Detection of extended-spectrum beta-lactamase genes among Escherichia coli isolates from urinary tract infection in Mashhad

Maryam Hafiz¹, Gholamreza Hashemi Tabar¹,*, Mehrnaz Rad¹

¹Department of Pathobiology, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

Abstract
Urinary tract infections (UTIs) are known as one of the most important infections around the world, and Escherichia coli is the most important cause of UTI. Also, the empiric treatment and misusing of antimicrobial agents has led to increasing multi-drug resistance around the world which is a worldwide concern. Extended-spectrum beta-lactamase (ESBLs) is an enzyme group that is produced by the Enterobacteriaceae family. The three main ESBLs enzyme are as follow: blaCTX-M, blaTEM, and blaSHV, additionally, there are several types of each of them by the same mechanism. This study was conducted to evaluate the prevalence of ESBL genes among E. coli isolated from UTI patients. A total of 105 isolates were collected from UTI patients at two hospitals in Mashhad from 2017 to 2019. Bacterial identification was performed by standard microbiologic methods. The assessment of antimicrobial susceptibility was accomplished by the disk diffusion method. The presence of ESBL genes was investigated by multiplex-PCR. The prevalence of UTI, among females, was identified more than males. Furthermore, the blaTEM and blaCTX-M genes were detected in all isolates, but only six isolates (5.7%) were harboring blaSHV. The considerable role of E. coli in UTI infection, as well as the presence of ESBL genes in E. coli strains, emphasize the need for surveillance of antimicrobial therapy to prevent the extension of resistance among clinical strains.

Keywords: Escherichia coli, ESBL, Urinary tract infection, Multiplex-PCR

1. Introduction
Urinary tract infections (UTIs) happen through community-acquired or nosocomial-acquired, which are one of the most prevalent types of human infection [1, 2]. Escherichia coli is known as the most important pathogen in UTIs, among both outpatient and inpatient [3, 4]. On the other hand, the empiric of treatment and misusing of antimicrobial agents such as, beta-lactams, beta-lactamase inhibitor, fluoroquinolones, and carbapenems, has led to increasing multi-drug resistance around the world which is a worldwide concern [5, 6]. Extended-spectrum beta-lactamase (ESBLs) is an enzyme group that is produced by the Enterobacteriaceae family, which are mostly isolated from inpatient cases [7]. The ESBL positive bacteria are able to inactivate several antimicrobial agents including an oxyimino-group such as cephalosporins (e.g. ceftazidime, and ceftriaxone), monobactam (e.g. aztreonam), but not able to inactivate cephamycin, and carbapenems [7, 8]. The three main ESBL enzymes are blaCTX-M, blaTEM, and blaSHV [9, 10]. These enzymes are encoded generally by genes located on plasmids. Actually, the mutation in these genes (particularly blaTEM and blaSHV) led to the change in the form of the enzyme near its active site to increment the affinity and...
hydrolytic capability of beta-lactamase for oxymino compounds [11]. Also, some of them are encoding on transposons which could transmit between organisms [8]. The growing up of ESBL producing strains brings up some problems including the transmission of resistance to other organisms, refusing treatment, and increase in mortality rate. So, more surveillance is needed to identify the rate of the problem and help to select appropriate antimicrobial agents to treat the infection. This study was conducted to evaluate the prevalence of *bla*<sub>TEM</sub>, *bla*<sub>CTX-M</sub>, and *bla*<sub>SHV</sub> genes by multiplex polymerase chain reaction (M-PCR) among *E. coli* isolated from UTI patients.

### 2. Materials and Methods

#### 2.1 Sample's collection

A total of 105 *E. coli* isolates were collected from UTI patients at Ghaem and Emamreza hospitals in Mashhad from 2017 to 2019. Also, the basic information of patients was gathered, including age and gender, UTI history, hospital visit reports, and reports of taken antimicrobial agents from patients. Isolates cultured on selective-differential media, MacConkey agar (MAC), and Eosin Methylene Blue agar (EMB), were incubated overnight at 37°C. Then, microscopically examination was done by Gram staining from lactose-positive colonies. Likewise, biochemical profiling, such as Oxidase/Catalase activity, Motility, Indole, Urease, Triple Sugar Iron agar (TSI), and Methyl Red-Voges Proskauer test (MR-VP) has been studied.

#### 2.2 Antimicrobial susceptibility test

The assessment of antimicrobial susceptibility was accomplished by the disk diffusion method recommended by the Clinical and Laboratory Standards Institute (CLSI, 2020) [13][12], against 15 antibiotic disks (Poddanteb, Iran) as follow: ceftazidime (30 μg), imipenem (10 μg), amikacin (30 μg), nalidixic acid (30 μg), ampicillin (10 μg), ciprofloxacin (5 μg), cefixime (5 μg), cefazoline (30 μg), ceftriaxone (30 μg), meropenem (10 μg), gentamicin (10 μg), nitrofurantoin (300 μg), ceftaxime (30 μg) and co-trimoxazole (1.25/23.75 μg). *E. coli* ATCC 25922 was used as a positive control for antimicrobial susceptibility tests.

#### 2.3 PCR amplification

DNA extraction was done by the boiling method as described earlier by Nazari et al. [13]. The prepared DNA samples were assessed for prevalence of *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, and *bla*<sub>CTX-M</sub> genes by M-PCR by using the MJ mini thermal cycler (Bio-Rad, Hercules, CA, USA) [14]. The PCR reaction was conducted in 25 volumes including 12.5 μl of PCR 2X MasterMix (Parstous company, Iran) containing Taq DNA Polymerase, reaction buffer, dNTPs mixture, a protein stabilizer, and the convenience for use was optimized by adding

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Primer sequence (5'-3')</th>
<th>Amplicon size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>bla</em>&lt;sub&gt;TEM&lt;/sub&gt;164.SE</td>
<td>TCG CCG CAT ACA CTA TTC TCA GAA TGA</td>
<td>445</td>
<td>14</td>
</tr>
<tr>
<td><em>bla</em>&lt;sub&gt;TEM&lt;/sub&gt;164.AS</td>
<td>ACG CTC ACC GGC TCC AGA TTT AT</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>bla</em>&lt;sub&gt;SHV&lt;/sub&gt;.SE</td>
<td>ATG CGT TAT ATT CGC CTG TG</td>
<td>724</td>
<td></td>
</tr>
<tr>
<td><em>bla</em>&lt;sub&gt;SHV&lt;/sub&gt;.AS</td>
<td>TGC TTT GTT ATT CGG GCC AA</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>bla</em>&lt;sub&gt;CTX&lt;/sub&gt;-M. U1</td>
<td>ATG TGC AGC ACC AGT AAA GTG ATG GC</td>
<td>593</td>
<td></td>
</tr>
<tr>
<td><em>bla</em>&lt;sub&gt;CTX&lt;/sub&gt;-M. U2</td>
<td>TGG GTA AAG TAA GTG ACC AGA ATC AGC GG</td>
<td></td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Age groups</th>
<th>&lt;10</th>
<th>11-20</th>
<th>21-30</th>
<th>31-40</th>
<th>41-50</th>
<th>51-60</th>
<th>&gt;60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>8</td>
<td>3</td>
<td>9</td>
<td>5</td>
<td>5</td>
<td>10</td>
<td>16</td>
</tr>
<tr>
<td>Male</td>
<td>5</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>6</td>
<td>5</td>
<td>28</td>
</tr>
<tr>
<td>Total &lt;sup&gt;a&lt;/sup&gt;</td>
<td>13</td>
<td>3</td>
<td>11</td>
<td>8</td>
<td>11</td>
<td>15</td>
<td>44</td>
</tr>
</tbody>
</table>

<sup>a</sup> Results presented as number of cases.
sediment for electrophoresis and 2x solution of loading dye, 1 μl of each primer (10 pM), 2 μl of DNA template (100 ng/reaction) and 4.5 μl of Nuclease-free water. PCR conditions were as follows: initial denaturation at 95°C for 15 seconds; followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 61°C for 40 seconds, and extension at 72°C for 2 minutes with a final extension at 72°C for 10 minutes [15]. Electrophoresis of amplicons was performed using 1.5% agarose gels. The primers’ information is listed in Table 1. Also, E. coli ATCC 35218 and distilled water were used as the positive and negative control, respectively.

3. Results

3.1 Prevalence of UTI in different ages and genders

Based on the results, it was determined that the prevalence of UTI among females with 56 (53.3%) cases was more than males with 49 (46.7%) cases. Most isolates were recovered from patients with >60 ages, and the lowest rate was observed in 11-20 age groups. The information of patients’ age groups was listed in Table 2.

3.2 Antimicrobial susceptibility test

According to the results, amikacin (98%), meropenem (93%), nitrofurantoin (89%), and imipenem (88%) revealed the highest activity against isolates, respectively. All results are listed in Table 3.

3.3 PCR results

The analysis of PCR products on gel electrophoresis revealed that the most prevalent ESBLs among studied isolates were blaTEM, and blaCTX-M genes (100%). While only six (5.71%) isolates were carried the blaSHV gene.

4. Discussion

UTI is known as one of the most highlighted healthcare infections. Our finding notes that women have a higher rate of UTI than men. This fact confirms earlier findings of the stress UTI in females [16-19]. Also, the most prevalent was observed in the >60 age group, which was similar to previous surveys [20, 21]. However, this result was in contrast with other observations, which reported the highest prevalence in 2-10 and 20-29 age groups, respectively [17, 22]. Our results revealed the highest resistance rate for

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>CAZ</th>
<th>IMP</th>
<th>AMK</th>
<th>NAL</th>
<th>AMP</th>
<th>CIP</th>
<th>CIP</th>
<th>CFM</th>
<th>MEM</th>
<th>CFZ</th>
<th>CRO</th>
<th>GEN</th>
<th>NIT</th>
<th>FEP</th>
<th>CTX</th>
<th>SXT</th>
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<tbody>
<tr>
<td>Susceptible</td>
<td>54</td>
<td>(51.4)</td>
<td>(88.5)</td>
<td>(98.1)</td>
<td>(93.8)</td>
<td>(65.3)</td>
<td>(47.6)</td>
<td>(47.6)</td>
<td>(47.6)</td>
<td>(6.7)</td>
<td>(6.7)</td>
<td>(6.7)</td>
<td>(6.7)</td>
<td>(6.7)</td>
<td>(6.7)</td>
<td>(6.7)</td>
</tr>
<tr>
<td>Intermediate-resistant</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Resistant</td>
<td>51</td>
<td>12</td>
<td>2</td>
<td>34</td>
<td>87</td>
<td>55</td>
<td>50</td>
<td>7</td>
<td>61</td>
<td>0</td>
<td>87</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
</tbody>
</table>

Abbreviation: CAZ, ceftazidime; IMP, imipenem; AMK, amikacin; NAL, nalidixic acid; AMP, ampicillin; CIP, ciprofloxacin; CFM, cefixime; MEM, meropenem; CFZ, cefazolin; CRO, ceftriaxone; GEN, gentamicin; NIT, nitrofurantoin; FEP, cefepime; CTX, cefotaxime; SXT, trimethoprim/sulfamethoxazole
ampicillin (82.8%) and cotrimoxazole (70.4%) which was consistent with previous surveys [17, 23-25]. However, another study recorded a lower resistance rate for ampicillin and cotrimoxazole, 67% and 45%, respectively [26]. On the other hand, the highest susceptibility was observed against amikacin, meropenem, nitrofurantoin, and imipenem. While the lower susceptibility for amikacin (61%) and nitrofurantoin (72%) has been reported in an earlier survey [27]. However, our finding for nitrofurantoin susceptibility was close to other studies [17, 28]. Also, the susceptibility rate of isolates to imipenem and amikacin in the current study was in line with previous surveys [17, 23, 29].

According to earlier reports, the high rate of ESBL- E. coli was noted from UTI among both inpatient and outpatient, whereas it was more significant among inpatient [24, 30].

Generally, the highest presence of ESBLs was recorded for blaTEM and blaCTX-M, which was in line with earlier results [9, 31-34]. Although, some studies reported a lower rate of blaCTX-M, 28%, and 32%, respectively [35, 36]. This discrepancy can be explained by different geography and the increase of genes transferring during the time. On the other hand, blaSHV was detected in only six isolates. Our experiment about the prevalence of the blaSHV gene was close to previous survey [33]. According to these results, the blaSHV gene is not predominant as the two other genes. However, new studies revealed a high frequency of blaSHV that might increase in the future [11, 37]. Certainly, further tests such as confirmatory ESBL test and statistical analysis are required to determine the relationship between these genes and resistant phenotype.

The considerable role of E. coli in UTI infection, as well as the presence of ESBL genes in E. coli strains, emphasize the need for surveillance of antimicrobial therapy to prevent the extension of resistance among clinical strains.

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Author contributions
All authors contributed equally to each part of the manuscript and approved the final version.

Conflict of interests
The authors claim that there is no conflict of interest.

Ethical declarations
This study was in accordance with the declaration of Helsinki and ethical permission was sought from the institutional Ethics Committee of Ferdowsi University of Mashhad, Mashhad, Iran. However, because we only used leftovers from clinical specimens, the local ethics committee waived the need for informed consent.

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References


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