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# Antibiotic Resistance Pattern and Prevalence of Virulence Factors among ESBLs-producing *Escherichia coli* Isolates Causing Urinary Tract Infections

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## ABSTRACT

**Background and Aim:** Urinary tract infections (UTIs) pose a significant global health threat, primarily attributed to bacteria producing extended-spectrum beta-lactamases (ESBLs), with limited treatment options available. The specific genotypes of ESBL-producing strains remain largely unexplored in Guilan (northern Iran). Hence, the objective of this study was to determine the prevalence of ESBL-producing extra-intestinal pathogenic *Escherichia coli* (ExPEC) among hospitalized patients in this area.

**Materials and Methods:** In this descriptive cross-sectional study, 269 isolates were examined phenotypically for ESBL production. All ESBLs producing isolates were screened for the presence of virulence and housekeeping genes by polymerase chain reaction (PCR) using specific primers. The antimicrobial susceptibility patterns were determined using the disk diffusion method according to the CLSI recommendation.

**Results:** Out of the 269 *E. coli* isolates, 107 cases (39.8%) were identified as producers of ESBLs. Antibiotic susceptibility testing conducted on the ESBL-producing isolates revealed the highest levels of resistance to cefotaxime, ampicillin, amoxicillin-clavulanate, cephalothin, cefixime, and ceftriaxone (100.0%). Nalidixic acid exhibited a resistance rate of 91.6%, followed by ceftazidime at 84.1%. On the other hand, the isolates showed the highest susceptibility to imipenem (94.4%), followed by nitrofurantoin (87.9%) and gentamicin (63.9%).

**Conclusion:** The frequency of ESBL-producing ExPEC isolated from UTIs is concerning in Guilan. The most suitable antibiotic with the lowest risk of resistance for the patient can be prescribed using the updated susceptibility and resistance patterns of the bacteria.

**Keywords:** *Escherichia coli*, Extended spectrum beta-lactamases (ESBLs), Antibiotic Resistance, Urinary Tract Infections (UTIs)

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## 1. Introduction

The rise of antibiotic resistance in pathogenic organisms has emerged as a worldwide issue, significantly impacting the treatment of infectious diseases (1). β-lactam antibiotics, when employed against various bacterial strains, regularly undergo rapid and continuous alterations in their β-lactamase enzymes, rendering them more resistant to new β-lactam antibiotics. These particular enzymes are referred to as ESBLs (2). Gram-negative bacteria that produce ESBLs have been increasingly identified as a leading cause of urinary tract infections (UTIs) (3). UTIs are characterized by an infection affecting the kidneys, urethra, bladder, or ureter, accompanied by symptoms of infection. UTIs affect approximately 400 million people each year and are associated with significant medical costs and considerable morbidity

(4). UTIs can be caused by various microorganisms, but the primary culprit in nearly 70-90% of cases is *E. coli*. The pathogenicity of *E. coli* stems from different strains possessing diverse virulence factors (5). Among these strains, extra-intestinal pathogenic *E. coli* (ExPEC) belongs to a large group of *E. coli* and is the most prevalent pathogen responsible for UTIs in humans (6-8). ExPEC is the leading cause of both community-acquired and healthcare-associated UTIs (8). Administering prompt and effective antibiotic therapy for UTIs plays a critical role in minimizing kidney scarring and associated complications, such as renal failure and hypertension (9).

With the increasing prevalence of resistant bacteria worldwide, it is crucial to have local information on resistance and susceptibility patterns to inform the selection of appropriate empirical antibiotic therapy. In light of this, the objective of this study was to investigate the antibiotic susceptibility pattern and the occurrence of virulence factors in ESBL-producing extra-intestinal pathogenic *E. coli* (ExPEC) isolated from patients admitted to hospitals in Guilan Province, located in northern Iran.

## 2. Materials and Methods

### 2.1. Sampling and Identification of Bacteria

This descriptive cross-sectional study involved the utilization of 269 *E. coli* isolates obtained from urine samples of hospitalized patients in Rasht, a city in northern Iran. The isolates underwent a series of laboratory procedures. Initially, Gram staining was performed, followed by inoculation on blood agar and EMB agar. The plates were then incubated at 37°C for 48 hours. Standard biochemical tests, including oxidase, citrate, urea, triple sugar iron, SIM, lysine decarboxylase, methyl red, and Voges-Proskauer tests from Merck Co., Darmstadt, Germany, were employed to identify the grown colonies as *E. coli*. To serve as a control, *E. coli* PTCC No. 1399 (ATCC 25922) was obtained from the Iran Research Organization for Science and Technology (IROST).

### 2.2. Phenotypic Confirmatory Disc Diffusion Test (PCDDT) For ESBL Detection

Each of the test isolates was individually inoculated on Mueller Hinton agar at a final concentration of 0.5 McFarland and then incubated at 37°C for 24 hours. Ceftazidime (30 µg) and ceftazidime-clavulanic acid (30 µg/10 µg) disks were employed in this test. An increase in the zone diameter of ≥5 mm in the presence of clavulanic acid, compared to ceftazidime alone, was considered indicative of ESBL production. (10).

### 2.3. Antimicrobial Susceptibility Testing

The ESBL-producing isolates were subjected to antimicrobial susceptibility testing using the Kirby-Bauer disk diffusion method. The following antibiotics were tested: Cefotaxime (30), Ampicillin (10), Ciprofloxacin (5), Cefoxitin (30), Aztreonam (30), Amoxicillin-clavulanate (30), Tetracycline (30), Ofloxacin (5), Cephalothin (30), Gentamicin (120), Nalidixic acid (30), Cotrimoxazole (25), Cefixime (5), Nitrofurantoin (300), Imipenem (10), Ceftriaxone (30), and Ceftazidime (300). The antibiotics used in the study were provided by Mast Co., Merseyside, UK.

For the testing, a bacterial suspension containing 1.5 × $10^8$ CFU/mL (0.5 McFarland standard) of each strain was prepared, and 0.1 mL of the suspension was cultured separately on Mueller-Hinton agar. The antibiotic-containing disks were then placed on the surface of the medium. To determine antibiotic susceptibility, *E. coli* PTCC No. 1399 (ATCC 25922) was used as a control.

Following incubation, the zones of inhibition surrounding the antibiotic disks were measured and compared to the standard guidelines for each antibiotic, as outlined in the Clinical and Laboratory Standards Institute (CLSI-2020-M100-S30) (11). This comparison was done against the bacteria being investigated.

### 2.4. Plasmid Extraction

The plasmid DNA from all ESBL-producing *E. coli* isolates was extracted from fresh colonies using the Gene JET Plasmid Miniprep Kit (Fermentas, Lithuania), following the instructions provided by the manufacturer. The DNA concentration of each extracted plasmid DNA was determined using Nanodrop. The extracted DNA was then stored at -20 °C and used as a template for subsequent amplifications.

### 2.5. PCR Amplification, Electrophoresis and S Sequencing

The PCR amplification was conducted using specific primers listed in Table 1, which were synthesized by Metabion Company (Germany). The PCR reaction mixture consisted of 10 µL of Master Mix (PCRBIO Taq Mix Red 2X-1ml-AgG), 8.5 µL of $ddH_2O$, 0.5 µL of each primer (10 pmol), and 0.5 µL (50-100 nmole) of plasmid DNA as the template. The amplification reaction was carried out according to the conditions outlined in Table 2.

All PCR products were subjected to electrophoresis on a 1.5% agarose gel containing SYBR Safe. The molecular weight of the observed bands was determined using a DNA size marker under a UV lamp. The PCR products were then sent to Topazgene Co.,

Alborz, Iran, for sequencing. Sequence analyses and comparisons were performed using programs available on the NCBI server [http://www.ncbi.nlm.nih.gov].

### 2.6. Statistical Analysis

The frequency of resistance and housekeeping genes were analyzed using SPSS™ software, 26.0 (IBM Corp., USA).

**Table 1.** Oligonucleotide primers designed to amplify the studied genes

| Region detected | Primer designation | Primer sequences (5'→3') | Size of product (bp) | Ref. |
|---|---|---|---|---|
| **CTX-M** | CTX-M-F | TTT GCG ATG TGC AGT ACC AGT AA | 543 | 12 |
| | CTX-M-R | CGA TAT CGT TGG TGG TGC CAT A | | |
| **CTX-M-14** | CTX-M-14-F | CTG ATG TAA CAC GGA TTG ACC | 871 | 13 |
| | CTX-M-14-R | CGA TTT ATT CAA CAA AAC CAG | | |
| **CTX-M-15** | CTX-M-15-F | AGA ATA AGG AAT CCC ATG GTT | 875 | 14 |
| | CTX-M-15-R | ACC GTC GGT GAC GAT TTT AG | | |
| **O25b-ST131** | O25-ST131-F | TCC AGC AGG TGC TGG ATC GT | 347 | 15 |
| | O25-ST131-R | GCG AAA TTT TTC GCC GTA CTG T | | |
| **O16-ST131** | O16-ST131-F | AAA ACC GCG CCG CGT TAC CT | 145 | 16 |
| | O16-ST131-R | CCA GAA ATC GCG CCC GCA TT | | |
| **ST131- H30** | ST131-H30-F | CCG CCA ATG GTA CCG CTA TT | 354 | 17 |
| | ST131-H30-R | CAG CTT TAA TCG CCA CCC CA | | |
| **ST131-H30-Rx** | ST131-H30-Rx-F | GGT TGC GGT CTG GGC A | 194 | 18 |
| | ST131-H30-Rx -R | CAA TAT CCA GCA CGT TCC AGG TG | | |
| ***adk*** | *adk*-F | ATT CTG CTT GGC GCT CCG GG | 583 | 19 |
| | *adk*-R | CCG TCA ACT TTC GCG TAT TT | | |
| ***fumC*** | *fumC*-F | TCA CAG GTC GCC AGC GCT TC | 806 | 19 |
| | *fumC*-R | GTA CGC AGC GAA AAA GAT TC | | |
| ***icd*** | *icd*-F | ATG GAA AGT AAA GTA GTT GTT CCG GCA CA | 878 | 19 |
| | *icd*-R | GGA CGC AGC AGG ATC TGT T | | |
| ***purA*** | *purA*-F | CGC GCT GAT GAA AGA GAT GA | 816 | 19 |
| | *purA*-R | CAT ACG GTA AGC CAC GCA GA | | |
| ***gyrB*** | *gyrB*-F | TCG GCG ACA CGG ATG ACG GC | 911 | 19 |
| | *gyrB*-R | ATC AGG CCT TCA CGC GCA TC | | |
| ***recA*** | *recA*-F | CGC ATT CGC TTT ACC CTG ACC | 780 | 19 |
| | *recA*-R | TCG TCG AAA TCT ACG GAC CGG A | | |
| ***mdh*** | *mdh*-F | ATG AAA GTC GCA GTC CTC GGC GCT GCT GGC GG | 932 | 19 |
| | *mdh*-R | TTA ACG AAC TCC TGC CCC AGA GCG ATA TCT TTC TT | | |

**Table 2.** Gene amplification program in PCR

| Gene | Cycle | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Pre-denaturation | | Denaturation | | Annealing | | Extension | | Final Extension | |
| | Temperature | Time | Temperature | Time | Temperature | Time | Temperature | Time | Temperature | Time |
| **CTX-M** | 95°C | 2 min | 95°C | 30 sec | 59.2°C | 30 sec | 72°C | 60 sec | 72°C | 5 min |
| **CTX-M-14** | 95°C | 2 min | 95°C | 30 sec | 58.5°C | 30 sec | 72°C | 90 sec | 72°C | 5 min |
| **CTX-M-15** | 95°C | 2 min | 95°C | 30 sec | 59.5°C | 30 sec | 72°C | 90 sec | 72°C | 5 min |
| **O25b-ST131** | 95°C | 2 min | 95°C | 30 sec | 63.7°C | 30 sec | 72°C | 40 sec | 72°C | 5 min |
| **O16-ST131** | 95°C | 2 min | 95°C | 30 sec | 63.8°C | 30 sec | 72°C | 20 sec | 72°C | 5 min |
| **ST131-H30** | 95°C | 2 min | 95°C | 30 sec | 59.3°C | 30 sec | 72°C | 40 sec | 72°C | 5 min |
| **ST131-H30-Rx** | 95°C | 8 min | 95°C | 30 sec | 68°C | 30 sec | 72°C | 40 sec | 72°C | 5 min |
| ***adk*** | 95°C | 2 min | 95°C | 30 sec | 65°C | 30 sec | 72°C | 30 sec | 72°C | 5 min |
| ***fumC*** | 95°C | 2 min | 95°C | 30 sec | 65°C | 30 sec | 72°C | 30 sec | 72°C | 5 min |
| ***icd*** | 95°C | 2 min | 95°C | 30 sec | 65°C | 30 sec | 72°C | 30 sec | 72°C | 5 min |
| ***purA*** | 95°C | 2 min | 95°C | 30 sec | 65°C | 30 sec | 72°C | 30 sec | 72°C | 5 min |
| ***gyrB*** | 95°C | 2 min | 95°C | 30 sec | 65°C | 30 sec | 72°C | 30 sec | 72°C | 5 min |
| ***recA*** | 95°C | 2 min | 95°C | 30 sec | 65°C | 30 sec | 72°C | 30 sec | 72°C | 5 min |
| ***mdh*** | 95°C | 2 min | 95°C | 30 sec | 65°C | 30 sec | 72°C | 30 sec | 72°C | 5 min |

## 3. Results

Out of the 269 *E. coli* isolates, 107 strains (39.8%) were identified as ESBL producers. Among these 107 ESBL-producing *E. coli* isolates, 67 (62.6%) were obtained from female patients and 40 (37.4%) from male patients. The mean age of the patients was 48.95 ± 25.87 years, ranging from 1 month to 87 years. Furthermore, 6 (5.6%) patients were less than 1 year old, 11 (10.3%) were between 1 and 15 years old, 6 (5.6%) were between 16 and 30 years old, 13 (12.1%) were between 31 and 45 years old, 25 (23.4%) were between 46 and 60 years old, and 46 (43.0%) were over 61 years old. Therefore, the most common age range observed was over 61 years.

Regarding antibiotic susceptibility, the highest resistance rates were observed against cefotaxime, ampicillin, amoxicillin-clavulanate, cephalothin, cefixime, and ceftriaxone (100%). This was followed by nalidixic acid (91.6%) and ceftazidime (84.1%). On the other hand, the highest susceptibility rates were observed for imipenem (94.4%), nitrofurantoin (87.9%), and gentamicin (63.9%). The complete results of the antibiotic resistance patterns of the isolates can be found in Table 3. Additionally, the frequency of multi-drug resistant (MDR) isolates was estimated to be 100%.

Molecular analysis demonstrated that among the examined housekeeping genes, *purA*, *gyrB*, and *recA* were the most prevalent, being detected in 95.3% of the *E. coli* isolates. This was followed by *adk*, which was present in 91.6% of the isolates. The genes *mdh*, *icd*, and *fumC* were found in 86.9%, 84.1%, and 79.6% of the isolates, respectively. In terms of virulence genes, CTX-M-14 and CTX-M-15 were the most commonly detected, present in 86.9% of the isolates. A comprehensive overview of the gene distribution in the *E. coli* isolates can be found in Table 4. Notably, the results indicated that the frequency of occurrence for all 14 genes (CTX-M, CTX-M-14, CTX-M-15, O25b-ST131, O16-ST131, ST131-H30, ST131-H30-Rx, *adk*, *fumC*, *icd*, *purA*, *gyrB*, *recA*, and *mdh*) was greater than 50% among the isolates.

**Table 3.** The relative frequencies of antibiotic resistance pattern according to ESBLs production (n=107)

| Antibiotic (µg/disc) | Susceptible No. (%) | Intermediate No. (%) | Resistant No. (%) |
|---|---|---|---|
| ***β-lactams:*** | | | |
| **Cefotaxime (30)** | 0 (0.0) | 0 (0.0) | 107 (100.0) |
| **Ampicillin (10)** | 0 (0.0) | 0 (0.0) | 107 (100.0) |
| **Cephalothin (30)** | 0 (0.0) | 0 (0.0) | 107 (100.0) |
| **Cefoxitin (30)** | 68 (63.6) | 20 (18.7) | 19 (17.8) |
| **Aztreonam (30)** | 7 (6.5) | 11 (10.3) | 89 (83.2) |
| **Amoxicillin-clavulanate (30)** | 0 (0.0) | 0 (0.0) | 107 (100.0) |
| **Cefixime (5)** | 0 (0.0) | 0 (0.0) | 107 (100.0) |
| **Imipenem (10)** | 101 (94.4) | 6 (5.6) | 0 (0.0) |
| **Ceftriaxone (30)** | 0 (0.0) | 0 (0.0) | 107 (100.0) |
| **Ceftazidime (300)** | 5 (4.7) | 12 (11.2) | 90 (84.1) |
| ***Non-β-lactams:*** | | | |
| **Gentamicin (120)** | 68 (63.9) | 4 (3.7) | 35 (32.7) |
| **Nalidixic acid (30)** | 7 (6.5) | 2 (1.9) | 98 (91.6) |
| **Cotrimoxazole (25)** | 16 (15) | 0 (0.0) | 9 (85) |
| **Nitrofurantoin (300)** | 94 (87.9) | 3 (1.9) | 11 (10.3) |
| **Ciprofloxacin (5)** | 18 (16.8) | 5 (4.7) | 84 (78.5) |
| **Tetracycline (30)** | 22 (20.6) | 2 (1.9) | 83 (77.6) |
| **Ofloxacin (5)** | 24 (22.4) | 0 (0.0) | 83 (77.6) |

**Table 4.** The relative frequencies of virulence factors and housekeeping genes according to ESBL production (n=107)

| Gene | Protein function | Negative No. (%) | Positive No. (%) |
|---|---|---|---|
| **CTX-M** | Extended-spectrum β-lactamase | 20 (18.7) | 87 (81.3) |
| **CTX-M-14** | Extended-spectrum β-lactamase | 14 (13.1) | 93 (86.9) |
| **CTX-M-15** | Extended-spectrum β-lactamase | 14 (13.1) | 93 (86.9) |
| **O25b-ST131** | Antigen | 39 (36.5) | 68 (63.5) |
| **O16-ST131** | Antigen | 53 (49.5) | 54 (50.5) |
| **ST131-H30** | Adhesin | 38 (35.5) | 69 (64.5) |
| **ST131-H30-Rx** | Adhesin | 42 (39.3) | 65 (60.7) |
| ***adk*** | Adenylate kinase | 9 (8.4) | 98 (91.6) |
| ***fumC*** | Fumarate hydratase | 22 (20.6) | 85 (79.4) |
| ***icd*** | Isocitrate/isopropylmalate dehydrogenase | 17 (15.9) | 90 (84.1) |
| ***purA*** | Adenylosuccinate dehydrogenase | 5 (4.7) | 102 (95.3) |
| ***gyrB*** | DNA gyrase | 5 (4.7) | 102 (95.3) |
| ***recA*** | ATP/GTP binding motif | 5 (4.7) | 102 (95.3) |
| ***mdh*** | Malate dehydrogenase | 14 (13.1) | 93 (86.9) |

## 4. Discussion

When it comes to treating infections with antibiotics, it is crucial to consider local experience regarding susceptibility and resistance patterns. This is because the susceptibility of microorganisms to antibiotics can vary over time and in different geographical regions. In this particular investigation, every isolate demonstrated multidrug resistance (MDR). For empirically treating urinary tract infections caused by ESBL-producing strains, the most effective antibiotics were found to be imipenem and nitrofurantoin.

It is important to note that cefotaxime, ampicillin, amoxicillin-clavulanate, cephalothin, cefixime, and ceftriaxone exhibited high rates of resistance and are not recommended for the treatment or prevention of these strains in the Guilan region. The high frequency of virulence factors and resistance genes observed in this study is significant as it can lead to increased pathogenicity and treatment failure.

Our findings align with the study carried out by Negeri *et al.* in 2021. In their research, they examined the molecular epidemiology of ESBL genes and the antibiotic resistance profile of 204 clinical *E. coli* isolates in Ethiopia. According to their data, all of the isolates demonstrated multidrug resistance (MDR). Furthermore, out of the 189 bacteria that had ESBL genes, all except one had CTX-M β-lactamases **(20)**.

In a separate investigation, Abdelrahim *et al.* (2021) demonstrated that O25-ST131 isolates exhibited a significantly higher frequency of ESBLs and MDR occurrence. These isolates also showed antibiotic resistance to a majority of antibiotic classes, in contrast to non-O25-ST131 isolates **(21)**.

Rasoulinasab *et al.* (2021) collected 215 *E. coli* isolates from urine samples obtained from women experiencing symptomatic urinary tract infections (UTIs) at referral university hospitals in Tehran, Iran. They specifically investigated ciprofloxacin-resistant ESBL-producing (CIPR/ESBL+) *E. coli*. The study reported a prevalence of 82% for the blaCTX-M-15 gene and resistance rates of 70% for ampicillin-sulbactam, 97% for aztreonam, and 61% for gentamicin **(22)**.

In our study, we found a lower resistance rate for gentamicin (32.7%) and higher resistance rates for ciprofloxacin (78.5%), ampicillin (100.0%), and aztreonam (83.2%) when compared to the findings of Rasoulinasab's study. Furthermore, the prevalence of CTX-M-15 in our study was 86.9%.

Namaei *et al.* (2017) reported on the dissemination of the O25b/ST131 clone that produces CTX-M-15 among *E. coli* isolates. This study provided the first documented frequency of O25b/ST131 *E. coli* producing CTX-M-15 in Birjand, Iran. The authors observed a comparatively high prevalence of O25b/ST131 *E. coli*, which exhibited significant levels of virulence factors and antibiotic resistance. Additionally, the majority of these isolates carried the CTX-M-15 gene and were capable of producing ESBLs. These findings highlighted the spread of ST131 *E. coli* as a significant drug-resistant bacterium and a new threat to public health **(23)**.

In our study, we found that the abundances of ST131-H30, O25b-ST131, ST131-H30-Rx, and O16-ST131 were 64.5%, 63.5%, 60.7%, and 50.5%, respectively, based on our findings.

In their study, Bulut *et al.* (2021) investigated the prevalence of high-risk *E. coli* ST131 clones among ESBL-positive *E. coli* isolates in Turkey. The study reported a high rate of ST131 clones within the population. The notable resistance observed in ST131 isolates against ciprofloxacin highlights the significant implications of the dissemination of these high-risk clones for the development of resistance **(24)**. These findings align with the results of our study.

Other studies have documented the presence of antibiotic resistance and virulence genes in uropathogenic *E. coli*. Dziri *et al.* (2020) conducted research on the uropathogenic O25b-ST131 clone, determining its frequency and identifying the molecular pathways responsible for cephalosporin resistance in clinical *E. coli* isolates. During the period spanning from April 2015 to August 2016, a total of 42 *E. coli* strains resistant to cephalosporins, which were linked to urinary tract infections, were gathered from the provincial hospital situated on an island in southeast Tunisia. Most of their isolates were found to contain the CTX-M gene, which was detected through molecular screening of genes encoding β-lactamases using PCR and sequencing. This investigation marks the first report of O25b-ST131 *E. coli* producing CTX-M-14 on the island of Tunisia **(25)**. Demirci-Duarte *et al.* (2020) conducted a study in Turkey to examine the frequency of the ST131 clone and the presence of CTX-M in 299 urine isolates. The researchers also analyzed the antimicrobial susceptibility. The study found that the ST131 clone was detected in 31.7% of the isolates that were resistant to ciprofloxacin, accounting for 34.7% of all isolates. Furthermore, it was observed that 81.8% of the ST131 clone belonged to the H30-R subclone, while 66.7% belonged to the H30-Rx subclone. However, the study revealed that 57.6% of isolates from the ST131 clone had CTX-M, in comparison to 62.9% in the H30-R subclone and 68.2% in the H30-Rx subclone. These findings suggest that the occurrence of CTX-M-positive ST131 clones is relatively high **(26)**. In their study, Hojabri *et al.* (2017)

assessed the prevalence of the O25b/O16 subgroups of the ST131 clone and the H30/H30-Rx lineages in *E. coli* isolates that cause extraintestinal infections. The aim was to understand their role in the dissemination of antimicrobial resistance. Additionally, PCR was used to evaluate the genetic diversity and virulence properties of the ST131 isolates. The findings of this study confirm the increasing presence of the H30 subclone in our region and suggest that this particular subclone is primarily responsible for the successful expansion of the ST131 clonal group.

Despite the lower frequency of the O16-ST131 clonal subgroup compared to O25b-ST131, it showed higher rates of resistance to most antibiotics. This observation highlights the significance of the O16-ST131 subgroup in the dissemination of multidrug-resistant *E. coli* **(27)**. Salehi *et al.* (2023) conducted a study in Mashhad, Iran, to determine the pattern of antibiotic resistance and the frequency of specific genes in *E. coli* isolates. The results of their research indicated that a significant portion of the isolates exhibited resistance to antibiotics and were identified as *E. coli* **(28)**. Several studies conducted in various provinces of Iran have demonstrated the presence of multidrug-resistant extended-spectrum beta-lactamase (MDR-ESBLs)-producing *E. coli* **(29-33)**. The findings from these studies corroborate the current study's findings regarding the prevalence of virulence genes and antibiotic resistance in ExPEC strains responsible for urinary tract infections (UTIs). However, it is worth noting that the results of these studies vary, which could be attributed to differences in sample characteristics, geographical locations, sample sizes, and other factors.

## 5. Conclusion

When comparing these data with those from other countries, significant variations in the susceptibility and resistance characteristics of the bacteria become apparent. As a result, the choice of antibiotics for empirical therapy should be guided by the resistance patterns of the uropathogens specific to each city. Moreover, it can be inferred that the increase in virulence factors has contributed to the emergence of multidrug-resistant extended-spectrum beta-lactamase (MDR-ESBLs)-producing ExPEC strains.

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## Ethics Approval

The study procedures adhered to the ethical guidelines outlined in the Declaration of Helsinki. It is important to note that the test microorganisms were obtained from Dr. Mojtahedi's previous research. However, to ensure the authenticity of each *E. coli* sample, microscopic and biochemical tests were performed. All methods were conducted in accordance with applicable guidelines and regulations.

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## Authors' Contribution

Conceptualization, methodology, resources, review, and editing: All authors; Investigation and original draft: Samaneh Kazemi; Supervision: Ali Mojtahedi & Farzaneh Hosseini.

## Conflict of Interest

The authors declared no conflict of interest.

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