Determination of antibiotic resistance genes and virulence factors in *Escherichia coli* isolated from Turkish patients with urinary tract infection


[1]. Department of Genetics and Bioengineering, Faculty of Engineering and Natural Sciences, Gumushane University, Gümüşhane, Turkey.
[2]. Department of Biotechnology, Institute of Natural Sciences, Gumushane University, Gümüşhane, Turkey.
[3]. Department of Nutrition and Dietetics, Faculty of Health Sciences, Artvin Coruh University, Artvin, Turkey.
[4]. Department of Medical Microbiology, Faculty of Medicine, Recep Tayyip Erdoğan University, Rize, Turkey.

Abstract

**Introduction**: *Escherichia coli* ranks among the most common sources of urinary tract infections (UTI). Methods: Between November 2015 and August 2016, 90 isolates of *E. coli* were isolated from patients at Rize Education and Research Hospital in Turkey. Antibiotic susceptibility was determined for all isolates using the Kirby–Bauer disk diffusion method. These *E. coli* isolates were also screened for virulence genes, β-lactamase coding genes, quinolone resistance genes, and class 1 integrons by PCR. Results: With respect to the antibiotic resistance profile, imipenem and meropenem were effective against 98% and 90% of isolates, respectively. A high percentage of the isolates showed resistance against β lactam/β lactamase inhibitor combinations, quinolones, and cephalosporins. PCR results revealed that 63% (57/90) of the strains carried class 1 integrons. In addition, a high predominance of extended-spectrum β-lactamases (ESBLs) was observed. The *qnrA*, *qnrB*, and *qnrS* genes were found in 24 (26.6%), 6 (6.6%), and 3 (3.3%), isolates, respectively. The most common virulence gene was *fim* (82.2%). The *afa*, *hly*, and *cnf1* genes were detected in 16.6%, 16.6%, and 3.3%, isolates, respectively. Moreover, we observed eleven different virulence patterns in the 90 *E. coli* isolates. The most prevalent pattern was *fim*, while *hly-fim, afa-aer-cnf-fim, aer-cnf, afa-aer*, and *afa-cnf-fim* patterns were less common. Conclusions: Most of the *E. coli* virulence genes investigated in this study were observed in *E. coli* isolates from UTI patients. Virulence genes are very important for the establishment and maintenance of infection.

Keywords: Quinolones. Virulence genes. UTI.

INTRODUCTION

*Escherichia coli* are among the most common etiological agents that cause urinary tract infections (UTI); this makes *E. coli* infection an important public health issue. Many virulence factors are responsible for the pathogenicity of *E. coli* strains. There are two main types of *E. coli* virulence factors; these include (i) virulence factors that are produced within the cell and released at the site of action, and (ii) virulence factors that are displayed on the surface of the cell.

The most important *E. coli* virulence factors are the surface virulence factors (adhesins). P fimbriae are encoded by *pap* genes and are the main adherence factors. S fimbrial adhesion factors, encoded by *sfa* genes, represent another type of virulence factor. A fimbrial adhesion factors in *E. coli* are encoded by *afa* genes. In addition, the main fimbrial subunit of type 1 fimbriae is encoded by *fimA* in *E. coli*. Toxins are another important type of virulence factor in *E. coli*. The α-hemolysin (HlyA) virulence factor, cytotoxic necrotizing factor, and aerobactin are encoded by the *hly, CNF1*, and *aer* genes, respectively.

The β-lactamases (enzymes that hydrolyze β-lactam antibiotics) are classified into four groups depending on their amino acid sequences: class A (e.g., KPC, CTX-M, and GES), class B (e.g., IMP, VIM, SPM, GMT, NDM, and SIM), class C (e.g., AmpC), and class D (e.g., OXA-type β-lactamase).
All four classes of β-lactamase have been identified in E. coli. Metallo-β-lactamases (MBLs) are disseminated worldwide and have been mainly identified in Enterobacteriaceae of the IMP and VIM types.

Quinolones are widely used to treat UTIs caused by E. coli. This extensive use of quinolones has led to increased resistance in E. coli. Target modification, and changes in membrane permeability can confer resistance to quinolones. Moreover, plasmid-mediated qnr (quinolone-resistance) genes can facilitate quinolone resistance, with the qnrA, qnrB, and qnrS groups comprising the major qnr determinants.

Integrons are mobile genetic elements that contribute to the spread of antibiotic resistance. Many gene cassettes emerged when class 1 integrons were first discovered in clinical strains. The role of integrons in promoting bacterial multidrug resistance is significant. A number of studies investigating the prevalence of integrons in E. coli isolates from UTI patients have reported a significant link between antimicrobial resistance and integrons.

The purpose of this study was to investigate the presence of virulence genes, β-lactamase coding genes, quinolone resistance genes, fosfomycin resistance genes, and class 1 integron gene cassettes in E. coli isolates from patients with UTI.

METHODS

A total of 90 E. coli isolates were investigated in this study. All strains were isolated at the Rize Education and Research Hospital in Turkey between November 2015 and August 2016. Urine samples were cultured on blood agar and Eosin Methylene Blue (EMB) agar, then incubated at 37°C for 18–24 h. Bacteria were identified using colony morphology and biochemical tests in urine cultures with high levels of viable bacteria (≥10⁵ CFU/mL). Antibiotic susceptibility of each isolate was determined by Kirby–Bauer disk diffusion and was based on the criteria recommended by the Clinical Laboratory Standards Institute (CLSI, 2014).

Genomic DNA was obtained from bacterial suspensions grown overnight in Luria Broth (LB) at 37°C. Bacterial suspensions were centrifuged. Pellets were resuspended in 500 μL of distilled water, then boiled in a water bath for 10 min. Boiled suspensions were centrifuged at 11,357 g for 5 min. Five hundred microlitres of each supernatant were used as a template for PCR assays.

All strains were isolated from adult patients with uncomplicated community-acquired UTIs. Ninety E. coli isolates were screened for genes encoding β-lactamases, quinolone resistance factors, fosfomycin resistance factors, and virulence factors via polymerase chain reaction (PCR). Primers for β-lactamase-encoding genes (bla<sub>IMP</sub>, bla<sub>CTX-M</sub>, bla<sub>NDM</sub>, bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-2</sub>, bla<sub>GES</sub>, bla<sub>SIM</sub>, bla<sub>AmpC</sub>, and bla<sub>SPM</sub>), quinolone resistance genes (qnrA, qnrB, and qnrS), fosfomycin resistance genes (fosA, fosC2, and fosA3), and virulence genes (pap, sfa, afa, hly, aer, cnf, and fim) were used in these experiments. All PCR results were analyzed by electrophoresis in 1% agarose containing 0.5 μg/mL ethidium bromide, followed by examination under UV light.

PCR was performed on all isolates to detect class 1 integron gene cassettes using the primers 5'-GGCATCCAAGCAAGCAAG-3' (5′CS) and 5'-AAGCAGACTTACCTGA-3' (3′CS). The PCR conditions were 3 min at 94°C for initial denaturation, followed by 34 cycles of 45 s at 94°C, 1 min at 55°C, and 3 min at 72°C, with a final extension at 72°C for 5 min.

RESULTS

Ninety E. coli isolates were investigated in this study. Of the 90 patients diagnosed with community-acquired UTIs, 62 (68.9%) were women and 28 (31.1%) were men. The extended-spectrum β-lactamase (ESBL) positivity rate was 18.9%. All 90 strains were isolated from urine samples. Results of antibiotic susceptibility test revealed that these isolates had low resistance rates for fosfomycin (2.7%), imipenem (3.2%), and meropenem (3.2%). However, resistance rates for ciprofloxacin (62.2%), trimethoprim sulfamethoxazole (75.6%), and ampicillin (61.1%) were high. Rates of resistance against amikacin, nitrofurantoin, ceftriaxone, ceftazidime, gentamycin, amoxicillin with clavulanic acid, aztreonam, cefazolin, and cepfepime were found to be 9.9%, 8.9%, 22.2%, 21.1%, 27.8%, 27.8%, 18.9%, 18.9%, and 20%, respectively.

More specifically, we found that fim was the most common virulence gene and was found in 74 isolates (82.2%). The afa and cnfI genes were detected in 16.6% of the isolates, and hly was found in only three (3.3%) of the 90 isolates. The sfa and pap genes were not detected. In addition, the aer gene was found in 33 (36.6%) of the isolates. PCR results revealed that 63% (57/90) of the strains carried class 1 integron gene cassettes. We also observed a high prevalence of ESBLs, with 52 strains (57%) carrying a CTX-M-2, and 52 isolates (57%) carrying a CTX-M-1 group β-lactamase. No other β-lactamase-encoding genes (bla<sub>IMP</sub>, bla<sub>VIM</sub>, bla<sub>NDM</sub>, bla<sub>GES</sub>, bla<sub>SIM</sub>, bla<sub>AmpC</sub>, or bla<sub>SPM</sub>) were identified. We also demonstrated that the qnrA, qnrB, and qnrS quinolone resistance genes—present on the plasmid—were present in 26.6% (24/90), 6.7% (6/90) and 3.3% (3/90) of the isolates, respectively. No fosfomycin resistance genes (fosA, fosC2, or fosA3) were found.

The prevalence of virulence factors differed among isolates that produced a class 1 integron, bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-2</sub>, qnrS, qnrA, and qnrB (Table 1). Class 1 integron and CTX-M harboring isolates were more commonly positive for fim than for other virulence factors.

Eleven different virulence factors were observed among the 90 E. coli isolates. The most common virulence factor was fim (n = 35 isolates; 8.9%); hly-fim, afa-aer-cnf-fim, aer-cnf, afa-aer, and afa-cnf-fim were less commonly observed. No virulence factor was detected in fourteen of the isolates (Table 2).

DISCUSSION

Urinary tract infections (UTIs) are a major public health problem worldwide. E. coli is the most prevalent etiologic agent of UTIs. The virulence of UTI inducing E. coli strains is due to their expression of virulence factors.

P fimbriae (pap), a fimbrial adhesin I (afaI), hemolysin (hly), cytotoxic necrotizing factor 1 (cnfI), aerobactin (aer), S fimbriae (sfa) and type 1 fimbriae (fimH) are the most
important virulence factor genes found in these *E. coli* strains. The bacterial adhesin *fimH* (which plays an integral role in the pathogenesis of *E. coli*) is a virulence factor that is located on the type 1 pili of *E. coli*. Of the seven virulence genes examined in this study, the *fim* gene was detected most frequently (82.2%). Kot et al. (2016) reported similar results. Moreover, the *fimH* adhesion gene was the most common virulence gene in both UTIs and asymptomatic bacteriuria (ABU) isolates studied by Yun et al. (2014). Their results showed that the *pap* gene family was an important virulence factor genes found in these strains. Prevalence of virulence factors and antibiotic resistance genes among strains.

<table>
<thead>
<tr>
<th>Antibiotic resistance genes and integrons</th>
<th>afa</th>
<th>hly</th>
<th>aer</th>
<th>cnf</th>
<th>fim</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>class 1 integron</em></td>
<td>9</td>
<td>3</td>
<td>25</td>
<td>11</td>
<td>48</td>
</tr>
<tr>
<td><em>bla</em>&lt;sub&gt;CTX-M-1&lt;/sub&gt;</td>
<td>7</td>
<td>-</td>
<td>11</td>
<td>7</td>
<td>42</td>
</tr>
<tr>
<td><em>bla</em>&lt;sub&gt;CTX-M-2&lt;/sub&gt;</td>
<td>7</td>
<td>-</td>
<td>16</td>
<td>7</td>
<td>44</td>
</tr>
<tr>
<td><em>qnrS</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td><em>qnrA</em></td>
<td>4</td>
<td>1</td>
<td>12</td>
<td>3</td>
<td>23</td>
</tr>
<tr>
<td><em>qnrB</em></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>

**TABLE 2**: Prevalence of virulence patterns among 90 *E. coli* isolates.

<table>
<thead>
<tr>
<th>Pattern codes</th>
<th>Virulence Patterns</th>
<th>Number of Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td><em>afa-aer-fim</em></td>
<td>6 (6.7%)</td>
</tr>
<tr>
<td>E2</td>
<td><em>fim</em></td>
<td>35 (38.9%)</td>
</tr>
<tr>
<td>E3</td>
<td><em>aer-fim</em></td>
<td>12 (13.3%)</td>
</tr>
<tr>
<td>E4</td>
<td><em>afa-fim</em></td>
<td>6 (6.7%)</td>
</tr>
<tr>
<td>E5</td>
<td><em>hly-fim</em></td>
<td>1 (1.1%)</td>
</tr>
<tr>
<td>E6</td>
<td><em>afa-aer-cnf-fim</em></td>
<td>1 (1.1%)</td>
</tr>
<tr>
<td>E7</td>
<td><em>hly-aer-cnf-fim</em></td>
<td>2 (2.2%)</td>
</tr>
<tr>
<td>E8</td>
<td><em>aer-cnf-fim</em></td>
<td>10 (11.1%)</td>
</tr>
<tr>
<td>E9</td>
<td><em>aer-cnf</em></td>
<td>1 (1.1%)</td>
</tr>
<tr>
<td>E10</td>
<td><em>afa-aer</em></td>
<td>1 (1.1%)</td>
</tr>
<tr>
<td>E11</td>
<td><em>afa-cnf-fim</em></td>
<td>1 (1.1%)</td>
</tr>
<tr>
<td>No virulence factor</td>
<td></td>
<td>14 (15.6%)</td>
</tr>
</tbody>
</table>

Plasmid-mediated quinolone resistance (PMQR) genes are usually found in association with the ESBL genes. CTX-M enzymes have been identified in both hospital and community settings and belong to one group of ESBLs. Co-expression of *bla*<sub>CTX-M</sub> and PMQR genes has been reported in *E. coli* isolated from UTIs. Multi drug resistance (MDR) rates were significantly higher in PMQR-positive *K. pneumoniae* and *E. cloacae* isolates (17-28 times) than in PMQR-negative isolates. This finding, which has been observed by other researchers, may indicate a link between *qnrB* and other antibiotic resistance genes. In this study, however, this association was not found in *E. coli* isolates retaining PMQR genes.

The pattern of virulence factors and antibiotic resistance genes is constantly changing in organisms isolated from UTIs, so this and similar studies are necessary to stay abreast of local and national antimicrobial resistance trends for the empirical treatment of UTIs.
Conflict of interest

The authors declare no conflicts of interest.

REFERENCES


