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Analysis of phylogroups, biofilm formation, virulence factors, antibiotic resistance and molecular typing of uropathogenic *Escherichia coli* strains isolated from patients with recurrent and non-recurrent urinary tract infections

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Abstract

Background Uropathogenic *Escherichia coli* (UPEC) is the predominant cause of urinary tract infections (UTIs), and the recurrence of these infections poses significant treatment challenges.

Objective This study aimed to compare the phylogroups, biofilm formation, virulence factors, and antibiotic resistance of UPEC strains in patients with recurrent versus non-recurrent UTIs in Hamadan City, Western Iran.

Materials and methods A total of 110 *E. coli* isolates were collected from urine cultures across three major hospitals and laboratories. The isolates were confirmed through biochemical tests, and their antibiotic resistance profiles were evaluated using the disk diffusion method. Biofilm production was assessed using the microtiter plate method, while virulence genes and phylogroup determination were analyzed via PCR. Real-time PCR was employed to compare the expression levels of the *pap* and *fim*H virulence genes.

Results The results indicated that 73% of isolates were from non-recurrent UTI patients, with a higher incidence in females and children under 10 years. A significant difference was detected in the underlying diseases and the expression of the *pap* between the recurrent and non-recurrent groups. Antibiotic resistance was notably significant, particularly against Ampicillin-sulbactam, Trimethoprim-Sulfamethoxazole, Nalidixic acid, and Ciprofloxacin, with 77% of strains classified as multi-drug resistant (MDR). Despite differences in the rates of ESBL production between recurrent (53%) and non-recurrent (42.5%) strains, no significant differences were observed in antibiotic resistance, biofilm formation, virulence factors, or phylogroups between the two groups. Phylogenetic analysis revealed a predominance of phylogroups B2 and D, with high genetic diversity among the isolates.

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Conclusion The study highlights the traits of UPEC strains in recurrent and non-recurrent UTIs, showing high antibiotic resistance and genetic diversity among isolates. The study found notable differences in underlying diseases and the expression of the *pap* gene between recurrent and non-recurrent groups, suggesting that these factors may play a crucial role in the recurrence of infections. Further investigation into these differences could enhance our understanding and management of recurrent UTIs.

Keywords Uropathogenic *Escherichia coli*, Recurrent urinary tract infection

Background

Urinary tract infections (UTIs) present significant challenges primarily due to high recurrence rates and resistance to first-line antibiotics [1]. UTIs are among the most prevalent infectious diseases globally, predominantly caused by uropathogenic *Escherichia coli* (UPEC) [2]. These infections can lead to various conditions, including asymptomatic/symptomatic, acute, chronic, and recurrent bacteriuria [3]. UPEC strains are the most common pathogens, accounting for 85% and 50% of community-acquired and hospital-acquired UTIs, respectively [4]. Risk factors for contracting this disease include gender (being female), previous UTIs, sexual activity, use of condoms/diaphragms/spermicides, vaginal infections, trauma, diabetes, obesity, and genetic susceptibility/anatomical abnormalities [5]. While UTIs can occur in both men and women, the incidence among female patients is significantly higher than that among male patients due to anatomical differences [6]. Recurrence in this disease presents a significant challenge in patient treatment [3]. Recurrent urinary tract infections (RUTI) are prevalent and create notable clinical difficulties. Although the term RUTI has been vaguely defined for an extended period, a consensus definition has developed in recent years. Thus, RUTI is defined as "three episodes of UTI with a positive urine culture in the past 12 months or two episodes within 6 months," and evidence indicates that RUTI may arise from one of two mechanisms: recurrent ascending infections or chronic infection/infection in the bladder. The pathogenesis of recurrent UTI involves either bacterial reinfection or bacterial persistence, with the former being more common [7]. The key characteristic of UPEC strains is their ability to colonize the surfaces of host uroepithelial cells, facilitated by adhesion factors and the production of hemolysin and pili. This colonization results in increased damage to cells and tissues. The likelihood of repeated relapses is likely due to bacterial defense mechanisms and antibiotic resistance [8]. The global rise in the prevalence of UPEC that produce broad-spectrum β-lactamase (ESBL), which contributes to the emergence of multidrug resistance, leads to an increase in the severity of urinary tract infections (UTIs). The resistance of UPEC to the commonly prescribed antibiotic Trimethoprim-sulfamethoxazole has risen in the last decade, necessitating the use of last-line antibiotics such as fluoroquinolones [8, 9]. Given the limited studies that have compared and investigated recurrent versus non-recurrent urinary infections, and considering the clinical significance of urinary infections caused by UPEC and the recurrence of infections from this bacterium, the aim of this study was to compare phylogroups, biofilm formation, virulence factors, and antibiotic resistance of UPEC strains in patients with recurrent and non-recurrent urinary tract infections. This research was conducted among hospitalized patients and those referred to medical laboratories outside the hospital in Hamadan, west of Iran.

Methods

UPEC isolates

In a cross-sectional study, urine cultures were collected from hospitalized patients and those referred to medical laboratories outside the hospital in Hamadan city, west of Iran, between May and November 2023. Urine samples were cultured in the microbiology laboratory, and *E. coli* isolates were identified and confirmed using standard microbiological and biochemical tests [10]. Demographic information of patients, including age, sex, history of urinary tract infections, prescribed antibiotics, and the number of visits to the hospital or specialist doctor's office, was recorded.

Antibiotic susceptibility

The susceptibility of *E. coli* strains to Ampicillin-sulbactam (10 / 10 µg), Ceftriaxone (30 µg), Nalidixic acid (30 µg), Ciprofloxacin (5 µg), Levofloxacin (30 µg), Amikacin (30 µg), Gentamicin (10 µg), Amoxicillin / clavulanate (20/10 µg), Trimethoprim/sulfamethoxazole (1.25/23.75 µg), and Nitrofurantoin (300 µg) (Mast, UK) was evaluated using the Kirby-Bauer disc diffusion method, following the Clinical Laboratory Standards Institute (CLSI 2023) guidelines [11].

ESBL producing detection

Phenotypic detection of *E. coli* strains that produce ESBL was conducted using the double-disc synergy test (DDST) in accordance with CLSI guidelines, as previously described [11].

Biofilm formation assay

The ability of *E. coli* strains to form biofilms was quantitatively assessed using a microtiter plate and the crystal

violet method, as described in previous studies [12]. The biofilm formation test was repeated three times to minimize error, and the average of these three trials was included in the calculations.

Virulence factor detection

Genomic DNA from freshly cultured *E. coli* colonies was extracted using the boiling method. All *E. coli* strains underwent gene identification for virulence factors, including adhesions (*fim*H, *pap*, and *sfa*) and hemolysin (*hly*A), via PCR with specific primers obtained from Metabion, Germany, following previously established protocols [13–16], as detailed in Supplementary file 1.

Phylogenetic grouping

The phylogenetic classification of all *E. coli* isolates was performed using a PCR assay to identify the primary *E. coli* phylogenetic groups (A, B1, B2, and D). This involved using primers that target the *chu*A (279 bp), *yja*A (211 bp), and *TspE4C2* (152 bp) genes [17].The PCR reaction consisted of an initial denaturation step at 95 °C for 5 min, followed by.

35 cycles of 60 s at 94 °C, an annealing step (at 55 °C for *chu*A and *TspE4C2* and 54 °C for *yja*A at 15 s), extension step at 72 °C for 30 s and a final extension step at 72 °C for 10 min.

ERIC-PCR

The genetic linkage of all *E. coli* isolates was assessed using the ERIC-PCR technique. This procedure utilized primers as previously described [18]. The ERIC-PCR band patterns on the agarose gel were compared using an online database (inslico.ehu.es). The band patterns were analyzed using the Dice coefficient and clustering was performed using the unweighted pair group method with arithmetic mean (UPGMA).

Real-time PCR

To compare the expression levels of *pap* and *fim*H genes in recurrent and non-recurrent strains of *E. coli*, the Real-time PCR method was employed. A total of 10 recurrent strains and 10 non-recurrent strains, both positive for these genes, were selected for analysis. Total RNA was extracted using the RNA extraction kit (RNX-Plus solution from SinaClon Co., Iran) and subsequently converted to cDNA using the cDNA synthesis kit (Parstous Co., Iran). The synthesized cDNA was stored at -20 °C for use as DNA templates in the Real-time PCR reactions. The quantification of cDNA in real-time was performed using the SYBR Green PCR master mix and a detection system provided by Roche, Germany. The optimized reaction mixture consisted of a 10X master mix, 1 µl of each primer (10 pmol) as previously detailed, 2 µl of cDNA (at a concentration of 100 µg/ml), and 6 µl of DEPC-treated water, achieving a final volume of 20 µl. The *16 S rRNA* gene primer served as the internal control [18].The amplification process involved 40 cycles, starting with an initial denaturation at 95 °C for 5 min, followed by denaturation at 95 °C for 10 s, annealing at 60 °C for 20 s for *16 S rRNA* and *fim*H and 63 °C for 20 s for *pap* gene, Elongation at 72 °C for 30 s, and a melting curve at 60 °C for 15 s, and finally, final denaturation at 94 °C for 15 s. Expression values (R) were determined using the ΔΔCt method [19]. The expressions of all genes were calculated using the $2^{-\Delta\Delta Ct}$ method (fold).

Statistical analysis

Qualitative variables were reported as frequencies and percentages, while quantitative variables were expressed as means and standard deviations. To compare phylogroups, biofilm formation, virulence factors, and antibiotic resistance between the two groups of relapsing and non-recurring patients, the chi-square test or Fisher's exact test was employed. The t-test was utilized to compare the expression levels of *fim*H and *pap* genes between the two groups. A significance level of 0.05 was set for all tests. Data analysis was performed using Stata 17 software.

Results

Demographic information of patients with UTI

In this study, a total of 110 *E. coli* isolates were collected from urine cultures of hospitalized patients and those referred to diagnostic laboratories outside the hospital. Among these 110 *E. coli* isolates, 30 (27%) were from patients with recurrent UTIs, while 80 (73%) were from patients with non-recurrent UTIs. Additionally, of these 110 isolates, 15 (13.6%) were collected from medical diagnostic laboratories outside the hospitals (community), and 95 (86.4%) were collected from the hospitals. The frequency of *E. coli* strains in females was 85 (77.27%) compared to 25 (22.73%) in males. Among female patients, 72% experienced non-recurrent UTIs, whereas 28% had recurrent UTIs. In male patients, 76% had non-recurrent UTIs, while 24% had recurrent UTIs. There was no relationship between recurrent and non-recurrent infections (CI:95%, $P = 0.676$). The number of recurrences in patients with recurrent urinary tract infections varied, with two occurrences in the last six months being more frequent than other recurrences (Fig. 1). The highest frequency of *E. coli* isolates occurred in the age group of 0–10 years, with 31 patients (28.18%), while the lowest frequency was found in the age group of 91–100 years, with 1 patient (0.9%). The distribution of *E. coli* infections in other age groups is detailed in Supplementary file 2.

The patients presented with various types of urinary infections, including acute cystitis in 65 cases (59%), chronic cystitis in 30 cases (27.3%), and acute

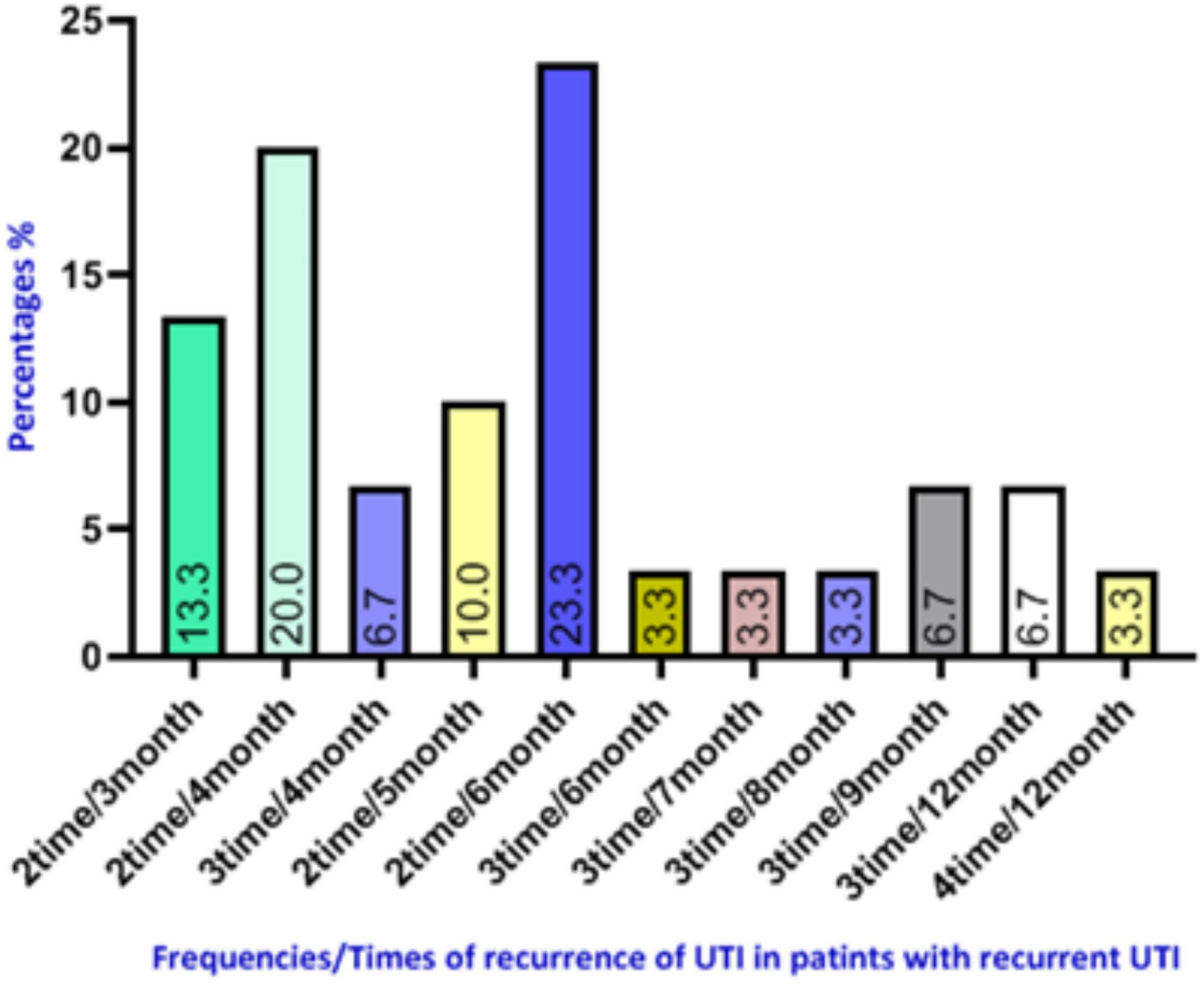

Fig. 1 Distribution of recurrence frequency in patients with recurrent urinary tract infection

pyelonephritis in 15 cases (13.7%). An examination of underlying conditions in patients with UTIs showed that 66 patients (60%) had one or more underlying health issues. Among these, eighteen patients (27.3%) had kidney-related problems, such as hydronephrosis, kidney stones, or transplant kidneys, ten patients (15.15%) had bladder-related issues, such as urinary reflux or urethral strictures. Nine patients (13.63%) had diabetes, while eight patients (12.12%) had other disorders, including rheumatism, asthma, and anemia. Additionally, six patients (9%) experienced prostate enlargement, and another six patients (9%) had high blood pressure. Four patients (6%) had gastrointestinal problems, and three patients (4.54%) suffered from heart problems (Fig. 2). A significant difference was observed in the underlying diseases between the recurrent and non-recurrent groups (CI: 95%, $P = 0.028$).

Antibiotic resistance patterns

The results of resistance to various antibiotics in *E. coli* isolates are presented in Table 1. The highest resistance was noted for Ampicillin-sulbactam (81.8%), followed by Nalidixic acid and Trimethoprim/sulfamethoxazole (71.8%). Over 60% of isolates exhibited resistance to Ciprofloxacin and Ceftriaxone, while more than 50% were resistant to Levofloxacin, and 40% showed resistance to Gentamicin. Additionally, the frequency of antibiotic resistance in recurrent and non-recurring strains is compared in Table 1. It was found that 85 (77%) strains were multi-drug resistant (MDR). Among these, 25 (83.3%) and 60 (75%) of the non-recurring and recurring strains displayed the MDR phenotype, respectively. A total of 50 (45.4%) strains were identified as ESBL-producing strains. Of these 50 ESBL-producing strains, 37 (33.63%) were from hospitalized patients and 13 (11.81%) from outpatients. In total, 42.5% of non-recurring strains and 53% of recurring strains were ESBL producers. No

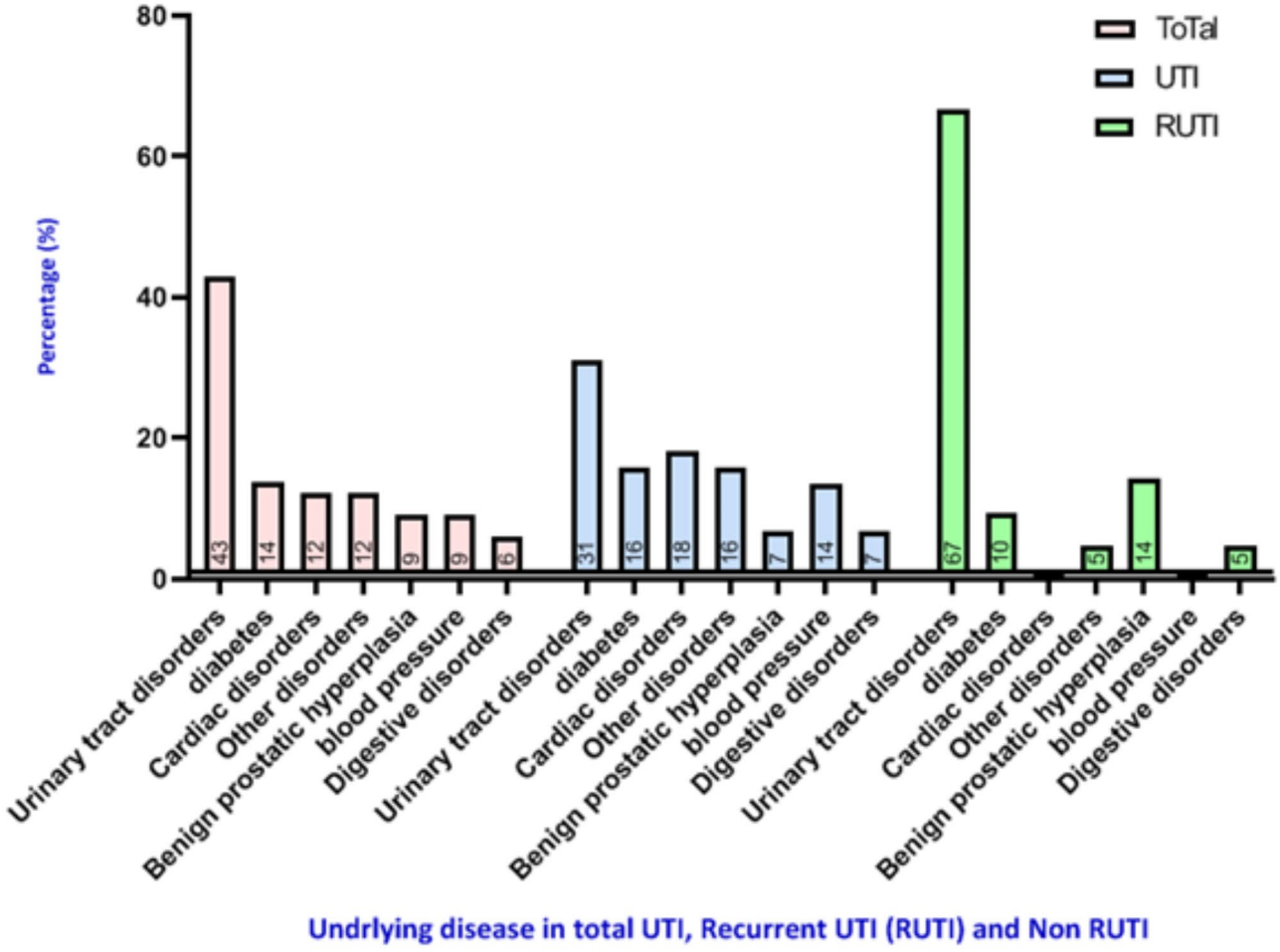

Fig. 2 Frequency distribution of underlying diseases in patients with recurrent and non-recurrent urinary tract infections

significant difference was observed in antibiotic resistance and ESBL frequency between the two groups of recurrent and non-recurrent strains (Table 1).

Biofilm phenotype of strains

Out of 110 *E. coli* isolates obtained from the urine of patients with urinary tract infections, 80 isolates (72.8%) were capable of forming biofilms. Among the 80 biofilm-forming isolates, 65 (81.2%) produced weak biofilms, while 15 (18.8%) produced moderate biofilms. No isolates exhibited strong biofilm formation. Of the 30 recurring strains, 10 isolates (33%) did not produce biofilm, 18 isolates (60%) were weak biofilm producers, and 2 isolates (7%) were moderate biofilm producers. Among the 80 non-recurring strains, 20 isolates (25%) did not produce biofilm, 47 isolates (59%) produced weak biofilms, and 13 isolates (16%) produced moderate biofilms (Table 1). The results indicated that there was no statistically significant difference in biofilm formation between the two groups of recurrent and non-recurrent strains (CI: 95%, $P = 0.362$).

Frequency of genes encoding virulence factors

PCR results of virulence genes from 110 *E. coli* isolates indicated a higher rate of *fim*H (88%) compared to other virulence genes, followed by *pap* (60%), *sfa* (20%), and *hly*A (11%). The frequency of virulence genes in the two groups, recurrent and non-recurrent, was investigated and compared. The frequency of the *fim*H gene was 80% in non-recurrent strains and 91% in recurrent strains. The frequency of the *pap* gene in non-recurrent strains was 56%, while in recurrent strains it was 61%. The frequency of the *sfa* gene was 20% in both groups, and the frequency of the *hly*A gene was 10% in non-recurrent strains and 11% in recurrent strains (Fig. 3). There was no relationship between virulence factors from recurrent and non-recurrent infections (Table 1).

Phylogenetic groups

The prevalence order of *E. coli* phylogroups in UTI was as follows: B2 (68%), D (20%), A (7%), and B1 (4%). The prevalence of phylogroups in non-recurring strains was investigated and compared to recurring strains. In non-recurring strains, the phylogroup distribution was B2

Table 1 Comparison of frequency of phylogroups, antibiotic resistance patterns, virulence factors, ESBL production and biofilm formation strength in uropathogenic *E. Coli* strains in recurrent and non-recurrent urinary tract infections

Variables	Recurrent (CI: 95%)	Non-recurrent (CI: 95%)	*p*-value
Phylogroup A	10% (3.22, 27.07)	6.25% (2.60, 14.28)	
Phylogroup B1	10% (3.22, 27.07)	2.5% (0.62, 9.58)	
Phylogroup B2	60% (41.75, 75.84)	71.25% (60.30, 80.17)	0.317
Phylogroup D	20% (9.19. 38.19)	20% (12.56, 30.32)	
Biofilm			
Weak biofilm	60% (41.75, 75.84)	58.75% (47.59. 69.08)	0.362
Moderate biofilm	6.67% (1.65, 23. 35)	16.25% (9.62, 26.16)	
fim H	80% (61.81, 90.81)	91.25% (82.64, 95.81)	0.104
sfa	20% (9.19, 38.19)	20% (12.56, 30.32)	1.000
pap	56.7% (38.65, 73.08)	61.25% (50.07, 71.35)	0.662
*hly*A	10% (3.22, 27.07)	11.25% (5.91, 20.36)	0.851
Ampicillin-sulbatam	76.6%(58.27, 88.55)	83.75% (73.87, 90.38)	0.126
Ceftriaxone	70% (51.44, 83.71)	57.5% (46.36, 67.93)	0.488
Nalidixic acid	86.7% (69.15, 94.96)	66.25% (55.13, 75.82)	0.094
Ciprofloxacin	70% (51.44, 83.71)	60% (48.83, 70.22)	0.500
Levofloxacin	53.33% (35.62, 70.24)	53.75% (42.7, 64.44)	0.838
Amikacin	30% (16.29, 48.56)	26.25% (17.7, 37.07)	0.710
Gentamicin	30% (16.28, 48.56)	43.75% (33.2, 54.87)	0.422
Trimethoprim/sulfamethoxazole	73.3% (54.82, 86.17)	71.25% (60.30, 80.17)	0.713
Nitrofurantoin	33.3% (18.84, 51.86)	28.75% (19.83, 39.7)	0.868
ESBL	53.3% (35.62, 70.24)	42% (32.07, 53.64)	0.310

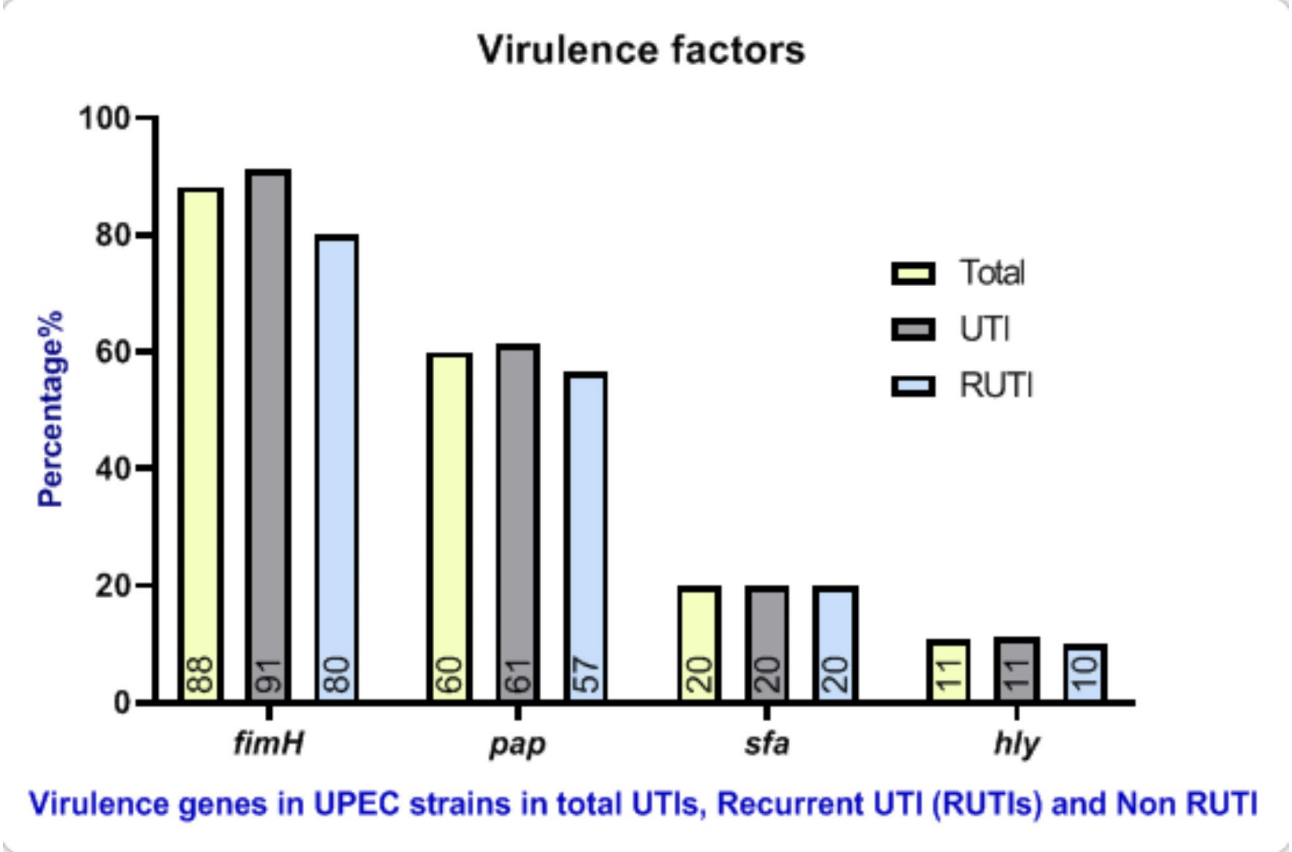

Fig. 3 Frequency distribution of virulence factor encoding genes in uropathogenic *E. coli* strains from all patients with a urinary tract infection, patients with recurrent and non-recurrent urinary tract infections

(71%), D (20%), A (2.6%), and B1 (2.5%), while in recurring strains, it was B2 (60%), D (20%), A (10%), and B1 (10%). The results indicated that phylogroup B2 is dominant in both groups (Table 1). There was no relationship between phylogroups from recurrent and non-recurrent infections (CI:95%, $P = 0.317$). The strength of biofilm formation in different phylogroups was examined and compared between the two groups of recurrent and non-recurrent urinary tract infections, and no significant relationship was determined.

Genetic linkages of *E. Coli* strains

The analysis of the results from the ERIC-PCR technique revealed a high genetic diversity among the various isolates of *E. coli* examined in this study. Out of 110 collected isolates, 103 could be typed, while ERIC bands were not observed in 7 isolates. In total, among the 103 typed isolates, 23 common types (22%) and 80 types with specific or unique patterns (78%) were identified. The largest common type comprised 8 isolates, shared between two Hospitals, indicating a genetic relationship. The next major common type included seven isolates, all from a same Hospital. Additionally, the results indicated that there is genetic similarity between *E. coli* strains isolated from outpatients (referring to laboratories outside the hospital) and those isolated from hospitalized patients. Of the 30 isolates related to recurrent infections, only 7 (23.3%) were classified as common types, meaning they had genetic similarity with isolates from the hospitals. The remaining isolates of recurrent infection were single type, meaning they had no genetic similarity with other isolates. It was notable that some isolates belonging to a common type exhibited differences in antibiotic resistance patterns, virulence gene profiles, phylogroups, and biofilm formation strength, despite having identical ERIC banding patterns. Overall, the ERIC results suggest the circulation of diverse strains or clones of *E. coli* within hospitals and the community (Fig. 4).

Expression level of virulence genes

The results obtained from Real-time PCR indicated that there was no significant difference in the expression level of the *fim*H gene between the two groups of patients with recurrent and non-recurrent urinary tract infections. However, the expression level of the *pap* gene was significantly different between these two groups (Fig. 5). A notable nine-fold increase in *pap* gene expression was observed in isolates from recurrent UTI patients in comparison to the control gene (CI: 95%, $P = 0.00$).

Discussion

There is a limited body of research on recurrent and non-recurrent urinary tract infections (UTIs) in patients. This cross-sectional study aims to provide an overview of the epidemiology of *E. coli*-induced UTIs in hospitalized and outpatient settings in a major city in western Iran. It offers valuable insights into the epidemiology, antibiotic resistance patterns, virulence factors, phylogroups, gene expression, and biofilm phenotypes of UPEC strains in patients with recurrent and non-recurrent UTIs in Iran. Previous studies have highlighted the prevalence of UPECs in UTIs [20–24]. UTIs are among the most common infections in women, often occurring alongside vaginal infections and typically caused by gastrointestinal pathogens [25]. Consistent with earlier findings, our study found a higher incidence of UTIs in females compared to males [24–26]. This disparity can be attributed to anatomical and physiological factors, including the shorter female urethra, its proximity to the anus, hormonal influences, sexual activity, contraceptive use, and pregnancy. Research in Iran on UTI recurrence is limited. Nearly half of women will experience a UTI in their lifetime, with 27–50% likely to have recurrent infections within six months [27]. Craig et al. reported that 64% of UTI patients were female, with 42% experiencing recurrent UTIs [28]. Kao et al. found that uropathogens in recurrent UTIs were more virulent among genetically similar *E. coli* strains [27].Given the significant impact of urinary infections on women's quality of life, accurate diagnosis and treatment are crucial. A comprehensive approach involving various diagnostic methods, treatment options, and prevention strategies is recommended to mitigate recurrent infections. Women with recurrent UTIs should seek evaluation and management from healthcare providers.

Urinary tract infections (UTIs) can be influenced by various underlying health conditions, such as diabetes, kidney stones, urinary retention, immunocompromised states, pregnancy, neurological disorders, and hygiene practices [29]. Our study revealed a significant association between these conditions and UTI occurrence, with urinary tract disorders and prostatic hyperplasia being more common in patients with recurrent UTIs. Research on underlying diseases in recurrent UTIs is limited. A study by Hsiao et al. in Taiwan found that hypertension was the most prevalent underlying condition among patients with UTIs, followed by upper UTIs and diabetes mellitus. No significant differences in clinical characteristics were noted between patients with and without bacteremia, except for a higher prevalence of hypertension and septic shock in the bacteremia group [30]. Recognizing the link between underlying diseases and UTIs is essential for effective prevention and management. Individuals with these risk factors should find guidance from healthcare providers for appropriate preventive measures.

Antibiotic resistance is on the rise, particularly in urinary tract infections (UTIs), prompting extensive research [31–39]. Our study shows that *E. coli* isolates

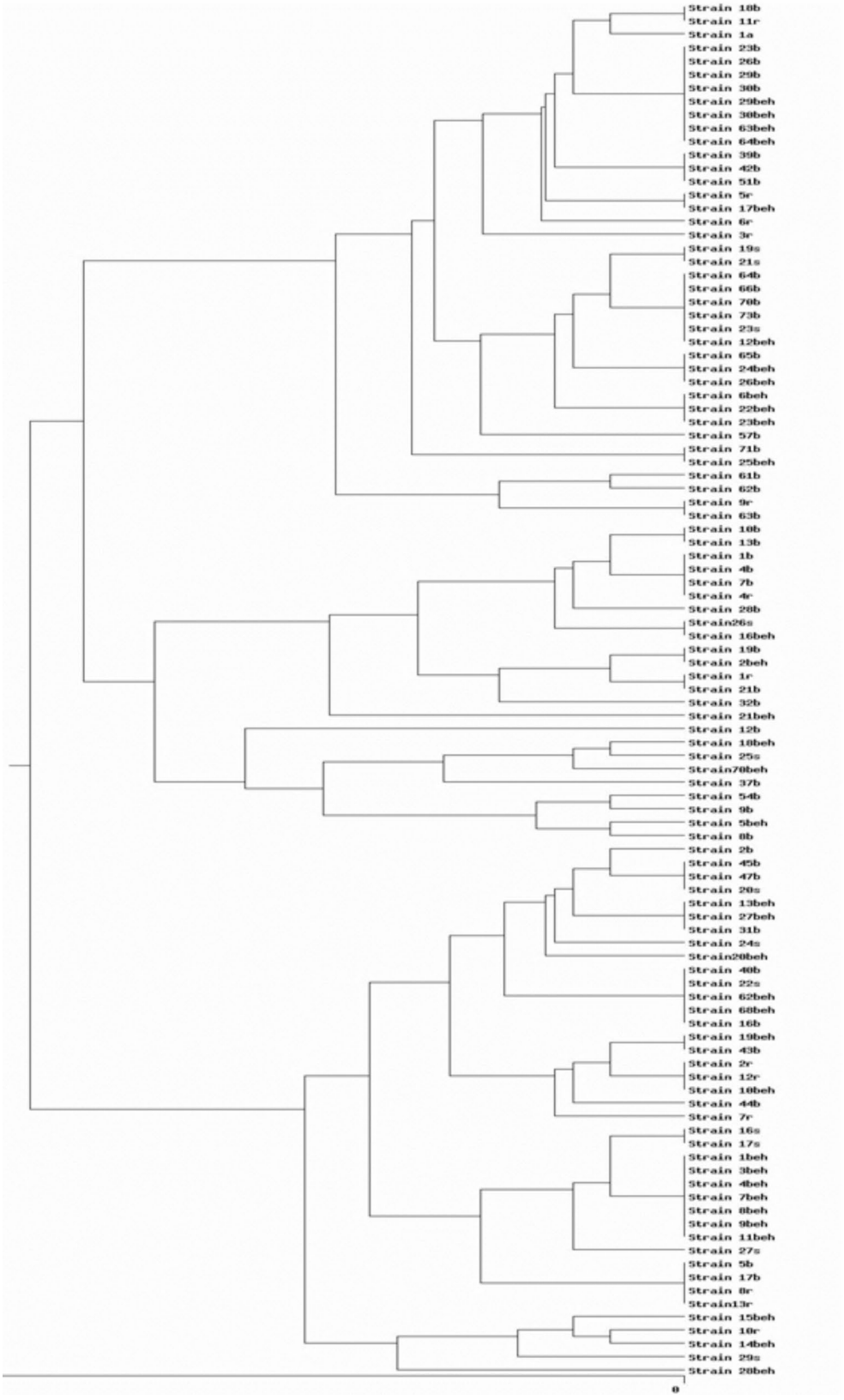

Fig. 4 Dendrogram of UPECs isolated from hospitalized patients and patients referred to medical diagnostic laboratories in Hamadan (**beh**: Beheshti Hospital, **b**: Besat Hospital: **s**: Sina Hospital, **r**: Razi Laboratory)

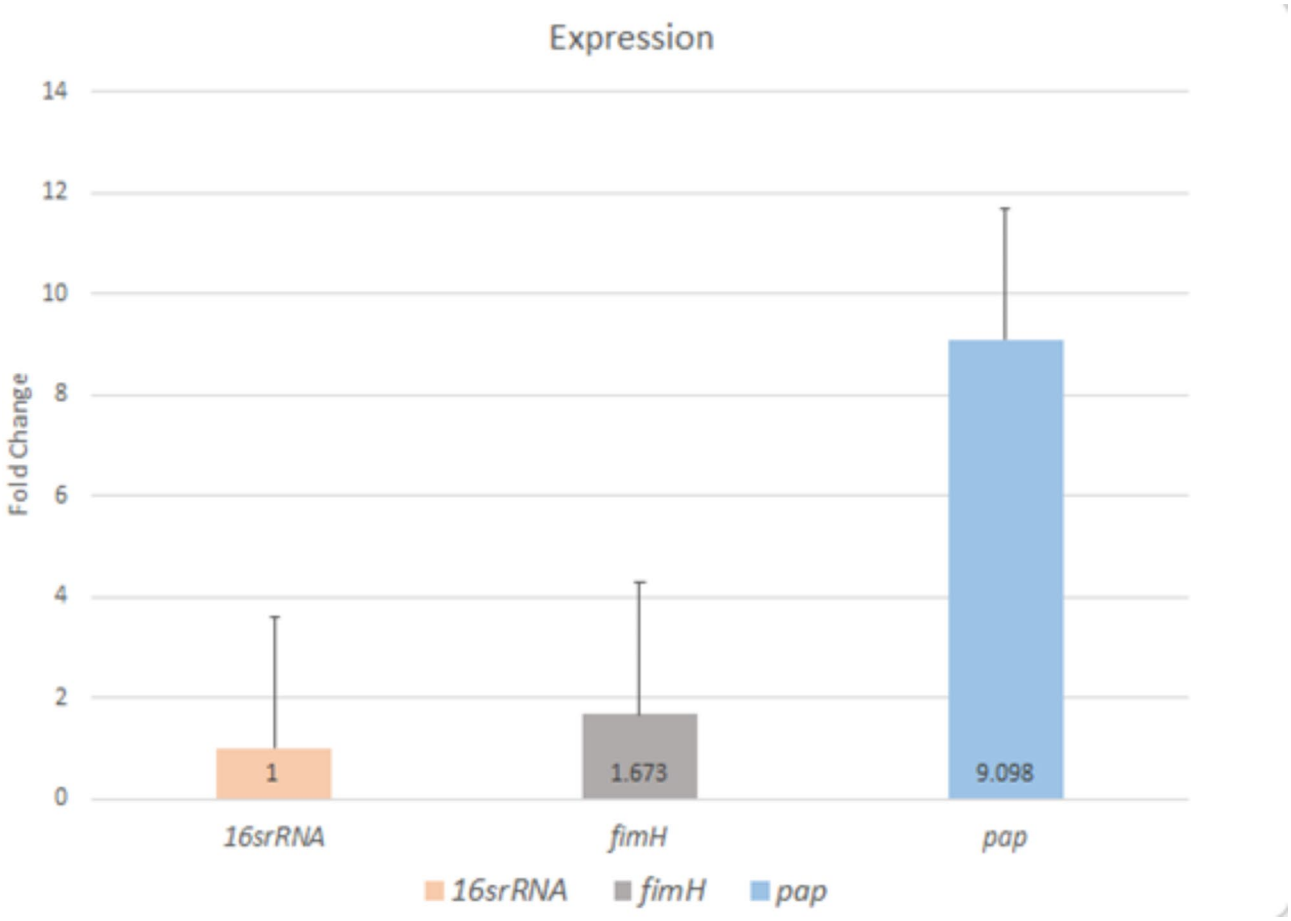

Fig. 5 Differences in gene expression based on fold change of virulence genes (*fim* H, *pap*) of uropathogenic *Escherichia coli* strains isolated from patients with recurrent and non-recurrent urinary tract infection

from UTIs exhibit significant resistance to beta-lactam, quinolone, and fluoroquinolone antibiotics, limiting treatment effectiveness. There is also a concerning 30% resistance rate to Nitrofurantoin and Amikacin, highlighting the need for cautious prescribing. Physicians should request antibiogram tests to evaluate bacterial sensitivity before prescribing antibiotics.

Research on antibiotic resistance in UPEC strains shows varied results globally, likely due to geographical differences and strain types, but most studies indicate a significant level of resistance. While Nitrofurantoin and Amikacin are more effective than other antibiotics [34–37], a study by Ormeño et al. reported resistance rates of 69.6% for Ciprofloxacin, 68.5% for Levofloxacin, and lower rates for Nitrofurantoin (6.9%) and Amikacin (2.7%), aligning with our findings. Ormeño et al. also found a significant relationship between resistance rates and patient demographics, with an RUTI incidence of 11.3% and high rates of multi-drug resistant (MDR) (46.5%) and extensively drug-resistant (XDR) strains (24.3%) [40]. Although our study did not find a significant link between antibiotic resistance and recurrent UTIs, larger studies may reveal its potential impact. With diminishing options for effective antibiotic treatment, alternative strategies such as combination therapy and the use of medicinal plant compounds and prebiotics are suggested to help restore normal urinary tract flora.

Extended-spectrum beta-lactamase (ESBL)-producing strains of *E. coli* pose a significant challenge in urinary tract infections (UTIs). In our study, the prevalence of ESBL-positive strains was 53% in patients with recurrent UTIs and 42.5% in those with non-recurrent UTIs. While there was no significant relationship between the two groups, the higher frequency of ESBL-positive strains in recurrent UTI patients is noteworthy. Additionally, we reported a 72% prevalence of multi-drug resistant (MDR) isolates. Most studies in Iran indicate an ESBL prevalence of around 40% among UPEC strains, with similarly high rates of MDR strains, though some exceptions exist [41–44]. A study in China reported a prevalence of 38.07% for ESBL-producing isolates in community-acquired UTIs, which is slightly lower than findings from Iran and Pakistan [45, 46]. Conversely, research from Peru found a 42.5% prevalence of ESBL-positive isolates in patients

with recurrent UTIs, aligning closely with our results [40]. Infections caused by these strains may present similarly to other UTIs but tend to be more complex, often requiring advanced treatment options. Patients with ESBL-producing infections typically experience longer symptom duration, higher hospitalization rates, and may need broader-spectrum antibiotics, such as carbapenems, for effective management [33–35, 41]. Identified risk factors for ESBL-producing bacteria in UTIs include older age, a history of previous UTIs, and prior antibiotic use (42, 43(.

UPEC strains are significant contributors to urinary tract infections (UTIs) worldwide. These bacteria can colonize the urinary tract and form biofilms, which complicate treatment by allowing them to persist and survive antibiotic therapy [47, 48]. In our study, 72.73% of UPEC strains produced biofilms, primarily weak and moderate, with no strong biofilm producers identified. Previous research indicates that strong biofilm-forming *E. coli* strains are often more resistant to beta-lactam antibiotics [49]. Despite high resistance to Ampicillin-sulbactam, and Ceftraixone, no strong biofilm formation was observed in our strains. Similar findings were reported in Iran, where weak biofilm phenotypes predominated [50, 51]. Studies in Finland and Denmark showed varying relationships between biofilm formation and recurrent UTIs, with no significant differences noted in some cases [51–53]. Further research with larger clinical samples is necessary to better understand the impact of biofilm formation on antimicrobial resistance in UPEC strains.

Virulence factors are specific traits that enable microorganisms to cause infections and evade the host's immune response. The UPEC strains, which deviate from harmless intestinal flora, possess various virulence factors that facilitate urinary tract infections (UTIs) [54]. Understanding these mechanisms is essential for developing targeted prevention and treatment strategies, particularly for individuals with recurrent infections [54, 55]. The *fim*H gene, a key virulence factor, is frequently found in UPEC and plays a crucial role in adhesion. FimH, located at the tip of type 1 pili, allows UPEC to bind to mannosylated receptors on bladder epithelial cells, promoting colonization and persistence [56]. Studies have shown that FimH-based formulations can significantly protect against UTIs in mouse models [57]. Following *fim*H, the *pap* gene is also prevalent among UPEC strains [30, 50, 58, 59]. A deeper exploration of the *pap* gene will be included in the gene expression results section. Understanding these virulence mechanisms is vital for developing effective strategies to prevent and treat UTIs, especially in recurrent cases.

Phylogroup classification is essential for understanding the evolutionary relationships and pathogenic potential of different *E. coli* strains, particularly in urinary tract infections (UTIs). *E. coli* is typically categorized into four main phylogroups: A, B1, B2, and D [17]. Our study provides valuable epidemiological insights into the distribution of phylogroups among UPEC isolates from Iranian patients. We found that phylogroups B2 and D are the most prevalent among UPEC strains, consistent with global observations [60–65]. Specifically, phylogroups B and D were more common in both recurrent and non-recurrent UTIs. A meta-analysis indicated a significant increase in the incidence of phylogroup B2, rising from 20% in 2014 to 83% in 2020 [65]. This increase correlates with a rise in UPEC infections linked to phylogroup B2, which is associated with multiple virulence factors that enhance adhesion to urinary tract tissues. Phylogroup D has also emerged as a significant strain, suggesting the colon may serve as a primary reservoir for UTI-causing strains. Additionally, phylogroup D is the second most prevalent among drug-resistant UPEC strains, showing a slight phylogenetic shift toward group B2 [64–66].

Given the high prevalence of phylogroups B2 and D, it is crucial to focus on these groups in UTI management. Effective infection control measures should be implemented to control the spread of these virulent strains, and strategies for monitoring antibiotic therapy need to be developed.

Given the high prevalence of UPEC strains, implementing effective infection control measures and monitoring strategies for antibiotic therapy is essential. By comparing genetic fingerprints, researchers can identify outbreaks, trace transmission routes, and track sources of infection and antibiotic-resistant strains [67]. One useful molecular typing technique is ERIC-PCR, which differentiates bacterial strains by assessing genetic diversity [68]. This method is particularly valuable in epidemiological studies and infection control related to UTIs [69, 70]. Our study found that *E. coli* isolates from UTI patients exhibited significant genetic diversity. Moreover, genetic similarities among strains from different hospitals and community settings suggest the circulation of similar clones across these environments. High genetic diversity was also observed in strains isolated from recurrent urinary tract infections. These findings align with studies conducted in Iran and globally, which also indicate high genetic diversity among uropathogenic strains identified using the ERIC technique [69–72]. This genetic variability poses challenges for the treatment and control of diverse *E. coli* strains associated with UTIs in both hospital and community settings.

In this study, we investigated the expression levels of two key adhesion genes, *fim*H and *pap*, which play crucial roles in bacterial binding and pathogenicity. Notably, we found no prior research examining the expression of these adhesion genes in *E. coli* strains isolated from recurrent versus non-recurrent urinary tract infections

(UTIs). Our results indicated no significant relationship in the expression of the *fim*H gene between UTI patients and the non-recurrent group. However, we observed a notable nine-fold increase in *pap* gene expression in isolates from recurrent UTI patients, highlighting the significance of the *pap* gene in strains associated with recurrent infections. The *pap* gene encodes P pili, which bind to specific receptors on uroepithelial cells, enhancing UPEC's ability to colonize the urinary tract. Strains expressing P-fimbriae are more likely to cause severe UTIs, including pyelonephritis (73–74), and the *pap* gene is often used as a marker for identifying potentially dangerous UPEC strains [75]. Previous studies have demonstrated a direct role of *pap*G in both adherence and biofilm formation in UPEC, suggesting that P-fimbriae could be a potential candidate for anti-virulence therapy targeting drug-resistant UPEC strains [75]. These findings underscore the need for further research into the role of these adhesions in recurrent UTIs Recent evidence also indicates that reducing UPEC adhesion to urinary tract tissues can decrease recurrence and improve recovery, positioning UPEC adhesions as promising therapeutic targets [6]. The findings regarding the adhesion genes, *fimH* and *pap*, provide valuable insights into their contribution to bacterial binding and persistence in the urinary tract. Given the correlation between increased *pap* expression and recurrent infections, targeting these adhesion mechanisms may present effective strategies for reducing UTI recurrence. Ultimately, understanding the genetic and functional traits of UPEC will be vital in developing innovative therapeutic approaches and infection control measures to combat UTI outbreaks effectively.

Conclusion

This study highlights various factors that can influence the occurrence, persistence, and recurrence of urinary infections. While some factors did not show a significant relationship with urinary infections or their recurrence, broader sampling could yield more conclusive results. Key factors identified include high antibiotic resistance, the presence of ESBL and MDR *E. coli* strains, female gender, and elevated expression of the *pap* gene, with phylogroup B2 being the most significant group associated with uropathogenic *E. coli*. The genetic diversity revealed through techniques like ERIC-PCR further emphasizes the complex challenges in managing UPEC infections, as similar clones circulate across different environments. Understanding the pathogenic mechanisms of these strains is crucial for developing targeted prevention and treatment strategies for urinary tract infections (UTIs), particularly in individuals with recurrent infections. A lack of understanding of these factors can lead to prolonged symptoms, extended hospital stays, and the need for broad-spectrum antibiotics. Additionally, awareness of underlying diseases related to UTIs is important for effective prevention and management, encouraging individuals with risk factors to consult healthcare providers for appropriate prophylaxis.

Abbreviations

UPEC	Uropathogenic E. coli
UTI	Urinary tract infections
RUTI	Recurrent urinary tract infections
ESBL	Extended-spectrum β-lactamases
MDR	Multi-drug resistant

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Author contributions

P.M, L.SH and MY.A designed the experiments and wrote the manuscript. P.M and L.SH conducted the experiments. H.EM helped in clinical consultation and completing patients' information. P.M and A.DI were analyzed the data. P.M and L.SH participated in the initial draft and the revision of the manuscript. L.SH and MY.A revised the final version of the manuscript. All authors read and approved the final manuscript.

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Data availability

All data supporting the findings of this study are available within the paper and its Supplementary Information.

Declarations

Ethics approval and consent to participate

The present study was ethically approved by the Hamadan University of Medical Sciences, Institutional Review Board (IR.UMSHA.REC.1402.325). The experiments in our study were conducted following the relevant guidelines and regulations, as well as the Declaration of Helsinki. Informed consent was obtained from all patients, as well as from the parents or legal guardians of any participating children.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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