



The Prevalence of CTX-M Beta-Lactamase Gene in Urinary Tract Infections in Patients Referring to Bonab City Hospitals

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Article Information

Article Subject:

Molecular Epidemiology



10.30699/ijmm.13.4.302

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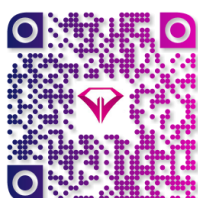
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Abstract

Background and Aims: Urinary Tract Infection (UTI) is one of the most common bacterial infections, and several studies have reported the prevalence of UTI caused by ESBL-producing *E. coli* strains in different cities of Iran. The aim of this study was to evaluate antibiotic resistant UTI factors in outpatients and determine the prevalence of *ctx-m* gene in ESBL-producing *E. coli* strains of Bonab (East Azerbaijan, Iran).

Materials and Methods: In 2015, 266 clinical isolates of *E. coli* were collected from clinical laboratories in Bonab. Phenotypic screening and confirmation tests for extended-spectrum beta-lactamases (ESBLs) were carried out using disk diffusion (Kirby Bauer) method. All of the ESBL producing isolates were tested by PCR using specific primers. A total of 10 PCR products were randomly selected and purified and subsequently sent for sequencing.

Results & Conclusion: The maximum resistance was seen regarding ampicillin (67.3 %) and the maximum sensitivity was seen regarding imipenem (92.5%). In this study, 45 % of isolates were multidrug resistance, which showed at least resistance for three antibiotics. Out of 154 isolates, 58 (37.7%) isolates were ESBL producers which 94.8% of isolates contained *ctx-m* gene. The prevalence of the *ctx-m* gene, as in other parts of Iran and the world, is high in Bonab. For the treatment of infections suspected to be an ESBL producer, appropriate antibiotics should be selected, and strains that have decreased their sensitivity to ceftazidime, cefotaxime, and cefetoxin should be considered for ESBL genes.

Keywords: *Escherichia coli*, UTI, ESBLs, CTX-M

Received: 2019/06/25

Accepted: 2019/09/11

Available online: 2020/01/1

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How to cite this article:

Mohammadzadeh R, Mohammadi-Gollou A, Salehabadi Y. The Prevalence of CTX-M Beta-Lactamase Gene in Urinary Tract Infections in patients referring to Bonab city Hospitals. Iran J Med Microbiol. 2019; 13 (4) :302-309

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Introduction

Urinary Tract Infection (UTI) is one of the most common bacterial infections and the second most common infection in patients referred to hospitals (1). Given the role of *Escherichia coli* in the infection of the urethra, it is important to know the pattern of susceptibility of this bacterium to various antibiotics. The use of broad-spectrum antibiotics is widely used in the treatment of bacterial infections today, and this has led to antibiotic resistance in this group of bacteria (2,3). The emergence of new antibiotics such as broad-spectrum cephalosporins and azetronam and their use in the treatment of bacterial infectious diseases has led to the expression of a new class of these enzymes called Extended Spectrum β lactamase (ESBL) (4).

Broad-spectrum antibiotics are transcribed by genes such as *ctx-m*, *tem*, *shv* and *per*. Due to the increasing number of different types of ESBL *bla* SHV, *bla* TEM (*bla* CTX-M) and their different effects on different antibiotics, accurate determination of different types of ESBL enzymes by molecular methods such as PCR is necessary to identify regional resistance. (5) Since there is no comprehensive research on this subject in Bonab city so far, in this study we investigated the drug resistance of ESBL-producing *Escherichia coli* strains and *ctx-m* gene in urinary tract isolates obtained from patients referred. We studied hospitals in Bonab city of East Azerbaijan province.

Materials and Methods

A total of 266 positive urine cultures of *E. coli* from hospital labs were collected and studied in 2015. Criteria for data entry in this study were sample patient satisfaction and urine culture positive for *E. coli* and data exclusion criteria were patient dissatisfaction, urine culture positive for *E. coli* and recorded or incomplete data. Blood agar (Difco, USA) was used as a primary medium for bacterial detection by API kit (Biomerieux France) and antibiogram antibacterial test. After bacterial inoculation in eosin methylene blue agar medium (Merck, Germany), MR / VP medium (Oxoid, UK) and SIM agar medium (Merck, Germany) to investigate bacterial motility and molar Hinton broth medium (Merck, Germany) Bacteria were used for antibiotic testing as well as the Mulherton

Agar medium (Merck, Germany) for antibiotic testing.

Screening tests for selected strains resistant to selected cephalosporins and other antibiotics were performed by disk diffusion method and the diameter of bacterial growth halo was measured. Finally, using Rosco's CLSI tables, the bacteria were divided into three groups of susceptible, intermediate, and resistant, and the results of each isolate were recorded for all discs used. Antimicrobial susceptibility to amikacin and ciprofloxacin were also determined. Antimicrobial susceptibility testing of hybrid discs was performed for confirmation of broad-spectrum beta-lactamase producing strains by disk diffusion method.

E. coli strain (ATCC 25922) were used as a negative control for antibiogram tests and *Klebsiella pneumoniae* strain (ATCC 7881) were used as positive control.

DNA extraction from the samples was accomplished by boiling and their concentration was measured by spectroscopy and DNA concentration determination. DNA extraction from The ESBL positive strains were performed amplified by PCR executed using specific primers (*Ctx* -F: 5'-TTTGCGATGTGCAGTACCAG-3') and (*Ctx* -R: 5'-GATATCGTTGGTGGTGCCAT-3') genes. *Ctx*-M was amplified and electrophoresis on agarose gel was used to evaluate the results of various experiments, including DNA extraction and PCR.

For statistical analysis of the relationship between the data obtained in different stages of this study, the data were entered into SPSS 21 (SPSS Inc., Chicago, Ill., USA) and the significance of the data was analyzed by Chi-square test.

Results and Discussion

A total of 266 specimens of suspected urine cultures of *E. coli* were collected from outpatients referred to hospitals. Bacterial isolates were cultured on eosin methylene blue agar medium, Triple Sugar Iron agar medium (TSI), Simon citrate agar, SIM and MR / VP. Acid-acidity in the TSI tube, citrate test negative, indole production, motility and H₂S production were confirmed for culture.

According to the statistical analysis, 99.26% of the isolates were identified as *E.coli* and only two

isolates were identified as *Enterobacter* and *Serachia*. The results of these kits indicate that in 99.26% of the cases the results are consistent with classical biochemical tests.

The isolates were evaluated for susceptibility to antibiotics ampicillin, ciprofloxacin, ceftraxation, imipim, amikacin, cefipim, azotrenam, cefotaxime and ceftazidime.

The maximum and minimum resistance for each of antibiotics were seen for ampicillin (67.3%) and imipim (5.3%) respectively. Also, the highest antibiotic susceptibility among the isolates was related to imipim (92.5%). Also, 45% of strains isolated from *E. coli* showed resistance to more than three antibiotics.

During the screening test out of 154 samples out of 264 confirmed *E. coli*, 112 were selected according to CLSI criteria and 42 others were selected according to resistance to different antibiotic groups. In the final test (combined disk), 58 *E. coli* strains (37.7%) out of 154 strains were

reported as ESBL producing bacteria. In addition, a total of 58 ESBL producers were selected in accordance with CLSI criteria.

The *ctx-m* gene as one of the ESBL-producing genes was investigated in this study. Of the 264 *Escherichia coli* samples, 68 (25.5%) had *ctx-m* gene, and of the 58 ESBL positive, 55 (94.8%) were *ctx-m* positive. The *ctx-m* gene had a 544 bp region. It should be noted that in some isolates lacking ESBL, the *ctx-m* gene was also identified ([Figure 1](#)).

Ampicillin resistance was very high (67.3%) among the studied isolates, which was in agreement with the results of other studies in Iran and elsewhere in the world. The frequency of resistance of *E. coli* isolates to ceferiaxone, cefotaxime and ceftazidime were 50.8%, 48.5% and 30.5%, respectively ([6-8](#)). The least antibiotic resistance was observed in imipenem (5.3%) and the highest susceptibility was to this antibiotic. Consistent with the results of other studies in Iran and somewhere else in the world that reported high sensitivity ([9-11](#)).

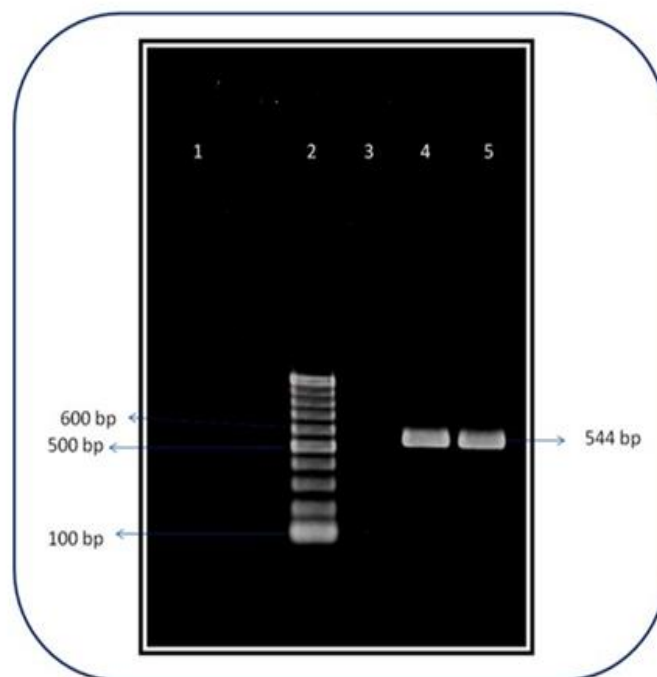


Figure 1. PCR product electrophoresis for the *ctx-m* gene. Lane 1: Blank, Lane 2: Marker (100bp DNA Ladder) Lane 3: Ctx-m negative control, Lane 4: Ctx-m positive control, Lane 5: PCR positive *ctx-m* gene.

Different prevalence of *ctx-m* gene in Iran and other countries can be due to cephalosporins and fluoroquinolones usage patterns in treatment of infections, transferability of *ctx-m* gene from animal reservoirs such as poultry and pets to humans, international travel, observance of control standards Nosocomial infection, primary origin of *ctx-m* gene from *Kluyvera*, transmission and movement of plasmids carrying *ctx-m* gene among different strains (12,13).

The prevalence of the *ctx-m* gene, as in other parts of Iran and the world, is high in Bonab. For the

treatment of infections suspected to be an ESBL producer, appropriate antibiotics should be selected, and strains that have decreased their sensitivity to ceftazidime, cefotaxime, and cefetoxin should be considered for ESBL genes.

Acknowledgments

The authors would like to express their gratitude and appreciation to the staff of the Diagnostic Laboratories of Bonab city Hospitals and the University of Maragheh Microbiology Laboratory.

Conflict of Interest

The authors declared no conflict of Interest.