Characterization of Uropathogenic *Escherichia coli*: Distribution of Adhesin-Encoding Genes and O-Serotypes Among Ciprofloxacin Susceptible and Resistant Isolates

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Abstract

**Background:** Some evidence indicates that there is a potential linkage between ciprofloxacin resistance and the prevalence of virulence factors in pathogenic *Escherichia coli* strains.

**Objectives:** The study was conducted to evaluate the association of eight putative adhesin-encoding genes and 12 O-serotypes among ciprofloxacin susceptible/resistant uropathogenic *E. coli* (UPEC) isolates.

**Methods:** A total of 100 *E. coli* isolates collected from symptomatic patients with urinary tract infection were surveyed for antimicrobial susceptibility test and polymerase chain reaction (PCR) to find the presence of eight putative adhesin-encoding genes and 12 O-serotype and their association with ciprofloxacin susceptibility and resistance.

**Results:** The highest and the lowest resistance rates were obtained against ampicillin (92%) and gentamycin (19%), respectively. However, the resistance rate to ciprofloxacin was detected in 43% of *E. coli* isolates. PCR results revealed the frequency of eight putative adhesin-encoding genes and 12 O-serotype and their association with ciprofloxacin susceptibility and resistance.

**Conclusions:** The results revealed that UPEC isolates of different geographical regions might have various properties. It is worthwhile to elucidate the differences that might result in producing valuable evidence based on clinical guidelines for the management of urinary tract infection.

**Keywords:** *Escherichia coli*, Adhesin-Encoding Genes, O-Serotyping, Ciprofloxacin

1. Background

Urinary tract infections (UTIs) are one of the most usual among community-acquired and nosocomial infections encountered by physicians (1). These infections are rarely directly associated with the death of the patients. However, they play an accelerating role in the risk of comorbidity and healthcare-associated costs. Such infectious diseases are also responsible for increased antibiotic prescription by physicians. Fluoroquinolones and quinolone antibiotics are broad-spectrum antibiotics that are used to treat several Gram-negative and Gram-positive bacterial infections. The quinolone antibiotics were first described in the early 1960s, have become prevalent in the treatment of urinary and urogenital *Escherichia coli* infections (2). Quinolone-resistant *E. coli* (QREC) has recently emerged in different geographical regions as an important cause of extraintestinal infections (3-5).

Uropathogenic *E. coli* (UPEC) harbors a range of virulence determinants that relate to its ability to colonize the urinary tract and cause disease. The most significant adhesin-encoding genes include *fimH*, *sfa*, *papC*, *foc*, *afa*, *papGI*, *papGII*, and *papGIII* and genes related to the adhesin systems. Several studies have shown that these adhesin factors have significant roles in the pathogenicity of UPEC strains because the strains are able to colonize and overcome host defense systems, thereby resulting in the disease (6, 7). The increase in the multi-drug resistant strains of *E. coli* in many countries has caused failure in the treat-
ment accompanied by a huge health burden (8).

A very limited range of antibacterial agents remains
due to the appearance of QREC isolates, warranting that a
simple case of UTI is increasingly a competing element (9).
Studies have suggested that fluoroquinolone and QREC
strains show fewer virulence genes and are less capable of
causing infection compared with susceptible strains (10-13). In various parts of Iran, it is important to perform re-
search studies to document the prevalence of UPEC and
determine the putative virulence genes and antibiotic re-
sistance of UPEC isolates. The complex interactions of the
virulence characteristics and the O-serotype background
similar to the antibiotic resistance of E. coli resulting from
their various relationships need further study.

2. Objectives

The objective of the present study is to clarify the type
of association of the acquisition of virulence factors (VFs)
with resistance alone and resistance depends on an O-
serotype or unknown factors. The antibiotic resistance, the
genotypic adhesin factors and O-serotype characterization
in UPEC isolates were assessed.

3. Methods

3.1. Case Definition

The UTIs are determined when the following symp-
toms appear: (1) bacteria with ≥ 10^4 CFU/mL count in mid-
stream urine, (2) the observation of ≥ 5 leukocytes per
high power field, and (3) the presence of symptoms of UTI
dysuria, frequency or urgency of urination in the host.

3.2. Experiment Setting

One hundred non-repetitive E. coli isolates were col-
lected from symptomatic UTI patients in a teaching hos-
pital in Zabol, southeast of Iran. Among the 100 UTI di-
agnosed patients, 61 (61%) were females and 39 (39%) were
males, with a mean age of 35.5 ± 18.3 (mean ± SD).

3.3. Antimicrobial Susceptibility Testing

The Kirby-Bauer’s disk diffusion method was applied
for antibiotic susceptibility test on Mueller Hinton agar
following the CLSI guidelines (14) using antibiotic discs as
follows: cefazolin (CZ), ampicillin (AM), ciprofloxacin (CP),
azithromycin (AZM), cefixime (CFM), ceftazidime (CAZ), ce-

toxime (CTX), and gentamycin (GM) (Padtan Teb, Iran).
Standard E. coli ATCC 25922 strain was used as the control.

3.4. Detection of Adhesin Genes

The DNA of overnight cultures of UPEC isolates was ex-
tracted by boiling method (15). The uniplex-PCR was used to
identify the presence of eight adhesin-encoding genes:
afa, fimH, foc, papC, papG alleles (papGI, papGII and papGIII),
and sfa in UPEC isolates. The predicted sizes and details of
the primer sequences of the amplified products are shown in
Table 1. Amplification reactions were performed in a to-
total volume of 25 µL, including 8.5 µL ddH2O, 12.5 µL of Mas-
ter Mix Red (amplicon), 2 µL of template DNA, and 1 µL of
each primer (30 pmol of each of the primers) (Pishgam, Iran).
PCR reactions were done in a Mastercycler gradient®
(pro (Eppendorf, Hamburg, Germany) using the following
conditions, initial denaturation for 4 min at 94°C followed
by 35 cycles of 30 s at 94°C, 50 s at 60°C, and 70 s at 72°C, with
a final extension step for 7 min at 72°C.

3.5. UPEC Sero-Grouping

The E. coli sero-grouping was determined by the ampli-
fication of the following target genes: O1, O2, O4, O5, O7, O12,
O15, O16, O18, O25, O75, and O57 as described by Clermont et
al. (16). In brief, the experiment was performed in two sepa-
rate PCR runs, including six reverse primers representative
de of six O-reverse and one universal forward primer (Table 1)
as described by Clermont et al. (16). Each 25 µl PCR mix-
ture contained 9.5 µL ddH2O, 0.2 mM/mL of each primer (1
µL) (Pishgam, Iran), 2 µL of genomic DNA and 12.5 µL of Taq
DNA Polymerase Master Mix Red (amplicon). The PCR am-
plification was done in the following condition: 95°C for 4
min, 30 cycles of 95°C for 40 s, 57°C for 30 s, and 72°C for 30
s, with a final extension of 72°C for 6 min. The PCR products
were visualized by 1.5% agarose gel (1 × TAE buffer).

3.6. Statistical Analysis

The analyses of data were done by appropriate de-
scriptive statistics. Statistical Package for the Social Sci-
ences (SPSS) V 16.0 software was used for statistical analy-
sis. Descriptive analysis of data was done by chi-square and
Fisher’s exact tests at the P value of ≤ 0.05.

4. Results

4.1. Antibiotic Resistance

The antibiotic susceptibility test showed that the 92%,
74%, 71%, 65%, 55%, 53%, 43%, and 19% of UPEC isolates were
resistant to ampicillin, cefazolin, cefixime, cefotaxime, cef-
tazidime, azithromycin, ciprofloxacin, and gentamycin,
respectively. According to in vitro findings, ciprofloxacin
and gentamycin were more effective antibiotics for the
treatment of UPEC isolates, while ampicillin, cefazolin and
cefixime had the least therapeutic effects. More than 70%
of 

\[ \text{E. coli} \]

isolates were resistant to the third generation of the tested cephalosporins and 57% were susceptible to ciprofloxacin (Table 2).

| Primer Sequence (5’-3’) | Size, bp |
|-------------------------|----------|
| fimH                    | 400      |
| GTGTTGCTCTTGCCCCTGTC   |          |
| TAAGGTGCACCAATCCAG     |          |
| papC                    | 328      |
| GACGGCGTCGACCGAGGGTGTGGCG |          |
| ATATCCTTTCGACGGATTGCAATA |          |
| sfa                     | 100      |
| CGTAAGAGTGCTGCGGAG     |          |
| AGCCAGTCTCG--GCCACCGG  |          |
| foc                     | 388      |
| GGTGGAACCGGACAAATATAC |          |
| GAGCCTGGAGGMAGAGTG     |          |
| afa                     | 750      |
| GCTGGGCGACGAAACTGATAACTCTC |        |
| CACGACGGTGTGTTGCTGGCCCG |          |
| papGII                  | 461      |
| TGCTGCTGACGGCCGATTTT   |          |
| TGGAACCCGACCAATITC     |          |
| papGIII                 | 190      |
| GGGATGACGGCGCCCTGTGAT  |          |
| CGGCGCCCGAACGACTCG     |          |
| papGII                  | 258      |
| GCCCTGGAATGGATTACGTCC  |          |
| CCACCAAGAGACGACCAAGAC  |          |
| gndb1s.F                |          |
| ATCCCGAGGCGCCCATCTG    |          |
| rfo1K.R                 | 189      |
| CCAGAATACACTGCGAGAC    |          |
| rfo25.K.R               | 247      |
| GCTAAATTCCGAAACTCCG    |          |
| rfo17.R                 | 360      |
| GAGATGCGCTACTATGCTTCCG |          |
| rfo16.R                 | 450      |
| GCTACTTATGCTGTAAGCC    |          |
| rfo16a.R                | 584      |
| AATGGGCGCCCACTTAC      |          |
| rfo7.R                  | 722      |
| CGAAGATCATCCAGGATCCG   |          |
| rfo4.R                  | 393      |
| AGGGCCATTGGACACCACCTC  |          |
| rfo12.R                 | 239      |
| GTGTTGACTGCTGGTACCC    |          |
| rfo25a.R                | 313      |
| GAGATCCAAAAAGCTTGTGT  |          |
| rfo7R5.R                | 491      |
| GTAAATATGCTGGCAAACC    |          |
| rfo15.R                 | 536      |
| TGTAATGACCAATCGAGG     |          |
| rfo17.R                 | 672      |
| TACGACAGAAGCTGTGGAG    |          |

Table 1. Primer Used In This Study

| Primer Sequence (5’-3’) | Size, bp |
|-------------------------|----------|
| fimH                    | 400      |
| GTGTTGCTCTTGCCCCTGTC   |          |
| TAAGGTGCACCAATCCAG     |          |
| papC                    | 328      |
| GACGGCGTCGACCGAGGGTGTGGCG |          |
| ATATCCTTTCGACGGATTGCAATA |          |
| sfa                     | 100      |
| CGTAAGAGTGCTGCGGAG     |          |
| AGCCAGTCTCG--GCCACCGG  |          |
| foc                     | 388      |
| GGTGGAACCGGACAAATATAC |          |
| GAGCCTGGAGGMAGAGTG     |          |
| afa                     | 750      |
| GCTGGGCGACGAAACTGATAACTCTC |        |
| CACGACGGTGTGTTGCTGGCCCG |          |
| papGII                  | 461      |
| TGCTGCTGACGGCCGATTTT   |          |
| TGGAACCCGACCAATITC     |          |
| papGIII                 | 190      |
| GGGATGACGGCGCCCTGTGAT  |          |
| CGGCGCCCGAACGACTCG     |          |
| papGII                  | 258      |
| GCCCTGGAATGGATTACGTCC  |          |
| CCACCAAGAGACGACCAAGAC  |          |
| gndb1s.F                |          |
| ATCCCGAGGCGCCCATCTG    |          |
| rfo1K.R                 | 189      |
| CCAGAATACACTGCGAGAC    |          |
| rfo25.K.R               | 247      |
| GCTAAATTCCGAAACTCCG    |          |
| rfo17.R                 | 360      |
| GAGATGCGCTACTATGCTTCCG |          |
| rfo16.R                 | 450      |
| GCTACTTATGCTGTAAGCC    |          |
| rfo16a.R                | 584      |
| AATGGGCGCCCACTTAC      |          |
| rfo7.R                  | 722      |
| CGAAGATCATCCAGGATCCG   |          |
| rfo4.R                  | 393      |
| AGGGCCATTGGACACCACCTC  |          |
| rfo12.R                 | 239      |
| GTGTTGACTGCTGGTACCC    |          |
| rfo25a.R                | 313      |
| GAGATCCAAAAAGCTTGTGT  |          |
| rfo7R5.R                | 491      |
| GTAAATATGCTGGCAAACC    |          |
| rfo15.R                 | 536      |
| TGTAATGACCAATCGAGG     |          |
| rfo17.R                 | 672      |
| TACGACAGAAGCTGTGGAG    |          |

4.2. Adhesin-Encoding Genes

The rate of the studied virulence genes is presented in Table 2. Regarding all studied adhesin factors, the fimH gene was the most common adhesin gene and was identified in 95% of the UPEC isolates followed by sfa (81%), papC (57%), papGII (34%), foc (16%), papGI (16%), afa (12%) and papGIII (4%) genes. The carriage of adhesin-encoding genes in ciprofloxacin-susceptible and -resistant isolates is also summarized in Table 2. Among the adhesin-encoding genes examined, the prevalence of fimH (55 vs. 40), sfa (47 vs. 34), papC (29 vs. 28), foc (10 vs. 6), afa (11 vs. 1), papGII (8 vs. 8), papGIII (19 vs. 15), and papGIII (2 vs. 2) was higher in susceptible isolates compared with resistant isolates. As far as adhesin genes are concerned, fimH, sfa, papC, papGI, and papGII were found in a large number of isolates of both ciprofloxacin susceptible and resistant isolates, whereas afa was almost exclusively found in the ciprofloxacin susceptible isolates (11 vs. 1). Almost all of ciprofloxacin-susceptible/resistance UPEC isolates were positive for adhesin production and there was no significant discrimination between ciprofloxacin-susceptible and -resistant isolates (Table 2).

All the ciprofloxacin susceptible and resistant studied isolates exhibited 20 and 21 adhesin genes patterns, corresponding to EC, respectively, of which the following gene associations occurred more frequently in ciprofloxacin susceptible isolates: fimH, sfa, papC, foc, afa, and papGII (1 isolate), fimH, sfa, papC and foc (3 isolate), and EC6 was identified by the presence of the fimH, sfa, papC and papGII only, and was the most prominent pattern found in susceptible isolates (Table 3). Among 43 ciprofloxacin-resistant isolates: fimH, sfa, papC and foc (1 isolate), fimH, sfa, papC, papGI, and papGII (4 isolates) and fimH, sfa, papC, papGII (4 isolates) and fimH, sfa (11 isolates), fimH, sfa, and papC (5 isolates) were the most noted pattern (Table 4). In addition, the fimH, sfa, and papC genes were more detected in the ciprofloxacin-susceptible isolates (31 vs. 17).

4.3. Serotype Structure

Of 100 UPEC isolates, 73 of the isolates belonged to the assessed 12 O-serogroups. Twenty-seven isolates could not be typed as they were either rough or non-typeable. The sero-groups O2, O9a, O107, O25, O4, O9, O16, O25, O7, and O2 were present in 16.43%, 16.43%, 13.69%, 10.95%, 9.58%, 8.21%, 6.84%, 4.10%, 2.73%, and 2.73% of UPEC isolates, respectively. There was no positive isolate for O15. Out of 73 O-serotyped UPEC isolates 69 (95%) and 59 (81%) were positive for fimH and sfa genes which were the most two prevalent genetic markers. In total, 73%, 73%, 72%, 63%, 92%, 81%, 79%, and 100% of fimH, sfa, papC, foc, afa, papGI, papGII, and papGIII were found in all O-serotyped isolates (Table 5). All of the detected adhesin-encoding genes were
Table 2. Pattern of Antibiotic Susceptibility and Distribution of Adhesin Genes Among Escherichia coli Isolates

| Pattern | CZ | CP | APM | GM | AM | CFM | CTX | CAZ |
|---------|----|----|-----|----|----|-----|-----|-----|
| fimH (95) | 26 | 69 | 0.87 | 55 | 40 | 1 | 45 | 50 | 1 | 77 | 18 | 1 | 7 | 88 | 1 | 29 | 65 | 0.87 | 35 | 60 | 0.88 | 44 | 51 | 0.88 |
| sfa (94) | 25 | 55 | 0.70 | 47 | 34 | 1 | 54 | 43 | 1 | 67 | 14 | 1 | 45 | 50 | 1 | 77 | 18 | 1 | 7 | 88 | 1 | 29 | 65 | 0.87 | 35 | 60 | 0.88 | 44 | 51 | 0.88 |
| papC (187) | 17 | 40 | 0.73 | 29 | 28 | 1 | 20 | 21 | 1 | 47 | 10 | 1 | 6 | 51 | 1 | 77 | 18 | 1 | 7 | 88 | 1 | 29 | 65 | 0.87 | 35 | 60 | 0.88 | 44 | 51 | 0.88 |
| foc (185) | 13 | 42 | 0.86 | 10 | 8 | 1 | 18 | 15 | 1 | 54 | 13 | 1 | 24 | 20 | 1 | 67 | 14 | 1 | 7 | 88 | 1 | 29 | 65 | 0.87 | 35 | 60 | 0.88 | 44 | 51 | 0.88 |
| papGI (182) | 11 | 44 | 0.60 | 8 | 6 | 1 | 28 | 24 | 1 | 47 | 10 | 1 | 6 | 51 | 1 | 77 | 18 | 1 | 7 | 88 | 1 | 29 | 65 | 0.87 | 35 | 60 | 0.88 | 44 | 51 | 0.88 |
| papGII (181) | 10 | 42 | 0.73 | 5 | 4 | 1 | 30 | 26 | 1 | 54 | 13 | 1 | 24 | 20 | 1 | 67 | 14 | 1 | 7 | 88 | 1 | 29 | 65 | 0.87 | 35 | 60 | 0.88 | 44 | 51 | 0.88 |
| papGIII (180) | 9 | 41 | 0.70 | 4 | 3 | 1 | 30 | 26 | 1 | 54 | 13 | 1 | 24 | 20 | 1 | 67 | 14 | 1 | 7 | 88 | 1 | 29 | 65 | 0.87 | 35 | 60 | 0.88 | 44 | 51 | 0.88 |

Table 3. Adhesin Gene Profile Pattern Among Ciprofloxacin-Susceptible Escherichia coli Isolates

| Pattern | fimH | sfa | papC | foc | afa | papGI | papGII | papGIII | Number of Strains |
|---------|------|-----|------|-----|-----|-------|--------|---------|-----------------|
| EC1 | + | + | + | + | - | + | - | - | 1 |
| EC2 | + | + | + | + | - | + | - | - | 3 |
| EC3 | + | - | + | - | - | + | + | - | 1 |
| EC4 | + | - | + | - | - | - | - | - | 1 |
| EC5 | + | + | + | - | - | - | - | - | 1 |
| EC6 | + | + | + | - | - | - | - | - | 1 |
| EC7 | + | - | + | - | - | - | - | - | 1 |
| EC8 | + | + | + | - | - | - | - | - | 1 |
| EC9 | + | + | + | - | - | - | - | - | 1 |
| EC10 | + | + | + | - | - | - | - | - | 1 |
| EC11 | + | + | + | - | - | - | - | - | 1 |
| EC12 | + | + | + | - | - | - | - | - | 1 |
| EC13 | + | + | + | - | - | - | - | - | 1 |
| EC14 | + | + | + | - | - | - | - | - | 1 |
| EC15 | + | + | + | - | - | - | - | - | 1 |
| EC16 | + | + | + | - | - | - | - | - | 1 |
| EC17 | + | + | + | - | - | - | - | - | 1 |
| EC18 | + | + | + | - | - | - | - | - | 1 |
| EC19 | + | + | + | - | - | - | - | - | 1 |
| EC20 | + | + | + | - | - | - | - | - | 1 |
| Total | 56 | 48 | 33 | 9 | 9 | 8 | 18 | 3 | 57 |

Abbreviations: AM, ampicillin; AZM, azithromycin; CAZ, ceftazidime; CZ, cefazolin; CFM, cefixime; CTX, cefotaxime; CP, ciprofloxacin; GM, gentamycin.

5. Discussion

A better knowledge of the frequency of genes coding for fimbrial adhesive systems such as fimH, papC, and papG alleles of UPEC strains, especially in ciprofloxacin-susceptible/resistant UPEC isolates allows the scientists to pursue the pattern of pathogenicity of strains causing the present alone or in combination with each other. Our results showed that sfa, fimH, papC, foc, afa, papGI, and papGII were found in 100%, 90%, 80%, 50%, 10%, 40%, and 60% of O18 positive isolates, respectively. Of various papG alleles, allele III were more prevalent in O16 isolates (3 out of 4 isolates) and allele II was harbored by 18%, 15%, 12%, 9%, 9%, 3%, and 3% of O18, O1, O157, O16, O2, O75, and O18 positive UPEC isolates, respectively (Table 5). Our data found that all O1, O2, and O25 positive isolates were negative for the three alleles of papG. The genetic marker for papC was found in 41 (56.16%) isolates belonging to different O-serotype, which was the third most prevalent adhesin gene.
UTIs (17, 18). Since ciprofloxacin is currently used in protocols to treat UTIs, we feel it is appropriate to have a stronger knowledge of the interaction between the ciprofloxacin-resistance and the modest changes in the prevalence of genes involved in pathogenicity, especially adhesin encoding genes. Therefore, as ciprofloxacin use in UTIs, the frequency of ciprofloxacin-resistant is increasing among clinical isolates (19), clinicians may begin to see a change in the patterns of urinary tract disease accompanied by modifications in the prevalence of *E. coli* virulence determinants.

Ciprofloxacin usually prevents the synthesis of bacterial DNA through the inhibition of two DNA gyrase enzymes and topoisomerase, which are essential for bacterial viability (20). However, the frequency of genes involved in virulence in both ciprofloxacin-sensitive and -resistant UPEC strains is not obvious.

It has been proposed that ciprofloxacin-resistant bacteria may lose some virulence genes due to decreased efficiency of gyrase and topoisomerase (21). In contrast, the development of resistance to some beta-lactam antibiotics, such as ampicillin, correlates with adhesin-encoding genes, which play an important role in bacterial colonization (22). In this study, 57 and 43 isolates were susceptible and resistant to ciprofloxacin, respectively that is consistent with the finding of Alishahi et al. in Estahban-Iran (23). In our study, as we expected, almost all the isolates (95%) harbored the *fimH* gene were consistent with some previous reports (24, 25). Conversely, in agreement with other published data in Iran (26), the prevalence of *fimH* was reported 64 percent. Moreover, the *fimH* gene was highly conserved (55%, 40%) in both ciprofloxacin-susceptible and-resistant isolates, emphasizing its important role during urinary tract colonization (27, 28). In addition, 34% of the isolates had *papG* II that is responsible for encoding PapG adhesion on the tips of P fimbriae. In other studies, it was realized that various classes of PapG adhesion were dominant in the *E. coli* strains isolated from UTIs (6, 29). In the current study, the frequency of *papGI* and *papGIII* were 16% and 4%, respectively. It was presented that class II papG allele was contributed to pyelonephritis cases, while *papGIII* was initially associated with UTIs in dogs and cats (6).

The *sfa* was the second most predominant adhesin gene (81%) in our isolates that was in agreement with a research carried out in Shiraz (Fars province, Iran) with a frequency of 79.4% (29). In previous reports, the prevalence of the *sfa* was conversely less than 30% (7). Nevertheless, the exact role of S-fimbriae is not clarified; therefore, the distribution of bacterium within the host tissue depends on this adhesin marker (29). In our isolates, the *papC* was the third most prevalent adhesin gene (57%) that was similar to a study conducted in Jahrom (Fars province, Iran) (30). The *PapC* usher protein was essential for the fimbriae P biogenesis regulation that was encoded by the *papC* gene (31) which was associated with pyelonephritis. Therefore, a
Table 5. Common Virulence Factors, Antibiotic Resistance, and Genetic Among O-Serotype *Escherichia coli* isolates of Human

| Genes    | O1 (6) | O2 (12) | O4 (6) | O6 (12) | O7 (2) | O12 (2) | O16 (50) | O18 (10) | O25 (3) | O75 (7) | O157 (8) | Total (73) |
|----------|--------|---------|--------|---------|--------|---------|----------|----------|---------|--------|---------|-----------|
| fimH     | 5 (5.26) | 20 (20.53) | 5 (5.26) | 20 (20.53) | 2 (2.20) | 2 (2.20) | 5 (5.26) | 10 (12.63) | 2 (2.10) | 8 (9.47) | 2 (2.00) | 7 (7.86) | 6 (6.98) | 69 (77.0) |
| Sfa      | 4 (4.65) | 50 (12.54) | 6 (7.40) | 10 (12.63) | 2 (2.46) | 2 (2.46) | 4 (4.93) | 10 (12.63) | 1 (1.23) | 8 (7.40) | 4 (4.93) | 10 (12.63) | 1 (1.23) | 6 (7.40) | 10 (12.63) | 9 (11.0) |
| papC     | -      | 50 (12.54) | 5 (5.26) | 5 (5.26) | 1 (1.23) | 2 (2.46) | 4 (4.93) | 10 (12.63) | 1 (1.23) | 8 (7.40) | 4 (4.93) | 10 (12.63) | 1 (1.23) | 6 (7.40) | 10 (12.63) | 9 (11.0) |
| foc      | -      | -       | 1 (6.25) | -       | -       | -       | 1 (6.25) | -       | -       | -       | -       | -       | 1 (6.25) | -       | -       | 1 (6.25) |
| afa      | 12 (12.63) | 12 (12.63) | -       | 12 (12.63) | -       | -       | 1 (6.25) | -       | -       | -       | -       | 12 (12.63) | 2 (2.10) | 8 (9.47) | 2 (2.00) | 40 (52.6) |
| papGI    | 16 (16.39) | -       | 1 (6.25) | -       | 1 (6.25) | -       | 1 (6.25) | -       | -       | -       | -       | 16 (16.39) | 5 (5.26) | 1 (1.23) | 8 (7.40) | 27 (37.2) |
| papGII   | 34 (41.0) | -       | 1 (6.25) | -       | 1 (6.25) | -       | 1 (6.25) | -       | -       | -       | 2 (3.50) | 34 (41.0) | 5 (5.26) | 1 (1.23) | 8 (7.40) | 21 (28.3) |
| papGIII  | 4 (4.93) | -       | 1 (6.25) | -       | 1 (6.25) | -       | 1 (6.25) | -       | -       | 2 (3.50) | -       | 13 (16.63) | 4 (4.93) | 1 (1.23) | 8 (7.40) | 13 (16.63) |

| Antibiotic resistance | AZM | AM | CZ | CFM | CTX | CAZ | GM | CP |
|-----------------------|-----|----|----|-----|-----|-----|----|----|
| S                     | 5   | 2  | 1  | 4   | 4   | 1   | 1  | 1  |
| R                     | 0   | 6  | 1  | 0   | 0   | 0   | 0  | 0  |
| S                     | 5   | 8  | 4  | 1   | 2   | 4   | 3  | 3  |
| R                     | 0   | 4  | 0  | 1   | 0   | 0   | 0  | 0  |
| S                     | 3   | 5  | 6  | 1   | 2   | 4   | 3  | 3  |
| R                     | 0   | 4  | 0  | 1   | 0   | 0   | 0  | 0  |
| S                     | 4   | 2  | 5  | 1   | 2   | 4   | 3  | 3  |
| R                     | 1   | 5  | 2  | 1   | 0   | 0   | 0  | 0  |
| S                     | 5   | 4  | 5  | 1   | 2   | 4   | 4  | 4  |
| R                     | 0   | 7  | 2  | 2   | 1   | 2   | 5  | 5  |
| S                     | 3   | 3  | 4  | 1   | 2   | 3   | 7  | 7  |
| R                     | 2   | 9  | 2  | 1   | 2   | 3   | 2  | 2  |
| S                     | 2   | 3  | 4  | 0   | 1   | 0   | 0  | 0  |
| R                     | 3   | 10 | 0  | 7   | 2   | 2   | 5  | 5  |
| S                     | 1   | 2  | 4  | 0   | 1   | 1   | 0  | 0  |
| R                     | 4   | 9  | 2  | 2   | 1   | 4   | 3  | 3  |

Abbreviations: AM, ampicillin; AZM, azithromycin; CAZ, ceftazidime; CZ, cefazolin; CFM, cefixime; CTX, cefotaxime; CP, ciprofloxacin; GM, gentamicin.

high percentage of over 50%, including both ciprofloxacin-resistant (29%) and -susceptible (28%) UPEC isolates collected from the Zabol (Sistan and Balouchestan province, Iran) population, has great potential to colonize kidneys and generate pyelonephritis (31). In the present study, the frequency of *afa* was 12% that might influence the development of chronic nephritis.

In addition, there is no significant correlation between the presence of adhesin-encoding genes in ciprofloxacin-resistant and -susceptible UPEC isolates, although the *afa* gene is higher in ciprofloxacin-susceptible isolates (11 vs 1) that is able to be linked to cystitis cases. This result is in accordance with the previous report by Lloyd et al. (32). In this research, it was shown that ciprofloxacin-resistant UPEC isolates presented a lower prevalence of fimbrial genes (*fimH*+, *sfa*+, *papC*+) compared to ciprofloxacin-susceptible ones (31 vs 17). As the same as the previous results (33), the outcomes declared that fimbrial genes had a lower prevalence in UPEC resistant extended-spectrum cephalosporins rather than susceptible isolates. A possible description was that the virulence genes like the resistance genes could be harbored on conjugative plasmids; then the incompatible resistance-encoding plasmids were outcompeting fimbrial factor encoding plasmids (33).

It was discovered that the acquisition of antibiotic resistance might lead to alterations in phenotypic and physiological properties that were referred to as "biological fitness cost". The biological fitness cost on antibiotic resistance generally causes to decrease growth rates. More un-
favorable phenotypic changes, including poor fimbral expression, were presented in an ampicillin-resistant mutant Acinetobacter spp. strain DRIA in comparison to the wild type strain DRI (34). Therefore, the declined fimbral genes and adherence ability in ciprofloxacin-resistant UPEC might also be a reason for fitness trade-off for the quinolones to escape antibiotics exposure. More research is needed to explain the lower prevalence of fimbral genes among resistant UPEC isolates. In this study, 73% of the UPEC isolates were included in the eleven O-serotype (O2, O6, O9, O27, O75, O1, O4, O46, O25, O2, and O12), which O2 and O6 were the most predominant (16.43%). Similar results have been reported recently too (15, 35).

The most usual antibacterial drugs in UTI’s treatment are trimethoprim-sulfamethoxazole, cephalosporins and semi-synthetic penicillins alone or with beta-lactamase inhibitors and quinolones (36). In the present study, no correlation was seen between the type of O antigen and antibiotic resistance. Few other studies have been conducted for the evaluation of the correlation between adhesin-encoding genes, antibiotic resistance, and O antigen expression (37, 38).

5.1. Conclusions

Altogether, E. coli that causes UTI in different patients varies in its’ their pathogenic capability and susceptibility to antimicrobial and O-serotype profile. Developing guidelines for the management of UTI should be considered. Periodical surveys and formulation of antibiotic consumption policy are required to control the transmission and acquisition of antibiotic resistance.

This is the first report of the E. coli serotyping in the patients with UTI from southeast of Iran and their relation to antibiotic resistance and adhesin-encoding genes. Further research is necessary for a better understanding of the interaction between different virulence factors at the molecular level as well as the majority of the UPEC isolates that simultaneously express several VFs. Consequently, these results reinforce the international knowledge about antimicrobial resistance and the high rate of adhesin-encoding genes that encourage society to be aware of the proper use of antimicrobials.

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Footnotes

Authors’ Contribution: Study design, data collection, and data interpretation: Masuod Rahdar. Study design, data collection, data interpretation, funds collection, literature review, and manuscript preparation: Ahmad Rashki. Study design, manuscript preparation, and data interpretation: Zahra Rashki Ghalehnoo. Study concept and design: Ahmad Rashki and Zahra Rashki Ghalehnoo. Data collection and literature review: Masuod Rahdar and Ahmad Rashki.

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