



Research article

Molecular characterization of ESBL-producing uropathogenic *Escherichia coli* isolates among kidney transplant patients: Emergence and spread of B2-ST131 clone type

Hassan Pourmoshtagh^a, Mehrdad Halaji^{b,c,**}, Sina Ranjbar^d, Reza Ranjbar^{e,*}

^a Department of Pediatrics, Loghman-Hakim Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

^b Infectious Diseases and Tropical Medicine Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran

^c Department of Medical Microbiology and Biotechnology, School of Medicine, Babol University of Medical Sciences, Babol, Iran

^d Department of Microbiology, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran

^e Molecular Biology Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran

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ABSTRACT

This study was conducted to identify the distribution of virulence determinants in uropathogenic *Escherichia coli* (UPEC) isolates obtained from kidney transplant (KTP) and non-transplant patients (non-KTP) with urinary tract infections (UTI). Additionally, the (GTG)₅ fingerprinting technique was used to investigate the genetic diversity of Extended-Spectrum B-Lactamase (ESBL)-positive isolates.

In this case-control study, 111 urine isolates were obtained from non-KTPs and KTPs, respectively. The presence of genetic markers encoding adhesion proteins, toxins and major *E. coli* phylogroups was assessed through PCR amplification. Molecular typing of ESBL-positive UPEC strains was performed using (GTG)₅ fingerprinting and Multilocus sequence typing (MLST) techniques.

Overall, 65 and 46 UPEC isolates were obtained from non-KTPs and KTPs, respectively. Among the studied isolates, *traT* (85.6%) gene was the most frequently observed virulence gene, followed by *kpsMT* (49.5%). Using the 80% cut-off point, all the 35 UPEC isolates were classified into four major clusters, namely A, B, C, and D. The majority of the Sequence Type (ST) 131 isolates belonged to cluster A. Additionally, three ST1193 isolates belonged to cluster A and phylogroup B₂. Moreover, ST38, ST131 and ST10 were in different cluster.

In general, we observed significant differences in the *papA*, *ompT*, *sat*, and *vat* genes between KTPs and non-KTPs. Furthermore, since all the isolates carried one or more virulence factors (VFs), these findings are concerning in the context of managing UTIs caused by the UPEC strain. Additionally, the distribution of ST and Clonal Complex (CC) among isolates in the main clusters revealed significant differences between MLST and (GTG)₅ fingerprinting analysis.

* Corresponding author.

** Corresponding author. Department of Microbiology, School of Medicine, Babol University of Medical Sciences, Babol, Iran.

E-mail addresses: mehrdad.md69@gmail.com (M. Halaji), ranjbarre@gmail.com (R. Ranjbar).

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1. Introduction

Transplantation of the kidney is the best treatment for patients with end-stage renal disease. This medical procedure not only enhances the quality of life but also extends the life expectancy of the patients [1,2]. However, transplantation is not without complications, and various life-threatening infections may develop in these patients [3]. The most frequent infectious complication suffered by kidney transplant patients (KTP) is a urinary tract infection (UTI), which can lead to graft failure, morbidity, and mortality [4,5]. Gram-negative pathogens account for more than 70% of UTIs. In this respect, uropathogenic *Escherichia coli* (UPEC) is a common etiological agent of UTIs, not only in the general population but also after renal transplantation [6]. The frequency of UTIs depends on various factors, such as anatomical factors, immune system functionality, and the pathogenicity of the infecting bacteria [7–9]. UTIs can be classified according to their location within the urinary tract, including pyelonephritis (in the kidney), cystitis (in the bladder), and bacteriuria (in the urine) [8]. The pathogenicity of UPEC strains is attributed to virulence factors that contribute to the development and progression of infection. The expression of virulence-encoding genes such as P (*pap*) and S (*sfa*) fimbriae in UPEC, causes tight and irreversible adherence of the bacterium to urothelial cells and subsequent invasion into the host cells [10–12]. The capacity of these strains to produce toxins like hemolysin (*hlyF*), vacuolate autotransporter toxin (*vat*), and secrete autotransporter toxin (*sat*) promotes bacterial dissemination. Consequently, nutrients are released from the host, the host cell is vacuolized, and thus immune effector cells are incapacitated [12,13].

However, other virulence genes, such as *kpsMT* (capsular antigens), *ompT* (outer membrane protein), *iroN* (Salmochelin), *traT* (Transfer protein), and *yfcV* (a major subunit of a putative chaperone-usher fimbria) are also known to be involved in the pathogenicity of UPEC [13,14]. Characterizing the local molecular epidemiology is essential for controlling the dissemination of UPEC strains in a different populations, especially among hospitalized transplant patients [15]. For this purpose, various molecular typing methods have been developed. For instance, PCR-based methods such as Repetitive Element PCR (REP-PCR), are rapid and reproducible, providing high discriminatory power for local typing of UPEC strains [16–18].

E. coli strains are primarily divided into seven phylogenetic groups (A, B1, B2, C, D, E, and F), each intertwined with a different pattern of virulence factors (VFs). For effective therapy, prevention, and control of UTI, understanding the phylogroup and virulence factors of the *E. coli* causing UTI is crucial. A previous phylogenetic study revealed that *E. coli* strains causing UTIs mainly belong to phylogenetic groups B₂ or D, which possess various VF genes [19].

Regarding the significance of kidney transplantation in Iranian patients and the lack of sufficient studies on infections in this populations, this study was conducted to explore the correlation between phylogenetic group distribution and virulence determinants of UPEC isolate obtained from kidney transplant and non-transplant patients with UTI. Additionally, to formulate infection control policies, the genetic diversity of ESBL-producing and MDR strains was investigated using (GTG)5 fingerprinting and Multilocus sequence typing (MLST).

2. Material and methods

2.1. Study design and setting

In this case-control study, a total of 111 non-repetitive UPEC isolates were collected, comprising 65 isolates from non-KTPs (as the control group) and 46 isolates. The isolates were obtained from two nephrology private clinics and a laboratory affiliated with Isfahan University of Medical Sciences (IUMS) between June 2019 and October 2019. Medical information was examined to obtain demographic and clinical data. All isolates were identified by standard biochemical tests and their confirmation was established in a previous study conducted by our team [20].

2.2. Molecular detection of genes encoding virulence factors

DNA extraction was performed using a standard method as previously described. The presence of genetic markers encoding adhesion proteins (*papA*, *papEF*), toxins (*hlyF*, *vat*, *sat*), capsule synthesis proteins (*kpsMTII*), outer membrane protein (*ompT*), Salmochelin (*iroN*), Transfer protein (*traT*), and the major subunit of a putative chaperone-usher fimbria (*yfcV*) was assessed by PCR amplification using specific primers according to previously described methods [21,22]. The virulence score was calculated based on the number of virulence genes detected in each isolate. The amplification reactions were performed using a BioRad thermal cycler (Goettingen, Germany) under the following conditions: initial denaturation at 95 °C for 5 min, followed by 35 cycles of: 30 s at 94 °C for, 30s at annealing for 55–57 °C at primer-specific temperatures, then 30 s at 72 °C, with a final extension step at 72 °C for 5 min. PCR products were resolved on a 1% agarose gel stained with safe stain loading dye (CinnaGen Co., Iran) and visualized using an ultraviolet (UV) transilluminator.

2.3. Phylogenetic analysis

UPEC isolates were assigned to one of the four main *E. coli* phylogroups (A, B1, B2, D) and sub-grouping schemes (E, F, C) using quadruplex PCR [19].

2.4. Molecular analysis of ESBL producing isolates

Molecular typing of ESBL and MDR-UPEC strains was carried out using the REP-PCR method. The (GTG)₅ fingerprinting was performed using the following primers: (5'-GTGGTGGTGGTGGTGGT-3') as described previously [23]. Using electrophoresis on 1.5% agarose gel dyed with safe red, the PCR amplification products were observed (CinnaGen Co., Tehran, Iran). The PCR amplification products were observed by electrophoresis on a 1.5% agarose gel dyed with safe red (CinnaGen Co., Tehran, Iran). Finally, the (GTG)₅ fingerprinting patterns and sizes were analyzed using the GelJ program, applying the Dice correlation coefficient and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm, as previously mentioned. Genotypes exhibiting a similarity coefficient equal to or above 80% were considered identical [24].

Based on our previous analysis, MLST was performed to investigate twenty-six MDR and ESBL-producing isolates, following the Achtman scheme, which involves amplification of seven housekeeping genes available on the pubMLST website (https://pubmlst.org/bigsub?db=pubmlst_escherichia_seqdef).

2.5. Statistical analysis

Categorical and continuous data were presented as mean ± Standard Deviation (SD) and frequency (percentage), respectively. Categorical information was compared among groups by using chi-squared test. Continuous variables (virulence factor (VF) scores) were compared between groups using One-Way ANOVA ("analysis of variance") and Gabriel post hoc test for pairwise comparisons. The level of statistical significance was considered as *P* value < 0.05. All statistical analyses were performed using SPSS 16 (IBM Corp., USA).

The VF score, as previously outlined, was determined by tallying the total number of distinct virulence factors (VFs) identified in each isolate, with each virulence factor being counted as one [25].

3. Results

According to demographic data, the mean, median and range of age of KTP and non-KTP were 50.8; 53; (15–82) and 45.42; (1–80), respectively. None of the non-KTP had history of antibiotic treatment in the month before sample collection, while, 31.6% of KTP had a history of antimicrobial therapy. Furthermore, 50%, 28.9%, 42.1% and 57.9% of KTP had a history of hypertension, diabetes mellitus, recurrent UTI and history of UTI. A history of hospitalization during the three months before participation was reported for 0% and 7.9% of KTP and non-KTP, respectively, as previously describe [20].

Based on the results of PCR assay, all UPEC isolates carried at least one of the VFs genes and the prevalence of individual VF genes ranged from 4.5% (*hlyF*) to 85.6% (*traT*). Overall, among all isolates, *traT* (85.6%) gene was the most common virulence factor, followed by *kpsMT* (49.5%) and *sat* (46.8%) genes. Meanwhile, *hlyF* (4.5%) and *iroN* (10.8%) were the least prevalent virulence genes (Supplementary file 1 Fig. S1).

On the other hand, our results revealed that among KTP and ESBL-positive isolates *traT* and *sat* were the most prevalent virulence genes, whereas among non-KTP and ESBL-negative isolates *traT* and *kpsMT* were the most prevalent virulence genes.

Additionally, the overall VF scores were highest among KTP isolates (median VF score 4.06 ± 1.1 (1–6)) than non- KTP isolates (median VFs score 2.75 ± 0.8, 3 (1–5)). The distribution of genes encoding VFs among KTP and non-KTP isolates is shown in Table 1.

Seventy-three different virulence profiles were identified (named 1–73). Nineteen of them grouped more than one isolate; profile 59 was the most predominantly found (n = 7), while profile 39, 9 and 5 showed the highest VFs (VFs: 6).

3.1. Correlations between phylogroups and VFs

Based on the VF distribution in phylogroups, *traT*, *kpsMT*, and *sat* tended to be more common among B2, D, and A group, followed

Table 1
Distribution of virulence-related genes in KTP and non-KTP isolates.

Function	Virulence factor	Total (n = 111) No. (%)	KTP (n = 46) No. (%)	Non-KTP (n = 65) No. (%)	P-value	ESBL-positive 35	ESBL-negative 76	P-value
Miscellaneous	<i>ompT</i>	32 (28.8)	22 (47.8)	10 (15.4)	0.001	11 (31.4)	21 (27.6)	0.6
Adhesions	<i>papA</i>	37 (33.3)	20 (43.5)	17 (26.2)	0.5	11 (31.4)	26 (34.2)	0.7
	<i>papEF</i>	23 (20.7)	10 (21.7)	13 (20)	0.8	7 (20)	16 (21.1)	0.8
	<i>kpsMT</i>	55 (49.5)	23 (50)	32 (49.2)	0.9	17 (48.6)	38 (50)	0.8
	<i>yfcV</i>	31 (27.9)	15 (32.6)	16 (24.6)	0.3	13 (37.1)	18 (23.7)	0.1
Toxins	<i>hlyF</i>	5 (4.5)	2 (4.3)	3 (4.6)	0.9	2 (5.7)	3 (3.95)	0.6
	<i>sat</i>	52 (46.8)	30 (65.2)	22 (33.8)	0.001	20 (57.1)	32 (42.1)	0.1
	<i>vat</i>	24 (21.6)	17 (37)	7 (10.7)	0.001	8 (22.8)	16 (21.1)	0.8
	<i>iroN</i>	12 (10.8)	8 (17.4)	4 (6.2)	0.6	4 (11.4)	8 (10.5)	0.8
Siderophores	<i>traT</i>	95 (85.6)	40 (87)	55 (84.6)	0.7	30 (85.7)	65 (85.5)	0.9
VF Score (mean, median, range)		3.29 ± 1.1, 3 (1–6)	4.06 ± 1.1, 5 (1–6)	2.75 ± 0.8, 3 (1–5)	0.001	3.5 ± 1.3, 4 (1–6)	3.2 ± 1.1, 3 (1–6)	0.18

by *traT*, and *papA*, which were more frequent among phylogroups B1 and E. Meanwhile, *traT*, *kpsMT* and *papEF* more common among phylogroup C members. The detailed distribution of VF genes among different phylogroups is presented in Table 2. In addition, there is a variation in the weight of VF score according to phylogenetic analysis. Accordingly, the highest median VF score (and range) were 3.6 ± 1.2 (1–6) among isolates; with phylogroup B2 being the most prevalent, followed by phylogroup D with 3.41%. (2–5), On the other hand, the lowest median and range VF scores were 2.5 ± 0.8 , (1–3) and 2.5 ± 1.3 , (1–4) for isolates belonging to phylogroup C and F. (GTG)5-PCR fingerprints of 35 ESBL and MDR-positive UPEC isolates have been indicated in Fig. 1. Accordingly, (GTG)5-PCR fingerprints generated 5 to 11 bands, with a range from 400 bp to more than 1800 bp. (GTG)5-PCR patterns revealed 32 (GTG)5 types (G1–G32) with 30 isolates of unique patterns (Fig. 1).

The (GTG)5 type G8 was the most frequent (GTG)5 type characterized by three isolates, all from KTP. The second most prevalent (GTG)5 type was G19 represented by two isolates. According to the 80% cut off point, all 35 UPEC isolates were majorly classified into 4 clusters, namely A, B, C and D and three single type. Cluster A contained 31.4% of isolates, followed by B (ten isolates, 15.7%) and D (nine isolates, 28.5%).

According to MLST results, most ST131 isolates belonged to cluster A. Additionally, three ST1193 isolates belonged to cluster A and phylogroup B₂. Moreover, ST38, ST131 and ST10 were in different cluster. Three singletons isolates belonged to ST8503, ST838 and 4516.

4. Discussion

Determining and identifying the virulence properties of UPEC strains isolated from UTIs is of great importance in understanding the pathogenesis and severity of UTIs, particularly in KTP [7]. The advent of potential UPEC strains with a multidrug-resistant phenotype is a warning event. These strains could potentially cause serious clinical issues related to the challenge of treating infections with antibiotic-resistant bacteria [26,27].

The present case-control study describes the distribution of VFs of UPEC isolates obtained from KTP and non-KTP in a referral hospital in Iran. These data allow the researcher to identify targets for vaccine and drug development. This research also provides evidence for the wide dissemination of the VFs genes and high level of VF scores among KTP and ESBLs-producing UPEC isolates. According to our analysis, the mean VF score among KTPs was significantly higher than that of non-KTP isolates. Moreover, the mean VF score among ESBL-producing isolates was relatively higher than the non-producing isolates. However, no differences were reported in the mean number of VFs among ESBL producing isolates compared to non-producing isolates. The results obtained from the current report indicate that *traT*, *sat*, and *kpsMT* were the most prevalent, while *hlyF* was the least prevalent among all UPEC isolates. The frequency of other VF genes was 10–33%. The results of the present research are similar to many reports that have shown that the majority of isolates carried *sat*, *traT*, and *kpsMT* genes [21,28]. In the current study, a high prevalence of *traT* (87 vs. 84.6), *sat* (65.2% vs. 33.8%), and *kpsMT* (50% vs. 49.2%) was observed in the UPEC isolated from KTP and non-KTP. This result is in line with previous reports in Iran [21,29,30]. Moreover, *papA*, *ompT*, *sat*, and *vat* were significantly higher among KTP isolates than non-KTP isolates. The significant prevalence of *papA*, *ompT*, *sat* and *vat* genes in our study may be attributed to the specific KTP-UPEC isolates obtained from this geographical area. The discrepancies between our study and different studies abroad may be due to difference in disease type, geographic area, methodology, and the studied population parameters such as age and history of antibiotic use [31]. It is important to target several classes of UPEC virulence agents as vaccine candidates for the prevention of UTIs. In this regard, a vaccine is being considered with possible candidates targeting bacterial surface polysaccharides and fimbrial adhesives [32]. Nevertheless, for a successful UTI vaccine, it is necessary to target more than one UPEC virulence factor to be effective against such a variety of pathogens [32,33]. Several previous phylogenetic analyses have reported that virulent UPEC strains belong to phylogroups B₂ and D and contain more VFs than strains from other phylogroups. Our findings are consistent with those of studies conducted in Germany [34], Ethiopia [35], and Pakistan [36], where it was found that the majority of virulent UPEC strains predominantly belong to phylogenetic group B₂. However, Khairy et al. showed that VFs were more prevalent in phylogroup A, which is accounted for as a pathogenic phylogroup [37]. In the present study, the prevalence of VFs was not significantly correlated with any of the phylogenetic groups and only *traT* and

Table 2
Distribution of VF genes among phylogenetic groupings.

Phylogenetic group	B ₂	D	A	B ₁	C	E	F	Unknown	P
Virulence factor	43	21	15	10	6	5	4	7	value
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	
<i>ompT</i>	12 (27.9)	6 (28.6)	5 (33.3)	3 (30)	0	1 (20)	2 (50)	3 (42.9)	0.7
<i>papA</i>	14 (32.6)	8 (38.1)	4 (26.7)	5 (50)	1 (16.7)	3 (60)	1 (25)	1 (14.3)	0.6
<i>papEF</i>	14 (32.6)	2 (9.5)	2 (13.3)	1 (10)	3 (50)	0	0	1 (14.3)	0.09
<i>kpsMT</i>	23 (53.5)	9 (42.9)	8 (53.3)	5 (50)	3 (50)	2 (40)	1 (25)	4 (57.1)	0.95
<i>yfcV</i>	16 (37.2)	7 (33.3)	4 (26.7)	1 (10)	2 (33.3)	0	0	1 (14.3)	0.3
<i>hlyF</i>	3 (7)	1 (4.8)	1 (6.7)	0	0	0	0	0	0.9
<i>sat</i>	26 (60.5)	12 (57.1)	6 (40)	3 (30)	1 (16.7)	2 (40)	1 (25)	1 (14.3)	0.1
<i>vat</i>	7 (16.3)	4 (19)	5 (33.3)	2 (20)	0	1 (20)	1 (25)	4 (57.1)	0.2
<i>iroN</i>	5 (11.6)	2 (9.5)	2 (13.3)	2 (20)	0	0	0	1 (14.3)	0.8
<i>traT</i>	35 (81.4)	21 (100)	11 (73.3)	9 (90)	5 (83.3)	5 (100)	4 (100)	5 (71.4)	0.2
VF Score (mean, median, range)	3.6 ± 1.2 , 4 (1–6)	3.4 ± 1 , 4 (2–5)	3.2 ± 1.1 , 3 (1–5)	3.1 ± 0.8 , 3 (2–5)	2.5 ± 0.8 , 3 (1–3)	2.8 ± 1.9 , 2 (1–6)	2.5 ± 1.3 , 2.5 (1–4)	3 ± 0.8 , 3 (2–4)	0.2

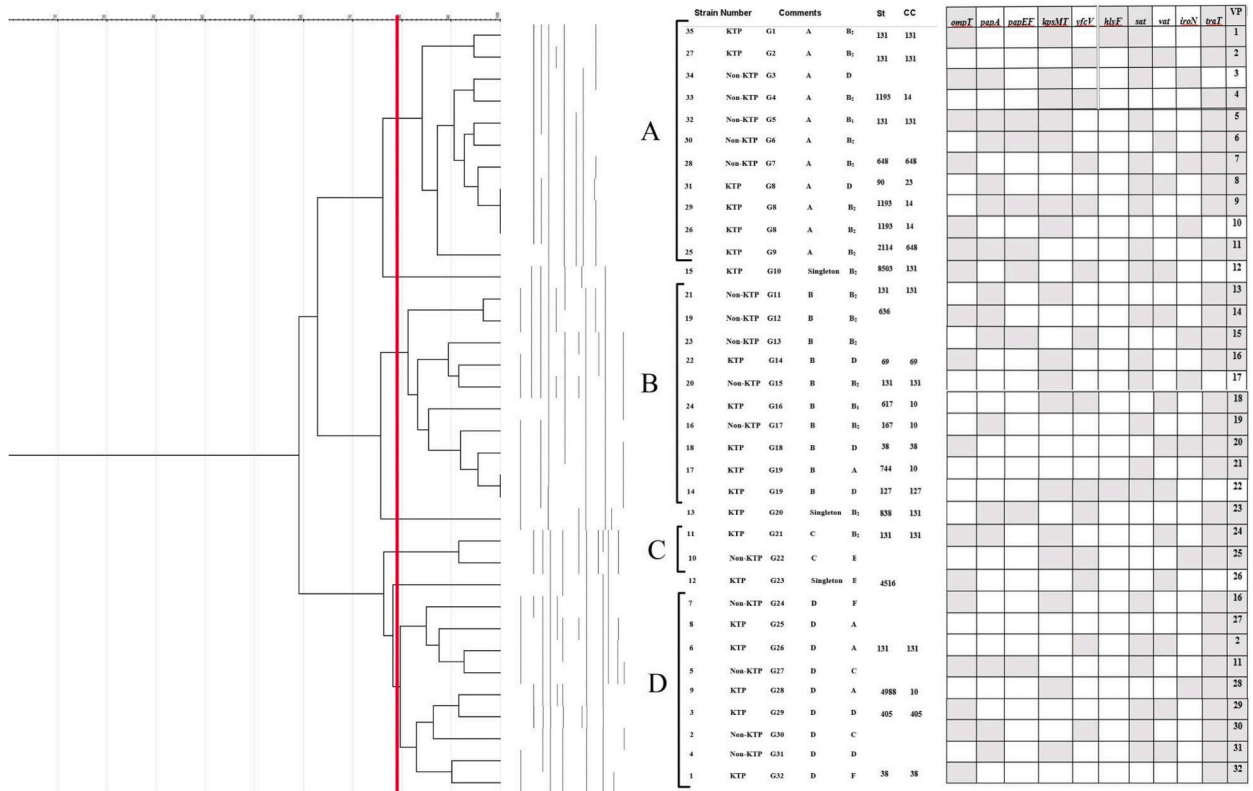


Fig. 1. Dendrogram based on (GTG)5-PCR fingerprints analysis and MLST information of 35 ESBL and MDR-positive UPEC isolates. (GTG)5-PCR fingerprints shows 5 to 11 bands ranging from 400 bp to more than 1800 bp. (GTG)5-PCR patterns revealed 32 (GTG)5 types (G1–G32) with 30 isolates of unique patterns. VP, virulence profile number; MLST: Multilocus sequence typing; ESBL: Extended-Spectrum B-Lactamase; UPEC: uropathogenic *Escherichia coli*; Gray box: gene positive; White box: Negative gene.

kpsMT were predominantly more prevalent in all phylogenetic groups. Moreover, *vat*, *ompT*, and *papA* were detected in 57.1, 50, and 60% of the strains belonging to the untypeable group and the phylogenetic groups F and E, respectively. This finding is explained by the fact that *E. coli* strains belonging to different phylogroups harbor single or various virulence gene combinations, as reported by other studies on UPEC isolates [38].

(GTG)5 is an effective fingerprinting method for molecular differentiation of a broad variety of bacteria, including Staphylococci, Enterococci, Salmonella, and *E. coli*. Accordingly, all the isolates were typable and classified using this method. About 91% (32 of 35) of the isolates produced unique genotypes, representing partially high genotypic diversity in UPEC among ESBL-positive isolates. In this regard, high levels of genetic diversity have been reported in other studies as well. Furthermore, *E. coli* isolates from the same phylogroup were dispersed on distinct clusters (GTG) in this investigation, suggesting the genotypic diversity of these isolates. According to the phylogenetic distribution group between clusters, 72.7, 50, 50, and 0% of phylogroup B₂ belonged to clusters A, B, C and D, respectively. This is in parallel with some previous studies by Khare et al., who demonstrated how *E. coli* isolates from the same phylogroup were dispersed in several Enterobacterial Repetitive Intergenic Consensus (ERIC)/(GTG)5 clusters [39].

In this respect, a study from Kentucky, USA, showed that Rep-fingerprints are not associated with phylotypes and other virulence genes [40]. Contrary to our findings, Bogartz et al. have reported that *E. coli* isolates with a genetic correlation based on the same PCR replications belong to different phylogroups [41]. According to distribution of ST and CC among isolates in the main clusters, significant differences between MLST and ERIC analysis were revealed. However, ERIC-PCR and MLST are both methods for measuring genetic diversity, but they are not interchangeable. ERIC-PCR displays a profile of different-sized DNA fragments based on the genomic positions of certain repetitive sequences, while MLST provides a nucleotide polymorphism measurement based on the DNA sequences of seven housekeeping genes.

We encountered several limitations in our study. Firstly, we were unable to obtain complete background details and physical exam information of patient history, which restricted our ability to draw conclusions about mortality based on the available data. Secondly, in order to achieve broader generalizability, it is crucial to replicate these findings in various hospitals. Therefore, due to the heterogeneity of the two patient groups, the results of our study may not entirely represent the comprehensive landscape of ESBL-producing uropathogenic *Escherichia coli* isolates among kidney transplant patients in Iran.

5. Conclusion

As a general conclusion, the main contribution of our study is reporting different VFs and their distribution among phylogroups that were less previously reported among UPEC isolates obtained from KTPs. The results of this study showed significant differences regarding the *papA*, *ompT*, *sat*, and *vat* genes from KTPs compared to non-KTPs. Therefore, the strains of *E. coli* isolated from KTP can be considered pathogenic. Since all isolates have one or more VFs, these findings are worrying not only due to the high risk of infection among KTPs but also because of the major indirect risk of possible horizontal transfer of genes to other pathogenic bacteria. Accordingly, (GTG) 5-PCR fingerprint analysis revealed relatively high variability in UPEC isolates obtained from ESBLs. Therefore, the present study focuses on the management and control of UTI caused by UPEC strain, especially among KTPs as one of the most important public health concerns. According to distribution ST and CC among isolates in main clusters, significant differences between MLST and ERIC analysis were revealed.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CRedit authorship contribution statement

Hassan Pourmoshtagh: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Mehrdad Halaji:** Writing – review & editing, Writing – original draft, Software, Project administration, Methodology, Investigation, Conceptualization. **Sina Ranjbar:** Software, Methodology, Investigation. **Reza Ranjbar:** Writing – review & editing, Writing – original draft, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

ESBL	Extended-spectrum b-lactamases
KTP	kidney transplant patients
SD	Standard Deviation
UPEC	Uropathogenic <i>Escherichia coli</i>
UTI	urinary tract infections
VFs	virulence factors
MDR	Multidrug resistant
MLST	Multilocus sequence typing

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2024.e27339>.

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