# Original Article

# Multiplex PCR Amplification for the Detection of Biofilm and Extended-spectrum Beta-Lactamase Resistance Genes and Antibiotic Resistance Patterns in Uropathogenic *E. coli*

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## ABSTRACT

The issue of urinary tract infections (UTIs), particularly those stemming from *Escherichia coli* belonging to the *Enterobacteriaceae* family, has received considerable critical attention and is evaluated as the second most common infection in humans. Uropathogenic *Escherichia coli* (UPEC), which is virulent, produces extended spectrum beta-lactamase (ESBL), as well as being multidrug-resistant (MDR), is considered to be a common growing public health issue worldwide. This phenomenon has been demonstrated to contribute to the escalation of UTIs to more severe states, the diminution in the efficacy of first-line antibiotics, and the consequent escalation in morbidity and mortality rates. The present experiment involved the isolation of 73 *Escherichia coli* strains from urine specimens, and the antibiotic susceptibility of the isolates was evaluated through the disc agar diffusion method. The resistance patterns exhibited by these isolates collectively constitute the underlying basis for MDR. The evaluation of three significant biofilm genes and antimicrobial resistance mechanisms in these isolates was conducted using ten typical antibiotic discs. The data was processed using SPSS statistical software, version 25. The investigation revealed that 73 isolates of *E. coli* were examined, with the *pap* gene present in 89% of isolates, the *fim*H gene present in 86.3% of isolates, and the *sfa* gene present in 69.9% of isolates. Furthermore, the beta-lactamase gene *bla*SHV, *bla*TEM, and *bla*CTX-M gene frequency was found to be 50.7%, 90.4%, and 79.5%, respectively. The results regarding antibiotic resistance patterns elucidated that a significant number of the isolates were resistant to Imipenem, Amoxicillin, and Ampicillin, respectively. This study posits that the rapid emergence of virulent ESBL-producing *E. coli* strains in such experiments necessitates the implementation of an antibiotic stewardship program, regional surveillance of extended-spectrum beta-lactamase (ESBL)-producing organisms and their associated virulence determinants for the purpose of rational antibiotic selection, or the development of novel UTI treatment strategies that involve the inactivation of essential virulence factors relating to UPECs.

**Keywords:** UPEC, *E. coli*, ESBL, UTIs, Biofilm.

## 1. Introduction

Uropathogenic *Escherichia coli* (UPEC) is a bacterium that is commonly associated with urinary tract infections (UTIs), particularly among female patients. It is therefore probable that numerous virulence factors (VFs) found in UPEC isolates have an impact on the frequency and severity of UTIs (1, 2). A number of these VFs contain genes that encode adhesins, including temperature-sensitive hemagglutinin (tsh), papA, curli fimbriae (csgA), papC-associated pili, a fimbrial adhesin (afa), and the type-1 fimbriae (fimH). These adhesins play a substantial role in the adherence of bacteria to the urinary tract, facilitating the initial stages of urinary tract infections (UTIs). Furthermore, the presence of toxins, such as α-hemolysin (hlyD), has been demonstrated to exert a deleterious effect on the host, thereby significantly accelerating the pathogenicity of the bacterial strain in question (3, 4). In addition, genes that encode invasins, such as ibeA, have been shown to facilitate the invasion of microvascular endothelial cells in the brain. Concurrently, genes that encode protective factors, including increased serum survival (iss), have been observed to contribute to the protection of *E. coli* from the bactericidal effects of serum. Furthermore, the presence of siderophores, such as the sitA iron transporter, facilitates bacterial growth, and the pathogenicity island marker malX is involved in the glucose and maltose transportation in terms of treating urinary tract infections. The escalating prevalence of antibiotic resistance poses a significant health risk (5,6). Bacterial communities known as biofilms adhere to surfaces and are unclasped by an extracellular polymeric substance (EPS) matrix. Such biofilms form in response to environmental signals, allowing a population of bacteria to grow and survive in the urinary tract (6). In controlled laboratory settings, a specific strain of *E. coli* has been shown to produce biofilms with varying characteristics based on factors such as nutrient composition, temperature, and flow dynamics (7). The synthesis of exopolysaccharides (e.g. cellulose) that form the biofilm matrix encompassing the bacteria is facilitated by autotransporter proteins that also play a role in this process (8). The process of adherence between bacteria and host cells is facilitated by specific bacterial adhesins such as P-fimbriae, which are expressed by the pyelonephritis associated pili gene or the *pap* gene. P-fimbriae plays an indispensable role in the colonization of the upper urinary tract by bacteria, their affixation to the renal vascular endothelium, and the subsequent development of pyelonephritis (9). It is imperative to consider the role of S-fimbriae in the context of adhesion, given its regulation by the S-fimbria adhesion gene (*sfa*) and the *sfa* gene. These genes, which are classified as mannose resistant adenosine, are located within a region of the chromosome designated as Pathogenicity Islands (10). The *sfa* and *pap* genes are frequently observed in *E. coli* strains isolated from urinary tract infections (UTIs), and are responsible for encoding pili that aid in the adherence of bacteria to the host tissues, resulting in the formation of antibiotic-resistant biofilms (11).

It is crucial to identify UPEC strains capable of producing biofilms in order to gain a deeper understanding of the pathogenicity and antibiotic resistance mechanisms exhibited by this bacterium in UTIs. The study's findings indicate that beta-lactam antibiotics are commonly prescribed for the treatment of urinary tract infections in clinical settings (12). Certain bacterial strains, notably those of Escherichia coli, are capable of producing extended-spectrum beta-lactamases (ESBLs), which are enzymes that can degrade penicillins, first to third-generation cephalosporin, and monobactams (aztreonam). Inhibition of the three primary ESBL enzyme groups - TEM, CTX-M, and SHV - has been demonstrated to be effective when using clavulanic acid, tazobactam, or sulbactam (13). It is important to note that the management of infections resulting from ESBL-producing *E. coli* can become increasingly challenging and restricted in cases where resistance to additional antibiotic categories is present concurrently. These categories include aminoglycosides, tetracyclines, chloramphenicol, trimethoprim-sulfamethoxazole, and fluoroquinolones, and are often found within the same plasmids containing the ESBL genes. This phenomenon has the potential to exert a substantial influence on the economic burden of morbidity and mortality associated with UTIs (14). The present study is thus focused on the examination of the prevalence of biofilm genes (*pap*, *fim*H, and *sfa*) in conjunction with extended-spectrum Beta-Lactamase resistance genes ($bla_{TEM}$, $bla_{CTX-M}$, and $bla_{SHV}$), and on the investigation of the antimicrobial resistance profiles of UPEC in Karaj, Iran.

## 2. Materials and Methods

### 2.1. Sample Collection and Isolation of *E. coli*

This investigation was meticulously planned, with a focus on 78 *E. coli* isolates found in the urine samples of outpatients suspected of UTIs, spanning a period of 9 months from October 2022 to June 2023.The investigation yielded 73 *E. coli* isolates, from which 59 of the 73 samples were drawn from female subjects and 14 from male subjects. These samples were then transported to the laboratory within two hours of collection in TSB transport medium. Following this, the samples were cultured on Blood agar (Ibresco, Iran), MacConkey agar (Ibresco, Iran), and EMB agar (Ibresco, Iran) plates. These plates were then incubated at 37°C for a duration of 24 hours. Finally, strains of *E. coli* were obtained by standard microbiological methods.

### 2.2. Antimicrobial Susceptibility of *E. coli* isolates

Samples were selected for the purpose of examining the susceptibility patterns of 10 antimicrobial agents, which belonged to a variety of classes. These were as follows: tetracycline (30μg), imipenem (10μg), ampicillin (10 μg), co-trimoxazole (25μg), amikacin (30μg) and cefixime. (5μg),Cefalexin (30μg), Amoxicillin (25μg), Ciprofloxacin (5μg), and Gentamicin (10μg) discs (Padtan Teb, Iran).The Kirby-Bauer method was employed to investigate

the susceptibility of *E. coli* isolates to antibiotics, with analysis based on the guidelines outlined by CLSI.

## 2.3. DNA Extraction

In this experiment, DNA was extracted from samples using the boiling method. A loopful of bacterial colonies was suspended in 300 µl of sterile distilled water and subsequently subjected to heating for a duration of 20 minutes. The liquid part was then used as a DNA sample in the PCR (Polymerase Chain Reaction) mixture after spinning it in a machine for 15 minutes at a high speed of 13,000 rpm (15).

## 2.4. Detection of Genes

The present study utilized PCR and electrophoresis techniques to ascertain the presence of the *pap*, *fim*H, *sfa*, *bla*$_{TEM}$, *bla*$_{CTX-M}$, and *bla*$_{SHV}$ genes. The amplification conditions applied to the pap, *fim*H, and *sfa* genes were as follows: denaturation at 95°C for five minutes, 30 cycles of 95°C for one minute, 56°C for 40 seconds, 72°C for 45 seconds, and a final extension at 72°C for five minutes (15–17). Conversely, the *bla*$_{TEM}$, *bla*$_{SHV}$, and *bla*$_{CTX-M}$ genes were amplified under the following conditions: a preliminary denaturation at 95°C for 15 minutes, followed by 30 amplification cycles. Each cycle comprised denaturation at 94°C for 30 seconds, annealing at 62°C for 90 seconds, and elongation at 72°C for 60 seconds. The protocol was then concluded with an elongation step at 72°C for 10 minutes (18–20). The molecular approach was optimized using *E. coli* ATCC 25922 as the control positive strain. The primer sequences and PCR conditions required to detect genes are displayed in Table 1.

## 2.5. Statistical Analysis

The correlation between sociodemographic factors and the frequency of *E. coli* isolation was ascertained using model selection log-linear analysis on categorical data. Additionally, the statistical analyses were all conducted using SPSS 25.0 software for Windows. The threshold for statistical significance was determined to be P<0.05.

## 3. Results

## 3.1. Antibiotic Susceptibility

The cumulative resistance of *E. coli* isolates to antimicrobial agents was found to be as follows: 95.9% for Ampicillin; 84.9% for Imipenem; 57.9% for Amoxicillin; and 32.9% for Cefalexin. The findings indicate that the highest levels of sensitivity were observed for Amikacin (86.3%), Gentamicin (78.1%), and Co-trimoxazole (75.3%), respectively. The antibiotic susceptibility pattern of strains to antimicrobial agents is presented in Table 2. Figure 1 demonstrates the percentage of antibiotic resistance by gender.

## 3.2. Gene pattern characterization

The findings obtained from this investigation demonstrate that the occurrence of biofilm-producing genes in urine samples was as follows: the *sfa*, *fim*H, and pap genes was 69.9%, 86.3%, and 89% respectively. Furthermore, the frequency of β-lactamase genes in clinical samples was as follows: *bla*$_{SHV}$, *bla*$_{TEM}$, and *bla*$_{CTX-M}$ genes was 50.7%, 90.4%, and 79.5%, respectively. The PCR products of genes from E. coli isolates are shown in Figures 2 and 3. A notable discrepancy was observed in the prevalence of biofilm genes (*pap* and *fim*H) in relation to gender (P<0.05). The statistical analysis revealed significant associations between the resistant against Amoxicillin (P=0.04) and tetracycline (P=0.05) of the *bla*$_{SHV}$ gene in the UPEC isolates. Furthermore, the statistical analysis indicated significant connections between the resistant against Amoxicillin (P=0.02) and Gentamicin (P=0.04) of the *bla*$_{CTX-M}$ gene, along with critical associations between Ampicillin (P=0.001) and *bla*$_{TEM}$ in the UPEC isolates.

**Table 1.** Primers sequences as per standard reference.

| Gene | Primer Sequences (5′ to 3′) | Product Size (bp) | annealing | Reference |
|---|---|---|---|---|
| *pap* | F: GCAACAGCAACGCTGGTTGCATCAT<br>R: AGAGAGAGCCACTCTTATACGGACA | 336 | 56°C | 15 |
| *fim*H | F: GAGAAGAGGTTTGATTTAACTTATTG<br>R: AGAGCCGCTGTAGAACTGAGG | 559 | 56°C | 16 |
| *sfa* | F: CTCCGGAGAACTGGGTGCATCTTAC<br>R: CGGAGGAGTAATTACAAACCTGGCA | 410 | 56°C | 17 |
| *bla*$_{CTX-M}$ | F: ATGTGCAGYACCAGTAARGTKATGGC<br>R: TGGGTRAARTARGTSACCAGAAYCAGCGG | 593 | 62 °C | 18 |
| *bla*$_{SHV}$ | F: CTT TATCGGCCCTCACTCAA<br>R: AGGTGCTCATCATGGGAAAG | 237 | 62 °C | 19 |
| *bla*$_{TEM}$ | F: CGCCGCATACACTATTCTCAGAAT GA<br>R: ACGCTCACCGGCTCCAGATTTAT | 445 | 62 °C | 20 |

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**Table 2.** Antimicrobial susceptibility pattern of *E. coli* in total isolates.

| Antibiotics | Sensitive (%) | Intermediate (%) | Resistance (%) |
|---|---|---|---|
| Tetracycline | 41 (56.2) | 11 (15.1) | 21 (28.8) |
| Amikacin | 63 (86.3) | 2 (2.7) | 8 (11) |
| Ampicillin | 1 (1.4) | 2 (2.7) | 70 (95.9) |
| Amoxicillin | 37 (36.8) | 9 (5.3) | 27 (57.9) |
| Cefalexin | 39 (53.4) | 10 (13.7) | 24 (32.9) |
| Co-trimoxazole | 55 (75.3) | 6 (8.2) | 12 (16.4) |
| Cefixime | 41 (56.2) | 14 (19.2) | 18 (24.7) |
| Imipenem | 8 (11) | 3 (8.2) | 62 (84.9) |
| Ciprofloxacin | 43 (58.9) | 9 (12.3) | 21 (28.8) |
| Gentamicin | 57 (78.1) | 1 (1.4) | 15 (20.5) |
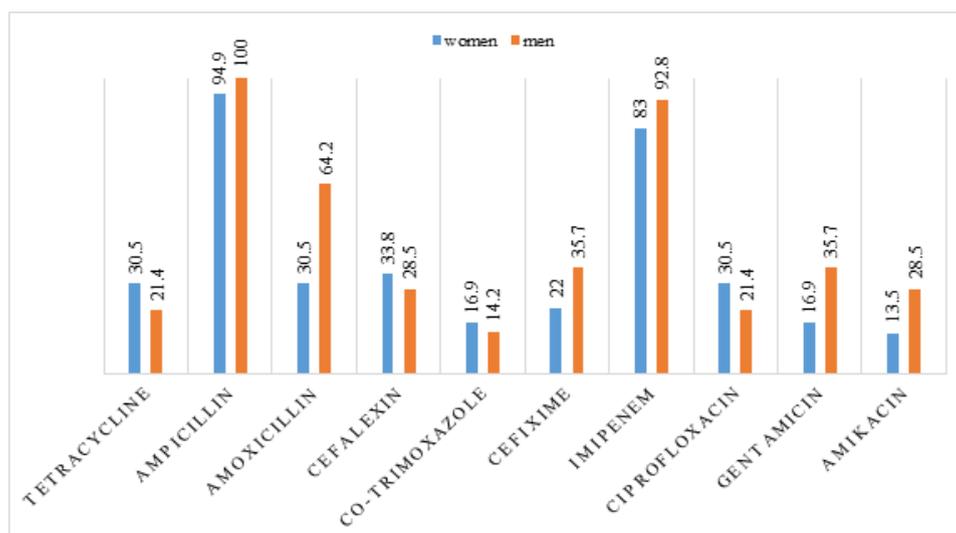


**Figure1.** The percentage of antibiotic resistance by gender.
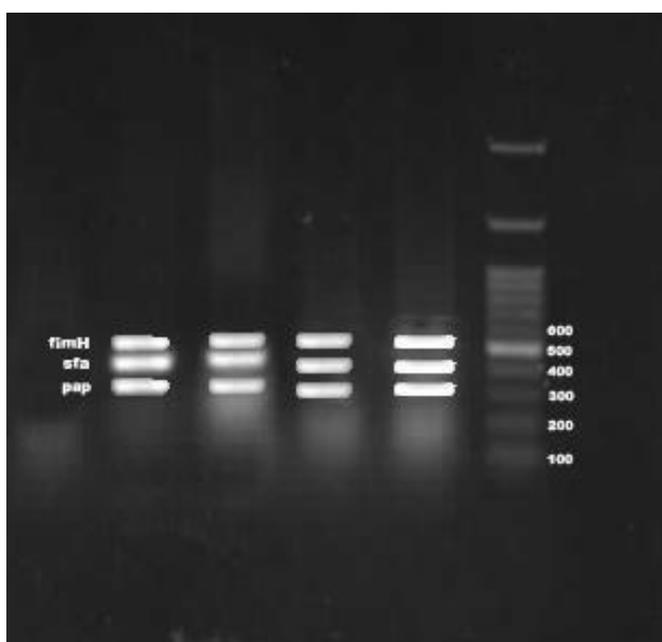


**Figure2.** Multiplex PCR Amplification of *pap*, *fimH*, *sfa* from *E.coli* isolates. 100bp DNA ladder.
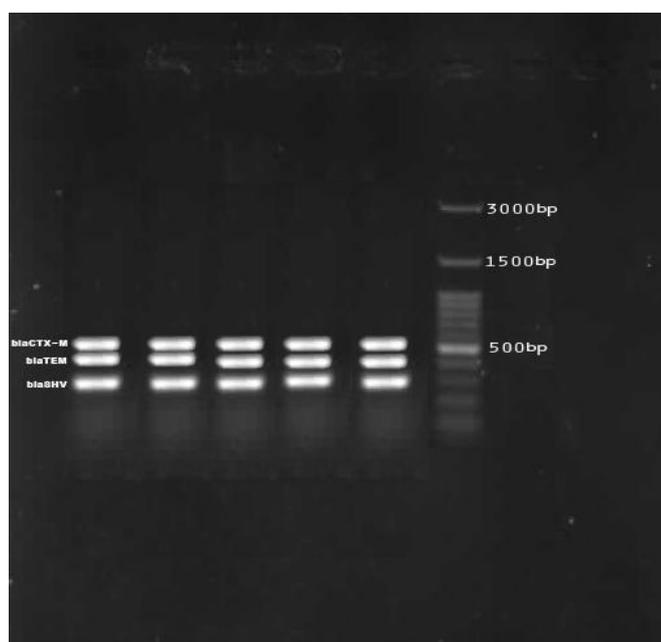


**Figure3.** Multiplex PCR Amplification of *bla$_{SHV}$*, *bla$_{TEM}$*, and *bla$_{CTX-M}$* genes from *E.coli* isolates. 100bp DNA ladder.

## 4. Discussion

Research has demonstrated that *E. coli* is the most prevalent factor in the occurrence of urinary tract infections (UTIs) among both ambulatory individuals and those hospitalized. This discrepancy can be attributed to the anatomical differences between the male and female genital tracts, particularly the shorter length of the female urethra, which facilitates access for pathogenic species such as *E. coli* to the bladder. In terms of microbial expansion, the position of the female urethral orifice, which is in close proximity to the vaginal and anal areas, contributes to the less demanding action by the microbes to reach the urinary tract area (21). In the present study, a comprehensive set of 73 isolates belonging to the *E. coli* species were analyzed, revealing the presence of the pap gene in 89% of isolates, the *fim*H gene in 86.3% of isolates, and the *sfa* gene in 69.9% of isolates. Furthermore, the prevalence of the beta-lactamase gene $bla_{SHV}$, $bla_{TEM}$, and $bla_{CTX-M}$ were found to be 50.7%, 90.4%, and 79.5%, respectively. The antibiotic resistance patterns exhibited that the majority of the isolates demonstrated resistance to Ampicillin, Imipenem, and Amoxicillin, respectively. In a report by Yazdi et al. (22), the frequency of the *fim*, *pap*, and *sfa* genes was 100%, 79%, and 69%, respectively, which was close to the results of the current investigation. In a further major study, Saki et al. (23) stated the prevalence of biofilm genes pap, *fim*H, and *sfa* as 96.6%, 93.3%, and 4.6%, respectively, among which the prevalence of *fim*H and *pap* genes are close to this paper's results. In a separate study by Naziri et al. (24), 100% of ESBL-producing strains were found to harbor $bla_{CTX-M}$ genes, 63% possessed $bla_{SHV}$ genes, and 11.1% carried $bla_{TEM}$ genes. Additionally, Habeeb et al. (25) found that 42.5% of isolates contained the $bla_{CTX-M}$ gene and 48.1% contained the $bla_{TEM}$ gene. Tiba et al. (26) conducted a study on 162 isolates of uropathogenic *E. coli* in Brazil, reporting frequencies of 27.8% for the *pap* gene and 6.2% for the *sfa* gene. In 2006, Arisoy et al. (27) published a paper in which 161 isolates of uropathogenic Escherichia coli were examined and the frequency of sfa and pap genes were calculated as 6.2% and 28.9%, respectively. Cristea et al. (28) observed that 19.7% of *E. coli* isolates contained the gene $bla_{SHV}$. Additionally, Sadeghi et al. (29) noted that 38.8% of isolates carried the gene $bla_{CTX-M}$. A plethora of studies have demonstrated that the prevalence of antibiotic-resistant *E. coli* is influenced by geographical location and biological patterns. Consequently, the prescription of common antibiotics for such bacterial infections varies from one geographical area to another, resulting in divergent study outcomes worldwide. Consequently, investigating the change of antibiotic resistance patterns over specific periods can be very efficient in the treatment of urinary tract infections (UTIs). The organization of pathogenicity and urinary contamination is a prerequisite for uropathogenic *Escherichia coli* strains to interact with their target location, and the fimbriae qualities play a critical role in this association. The study shows that out of the three examined qualities, the *fim*H and *pap* qualities were the most prevalent in this study. As urinary tract infection is a prevalent infection in healthcare facilities, it is crucial to conduct in-depth research on antimicrobial resistance due to the overuse of antimicrobials. This research aims to develop a vaccine against such fimbriae.

## Authors' Contribution

Literature review and research writing, including reviewing and editing, supervision, methodology, project administration, conceptualization, studies analysis, and investigation: A.S.B., S.H.N., N.S.
Writing of the original draft, preparation, reviewing and editing, and methodology: N.S., N.O., investigation.
Validation and reviewing: M.V., M.M.

## Ethics

Not Applicable.

## Conflict of Interest

The authors have declared that there is no conflict of interest.

## Data Availability

All findings derived or examined throughout the course of this investigation have been encompassed within the confines of this published article.

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