

Frequency of *bla* TEM, *bla* SHV, *bla* CTX-M, and *qnrA* Among *Escherichia coli* Isolated From Urinary Tract Infection

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Background: *Escherichia coli* is the most important as well as the most common bacteria causing urinary tract infections (UTIs) and its resistance to common antibiotics is increasing. Extended-spectrum beta-lactamase (ESBL) producer *E. coli* strains can resist against the third-generation and fourth-generation cephalosporins.

Objectives: This study aimed to evaluate the resistance profile of *E. coli* isolated from patients with UTIs referred to Imam Khomeini and Baqiyatallah Hospitals, Tehran, Iran, through phenotypic and molecular methods.

Materials and Methods: During 2010-2011, 180 urine samples of patients with UTIs from Imam Khomeini and Baqiyatallah Hospitals were collected. Based on the standard bacteriologic tests, *E. coli* isolates were identified. Resistance to common antibiotics was tested by the Kirby-Bauer method and reconfirmed by determining minimum inhibitory concentration (MIC) through microdilution method. Further phenotypic double-disk synergy test (DDST) was performed to screen the ESBL producer strains. Resistance genes related to ESBL and *qnrA* were evaluated by Polymerase chain Reaction (PCR).

Results: A total of 100 *E. coli* strains were examined by antibiogram and the rates of resistance to the tested antibiotics were as follows: 100% to penicillin and amoxicillin, 77% to amoxicillin-clavulanic acid, 72% to ceftazidime, 69% to cefotaxime, 47% to cefoxitin, 46% to ceftriaxone, 43% to cephalixin, 27% to aztreonam, 53% to nalidixic acid, 51% to ciprofloxacin, and 2% to imipenem. The MIC to ciprofloxacin, cefazolin, and ceftriaxone were ≥ 0.249 , ≥ 0.508 , and ≥ 0.044 , respectively. Moreover, 20% of *E. coli* isolates were ESBL-producing isolates by DDST. The frequency of *bla* CTX-M, *bla* TEM, *bla* SHV, and *qnrA* genes was 87%, 82%, 65%, and 39%, respectively.

Conclusions: Considering the high prevalence of ESBL genes (*bla* CTX-M, 87%; and *bla* TEM, 82%), fluoroquinolones may be used as an alternative drug in treatment, although resistance to this family is increasing as well. As a result, this increasing trend should be prevented using appropriate guidelines for prescription.

Keywords: *Escherichia coli*; *bla* CTX-M; *bla* TEM; beta-Lactamases; *bla* SHV

1. Background

Escherichia coli is the most common bacterial agent causing urinary tract infections (UTIs). Cephalosporins, especially the third-generation ones, are used as a routine treatment for UTIs and fluoroquinolones are used increasingly as new alternative treatment of choice (1). During the recent years, these bacteria have undergone mutation and became resistant to many antibiotics by exchanging the resistant genes (2). One of the resistance mechanisms is producing enzymes like beta-lactamase and extended-spectrum beta-lactamases (ESBLs) that can hydrolyze the beta-lactam ring and inactivate these drugs that contain this structural element. Major reports

of ESBL production are related to *bla* CTX-M, *bla* TEM and *bla* SHV as plasmid genes, which can simultaneously carry other resistant genes (3-7). As mentioned before, the fluoroquinolones are another choice for treatment of beta-lactam-resistant bacteria (8, 9). Similarly, some plasmid genes like *qnrA* are responsible for quinolone resistance.

2. Objectives

The aim of this study was to evaluate the resistance profile of *E. coli*, isolated from urine samples of the patients with UTIs, to routine antibiotics especially beta-lactams and ciprofloxacin.

Implication for health policy/practice/research/medical education:

According to the results of this study, the rate of resistance to beta-lactam antibiotics as well as fluoroquinolones is increasing and treatment is difficult. In addition, synchronous resistance to these two groups of antibiotics has been seen in most specimens. As a result, we recommended antibiogram test before prescribing any antibiotic in order to prevent the increasing trend of resistance to fluoroquinolones and beta-lactam drugs.

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3. Materials and Methods

In this study, 180 urine samples were collected from patients with UTI referred to Imam Khomeini and Baqiyatallah Hospitals, Tehran, Iran, during September 2010 to March 2011. After special identification tests including EMB (eosin-methylene blue agar), TSI (Triple Sugar Iron agar), SIM (SH₂, Indole and Motility), MR-VP (Methyl Red Voges-Proskauer Broth), and Citrate, 100 *E. coli* isolates were randomly selected for further investigation. Their resistance to common antibiotics, namely, imipenem, ceftriaxone, penicillin, amoxicillin, amoxicillin-clavulanic acid, ciprofloxacin, nalidixic acid, cefixime, cephalixin, cefotaxime, ceftazidime, and aztreonam discs (all from Himedia Company, India) was assessed according to the Clinical and Laboratory Standards Institute (CLSI) protocol and by the Kirby-Bauer method (10). In further step, minimal inhibitory concentration (MIC) was determined by microdilution method for ceftriaxone, cefazolin, and ciprofloxacin in accordance with CLSI protocol (10). Simultaneously, *E. coli* ATCC 25922 was used as the control. In the next step, ESBL production was assessed by double-disk synergy test (DDST) and using cefotaxime with and without clavulanic acid and ceftazidime with and without clavulanic acid discs. In this test, the antibiotic disks alone and with clavulanic acid were placed 25 mm apart from each other (10). More than or equal to 5 mm increase in the diameter of the growth inhibition zone of the antibiotic with clavulanic acid in comparison to the disk alone showed positive results for the ESBL production (11, 12). For molecular investigation, DNA extraction was done using DNA Extract Kit (Taif Ara, Iran) and consequently polymerase chain reac-

tion (PCR) method was employed to assess the frequency of resistance genes including *bla CTX-M*, *bla SHV*, and *bla TEM* for ESBLs genotyping and *qnrA* gene for ciprofloxacin resistance study (13, 14). More details about the sequence of used primers and PCR programming are shown in Tables 1 and 2. The PCR products were analyzed after gel electrophoresis, stained by ethidium bromide, and exposed to the UV radiation. During PCR, ESBL-positive *Klebsiella pneumoniae* ATCC 700603 was used as the positive control.

4. Results

According to the identification tests, 100 confirmed *E. coli* isolates were selected for the experiment; 79% of the samples were collected from adults (females, 51%; and males, 28%) and 21% from children. The frequency of the samples based on the hospital wards was as follows: gynecology, 36%; urology, 15%; neurology, 14%; miscellaneous wards, 10%; and outpatient clinics, 25%. According to the antibiogram test, the rates of resistance of the bacteria to antibiotics was as follows: amoxicillin and penicillin, 100%; ceftazidime, 72%; cefotaxime, 69%; nalidixic acid, 53%; ciprofloxacin, 51%; ceftioxin, 47%; cephalixin, 43%; aztreonam, 27%; and imipenem, 2% (Figure 1). In microdilution test, the MIC of resistant strains to ceftriaxone, cefazolin, and ciprofloxacin was ≥ 0.044 , ≥ 0.508 , and ≥ 0.249 , respectively. By DDST test, 20% of the *E. coli* isolates showed ≥ 5 mm increase in the diameter of the growth inhibition zone around the clavulanic acid disks and were marked as ESBL-positive isolates (Figure 2). In the PCR test, after preparing the required conditions, the frequency of *bla CTX-M*, *bla TEM*, *bla SHV*, and *qnrA* genes was 87%, 82%, 65%, and 40%, respectively (Figures 3 and 4).

Table 1. The Sequence of Primers Used in This Study^a

Primers	Sequences
<i>bla TEM</i>	F: 5' - ATAAAATTCTTGAAGACGAAA - 3' R: 5' - GACAGTTACCAATGCTTAATCA - 3'
<i>bla CTX-M</i>	F: 5' - ACGCTGTTGTTAGGAAGTG - 3' R: 5' - TTGAGGCTGGGTGAAGT - 3'
<i>bla SHV</i>	F: 5' - GGGTTATTCTTATTGTGCG - 3' R: 5' - TTAGCGTTGCCAGTGCTC - 3'
<i>qnrA</i>	F: 5' - ATTTCTCAGCCAGGATTG - 3' R: 5' - GATCGCAAAGTTAGGTCA - 3'

^a Abbreviations: TEM, Temineira; CTX-M, cefotaxime-Munich; SHV, Sulphydryl variable; qnrA, plasmid mediated Quinolone resistance determinants.

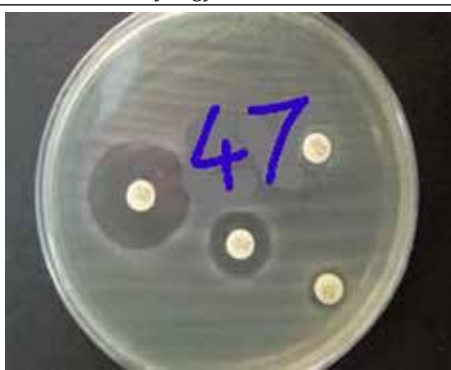
Table 2. Polymerase Chain Reaction Programs Used in This Study^a

<i>bla SHV</i> Gene	<i>bla TEM</i> Gene	<i>bla CTX-M</i> Gene	<i>qnrA</i> Gene
3 Minutes 94°C initial denaturation	3 Minutes 94°C initial denaturation	3 Minutes 94°C initial denaturation	3 Minutes 94°C initial denaturation
1 Minute 94°C denaturation	1 Minute 94°C denaturation	1 Minute 94°C denaturation	1 Minute 94°C denaturation
30 Seconds 58°C annealing	53 Seconds 30°C annealing	1 Minute 55.5°C annealing	1 Minute 55°C annealing
1 Minute 72°C extension	1 Minute 72°C extension	1 Minute 72°C extension	1 Minute 72°C extension
10 Minutes 72°C final extension	5 Minutes 72°C final extension	10 Minutes 72°C final extension	10 Minutes 72°C final extension
30 Cycles	30 Cycles	30 Cycles	30 Cycles

^a Abbreviations: bla CTX-M, cefotaxime-Munich; bla SHV, sulphydryl variable; bla TEM, Temoneira; qnrA, plasmid mediated Quinolone resistance determinants.

Figure 1. The Antibiogram Test

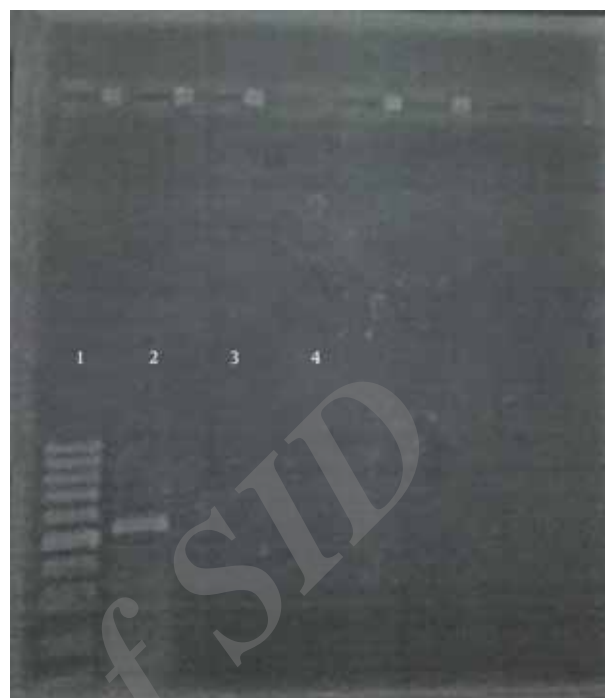
This *Escherichia coli* isolate was resistant to all tested antibiotics.

Figure 2. The Double-Disk Synergy Test

Increase of ≥ 5 mm in the growth inhibition zone around the disk containing cefotaxime with clavulanic acid versus cefotaxime for *Escherichia coli*. No 47 showed the extended-spectrum beta-lactamases-positive strains in this case.

Figure 3. The Frequency of *bla*_{C_{ITX-M}, *bla*_{TEM}, *bla*_{SHV}, and *qnrA* Genes Among *Escherichia coli* Isolates}

Lane 1, the 857 bp PCR product is related to *bla*_{C_{ITX-M} gene; lane 2, positive control (*Klebsiella pneumonia* ATCC 700603); lane 3, negative control (*Escherichia coli* ATCC 25922); lane 4, negative sample; lane 5, ladder (100-1000) Fermentas, UK; lane 6, The 615 bp PCR product of *bla*_{SHV} gene; lane 7, positive control (*K. pneumonia* ATCC 700603); lane 8, negative control (*E. coli* ATCC 25922); lane 9, ladder (100-1000) Fermentas, UK; lane 10, the 1150 bp *bla*_{TEM} gene in clinical samples; lane 11, positive control (*K. pneumonia* ATCC 700603); lane 12, negative control; and lane 13, ladder (100-1000) Fermentas, UK.}

Figure 4. The Frequency of *qnrA* Gene by Polymerase Chain Reaction

Lane 1, ladder (100-1000) Fermentas, UK; lane 2, the 516 bp PCR product of *qnrA*; lane 3, negative control (*Escherichia coli* ATCC 25922); and lane 4, a clinically negative case.

5. Discussion

In this study, the rate of resistance of the isolated *E. coli* strains to ampicillin and penicillin was 100% and this is an alarm for clinicians to pay more attention to the amount of consumption and prescription of these antibiotics. From 2002 to 2004, in Minnesota, USA, 931 specimens of *E. coli* were isolated from the urine samples of two groups, i.e. patients and controls, and their resistance to the fluoroquinolones was studied. In addition, the existence of resistance genes to fluoroquinolones, trimethoprim/sulfamethoxazole, and cephalosporins as well as the ability of ESBL production were analyzed by molecular PCR method. According to the results, the resistance rate to fluoroquinolones was higher in the hospitalized patients than in controls (15). Resistance of these bacteria was 100% to penicillin and amoxicillin, 43% to cephalexin, 46% to ceftriaxone, and 2% to imipenem; these results revealed a high trend of resistance to the common antibiotics used in the routine treatment of UTIs. In addition, Warburg et al. studied the resistance rate of *E. coli* isolates to fluoroquinolones and screened the frequency of *qnrA*, *qnrB* and *aac(6)-Ib-cr* genes by using PCR during 1991-2005; they found that not only is the resistance to ciprofloxacin growing but also most of these strains produce ESBL. Therefore, gene translocation by plasmids plays an important role in this regard. Moreover, taking the results into account, a hypothesis suggests that there

is a correlation between resistance to fluoroquinolones and synchronous ability of ESBL production because of the relation between these two plasmids (16). For this reason, the existence of *qnrA* gene among ESBL-positive *E. coli* strains was evaluated and based on its results, special attention must be paid to these strains during therapy.

In a five-year study in Houston, Texas, USA, the relation between resistance to fluoroquinolones and age as well as sex was studied and it revealed that the increase in age and duration of hospitalization accelerated the rate of resistance to fluoroquinolones in males (17). In another study in Norway, resistance of *E. coli* strains, taken from the urine of the patients with UTI, to ciprofloxacin and nalidixic acid by disk diffusion method showed 1% increase during a four-year period. In the mentioned study, most specimens were taken from women (83%). Then resistance to ciprofloxacin, trimethoprim, ampicillin, sulfonamide, nitrofurantoin, nalidixic acid, chloramphenicol, tetracycline, and gentamicin was studied by the phenotypic method and finally by using PCR and pulse-field method and the presence of genes involved in resistance was determined (18). Resistance to two routine drugs of the fluoroquinolones group, namely, nalidixic acid and ciprofloxacin was determined respectively to be 14% and 2% by using the disk diffusion method. In our study, resistance rate to nalidixic acid and ciprofloxacin were 53% and 51%, respectively. In a study by Johnson et al. in Canada, the changes in resistance to ofloxacin, a fluoroquinolone, were evaluated; they found that the resistance rate to this drug had increased from 1% to 9% within a five-year period. They concluded that factors like duration of hospitalization and irregular use of these drugs had led to increased resistance (19). Similarly, increase in resistance of *E. coli* isolated from the urine of patients with UTIs to ciprofloxacin was attributed to the irregular consumption of fluoroquinolones in Switzerland (20).

According to the results of our study, the rate of resistance to beta-lactam drugs is increasing and treatment is difficult. This increasing trend of resistance has been seen with fluoroquinolones and synchronous resistance to these two antibiotic groups has been seen in most strains. As a result, we recommend to perform antibiogram test before prescribing any drug in order to prevent the increasing trend of resistance to fluoroquinolones and beta-lactam drugs.

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Authors' Contributions

The study concept and design was done by Mojdeh Hakemi Vala and Reza Ranjbar. Analysis and interpretation was done by Shima Abdi and Mojdeh Hakemi Vala.

Administration of technical and material supports was done by Shima Abdi, Ozra Baghery Bejestani, and Fate-meh Baghery Bejestany. Mojdeh Hakemi Vala and Reza Ranjbar supervised the study. Statistical analysis was done by Shima Abdi. Drafting of the manuscript, critical revision of the manuscript, and intellectual contents was done by Mojdeh Hakemi Vala.

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