in *E. coli* strains isolated from UTI in women.

## 2. Materials and Methods

### 2.1 Bacterial strain collection and identification

In this descriptive-analytical study, 200 *E. coli* isolates with urinary tract infection were collected from women with UTI in two groups of inpatients (n=100) and outpatients (n=100) in teaching hospitals from Shahrekord, Iran from 2016 to 2017. In this study, patients who were diagnosed with UTI 48-72 hours after hospitalization were considered as inpatient samples. Patients who have not been hospitalized in the past month were also used as outpatient samples. Isolates were detected using standard tests including Gram stain, microscopy analysis and culture in EMB media, MacConkey agar, and Blood agar (Merck, Germany), and other biochemical tests.

### 2.2 Determination of MIC and ESBL production

The minimum inhibitory concentration (MIC) test was done by the Epsilon test (E-test) of Liofilchem Company (Italy) with a concentration range of 0.002-32  $\mu$ g/mL to meropenem and ceftazidime. Evaluation and determination of inhibition zones were performed after culturing and incubating the plates at 37 °C overnight, using the CLSI guidelines. The phenotypic combined disc method was also used to determine ESBL enzymes [8]. *E. coli* ATCC 25922 and *Klebsiella pneumonia* ATCC 1705 were considered as negative and positive controls.

### 2.3 MHT and EDS test

In this study modified Hodge test (MHT) and EDTA disk synergy test (EDS) were performed to detect MBLs enzymes. Further, *E. coli* ATCC 25922 and *Klebsiella pneumonia* ATCC1705 were considered as negative and positive controls for running MHT, respectively [17,18].

### 2.4 DNA extraction and PCR

At this stage, after culturing samples on media, DNA was extracted using the boiling method [8]. The Single-PCR technique was also used to detect NDM-1 and 16s rRNA genes. Primers for this study are shown in Table 1 [19-21]. The presence of the 16srRNA gene (internal gene for *E. coli*) was investigated to approve the phenotypic tests for *E. coli* detection.

### 2.5 Statistical analysis

The statistical analysis was performed using SPSS, version 23.0 (SPSS IBM, NY, USA). The results are presented as descriptive statistics in terms of relative frequency

Table 1. Primers used in this study

Target	Primer sequence (5' to 3')	Size (bp)
NDM-F	GGTTTGGCGATCTGGTTTTTC	624 bp
NDM-R	CGGAATGGCTCATCACGATC	
16s rRNA-F	AGGCCTTCGGGTTGTAAAGT	420 bp
16s rRNA-R	ACCTCCAAGTCGACATCGTT	

## 3. Results

In this study, the age range of patients was 2-88 years. The maximum and minimum prevalence of isolates were allocated to the internal wards and intensive care units (ICU) respectively. In this study, the result of resistance to ceftazidime was 73% for inpatients and 25% for outpatients. Resistance to meropenem was 28% for inpatients and 10% for outpatients. From 100 isolates from inpatients, 70 isolates (70%), and from 100 isolates from outpatients, 23 (23%) isolates produce ESBLs. The results of the MHT and EDS tests were positive in 21% and 20% of inpatient samples and 8% and 8% of outpatient samples, respectively. After the electrophoresis of PCR products, NDM-1 gene was not found in any of the PCR.

#### 4. Discussion

Resistant *E. coli* is considered a health-threatening pathogen and causes many diseases in humans, especially those with underlying diseases and immune deficiencies. The infections owing to *E. coli* are treated with broad-spectrum antibiotics [22-24]. Antibiotic resistance in these bacteria is increasing and is spreading all over the world [2]. Carbapenem and  $\beta$ -lactam antibiotics are drugs of choice for *E. coli* infections. It is very valuable to early detection of ESBL and MBL in bacteria to control and prevent the spread of resistance strain in society and hospitals. These results and information can lead to a proper prognosis and selection of the appropriate medication by the physician [25,26]. We observed that 93 isolates produce ESBL enzymes. Also, 97 isolates resistance to ceftazidime and 38 isolates resistance to meropenem. NDM gene was not found in the patients. In a similar study, in Pakistan, it was observed that among 116 *E. coli* isolates from UTIs, 98 isolates were resistant to meropenem and imipenem and a total of 66 isolates were found to be ESBL producers [27]. A study in India showed that imipenem and ceftazidime resistance in clinical isolates of *E. coli* were 9%, and 62.1%, respectively. They also showed 61.1% of *E. coli* isolates were ESBLs [28]. Jamil et al. from Pakistan reported that among 25 carbapenem-resistant *E. coli*, 16% contained MBL by phenotypic test [29]. In another study by Qamar et al. of 100 *E. coli* isolates, 81% were MBL producers and 18.4% were NDM-1 positive [30]. In a similar study by Koraei et al. from southwest of Iran, in 376 *E. coli* isolates, NDM-1 gene was not found in any isolates [31].

In this study, we had some limitations. The main limitations were the study of two medical centers and the small sample size.

The emergence and spread of antibiotic resistance by ESBL and MBL producer isolates have been a global problem that indicates the excessive use of antibiotics. These types of antibiotic resistance are a serious problem in clinical therapeutic selections in the hospital as well as in the community. So, it is necessary to report ESBL/MBL production along with the daily sensitivity reporting, which will help the clinicians in determining correct antibiotics.

#### Acknowledgments

We thank all the people and Staffs who assisted us in conducting this work.

#### Author Contributions

FK, AG, BZ: design of study; FK, AG, BZ, MHR: acquisition of data; FA, AR, MM and JF: evaluation of data, preparation of the manuscript; FK, BZ, AG and JF: assessment of data. All authors have read and approved the manuscript.

#### Conflict of Interests

We have no competing interests.

#### Ethical declarations

The study protocol was obtained by the ethics code of IR.SKUMS.REC.1395.215 from Shahrekord University of Medical Sciences. However, we only used leftovers from clinical specimens.

#### Financial Support

This research was supported by the Shahrekord University of Medical Sciences, Shahrekord, Iran (Grant No. 2540).

#### References

1. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nat Rev Microbiol*. 2015; 13(5):269-84.
2. Kakian F, Zamanzad B, Gholipour A, Zamanzad K. Determination of the carbapenem resistance in *Escherichia coli* isolated from samples obtained from Shahrekord hospitals and determination of their minimum inhibitory concentration. *J Shahrekord Univ Med Sci*. 2019; 21(6):280-3.
3. Sedighimehr N, Dehghan Manshadi F. Electrical Stimulation for Lower Urinary Tract Dysfunction in People with Multiple Sclerosis: A Systematic Review. *J Clin Physiother Res*. 2018; 3(2):48-53.
4. Nickel JC. Inflammation and benign prostatic hyperplasia. *Urol Clin North Am*. 2008; 35(1):109-15; vii.
5. Melzer M, Petersen I. Mortality following bacteraemic infection caused by extended spectrum beta-lactamase (ESBL) producing *E. coli* compared to non-ESBL producing *E. coli*. *J Infect*. 2007; 55(3):254-9.
6. Jamborova I, Dolejska M, Vojtech J, Guenther S, Uricariu R, Drozdowska J, et al. Plasmid-mediated resistance to cephalosporins and fluoroquinolones in various *Escherichia coli* sequence types isolated from rooks wintering in Europe. *Appl Environ Microbiol*. 2015; 81(2):648-57.
7. Evans BA, Amyes SG. OXA  $\beta$ -lactamases. *Clin Microbiol Rev*. 2014; 27(2):241-63.
8. Kakian F, Shahini Shams Abadi M, Zamanzad B, Najafi H, Amiri M, Gholipour A. Frequency Assessment of OXA-10 and PER  $\beta$ -Lactamase Genes and Determination of Minimum Inhibitory Concentration in *Klebsiella* Strains Isolated from Urinary Tract Infections. *Jundishapur J Microbiol*. 2019; 12(2):e65500.
9. Hawkey PM, Livermore DM. Carbapenem antibiotics for serious infections. *Bmj*. 2012; 344:e3236.

10. Lee CH, Su LH, Tang YF, Liu JW. Treatment of ESBL-producing *Klebsiella pneumoniae* bacteraemia with carbapenems or flomoxef: a retrospective study and laboratory analysis of the isolates. *J Antimicrob Chemother.* 2006; 58(5):1074-7.
11. Hashemi B, Afkhami H, Khaledi M, Kiani M, Bialvaei AZ, Fathi J, et al. Frequency of Metallo beta Lactamase genes, bla IMP1, INT 1 in *Acinetobacter baumannii* isolated from burn patients North of Iran. *Gene Rep.* 2020; 21:100800.
12. Doyle D, Peirano G, Lascols C, Lloyd T, Church DL, Pitout JD. Laboratory detection of Enterobacteriaceae that produce carbapenemases. *J Clin Microbiol.* 2012; 50(12):3877-80.
13. Chika E, Malachy U, Ifeanyichukwu I, Peter E, Thaddeus G, Charles E. Phenotypic detection of metallo- $\beta$ -lactamase (MBL) enzyme in Enugu, Southeast Nigeria. *Am J Biol Chem Pharm Sci.* 2014; 2(2):1-6.
14. Bocanegra-Ibarras P, Garza-González E, Morfín-Otero R, Barrios H, Villarreal-Treviño L, Rodríguez-Noriega E, et al. Molecular and microbiological report of a hospital outbreak of NDM-1-carrying Enterobacteriaceae in Mexico. *PLoS One.* 2017; 12(6):e0179651.
15. Poirel L, Lagrutta E, Taylor P, Pham J, Nordmann P. Emergence of metallo- $\beta$ -lactamase NDM-1-producing multidrug-resistant *Escherichia coli* in Australia. *Antimicrob Agents Chemother.* 2010; 54(11):4914-6.
16. Nordmann P, Boulanger AE, Poirel L. NDM-4 metallo- $\beta$ -lactamase with increased carbapenemase activity from *Escherichia coli*. *Antimicrob Agents Chemother.* 2012; 56(4):2184-6.
17. Amjad A, Mirza I, Abbasi S, Farwa U, Malik N, Zia F. Modified Hodge test: A simple and effective test for detection of carbapenemase production. *Iran J Microbiol.* 2011; 3(4):189-93.
18. Lee K, Chong Y, Shin HB, Kim YA, Yong D, Yum JH. Modified Hodge and EDTA-disk synergy tests to screen metallo-beta-lactamase-producing strains of *Pseudomonas* and *Acinetobacter* species. *Clin Microbiol Infect.* 2001; 7(2):88-91.
19. Damavandi MS, Gholipour A, Latif Pour M. Prevalence of Class D Carbapenemases among Extended-Spectrum  $\beta$ -Lactamases Producing *Escherichia coli* Isolates from Educational Hospitals in Shahrekord. *J Clin Diagn Res.* 2016; 10(5):Dc01-5.
20. Nordmann P, Poirel L, Carrère A, Toleman MA, Walsh TR. How to detect NDM-1 producers. *J Clin Microbiol.* 2011; 49(2):718-21.
21. Qi C, Malczynski M, Parker M, Scheetz MH. Characterization of genetic diversity of carbapenem-resistant *Acinetobacter baumannii* clinical strains collected from 2004 to 2007. *J Clin Microbiol.* 2008; 46(3):1106-9.
22. Kaesbohrer A, Schroeter A, Tenhagen BA, Alt K, Guerra B, Appel B. Emerging antimicrobial resistance in commensal *Escherichia coli* with public health relevance. *Zoonoses Public Health.* 2012; 59 Suppl 2:158-65.
23. Sanz-García M, Fernández-Cruz A, Rodríguez-Crèixems M, Cercenado E, Marín M, Muñoz P, et al. Recurrent *Escherichia coli* bloodstream infections: epidemiology and risk factors. *Medicine (Baltimore).* 2009; 88(2):77-82.
24. Fathi J, Ebrahimi F, Nazarian S, Tarverdizade Y. Purification of Shiga-like Toxin from *Escherichia coli* O157: H7 by a Simple Method. *J Appl Biotechnol Rep.* 2017; 4(4):707-11.
25. Rahamathulla M, Harish B, Mataseje L, Mulvey M. Carbapenem resistance mechanisms among blood isolates of *Klebsiella pneumoniae* and *Escherichia coli*. *Afr J Microbiol Res.* 2016; 10(2):45-53.
26. Teethaisong Y, Eumkeb G, Chumnarnsilpa S, Autarkool N, Hobson J, Nakouti I, et al. Phenotypic detection of AmpC  $\beta$ -lactamases, extended-spectrum  $\beta$ -lactamases and metallo- $\beta$ -lactamases in Enterobacteriaceae using a resazurin microtitre assay with inhibitor-based methods. *J Med Microbiol.* 2016; 65(10):1079-87.
27. Ullah F, Malik SA, Ahmed J. Antibiotic susceptibility pattern and ESBL prevalence in nosocomial *Escherichia coli* from urinary tract infections in Pakistan. *Afr J Biotechnol.* 2009; 8:3921-6.
28. Singh N, Pattnaik D, Neogi DK, Jena J, Mallick B. Prevalence of ESBL in *Escherichia coli* Isolates Among ICU Patients in a Tertiary Care Hospital. *J Clin Diagn Res.* 2016; 10(9):Dc19-dc22.
29. Jamil J, Haroon M, Sultan A, Khan MA, Gul N. Prevalence, antibiotic sensitivity and phenotypic screening of ESBL/MBL producer *E. coli* strains isolated from urine; District Swabi, KP, Pakistan. *J Pak Med Assoc.* 2018; 68(11):1704-7.
30. Qamar MU, Mustafa G, Qaisar U, Azeem F, Shahid M, Manzoor I, et al. Molecular detection of blaNDM and blaVIM in clinically isolated multi-drug resistant *Escherichia coli* in Pakistan. *Pak J Pharm Sci.* 2019; 32(S5):2305-9.
31. Koraei M, Moosavian M, Saki M. Investigation of New Delhi Metallo-Beta-Lactamase 1 (NDM-1) in Clinical Enterobacteriaceae Isolates in Southwest Iran. *J Res Med Dent Sci.* 2018; 6:1-5.