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Prevalence of carbapenem-resistant Metallo-Beta-Lactamase-producing *Escherichia coli* strains isolated from urinary tract infections

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Abstract

Background: Urinary tract infection (UTI) is one of the most prevalent bacterial diseases worldwide. *Escherichia coli* is a well-known etiological agent of UTI. The emergence and spread of metallo-beta-lactamase (MBL)-producing *E. coli* is a serious threat to public health.
This study aimed to investigate the antibiotic resistance pattern and prevalence of MBL-producing *E. coli* isolated from UTI.

Methods: From January 2020 to June 2021, 1200 urine specimens were collected from patients suspected of having UTI. Antibiotic susceptibility testing was carried out by the disk diffusion method. The prevalence of MBL (*bla*VIM, *bla*IMP, *bla*SPM, and *bla*NDM) genes was determined by the polymerase chain reaction (PCR) method.

Results: The highest susceptibility was observed against amikacin (96%) and gentamicin (95%). The isolates were mostly resistant against ampicillin (72%) and cephalothin (60%). All carbapenem-resistant isolates were MBL-positive. Based on the results of PCR, 75% of the isolates were *bla*NDM-positive.

Conclusion: Resistance to some antibiotics, such as ampicillin and cephalothin, was high, and their prescription must be restricted. The prevalence of MBL-producing isolates was not high; however, due to the high level of resistance against other antibiotics, continuous monitoring of MBL-producing isolates is highly essential.

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Introduction

Escherichia coli is a well-known Gram-negative bacillus and a member of the *Enterobacteriaceae* family, which is responsible for different types of infections, including bloodstream infections (BSIs), wound infections, respiratory tract infections, and particularly, urinary tract infections (UTIs) (1). Urinary tract infections are among the most common bacterial infections that are reported in hospitalized and non-hospitalized patients worldwide (1). Generally, UTIs are divided into 2 categories: complicated and uncomplicated infections. Complicated UTIs usually occur in hospitalized patients with structural abnormalities, including infected cysts, renal or bladder obsesses, and calculi (2). However, uncomplicated UTI occurs in outpatients, usually young sexually active women, who have a normal genitourinary tract and have no history of recent hospitalization (3).

The emergence and spread of antibiotic-resistant bacteria have been an increasing problem for healthcare providers worldwide. Due to different antibiotic resistance mechanisms identified in bacteria, treatment of UTIs has become more difficult every year. Mutation, site-specific modification, inactivation of drugs by a wide variety of enzymes, and efflux pump overexpression are among well-known mechanisms (4).

Extended-spectrum beta-lactamase enzymes (ESBLs) are very important in the dissemination of antimicrobial resistance genes among Gram-negative bacteria ESBL genes are usually located on mobile genetic elements such as plasmid and integron. These mobile genetic elements can harbor genes conferring resistance against different classes of antibiotics, including aminoglycosides and beta-lactamases (5, 6).

Beta-lactam antibiotics such as penicillins and cephalosporins were good choices for the treatment of infections caused by *E. coli*; however, due to the emergence and evolution of different resistance mechanisms, they have lost their effectiveness.[7] Carbapenems play an important role in our antibiotic armamentarium and have been used as last-line antibiotics for the treatment of multi-drug resistant *E. coli* infections. However, the development of different resistance mechanisms, in particular, the production of ESBLs, has compromised their effectiveness (7, 8).

According to the Ambler classification, ESBL enzymes have been divided into 4 types: class A, B, C, and D, which are divided into different subclasses based on the protein homology and molecular structure. Class B beta-lactamase, known as metallo-beta-lactamases or MBL (IMP, NDM, SIM, SPM, and VIM), class A (KPC type), and class D (OXA-48-like) are the most clinically important carbapenemase enzyme genes (9).

Different studies showed that carbapenem-resistant and MBL-producing *E. coli* are resistant to different classes of antibiotics and are associated with high mortality and morbidity (10, 11).

Given the poor outcomes of patients infected by carbapenem-resistant and MBL-producing *E. coli* and the need for identifying resistance mechanisms to launch successful dissemination of infection prevention programs, we aimed to investigate the antibiotic resistance pattern and prevalence of MBL-positive *E. coli* isolated from UTIs.

Methods

A total of 1,200 urine specimens were collected from patients suspected of having UTIs referred to a teaching hospital affiliated with Zabol University of Medical Sciences (ZBMU), Iran, from January 2020 to June 2021. This teaching hospital is the only referral hospital in the region, serving about 400 000 people. Midstream urine samples were collected and cultured on blood agar (HiMedia, India) and eosin-methylene blue (EMB) agar (HiMedia, India). The UTIs were diagnosed based on previously described guidelines (12). In brief, a combination of positive urine analysis (culture, $\geq 10^5$ cfu/mL) and clinical symptoms was applied. All *E. coli* isolates were identified with standard microbiology tests, including Gram-staining, catalase, oxidase, lactose fermentation, indole, methyl red, motility, gas production, citrate, urease, and Voges-Proskauer (13). This study was approved by the Ethics Committee of Zabol University of Medical Sciences (IR.ZBMU.REC.1400.027)

Resistance to the following antibiotics (Padtan Teb, Iran) was evaluated using Kirby-Bauer's disk diffusion method and guidelines of the Clinical and Laboratory Standards Institute (14); ampicillin (AMP, 10 µg), ciprofloxacin (CIP, 5 µg), meropenem (MEM, 10 µg), imipenem (IMP, 10 µg), amikacin (AMK, 30 µg), ceftriaxone (CRO, 30 µg), cephalothin (CF, 30 µg), trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 µg), and gentamicin (GM, 10 µg). Multi-drug-resistant (MDR) isolates were defined as resistant to at least 1 antibiotic in 3 categories (14). *E. coli* 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as the quality controls.

The combined disk test (CDT) was used to detect MBL-positive *E. coli* isolates. For this purpose, 2 disks of imipenem and imipenem plus EDTA 0.5 M (ethylenediaminetetraacetic acid) were placed on the cultured plate. After incubation at 35 °C for 16-18 h, an increase of ≥7 mm in the inhibition zone between the imipenem-EDTA disk and imipenem alone was considered as MBL-producing isolates (15). Besides, clinical MBL-positive isolates of *Klebsiella pneumoniae* and *P. aeruginosa* were used as the quality control strains.

The boiling method based on previous instructions was used for genomic DNA extraction (16). In brief, the carbapenem nonsusceptible isolates were cultured on blood agar at 37 °C. Then, 2 fresh colonies (24 h) of *E. coli* were completely dissolved in 200 µL of sterile distilled water. The suspension was heated at 100 °C for 8 min. Finally, after cooling and centrifugation at 13 000 g for 15 min, the supernatant was used for PCR. The oligonucleotide primers used

in this study are listed in Table 1 (17). The PCR amplification, using the Ampliqon (Denmark) ready-to-use master mix, was performed on the extracted DNAs for the detection of MBL genes (*bla*VIM, *bla*IMP, *bla*SPM, and *bla*NDM) (17). For each gene, the following PCR reaction (final volume 20 µL) was used: 15 µL of the ready-to-use master mix, 2 µL of the extracted DNAs, and 1.5 µL of forward and reverse primers (100 pmol). The following PCR program was used for the amplification of MBL genes: denaturation at 95 °C for 5 min, 30 cycles of denaturation at 94 °C for 55 sec, annealing at 51 °C, 52 °C, 53 °C, and 51 °C (IMP, VIM, SPM and NDM), respectively, for 45 sec, extension at 72 °C for 1 min, and a final extension at 72 °C for 5 min. The PCR products were separated using agarose gel electrophoresis (1% w/v), stained with Sybr safe (Thermo Fisher Scientific Inc.), and then visualized by the gel documentation system.

Table 1. Sequence of oligonucleotide primers used in this study

Genes	Sequence (5…3)	Amplicon	Reference
*bla*VIM	F- GATGGTGTTTGGTCGCATA R- CGAATGCGCAGCACCAG	390	(17)
*bla*NDM	F- GGTTTGGCGATCTGGTTTTC R- CGGAATGGCTCATCACGATC	621	(17)
*bla*IMP	F- GGAATAGAGTGGCTTAAYTCTC R- GGTTTAAYAAAACAACCACC	232	(17)
*bla*SPM	F- AAAATCTGGGTACGCAAACG R- ACATTATCCGCTGGAACAGG	271	(17)

Results

Out of 1 200 analyzed urine samples, 100 *E. coli* isolates were collected. Of these, 65 isolates (65%) were collected from women and 35 (35%) from men. Meanwhile, out of 100 isolated bacteria, 90 (90%) were from outpatients and 10 (10%) were from inpatients.

The highest susceptibility was observed against amikacin (n = 96; 96%), gentamicin (n = 95; 95%), meropenem (n = 93; 93%), and imipenem (n = 92; 92%), respectively (Table 2). Besides, according to the antibiotic susceptibility results, 30 isolates (30%) were known to be MDR. All carbapenem-resistant isolates were MBL-positive. Based on the results of PCR, 6 (75 %) isolates were *bla*NDM-positive (Figure 1 and Table 3). The other investigated genes were not detected.

Table 2. Antibiotic susceptibility patterns of isolates

Antibiotics	Abbreviation, (µg)	Resistant, N (%)	Intermediate, N (%)	Susceptible, N (%)
Ampicillin	AMP, (10)	72 (72)	0 (0)	28 (28)
Ciprofloxacin	CIP, (5)	30 (30)	2 (2)	68 (68)
Meropenem	MEM, (10)	7 (7)	0 (0)	93(93)
Imipenem	IMP, (10)	8 (8)	0 (0)	92 (92)
Amikacin	AMK, (30)	3 (3)	1 (1)	96 (96)
Ceftriaxone	CRO, (30)	25 (25)	5 (5)	70 (70)
Trimethoprim-sulfamethoxazole	SXT, (1.25/23.75)	41 (41)	0 (0)	59 (59)
Gentamicin	GM, (10)	5 (5)	0 (0)	95 (95)
Cephalothin	CF, (30)	60 (60)	5 (5)	35 (35)

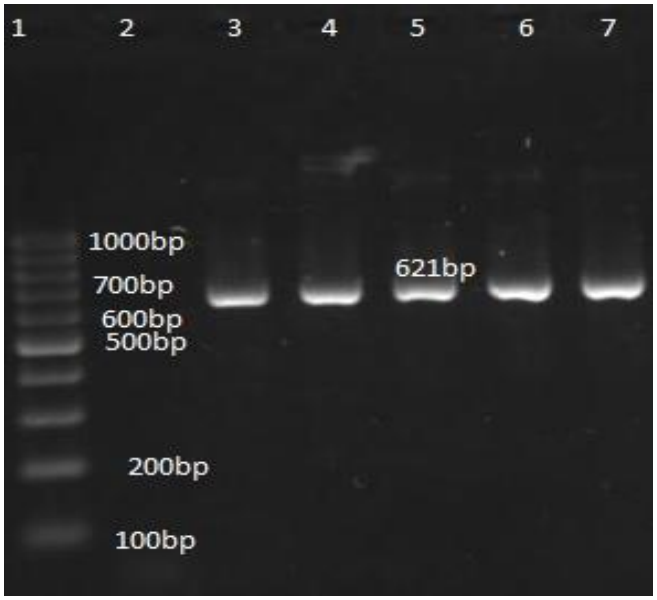

Figure 1. Electrophoresis image of blaNDM PCR, Lane 1: 100bp DNA Ladder, Lane 2: negative control, Lane3: positive control, Lanes 4-7: positive clinical isolates

Table 3. Characteristics of carbapenem-resistant *bla*NDM-producing isolates

N	Patients	Antibiotic resistance profiles [a]	CDT [b]
1	Inpatient	AMP, CF, CIP, IMP, CRO, SXT, GM, AMK, MEM	+
2	Outpatient	AMP, CF, CIP, IMP, CRO, SXT, GM, MEM	+
3	Outpatient	AMP, CF, CIP, IMP, CRO, SXT, MEM	+
4	Inpatient	AMP, CF, CIP, IMP, CRO, SXT, GM, AMK, MEM	+
5	Outpatient	AMP, CF, CIP, IMP, CRO, SXT, GM, AMK, MEM	+
6	Outpatient	AMP, CF, CIP, IMP, CRO, SXT, AMK, MEM	+

[a] AMP, Ampicillin; CF, Cephalothin; CIP, Ciprofloxacin; IMP, Imipenem; CRO, Ceftriaxone; SXT, Trimethoprim-sulfamethoxazole; GM, Gentamicin; AMK, Amikacin; MEM, Meropenem.
[b] Combined Disk Test

Discussion

Bacterial resistance to antibiotics is rapidly growing, and investigation of antibiotic resistance profiles and mechanisms is necessary in each region to control the spread of resistant bacteria. Urinary tract infections caused by *E. coli* strains are among the most important and prevalent infections that should be considered a serious threat to human health worldwide. In addition, treatment of infections caused by *E. coli* is becoming more difficult due to different antibiotic resistance mechanisms that are being developed (18). Therefore, in this study, we investigated the antibiotic resistance profiles and prevalence of *bla*VIM, *bla*IMP, *bla*SPM, and *bla*NDM genes among *E. coli* strains isolated from patients with UTI.

In this study, *E. coli* showed different levels of resistance to the tested antibiotics, revealing that carbapenem and aminoglycoside are the most effective antibiotics, with almost more than 90% of all isolates being susceptible. However, resistance to beta-lactam antibiotics such as ampicillin (72%) and cephalothin (60%) was high.

Based on the results of a comprehensive study conducted in Iran, the prevalence of ampicillin-resistant *E. coli* in Tehran, the capital of Iran, was about 90%. Also, resistance to cephalosporins in the majority of Iranian cities was reported to be high, e.g., in Rasht (north of Iran) 60%, Kermanshah (west of Iran) 73%, Hamadan (west of Iran) 85%, and Isfahan (central Iran) 59% (19). This high level of resistance to beta-lactam antibiotics such as ampicillin and cephalosporin may be due to the misuse of antibiotics in different regions of Iran because antibiotics are easily available in drug stores and usually prescribed at the request of patients.

Carbapenems (imipenem and meropenem) and aminoglycosides (gentamicin and amikacin) are last-line antibiotics, which are usually used to treat infection caused by resistant isolates. Our results revealed that the isolates were mostly susceptible to carbapenem (93%), which is significantly higher than the report from Babol, north of Iran (56.1%) (20). However, it is in agreement with most provinces of Iran, reporting a 95-100% susceptibility to carbapenems (19).

Noteworthy, our findings revealed that resistance to carbapenems and aminoglycosides is in agreement with some European countries such as Finland (0 and 5%), the Netherlands (0.1 and 5.6%), and Denmark (0.1 and 6%), respectively (21).

It has been reported that the frequency of antibiotic resistance can be affected by some factors, including antibiotic stewardship programs, widespread use and misuse of antibiotics, and the presence of some risk factors such as long-term stays at intensive care units (ICU) and a history of recent hospitalization, poor infection control policies, and differences in geographical locations (22, 23).

In this study, 8% of all investigated isolates were MBL-positive. The prevalence of MBL-producing *E. coli* in Iran varies between 0.3% in Isfahan and 16.8% in Golestan (24, 25).

The identification of MBL-harboring isolates is of paramount importance because they carry mobile genetic elements with multiple antibiotic-resistance genes and a substantial ability to spread, conferring resistance against different antibiotics. Hence, early detection of these isolates is necessary to prevent their spread in community and hospital settings and establish appropriate antimicrobial prescriptions (26). In addition, due to the simultaneous resistance against different antimicrobial drugs, it has been reported that the spread of MBL-positive isolates contributes to poor patient outcomes and an increase in mortality and morbidity (26-28).

The present study investigated the occurrence of *bla*VIM, *bla*IMP, *bla*SPM, and *bla*NDM among *E. coli* strains recovered from patients with UTIs. Our results revealed that *bla*NDM are mostly distributed genes (75%), while other MBL genes were not detected. In accordance with the findings of this study, there are many independent studies in Iran and other Asian countries, such as China, which have reported *bla*NDM as the prevalent MBL gene (26-30). For instance, the results of a study from Kuwait showed that 34.4% of isolates were positive for *bla*NDM (29). Likewise, Aung et al. from Myanmar reported the high prevalence of *bla*NDM-producing *E. coli* isolates, with 74% of carbapenem-resistant isolates being positive (31).

For the first time, in 2009, *bla*NDM was reported from a Swedish patient who traveled to New Delhi and acquired UTI due to MDR-*Klebsiella pneumoniae* (32, 33). Following the initial report, sporadic cases of infection with *bla*NDM-positive strains were reported from different countries, including

England, Denmark, Norway, Belgium, the United States of America, Algeria, Egypt, South Korea, Japan, and Australia (28).

In this study, all 8 imipenem-resistant isolates were MBL-positive. Among these, 6 isolates were found to be *bla*NDM-positive; however, the other MBL genes, including *bla*VIM, *bla*IMP, and *bla*SPM, were not detected. Different mechanisms, such as outer membrane protein mutations, efflux pumps, and class D beta-lactamases, are documented to be involved in the resistance against carbapenems (32).

Conclusion

Based on our findings, the prevalence of carbapenem-resistant isolates and MBL genes was not high. Nevertheless, the spread of carbapenem-resistant MBL-positive isolates must be evaluated constantly. Based on the results of the present study, the rate of resistance to ampicillin, cephalothin, and trimethoprim-sulfamethoxazole was high, and their prescription has to be restricted, whereas the prevalence of resistance to meropenem and gentamicin was low.

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Ethical statement

This study was approved by the Ethics Committee of Zabol University of Medical Sciences (IR.ZBMU.REC.1400.027).

Conflicts of interest

The authors declare no conflict of interest.

Author contributions

Study design: Hamid Vaez and Fatemeh Rashidi; Data collection: Zahra Yazdanpour, Fatemeh Rashidi, and Hamid Vaez; Interpretation of data: Hamid Vaez, Zahra Yazdanpour, and Farzad Khademi; Manuscript preparation: Hamid Vaez and Farzad Khademi.

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