Introduction

*Escherichia coli* strains belong to the *Enterobacteriaceae* family and are responsible for different types of infections, including urinary tract infections (UTIs), blood stream infections, wound infections, and respiratory tract infections.\(^1\) UTIs are amongst the most prevalent infections in community, and *E. coli* is responsible for 70%-90% of all causes.\(^2\)

Extended-spectrum beta-lactamase (ESBL)-producing *E. coli* is a health-care concern worldwide because these enzymes by hydrolyzing the structure of carbapenem and cephalosporines confer resistance to different types of antibiotics.\(^3\) These genes along with other drug-resistance genes are usually located at the transposable genetic elements (integrons and plasmids), therefore the dissemination of ESBL-producing isolates in hospitals can result in the spread of multi-drug resistance infections.\(^3,5\)

Based on structural features, ESBL genes are divided into four groups (A, B, C and D). Group A confers resistance against penicillin and cephalosporines, and the *blaCTXM*, *blaSHV*, and *blaTEM* genes are the most important genes in this group.\(^5,6\)

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**Prevalence of Extended-Spectrum Beta Lactamase *blaCTXM*, *blaSHV* and *blaTEM* Genes in *Escherichia coli* Strains Isolated from Clinical Samples of Patients With Urinary Tract Infections**

*Mozhgan Saeid*\(^1\), *Zahra Yazdanpour*\(^2\), *Farzad Khademi*\(^3\), *Hamid Vaez*\(^2*\)

\(^1\)School of Medicine, Zabol University of Medical Sciences, Iran

\(^2\)Department of Microbiology, School of Medicine, Zabol University of Medical Sciences, Iran

\(^3\)Department of Microbiology, School of Medicine, Ardabil University of Medical Sciences, Iran

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**Abstract**

**Introduction:** Urinary tract infections (UTIs) are the most prevalent infections in patients worldwide. *Escherichia coli* is considered as the most prevalent etiological agent of UTIs. The prevalence of extended-spectrum beta-lactamase (ESBL)-producing isolates is a health care concern worldwide. The aims of this study was to determine antibiotic resistance profiles and the prevalence of the *blaCTXM*, *blaSHV*, and *blaTEM* genes in *E. coli* isolated from clinical samples obtained from patients with UTIs.

**Methods:** From September 2019 to March 2021, a total of 1200 urine samples were collected and analyzed from patients suspected of having UTI referred to Amir-Al-Momenin hospital of Zabol, Sistan and Baluchistan province, south-east of Iran. Antibiotic resistance patterns and the prevalence of the *blaCTXM*, *blaSHV* and *blaTEM* genes were determined using the disk diffusion method and PCR, respectively.

**Results:** The isolates were mostly resistance against ampicillin and trimethoprim-sulfamethoxazole, with 66% and 54% of all isolates being resistant. Also, the isolates investigated were highly susceptible against meropenem and gentamycin (95%). The prevalence of the *blaCTX*, *blaSHV*, and *blaTEM* genes was 22%, 30%, and 24%, respectively.

**Conclusion:** Resistance against ampicillin and trimethoprim-sulfamethoxazole was high, therefore their prescription must be restricted. In addition, the expressions of the *blaCTXM*, *blaSHV*, and *blaTEM* genes were alarmingly high. In order to control the spread of infections by these isolates, constant monitoring of antibiotic resistance patterns is necessary.

**Keywords:** *E. coli*, Extended-spectrum beta-lactamase, Urinary tract infection, Antibiotic resistance
In recent years, infections caused by ESBL-producing *E. coli* have become more prevalent, causing serious health threats. For example, independent studies performed in USA, Europe, and Asia showed that 17-60% of isolated strains were ESBL positive.\(^7,8\)

Given the poor outcomes of patients infected by ESBL-producing *E. coli* and the necessity of identifying resistance mechanisms in each region for implementing successful infection prevention programs, the aim of this study was to investigate the antibiotic resistance profiles and the prevalence of ESBL-producing *E. coli* isolated from the clinical samples obtained from patients with UTIs.

**Methods**

**Sample Collection**

Totally, 1200 urine specimens were collected from patients suspected of having UTIs referred to Amir-Al-Momenin hospital, Zabol, Iran, from Sep 2019 to Mar 2021. Based on standard microbiology laboratory guidelines and procedures, midstream urine samples were collected and cultured on EMB agar (HiMedia, India) and Blood agar (HiMedia, India).\(^9,10\) The diagnosis of UTIs was made using previously described guidelines.\(^9,10\) In order to identify *E. coli* isolates, conventional microbiology tests, including gram-staining, oxidase, catalase, indole, lactose fermentation, methyl red, gas production, motility, urease, citrate, and Voges Proskauer, were used.\(^10\)

**Antibiotic Susceptibility Testing**

Kirby-Bauer’s disk diffusion method and Clinical and Laboratory Standardization Institute’s guidelines were used to evaluate antibiotic resistance patterns.\(^11\) The antibiotics used (Padtan Teb, Iran) were as follows; trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 µg), ampicillin (AMP, 10 µg), cephalothin (CF, 30 µg), meropenem (MEM, 10 µg), ceftriaxone (CRO, 30 µg), imipenem (IMP, 10 µg), ofloxacin (OFX, 5 µg), cefotaxime (CTX, 30 µg), cefazidime (CAZ, 30 µg), amikacin (AMK, 30 µg), and gentamicin (GM, 10 µg). *E. coli* 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality controls.

**Detection of ESBL-Producing Isolates**

Phenotypic detection of the *blaCTXM*, *blaSHV*, and *blaTEM* enzymes was performed according to previously described procedures using cefotaxime and cefazidime disks alone and in combination with clavulanic acid.\(^12\)

Genomic DNAs of the isolates collected were extracted by the boiling method.\(^13\) Briefly, cefotaxime or cefazidime non-susceptible isolates were cultured on blood agar at 37°C. Then the suspension was prepared by dissolving three colonies of *E. coli* in 300 µL of sterile distilled water. The suspension was heated at 100°C for 10 minutes. Finally, after cooling at room temperature and centrifugation at 1200 g for 20 minutes, the supernatant was used for PCR. Oligonucleotide primers used in this study have been provided in Table 1.\(^14,15\) Ampliqon (Denmark) ready to use master mix was used for the detection of ESBL genes (*blaCTXM*, *blaSHV*, and *blaTEM*). The PCR reaction (final volume: 20 µL) contained 14 µL ready to use master mix, 4 µL extracted DNAs, and 1 µL of each of forward and reverse primers (100 pmol). The following PCR program was used; denaturation at 95°C for seven minutes, 30 cycles of denaturation at 94°C for 60 seconds, annealing at 55°C, 56°C, and 54°C (for the *blaCTXM*, *blaSHV*, and *blaTEM* genes, respectively) for 50 seconds, extension at 72°C for 2 minutes, and a final extension at 72°C for 10 minutes. Agarose gel electrophoresis was used to separate PCR products, and after staining by SYBR Safe (Thermo Fisher Scientific Inc.), separated bands were visualized by gel documentation system.

**Statistical Analysis**

All data were analyzed by SPSS (V16, Chicago) and the chi-square and Fisher’s exact tests. Results were considered statistically significant if P value was ≤ 0.05.

**Results**

Out of 1200 urine samples analyzed; 100 non-duplicate *E. coli* isolates were identified. Of these, 32 (32%) were collected from male and 68 (68%) from female patients. The isolates were mostly resistance against ampicillin (n = 66; 66%), trimethoprim- sulfamethoxazole (n = 54; 54%), and cephalothin (n = 40; 40%) (Table 2). Compared with ESBL negative isolates, the prevalence of resistance to other antibiotics among ESBL positive isolates was significantly high (Table 2). In addition, 37% of the isolates were ESBL positive. The expression of the *blaCTXM*, *blaSHV*, and *blaTEM* genes was detected in 22%, 30%, and 24% of the isolates, respectively (Figures 1, 2, and 3).

**Discussion**

The increasing prevalence of antibiotic resistance in gram-negative bacilli, such as *E. coli*, is one of the most important problems in healthcare systems worldwide.\(^16\) In this study, the antibiotic resistance patterns of 100 *E. coli* isolated from UTI samples were investigated. Based on the findings of this study, isolates were mostly resistance against ampicillin (n = 66; 66%), trimethoprim-

**Table 1. Sequence of Oligonucleotide Primers Used in This Study**

<table>
<thead>
<tr>
<th>Genes</th>
<th>Sequence (5'-3')</th>
<th>Amplicon Length</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>blaTEM</em></td>
<td>F: 5’ ATACGCAATAAACCCACCC-3’, R: 5’ CCCCAGAAGACCTTTC-3’</td>
<td>516</td>
<td>15</td>
</tr>
<tr>
<td><em>blaSHV</em></td>
<td>F: 5’TCTCTCCAGATAAGGACATCC-3’, R: 5’ CGTTTCCCGCTATGAC-3’</td>
<td>866</td>
<td>14</td>
</tr>
<tr>
<td><em>blaCTXM</em></td>
<td>F: 5’TCTTCCAGATAAGGACATCC-3’, R: 5’ CGTTTCCCGCTATGAC-3’</td>
<td>909</td>
<td>14</td>
</tr>
</tbody>
</table>
Sulfamethoxazole (n = 54; 54%), and cephalothin (n = 40; 40%) (Table 2). These findings are in agreement with other studies conducted in different parts of Iran. For example, in a study conducted by Jamali et al in the north of Iran, as well as in a study by Pourakbari et al in Qom, 84% & 94% and 61% & 80% of E. coli isolates were resistant against ampicillin and trimethoprim-sulfamethoxazole, respectively. 17,18 Compared with European countries, this rate of resistance is high. For instance, based the report of the European Antimicrobial Resistance Surveillance Network, resistance to ampicillin in Finland, Norway, Iceland, Denmark, Germany, and Austria varied between 35%-50%. 19

Aminoglycosides (amikacin and gentamicin) and carbapenems (meropenem and imipenem) are known as bottom-line antibiotics, which are usually being used to treat infections caused by resistant isolates. Our findings showed that the isolates were mostly susceptible to imipenem and meropenem (95%), which is in agreement with most studies in Iran’s provinces, reporting 95%-100% susceptibility to carbapenem; however, it is significantly higher than the report from Babol, north of Iran (56.1%). 17,18

Similar to our findings, in Europe, the frequency of resistance to carbapenems in many countries such as Spain, Sweden, French, and England was reported to be close to zero. In addition, resistance to aminoglycosides has been reported to be between 5% and 10%. Different factors can affect the prevalence of antibiotic resistance, including easy access to antibiotics, arbitrary use of antibiotics regardless of prescription, indiscriminate use of antibiotics in livestock and poultry farms, and non-

Table 2. Antibiotic Susceptibility Patterns of Isolates

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>ESBL + No. (%)</th>
<th>ESBL- No. (%)</th>
<th>Total No. (%)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>R: 37 (37)</td>
<td>S: 0 (0)</td>
<td>29 (29)</td>
<td>34 (34)</td>
</tr>
<tr>
<td></td>
<td>R: 32 (32)</td>
<td>S: 5 (5)</td>
<td>22 (22)</td>
<td>41 (41)</td>
</tr>
<tr>
<td></td>
<td>R: 27 (27)</td>
<td>S: 10 (10)</td>
<td>13 (13)</td>
<td>50 (50)</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>R: 2 (2)</td>
<td>S: 35 (35)</td>
<td>3 (3)</td>
<td>40 (40)</td>
</tr>
<tr>
<td></td>
<td>R: 2 (2)</td>
<td>S: 35 (35)</td>
<td>3 (3)</td>
<td>40 (40)</td>
</tr>
<tr>
<td></td>
<td>R: 6 (6)</td>
<td>S: 35 (35)</td>
<td>3 (3)</td>
<td>40 (40)</td>
</tr>
<tr>
<td></td>
<td>R: 35 (35)</td>
<td>S: 2 (2)</td>
<td>2 (2)</td>
<td>5 (5)</td>
</tr>
<tr>
<td></td>
<td>R: 35 (35)</td>
<td>S: 3 (3)</td>
<td>3 (3)</td>
<td>5 (5)</td>
</tr>
<tr>
<td></td>
<td>R: 6 (6)</td>
<td>S: 31 (31)</td>
<td>6 (6)</td>
<td>12 (12)</td>
</tr>
<tr>
<td></td>
<td>R: 25 (25)</td>
<td>S: 6 (6)</td>
<td>2 (2)</td>
<td>5 (5)</td>
</tr>
<tr>
<td></td>
<td>R: 25 (25)</td>
<td>S: 6 (6)</td>
<td>2 (2)</td>
<td>5 (5)</td>
</tr>
<tr>
<td></td>
<td>R: 5 (5)</td>
<td>S: 5 (5)</td>
<td>5 (5)</td>
<td>3 (3)</td>
</tr>
<tr>
<td></td>
<td>R: 5 (5)</td>
<td>S: 5 (5)</td>
<td>5 (5)</td>
<td>2 (2)</td>
</tr>
<tr>
<td></td>
<td>R: 5 (5)</td>
<td>S: 5 (5)</td>
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<td>2 (2)</td>
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<tr>
<td></td>
<td>R: 5 (5)</td>
<td>S: 5 (5)</td>
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<tr>
<td></td>
<td>R: 5 (5)</td>
<td>S: 5 (5)</td>
<td>5 (5)</td>
<td>2 (2)</td>
</tr>
<tr>
<td></td>
<td>R: 5 (5)</td>
<td>S: 5 (5)</td>
<td>5 (5)</td>
<td>2 (2)</td>
</tr>
<tr>
<td></td>
<td>R: 5 (5)</td>
<td>S: 5 (5)</td>
<td>5 (5)</td>
<td>2 (2)</td>
</tr>
</tbody>
</table>

R, resistance; S, susceptible.

Figure 1. Electrophoresis Image of blaSHV PCR, Line 1: 100bp DNA Ladder, Line 2: positive control, Lines 3-11: positive clinical isolates, Line 12: negative control

Figure 2. Electrophoresis Image of blaCTXM PCR, Line 1: 100bp DNA Ladder, Line 2: positive control, Lines 3-4: positive clinical isolates, Line 5: negative control
Continuous monitoring of the antibiotic resistance pattern and the mechanisms causing antibiotic resistance should be considered. The alarming prevalence of the blaCTXM, blaSHV, and blaTEM genes and in order to prevent the spread of these drug-resistant infections, constant monitoring of antibiotic resistance patterns and mechanisms should be considered.

Conclusion
Based on the results of this study, resistance to some antibiotics such as ampicillin, trimethoprim-sulfamethoxazole, and cephalothin was high, suggesting that their use should be restricted. Also, due to the alarming prevalence of the blaCTXM, blaSHV, and blaTEM genes and in order to prevent the spread of these drug-resistant infections, constant monitoring of antibiotic resistance patterns and mechanisms should be considered.

Acknowledgements
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Authors’ contributions
Study design: HV and MS. Data collection: ZY, MS, and HV. Interpretation of data: HV and FK. Manuscript preparation: HV and FK.

Competing interests
The authors declare no conflict of interest.

Ethical Approval
This study was approved by ethics committee of Zabol University of Medical sciences (IR.ZBMU.REC.1400.026).

References


