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*

Saeedi, N[1], Vatani, M[2], Nejat, SH[3], Omidi, N[4], Moosavi, M[5], Soltani Borchaloee, A[6*]

1. *Department of Microbiology, Faculty of Biological Sciences, Islamic Azad University, North Tehran Branch, Tehran, Iran.*
2. *Department of Microbiology, Faculty of Biological Sciences, Falavarjan Branch, Islamic Azad University, Isfahan, Iran.*
3. *Department of Microbiology and Microbial Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran.*
4. *Department of Biology, Miyaneh Branch,Islamic Azad University, Miyaneh, Iran.*
5. *Department of physiology, Faculty of animal Sciences, Tarbiat modares University,Tehran, Iran.*
6. *SabaBiomedicals Science-Based Company, Tehran, Iran.*

How to cite this article: **Saeedi N, Vatani M, Nejat SH, Omidi N, Moosavi M, Soltani Borchaloee A. Multiplex PCR Amplification for the Detection of Biofilm and Extended-spectrum Beta-Lactamase Resistance Genes and Antibiotic Resistance Patterns in Uropathogenic *E. coli*. Archives of Razi Institute. 2025;80(1):125-.** DOI: 10.32592/ARI.2025.80.1.125

Copyright © 2023 by

Razi Vaccine & Serum Research Institute

Article Info:
Received: 31 March 2024
Accepted: 24 April 2024
Published: 28 February 2025

Corresponding Author's E-Mail:
a.soltaniborchaloee@gmail.com

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 (Jones et al., 2022).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 (Smith et al., 2021). 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 fimH gene present in 86.3% of isolates, and the sfa gene present in 69.9% of isolates. Furthermore, the beta-lactamase gene blaSHV, blaTEM, and blaCTX-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 programme, 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 enclasped 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 (Jones et al., 2022).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 Sfimbriae in the context of adhesion, given its regulation by the S-fimbrial adhesion gene (Sfa) and the sfa gene. These genes, which are classified as mannoseresistant 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, fimH, and sfa) in conjunction with extended-spectrum Beta-Lactamase resistance genes (blaTEM, blaCTX-M, and blaSHV), 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 (30 µg), imipenem (10 µg), ampicillin (10 µg), co-trimoxazole (25 µg), amikacin (30 µg) and cefixime. (5 µg), Cefalexin (30 µg), Amoxicillin (25 µg), Ciprofloxacin (5 µg), and Gentamicin (10 µg) discs (Padtan Teb, Iran).The Kirby-Bauer method was employed to investigate

the susceptibility of S. aureus 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, fimH, sfa, blaTEM, blaCTX-M, and blaSHV genes. The amplification conditions applied to the pap, fimH, 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 blaTEM, blaSHV, and blaCTX-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, fimH, and pap genes was 69.9%, 86.3%, and 89% respectively. Furthermore, the frequency of ß-lactamase genes in clinical samples was as follows: blaSHV, blaTEM, and blaCTX-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 fimH) in relation to gender ($P<0.05$).The statistical analysis revealed significant associations between the resistantly against Amoxicillin ($P=0.04$) and tetracycline ($P=0.05$) of the blaSHV gene in the UPEC isolates. Furthermore, the statistical analysis indicated significant connections between the resistantly against Amoxicillin ($P=0.02$) and Gentamicin ($P=0.04$) of the blaCTX-M gene, along with critical associations between Ampicillin ($P=0.001$) and blaTEM 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 R: AGAGAGAGCCACTCTTATACGGACA	336	56°C	15
*fim*H	F: GAGAAGAGGTTTGATTTAACTTATTG R: AGAGCCGCTGTAGAACTGAGG	559	56°C	16
sfa	F: CTCCGGAGAACTGGGTGCATCTTAC R: CGGAGGAGTAATTACAAACCTGGCA	410	56°C	17
blaCTX-M	F: ATGTGCAGYACCAGTAARGTKATGGC R: TGGGTRAARTARGTSACCAGAAYCAGCGG	593	62 °C	18
blaSHV	F: CTT TATCGGCCCTCACTCAA R: AGGTGCTCATCATGGGAAAG	237	62 °C	19
blaTEM	F: CGCCGCATACACTATTCTCAGAAT GA R: ACGCTCACCGGCTCCAGATTTAT	445	62 °C	20

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)

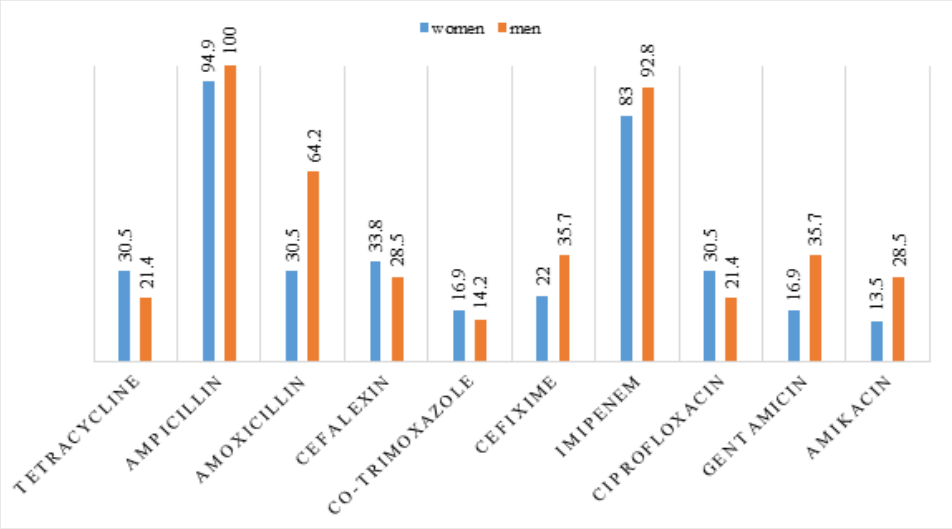

Figure1. The percentage of antibiotic resistance by gender.

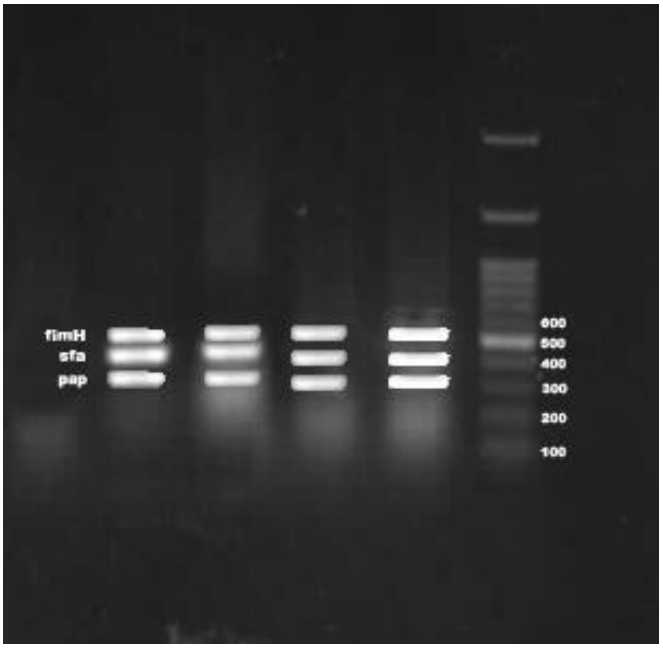

Figure2. Multiplex PCR Amplification of *pap*, *fimH*, *sfa* from *E.coli* isolates. 100bp DNA ladder.

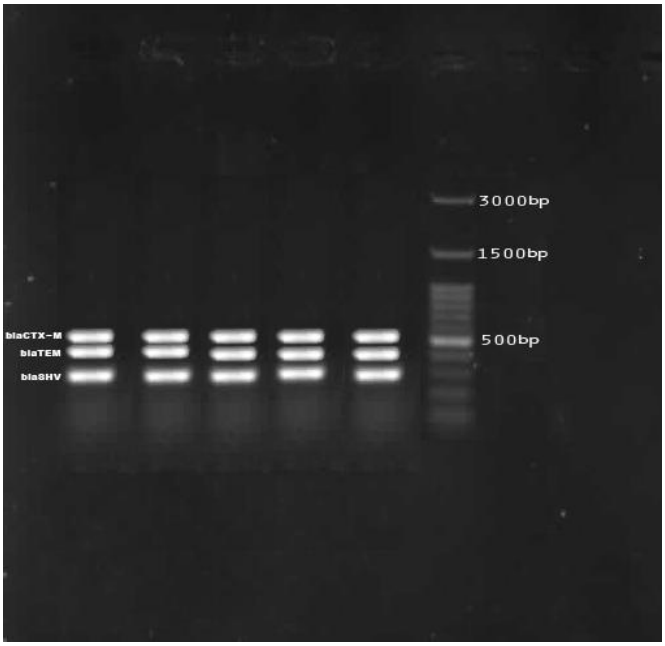

Figure3. Multiplex PCR Amplification of *bla*SHV, *bla*TEM, and *bla*CTX-M genes from *E.coli* isolates. 100bp DNA ladder.

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 fimH gene in 86.3% of isolates, and the sfa gene in 69.9% of isolates. Furthermore, the prevalence of the beta-lactamase gene blaSHV, blaTEM, and blaCTX-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, fimH, and sfa as 96.6%, 93.3%, and 4.6%, respectively, among which the prevalence of fimH 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 blaCTX-M genes, 63% possessed blaSHV genes, and 11.1% carried blaTEM genes. Additionally, Habeeb et al. (25) found that 42.5% of isolates contained the blaCTX-M gene and 48.1% contained the blaTEM gene.Tiba et al. (26) conducted a study on 162 isolates of uropathogenic Escherichia 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 blaSHV. Additionally, Sadeghi et al. (29) noted that 38.8% of isolates carried the gene blaCTX-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 organisation 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 fimH 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.

Acknowledgment

None.

Authors' Contribution

Literature review and research writing, including reviewing and editing, supervision, methodology, project administration, conceptualisation, 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.

References

1.Munkhdelger Y, Gunregjav N, Dorjpurev A, Juniichiro N, Sarantuya J. Detection of virulence genes, phylogenetic group and antibiotic resistance of uropathogenic *Escherichia coli* in Mongolia. The Journal of Infection in Developing Countries. 2017;11; 51-57.
2.Sarowska J, Futoma-Koloch B, Jama-Kmiecik A, Frej-Madrzak M, Ksiazczyk M, Bugla-Ploskonska G, Choroszy-Krol I. Virulence factors, prevalence and potential transmission of extraintestinal pathogenic *Escherichia coli* isolated from different sources: recent reports. Gut pathogens. 2019;11;1-6.
3.Borchaloee, A. S. Investigating Differences between Virulence Factors of *Escherichia coli* and *Pseudomonas aeruginosa* in Causing Urinary Tract Infections. I J Infectious Disea; 2023; 4; 1-8.
4.Baby S, Karnaker VK, Geetha R. Adhesins of uropathogenic *Escherichia coli* (UPEC). Int J Med Microbiol Trop Dis. 2016;1;10–18.
5.Dadi BR, Abebe T, Zhang L, Mihret A, Abebe W, Amogne W. Distribution of virulence genes and phylogenetics of uropathogenic *Escherichia coli* among urinary tract infection patients in Addis Ababa, Ethiopia. BMC infectious diseases. 2020;20;1-2.

6.Frömmel U, Lehmann W, Rödiger S, Böhm A, Nitschke J, Weinreich J, Groß J, Roggenbuck D, Zinke O, Ansorge H, Vogel S. Adhesion of human and animal *Escherichia coli* strains in association with their virulence-associated genes and phylogenetic origins. Applied and environmental microbiology. 2013;79;5814-5829.
7.Sadiq FA, Burmølle M, Heyndrickx M, Flint S, Lu W, Chen W, Zhao J, Zhang H. Community-wide changes reflecting bacterial interspecific interactions in multispecies biofilms. Crit Rev Microbiol. 2021;47:338–58.
8.Naziri Z, Kilegolan JA, Moezzi MS, Derakhshandeh A. Biofilm formation by uropathogenic *Escherichia coli*: a complicating factor for treatment and recurrence of urinary tract infections. Journal of Hospital Infection. 2021;117;9-16.
9.Usein CR, Damian M, Tatu-Chitoiu D, Capusa C, Fagaras R, Tudorache D, Nica M, Le Bouguénec C. Prevalence of virulence genes in *Escherichia coli* strains isolated from Romanian adult urinary tract infection cases. J Cell Mol Med. 2001; 5;303-310.
10. Schwartz DJ, Conover MS, Hannan TJ, Hultgren SJ. Uropathogenic *Escherichia coli* superinfection enhances the severity of mouse bladder infection. PLoS Pathog. 2015; 11;e1004599
11. Abe CM, Salvador FA, Falsetti IN, Vieira MA, Blanco J, Blanco JE, Blanco M, Machado AM, Elias WP, Hernandes RT, Gomes TA. Uropathogenic *Escherichia coli* (UPEC) strains may carry virulence properties of diarrhoeagenic *E. coli*. FEMS Immunol Med Microbiol. 2008; 52;397-406.
12. Nys S, Van Merode T, Bartelds AI, Stobberingh EE. Antibiotic treatment and resistance of unselected uropathogens in the elderly. International journal of antimicrobial agents. 2006;27;236-241.
13. Pitout JD, Nordmann P, Laupland KB, Poirel L. Emergence of Enterobacteriaceae producing extended-spectrum β-lactamases (ESBLs) in the community. Journal of antimicrobial chemotherapy. 2005;56;52-9.
14. Demirel I, Kinnunen A, Önnberg A, Söderquist B, Persson K. Comparison of host response mechanisms evoked by extended spectrum beta lactamase (ESBL)-and non-ESBL-producing uropathogenic *E. coli*. BMC microbiology. 2013;13;1-9.
15. Yamamoto S, Terai A, Yuri K, Kurazono H, Takeda Y, Yoshida O: Detection of urovirulence factors in *Escherichia coli* by multiplex polymerase chain reaction. FEMS Immunol Med Microbiol 1995, 12;85–90.
16. Struve C, Krogfelt KA: In vivo detection of *Escherichia coli* type 1 fimbrial expression and phase variation during experimental urinary tract infection. Microbiol 1999; 145; 2683–2690
17. Le Bouguenec C, Archambaud M, Labigne A: Rapid and specific detection of the pap, afa, and sfa adhesin-encoding operons in uropathogenic *Escherichia coli* strains by polymerase chain reaction. J Clin Microbiol 1992; 30; 1189–1193.
18. Shah HA, Syed A, Bhat MA, Kakru DK, Farooq S, Qureshi S, et al. Genomic Determinants and Antimicrobial Resistance Pattern of Clinical Isolates of Extended Spectrum Beta Lactamase (ESBL) Producing *Escherichia coli*. J Pure Appl Microbiol. 2023;17(3):1679-1690.
19. Fang H, Lundberg C, Olsson-Liljequist B, et al. Molecular epidemiological analysis of *Escherichia coli* isolates producing extended-spectrum b-lactamases for identification of nosocomial outbreaks in Stockholm, Sweden. J Clin Microbiol. 2004;42;5917-5920
20. Monstein HJ, Ostholm-Balkhed A̧, Nilsson MV, Nilsson M, Dornbusch K, Nilsson LE. Multiplex PCR amplification assay for the detection of *blaSHV*, *blaTEM* and *blaCTX-M* genes in Enterobacteriaceae. Apmis. 2007;115;1400-1408.
21. Rahdar M, Rashki A, Miri HR, Ghalehnoo MR. Detection of pap, *sfa*, *afa*, *foc*, and *fim* adhesin-encoding operons in uropathogenic *Escherichia coli* isolates collected from patients with urinary tract infection. Jundishapur J Microbiol. 2015; 8; e22647.
22. Yazdi M, Bouzari M, Ghaemi EA. Detection of *fim*, *pap*, *sfa* and *afa* adhesin-encoding operons in *Escherichia coli* strains isolated from urinary tract infections. Medical Laboratory Journal. 2018;12;10-15.
23. Saki A, Mirzaei M. Frequency of fimberial virulence genes (*fim*, *pap*, *sfa*) in *Escherichia coli* isolated from the patients with urinary tract infections from selective hospitals of Tehran, Boroujerd and sanandej citu in 2015-2016. Journal of Shahid Sadoughi University of Medical Sciences. 2017;24; 913-923.
24. Naziri Z, Derakhshandeh A, Borchaloee AS, Poormaleknia M, Azimzadeh N. Treatment failure in urinary tract infections: a warning witness for virulent multi-drug resistant ESBL-producing *Escherichia coli*. Infection and drug resistance. 2020;1839-1850.
25. Habeeb MA, Sarwar Y, Ali A, Salman M, Haque A. Rapid emergence of ESBL producers in E. coli causing urinary and wound infections in Pakistan. Pak J Med Sci. 2013;29; 540-544.
26. Tiba MR, Yano T, Leite DDS. Genotypic characterization of virulence factors in Escherichia coli strains from patients with cystitis. Rev Inst Med Trop. 2008; 50 ;255-260.
27. Arisoy M, Ayser D, Ekim M, Ozel D, Kose S K, Ozsoy E D, et al. Detection of virulence factors of *E.coli* from children by Duplex PCR. Int J Clin Pract. 2005; 60; 170-73.
28. Cristea VC, Gheorghe I, Barbu IC, et al. Snapshot of phylogenetic groups, virulence, and resistance markers in Escherichia coli uropathogenic strains isolated from outpatients with urinary tract infections in Bucharest, Romania. BioMed Res Int. 2019;5712371.
29. Sadeghi M, Sedigh Ebrahim-Saraie H, Mojtahedi A. Prevalence of ESBL and AmpC genes in E. coli isolates from urinary tract infections in the north of Iran. New Microbes New Infect. 2021;45; 100947.