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Original Article

# Frequency of Various Types of Beta-Lactamase Enzymes in *Escherichia coli* Strains Isolated From Urine Samples in Aliabad, North-east of Iran

**Seyed Asghar Hosseini[1], Ania Ahani Azari[1*], Ahmad Danesh[2]**

[1]Department of Microbiology, Gorgan Branch, Islamic Azad University, Gorgan, Iran
[2]Infectious Diseases Research Center, Golestan University of Medical Sciences, Gorgan, Iran

*Corresponding author:
Ania Ahani Azari, Tel: 0098(17) 321353000, 00989111777377
Email: ania_783@yahoo.com

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**Abstract**

**Background:** Beta-lactam resistance is rising in gram-negative bacilli. The aim of this study was to assess the frequency of β-lactamase enzymes, including extended-spectrum β-lactamases (ESBLs), metallo-β-lactamase (MBL), and AmpC beta-lactamase in *Escherichia coli* isolated from urine samples of the patients referred to medical laboratories in Aliabad.

**Methods:** A total of 780 urine samples were collected from patients suspected of having urinary tract infection (UTI). In positive urine samples, *E. coli* strains were identified by biochemical tests. The antibiotic resistance pattern was determined by disk diffusion method and phenotypic confirmatory test was performed for detecting ESBLs, MBL and AmpC beta-lactamases producers.

**Results:** Out of 780 urine samples, 250 *E. coli* strains were isolated from the positive samples. The majority of the isolates (more than 90%) were resistant to ampicillin and amoxicillin. However, imipenem was an effective antibiotic among the isolates. The frequency of the ESBLs, MBL and AmpC beta-lactamases producers were determined to be 40%, 16.8%, and 30%, respectively.

**Conclusions:** In this study, the high frequency of MBL and AmpC beta-lactamases may suggest an increasing trend of resistance to cephalosporins and carbapenems. This could have a great impact on the management of UTI cases in and out of hospital. It seems that continuous monitoring is highly essential in detecting resistant cases.

**Keywords:** Beta-lactamases, *Escherichia coli*, Frequency, Urine, Antibiotic

## Background

Beta-lactam antibiotics have long been used to treat bacterial infections, but resistance to these antibiotics is now a concern. Overuse and misuse of these antibiotics have caused difficulties in treating many infectious diseases (1). Production of beta-lactamase enzymes among clinical isolates of *Escherichia coli* is one of these concerns. *E. coli* is a common cause of urinary tract infections (UTIs) and some nosocomial infections such as sepsis, wound infection, gastroenteritis and neonatal meningitis. Antibiotic resistance of *E. coli* has been reported worldwide and its resistance to antibiotics has raised a great deal of concern in both developing and developed countries. Therefore, identification of the antibiotic resistance pattern of *E. coli* is highly important from clinical perspective (2).

Production of beta-lactamase enzymes is the most common resistance mechanism in Gram-negative bacteria. This enzyme hydrolyzes beta-lactam drugs such as cephalosporins and penicillins. Over the past two decades, new beta-lactam antibiotics have been developed that are specifically resistant to beta-lactamase enzymes. However, gram-negative bacteria have developed new strategies to inactivate these novel antibiotics by producing new beta-lactamases such as AmpC, extended-spectrum β-lactamases (ESBLs), and metallo-β-lactamases (MBLs). AmpC β-lactamases that are called cephalosporinase are also partially capable of hydrolyzing other beta-lactams. These enzymes hydrolyze broad-spectrum cephalosporins such as ceftazidime, ceftriaxone, cefepime, and monobactams (i.e., aztreonam and cephamycin), but they are not inhibited by common inhibitors such as clavulanate (3).

ESBLs are enzymes that, in addition to resistance to penicillins, mediate the development of resistance to a wide range of cephalosporins (e.g., ceftazidime, cefotaxime, and ceftriaxone) and monobactam (e.g., aztreonam). Beta-lactamase inhibitors such as clavulanic acid and sulbactam tazobactam have inhibitory effects on the function of these enzymes (1). The rate of ESBL production by Enterobacteriaceae varies worldwide. Among Enterobacteriaceae, *E. coli* is the highest ESBL producers followed by *K. pneumoniae*. MBLs can hydrolyze a range of beta-lactam antibiotics, including penicillins, cephalosporins, carbapenems, and cephamycins, but they cannot hydrolyze aztreonam. In addition, their catalytic activity is not inhibited by beta-lactamase inhibitors. MBLs commonly exist in *Pseudomonas aeruginosa* and *Acinetobacter* species but have recently been increasing in members of the Enterobacteriaceae. Although production of MBLs is a therapeutic challenge, little is known about their frequency (4).

Since UTI treatment is mostly experimental and is usually based on the frequency of resistant cases, epidemiological studies on the pattern of resistance can help us to better treat UTIs as outpatient cases (5). In addition, there is little information available about the frequency of different types of beta-lactamase enzymes in clinical isolates in Iran. Therefore, the present study was conducted to determine antibiotic resistance pattern and frequency of different types of beta-lactamase enzymes (ESBLs, AmpC, and MBLs) in *E. coli* strains isolated from urine samples in Aliabad, Golestan province in the north-east of Iran.

## Materials and Methods

### Sampling and Identification of Isolates

This was a cross-sectional study on urine samples of the patients referred to medical laboratories of Aliabad from March to June 2017. Midstream urine samples were collected in sterile containers and evaluated for the presence of leucocytes and/or bacteriuria. It should be noted that written informed consent was obtained from all patients. The patients' data were recorded anonymously and coded. Urine samples were cultured on blood agar and Eosin Methylene Blue media (purchased from Himedia Company, India) using standard wire loop (0.001 mL). After incubation at 35°C for 18-24 hours, all cultures showing a growth of ≥$10^5$ CFU/mL were considered positive for UTI and included in the study. Biochemical tests were performed on pure colonies for the identification of the isolates. Gram-negative bacteria were identified by gram stains and a series of biochemical tests including triple sugar iron agar, indole, Simon's citrate agar, lysine iron agar, urea, methyl red, Voges–Proskauer, and motility (6).

### Antibiotic Susceptibility Test

Antibiotic sensitivity pattern of isolates to commonly used antibiotics for treating UTI was determined by the Kirby Bauer disk diffusion method on Mueller-Hinton agar. Antimicrobial susceptibility tests of the isolates were performed according to the Clinical and Laboratory Standards Institute (CLSI) (7) guidelines by the Kirby Bauer disk diffusion method on Mueller-Hinton agar. *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were used for quality control as recommended by the CLSI. The plates were incubated at 35°C for 18 hours. The results were interpreted after measuring the zone of inhibition and comparing it with the standards. The susceptibility of the test isolate to each antibiotic was interpreted as sensitive (S), intermediate resistant (I) or resistant (R) by measuring the zone diameter of inhibition. The antibiotics disks used in this study were purchased from MAST Company.

### Detection of Extended-Spectrum Beta-Lactamases

The isolates showing resistance to ceftazidime or cefotaxime were screened by double disk-diffusion test for ESBL production. The zones of inhibition of each isolate were examined on Mueller-Hinton agar plates containing the inoculum with the disks containing 30 µg of ceftazidime and cefotaxime alone and in combination with 10 µg of clavulanic acid, respectively. If the zone of inhibition surrounding at least one combination disk was 5 mm larger than that produced by the corresponding antimicrobial disk without clavulanic acid, the isolate was considered an ESBL producer (7,8).

### Detection of AmpC β-Lactamases

The isolates showing resistance to cefoxitin (30 µg) were screened for AmpC β-lactamases production by boronic acid double disk-diffusion test. To prepare disks containing boronic acid, 120 mg of phenylboronic acid (benzeneboronic acid and Sigma-Aldrich) was dissolved in 3 mL of dimethyl sulfoxide. Then, 3 mL of sterile distilled water was added to this solution. Afterwards, 20 µL of the stock solution was dispensed onto cefoxitin disk (30 µg). Disks were allowed to dry for 30 minutes and used immediately. A 0.5 McFarland inoculum was swabbed on Muller-Hinton agar plates. Cefoxitin (30 µg) and cefoxitin + boronic acid (30/400 µg) disks were placed on Muller-Hinton agar plates. After incubation for 24 hours at 37°C, if the diameter of the inhibition zone surrounding the cefoxitin + boronic acid disk was 5 mm greater than the diameter of the inhibition zone around the cefoxitin disk alone, the AmpC production was considered positive (10).

### Detection of Metallo β-Lactamases

The isolates showing resistance to imipenem (10 µg) were screened for the presence of MBL by the IMP-EDTA double disk-diffusion test (DDDT). To prepare Disk containing EDTA, 186.1 g of EDTA (ethylene diamine tetra acetic acid) was dissolved in 1000 mL of distilled water; pH was adjusted to 8.0 using NaOH and sterilization was done by autoclaving. Then, 10 µL of stock solution was added to imipenem disk (10 µg) and allowed to dry for 30 minutes and used immediately. A 0.5 McFarland culture was swabbed on Muller-Hinton agar plates and imipenem (10 µg) and imipenem + EDTA (10 µg/750 µg) disks were placed on agar surface and incubated for 24 hours at 37°C. MBL production was considered positive if the diameter of the inhibition zone around the imipenem + EDTA disk was 5 mm greater than the diameter of the inhibition zone surrounding the imipenem disk alone (11).

## Results

### The Results of Sampling and Identification of Isolates

A total of 780 urine samples were collected from March to June 2017. Among them, 378 samples (49.61%) with colony count ≥$10^5$ CFU/mL were considered positive for UTIs. Additionally, 270 (71.42%) samples were obtained from female patients and 108 (28.57%) samples from male patients. Some personal and health-related information of the patients is given in Table 1. The mean age of the patients was 45 years (SD=±20.21). Of a total of 378 samples, 250 samples (66.13%) were positive for *E. coli*.

**Table 1.** Summary of Patient Demographics

| Characteristics | Number | Percent | Total number |
|---|---|---|---|
| Male | 108 | 28.57 | 378 |
| Female | 270 | 71.42 | 378 |
| Pregnancy state | 159 | 58.8 | 270 |
| UTI history | 106 | 28 | 378 |
| Diabetes | 53 | 14 | 378 |
| Symptoms of prostatitis | 17 | 15.74 | 108 |
| History of antibiotic use in the past year | 90 | 23.8 | 378 |
| Marital status | 306 | 80.95 | 378 |
| Median age | 45 | - | |

### The Results of Antibiotic Susceptibility Test

Resistance pattern of the *E. coli* isolates to 12 antimicrobial agents are shown in Table 2. The majority of isolates showed a high degree of resistance to ampicillin (95.2%) followed by amoxicillin (92.8%) and tetracycline (70%). In this study, the majority of the samples were sensitive to imipenem (74%) and gentamicin (64%). Additionally, 73% of the isolates exhibited a multidrug resistance phenotype.

### The Results of Detection of Extended-Spectrum β-Lactamases

A total of 109 (43.6%) *E. coli* isolates, which were resistant to ceftazidime and cefotaxime, were examined for possibility of positive ESBLs by combined disk assay. Among 109 screened isolates, 100 (91.74%) isolates were detected as ESBLs producers.

### The Results of Detection of AmpC β-Lactamases

Out of the 80 isolates that were resistant to cefoxitin, 75 isolates (93.75%) were positive for AmpC production. This is done by its respective phenotypic confirmatory test using combined disc method. Fifty-two isolates (20.8%) were positive for both ESBL and AmpC.

### The Results of Detection of Metallo β-Lactamases

The imipenem resistant isolates were selected for the detection of MBL production. It was revealed that 42 (84%) of the imipenem-resistant isolates were MBL positive. Among them, 12 isolates (4.8%) were positive for both ESBL and MBL. The Frequency of ESBLs, AmpC, and MBL production within the selected *E. coli*

**Table 2**. Pattern of Resistance to 12 Antimicrobial Agents Among 250 *Escherichia coli* Isolate

| | Resistant, No. (%) | Intermediate, No. (%) | Sensitive, No. (%) |
|---|---|---|---|
| Imipenem (10 µg) | 50 (20) | 15 (6) | 185 (74) |
| Ciprofloxacin (5 µg) | 113 (45.2) | 20 (8) | 117 (46.8) |
| Gentamicin (10 µg) | 63 (25.2) | 17 (6.8) | 160 (64) |
| Cefotaxime (30 µg) | 109 (43.6) | 29 (4.8) | 121 (48.4) |
| ceftazidime (30 µg) | 109 (43.6) | 15 (6) | 135 (54) |
| Cefoxitin (30 µg) | 80 (32) | 30 (12) | 145 (58) |
| Co-trimoxazole (1.25 µg) | 163 (65.2) | 7 (2.8) | 80 (32) |
| Tetracycline (30 µg) | 175 (70) | 5 (2) | 70 (28) |
| Amikacin (30 µg) | 73 (29.2) | 22 (8.8) | 155 (62) |
| Nalidixic acid (30 µg) | 195 (78) | 10 (4) | 45 (18) |
| Amoxicillin (30 µg) | 232 (92.8) | 0 (0) | 18 (7.2) |
| Ampicillin (10 µg) | 238 (95.2) | 0 (0) | 12 (4.8) |

isolates is presented in Table 3. The positive isolates for all three enzymes were 100% resistant to ampicillin and amoxicillin. In addition, MBL-positive isolates were 100% resistant to tetracycline.

## Discussion

The present study was performed to determine antibiotic resistance pattern and frequency of different types of beta-lactamase enzymes (ESBLs, AmpC, and MBLs) in *E. coli* strains isolated from urine samples in Aliabad, Golestan province in the north-east of Iran. Based on the results, 66.13% of the urine samples were positive for *E. coli*. The majority of isolates showed a high degree of resistance to ampicillin, amoxicillin, and tetracycline that was consistent with similar studies in the past (12-17). The highest susceptibility rate of the bacterium was observed for imipenem followed by gentamicin. Susceptibility to imipenem was in line with findings from some other studies in Iran (12-14). However, it seems that susceptibility to gentamicin is not a consistent finding (17-21).

The prevalence of ESBL-producing *E. coli* varies in different regions. It could be as low as 1.5% and 5% in Denmark and Canada, and as high as 36.7% and 69% in Turkey and India (22). In Iranian studies, the frequency of ESBLs varies between 27% in Kermanshah (western part of Iran) and 97.56% in Tabriz (19,23); however, it was 40% among the *E. coli* isolates in our study.

Previous studies have shown that the prevalence of AmpC-producing *E. coli* differs across different geographical regions. It varies from 5% in Zahedan (south-eastern Iran) to 34% in India (South Bangalore) (24,25). In our study, the frequency of AmpC production among *E. coli* isolates was 30%, which is higher than similar studies conducted in Iran (24,26,27). The prevalence of MBL-producing *E. coli* in Iran varies between 0.3% in Isfahan (central part of Iran) and 3.12% in Qom (south of Tehran) (28,29), but it was 16.85 in our study.

Therefore, the results of our study showed the *E. coli* isolates had the highest resistance rate to ampicillin and amoxicillin and the resistance rate to imipenem was 26%, which was very high compared to the results from other studies (19,20,30). The frequency of ESBLs in this study was 40%, which is similar to the results of other studies in this field, but the frequency of AmpC and MBL enzymes in this study is significantly different from the frequency of these enzymes reported by other researchers. Therefore, the knowledge of these organisms and their detection are important in controlling them and will help physicians to choose appropriate treatment. Currently, carbapenems are the most sensitive and reliable treatment options for infections caused by ESBL, AmpC and MBL producing isolates. However, the irrational use of carbapenems may lead to resistant organisms.

Finally, antibiogram testing prior to antibiotic prescription by physicians, rational use of antibiotics and avoiding self-medication are inevitable necessities.

**Table 3.** Frequency of ESBLs, AmpC, and MBL Production Among Total and Selected *E. coli* Isolates

| Enzymes | Number (%) of the Total Isolates | Number (%) of the Selected Isolates |
|---|---|---|
| ESBLs | 250 (40) | 109 (43.6) |
| AmpC | 250 (30) | 75 (93.75) |
| MBL | 250 (16.8) | 42 (84) |
| ESBLs & AmpC | 250 (20.8) | 52 (69.3) |
| ESBLs & MBL | 250 (4.8) | 12 (28.57) |

## Conclusions

In our study, the high prevalence of AmpC and MBL producing *E. coli* isolates may indicate an increasing trend of resistance to carbapenem and cephalosporins. This can have major impact on the management of UTI cases in and out of hospital. Therefore, restricting the use of carbapenem and third-generation cephalosporins, along with application of infection control measures, is the most effective means of controlling and reducing the spread of ESBLs, AmpC, and MBL isolates.

### Conflict of Interests Disclosures

No competing interest was declared by any of the authors.

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