

Original Article

Phylogenetic Groups and Antimicrobial Resistance among Uropathogenic *Escherichia coli* Isolates from Hospitalized Patients in Tehran

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Abstract

Background: The presence of *Escherichia coli* among uropathogens is increasing significantly worldwide. It accounts for a considerable amount of morbidity and high medical costs and also can lead to mortality. The current research aims to investigate *E. coli* antimicrobial susceptibility patterns and the molecular causes of *E. coli* resistance trends and virulence factors among phylogenetic groups of Uropathogenic *Escherichia coli* (UPEC) in Urinary tract infection (UTI) patients in a hospital in Tehran, Iran. The antimicrobial susceptibility of urinary *E. coli* isolates.

Materials and Methods: The antimicrobial susceptibility of urinary *E. coli* isolates was tested using the Kirby-Bauer agar disc diffusion method. In addition, resistance and virulence genes were monitored by polymerase chain reaction (PCR), and the clonal relation of isolates was studied by pulsed-field gel electrophoresis (PFGE).

Results: Studied isolates showed the highest susceptibility rates to MEM (95.7%), followed by TZP (90%). In contrast, resistance rates were found for AMP (100%), SXT (74%), and CIP (51.5%). ESBL-producing isolates were positive for *bla*TEM, *bla*CTX-M, and *bla*SHV by PCR, respectively. According to the adhesion gene analyses, *fimH* (85.8%) was the most prevalent among *E. coli* isolates, followed by *aer* (49.7%), *hlyA* (46.1%), and *pap* (38.9%). A total of 57 PFGE patterns and three clusters (A–C) were identified by the PFGE method. (cluster A: Non-ESBL & Sensitive to all Antibiotics use; Cluster B: The most common cluster in terms of TEM, CTX-M, or both; Cluster C: containing CTX-M gene and resistant to ceftriaxone and ciprofloxacin).

Conclusion: Our data showed ESBL rates were high in UTI *E. coli* isolated in the studied hospital. The UPEC isolates exhibited a high resistance rate to first- and second-generation cephalosporins and fluoroquinolone, which could result in serious public health risks. The relationship between virulence factors and resistance genes is complex and needs more studies specific to each area.

Keywords: *E. coli*, Virulence, Resistance, Urine tract infection, PFGE

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Introduction

The urinary tract infection (UTI) is a prevalent bacterial infection that results in significant morbidity, high medical expenses, and mortality. UTI is commonplace among women, except in early infancy, although it can impact both men and women.

E. coli accounts for approximately 90% of all UTIs¹⁻³. The prevalence of tract infection in positive cultures in Iran has been demonstrated in numerous studies to be approximately 10 to 12%⁴. UTI may be complicated or straightforward, and asymptomatic bacteriuria is significant due to the absence of symptoms⁵. Every year, UTIs and their consequences kill around 150 million people globally⁶. Increasing age, diabetes, a history of UTI, urinary tract diseases, and immunological inadequacy may all increase the risk of UTI in pregnant women⁷. According to the literature, women should be screened for bacteriuria during pregnancy⁸. Recent studies in Iran have reported a high prevalence of ESBL-producing *Escherichia coli* isolates. For instance, 52.8% of isolates were ESBL-positive⁵, while studies in northern Iran (2022) and Rafsanjan reported rates of 46%⁹ and 45.6%¹⁰, respectively. These figures are notably higher than the global average reported in a recent systematic review from 2024, which found an overall ESBL prevalence of 25.4% in *E. coli* isolates worldwide¹¹.

UPEC contains several virulence characteristics, including fimbrial adhesins (fim), S-fimbrial adhesins (sfa), fimbrial adhesins (afa), P-fimbrial adhesins (pap), and siderophore that enhance its capacity to colonize and thrive in the urinary system^{12, 13}. In order to develop strategies to manage and prevent UPEC infections, it is necessary to investigate the genetic characteristics of the isolates above¹⁴. Furthermore, assessing the gene distribution in clonally correlated strains is imperative, as the phylogenetic background can predict pathogenic behavior and reflect vertical or horizontal gene transfer^{15, 16}. Clarifying the correlations between drug resistance, virulence factors, and phylogenetic background would benefit clinicians, enabling them to predict other pathogenic behaviors of these bacteria that are restricted in detection.

Strains that generate hemolysin, express P fimbriae, and contain specific virulence genes such as *fimH*, *pap*, and

cnf1 are some of the frequent forms of UPEC. These UPEC types are often linked to various pathogenic pathways and clinical consequences in cases of UTIs.

CTX-M: This is the most widespread ESBL gene, occurring up to 90% of the time worldwide in some areas. It is thought to be the source of up to 30% of hospital-acquired illnesses in Europe. The frequency of bacteria in Iran that produce CTX-M varies depending on the kind of bacteria and the geography. Research conducted on 140 *E. coli* isolates from hospitals in Tehran revealed that 56 % of them produced CTX-M.

SHV: Though less common than CTX-M, SHV-producing bacteria are relatively common in certain areas. For example, up to 25% of hospital-acquired infections in Asia have been observed to have *E. coli* that produces SHV. Although less common than CTX-M, SHV-producing bacteria are common in specific Iranian locations. Twenty-four percent of the one hundred *E. coli* isolates from Mashhad hospitals were confirmed to be SHV-producing.

TEM: Though less common than CTX-M and SHV, TEM-producing bacteria are problematic in certain areas. For example, up to 15% of hospital-acquired infections in Latin America have been documented to have TEM-producing *E. Coli*. Although less common than CTX-M and SHV, TEM-producing bacteria are problematic in specific Iranian locations. Twelve percent of the one hundred *E. Coli* isolates from Tabriz hospitals that were studied produced TEM¹⁷.

The characteristics of typing methods, including discriminatory power, simplicity of performance, reproducibility, ease of interpretation, and cost, are crucial for obtaining the appropriate results^{18, 19}. PFGE is a frequently employed technique for the generation of DNA fingerprints, even though a wide variety of methodologies are employed to type *E. coli*. In Gram-negative bacilli bacterium typing, PFGE is considered the gold standard technique^{20, 21}.

This investigation presents substantial information concerning the distribution and correlation of the antimicrobial susceptibility, virulence genes, and clonal relationship of UPEC isolates from a general hospital.

Methods

Collection and identification of uropathogenic *E.*

coli: We collected 310 urine isolates from patients with UTI indications who were hospitalized at Milad Hospital (Tehran, Iran), a renowned general hospital in Tehran, Iran, between 2018 and 2023. Clinical data regarding the type of urinary tract infection (UTI) were collected from patient records. Among the 310 UPEC cases, 120 (38.7%) were diagnosed with cystitis, 85 (27.4%) with pyelonephritis, and 30 (9.6%) were classified as asymptomatic bacteriuria. In 75 (24.1%) cases, the specific UTI type could not be determined from available documentation. Only one isolate per patient was selected and included in the study to avoid duplication bias. Additionally, we collected the following patient data: gender and age. Microbial testing was conducted to corroborate the presence of cystitis, pyelonephritis, and urethritis symptoms in patients. The complete isolates were stored at -75°C in trypticase soy broth containing 15% glycerol for subsequent processing.

Antimicrobial susceptibility testing: The Clinical and Laboratory Standards Institute²² recommendations employed the Kirby-Bauer method to conduct antimicrobial susceptibility testing. The following antibiotic disks and their respective concentrations were used in the Kirby-Bauer disk diffusion method: ampicillin (10 µg), tazobactam-piperacillin (100/10 µg), ceftriaxone (30 µg), ceftazidime (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), sulfamethoxazole-trimethoprim (1.25/23.75 µg), and meropenem (10 µg). The subsequent antimicrobial agent classes were evaluated: aminoglycosides (gentamicin), penicillins (ampicillin (AMP)), penicillins with b-lactamase inhibitors (tazobactam-piperacillin (TZP)), cephalosporins (ceftazidime (CAZ), ceftriaxone (CRO)), carbapenems (meropenem (MEM)), fluoroquinolones (ciprofloxacin (CIP)), and folate pathway inhibitors (sulfamethoxazole-trimethoprim (SXT)). The Clinical and Laboratory Standards Institute guidelines were followed to screen for the production of ESBLs using 30 mg cefotaxime and ceftazidime disks with or without 10 mg clavulanic acid (Negative control: *E. coli* ATCC 25922 and Positive control: CTX-M). *Escherichia coli* (*E. Coli*) ATCC 35218, TEM: *Escherichia coli* (*E. Coli*) ATCC 35461, SHV: *Klebsiella pneumoniae* ATCC 700603, *Escherichia coli* (*E. Coli*) ATCC 25922:

Amoxicillin/Clavulanic acid, *Pseudomonas aeruginosa* (*P. Aeruginosa*) ATCC 27853:piperacillin/tazobactam, *Escherichia coli* (*E. Coli*) ATCC 25922:ceftazidime, *Escherichia coli* (*E. Coli*) ATCC 35218:ceftriaxone, *Klebsiella pneumoniae* (*K. Pneumoniae*) ATCC 700603:meropenem, *Escherichia coli* (*E. Coli*) ATCC 25922:gentamicin, *Escherichia coli* (*E. Coli*) ATCC 25922:ciprofloxacin, *Staphylococcus aureus* (*S. Aureus*) ATCC 29213: sxt)^{17, 23}.

PCR for antimicrobial resistance and virulence genes: PCR performed virulence gene analysis. We identified five virulence genes that have been primarily linked to UPEC. The adhesion genes *fimH*, *pap*, *afa*, and *SFA* represent molecular adhesions. PCR was used to examine the genes of the toxins, including *hly* and *cnf*, which are known to be associated with antibiotic resistance²⁴. The PCR thermal cycling conditions were as follows: initial denaturation at 94 °C for 5 minutes followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 55–60 °C (depending on primer) for 30 seconds, and extension at 72 °C for 1 minute; with a final extension at 72 °C for 7 minutes.

We found three antimicrobial resistance genes (*CTX-M*, *TEM*, and *SHV*) primarily linked to ESBL bacteria. Positive and negative controls were included in all PCR assays. *Escherichia coli* ATCC 25922 was used as a negative control, and previously confirmed positive isolates were used as positive controls for each target gene. Table 1 lists the primers utilized²⁵.

Pulsed-field gel electrophoresis: Using the restriction enzyme *XbaI* and pulsed-field gel electrophoresis

Table 1. Primers for uropathogenic *Escherichia coli* virulence genes PCR assay.

Gene	Sequence (5'-3')	Size (bp)
<i>pap</i>	F GCAACAGCAACGCTGGTTGCATCAT	336
	R AGAGAGAGCCACTCTTATACGGACA	
<i>afa</i>	F CGGCTTTTCTGCTGAACTGGCAGGC	672
	R CCGTCAGCCCCACGGCAGACC	
<i>aer</i>	F TACCGGATTGCATATGCAGACCGT	602
	R AATATCTTCTCCAGTCCGGAGAAG	
<i>fimH</i>	F AACAGCGATGATTTCCAGTTTGTGTG	465
	R ATTGCGTACCAGCATTAGCAATGTCC	
<i>hlyA</i>	F AACAAAGGATAAGCACTGTTCTGGCT	1177
	R ACCATATAAGCGGTCATTCCCGTCA	

(PFGE) by Pulsenet recommendations, isolates that produced ESBLs were examined for clonal connections^{13, 14}. In summary, the electrophoresis operating conditions were used: a switch time of 2.16 to 54.17 s at a gradient of 6 V cm⁻¹ and an included angle of 120° for 19 h at 14 °C. The agarose used for the electrophoresis was 1% pulse field certified, and the Tris-borate-EDTA was 0.5%. The *Salmonella* enteric serovar Braenderup H9812 universal marker was used as a size marker standard. The PFGE patterns were analyzed using BioNumerics version v.6.6 (Applied Maths, SintMartens-Latem, Belgium). The similarity between DNA banding patterns was calculated using the Dice similarity coefficient, which considers the presence or absence of bands. The position tolerance was set at 1.5% and optimization at 1% to account for minor variations in band migration. Clustering was performed using the unweighted pair group method with arithmetic mean (UPGMA). An 80% similarity cutoff was applied to define clusters, a threshold commonly used in epidemiological studies of *Escherichia coli* to indicate clonal relatedness. The Dice similarity coefficient was applied using average linkages and the unweighted-pair group technique, with a 1.5% band tolerance. A cluster was defined as isolates sharing 80% of the same characteristics. *Salmonella* enterica serotype Braenderup H9812 was used as a molecular size marker and internal positive control to normalize banding patterns across different PFGE gels by CDC PulseNet protocols²⁶ (Ethical Code: IR.SBMU.MSP.REC.1403.220).

Results

Statistical analysis: IBM Corporation, Armonk, New York, USA's SPSS software version 20.0 was used for the statistical study. A Chi-test was used to examine variations in the distribution of virulence-related genes and ESBLs and the connections between antimicrobial drugs, individual patients, and clusters.

Bacterial collection: From the urine samples of UTI patients, we obtained 310 viable isolates, which biochemical testing verified as *E. coli*. The average age was 69±14 years old, while the median age was 58 years old. The mean age of the patients was 69 years, with a standard deviation of 14 years, indicating that most patients were aged between 55 and 83 years.

Among the 310 UPEC isolates, 216 were obtained from female patients and 94 from male patients. Most isolates originated from renal transplants and emergency wards, followed by pediatrics, CCU, and NICU. However, for 72 isolates, it remained unclear whether they were collected from inpatients or outpatients, as this information was not recorded in the

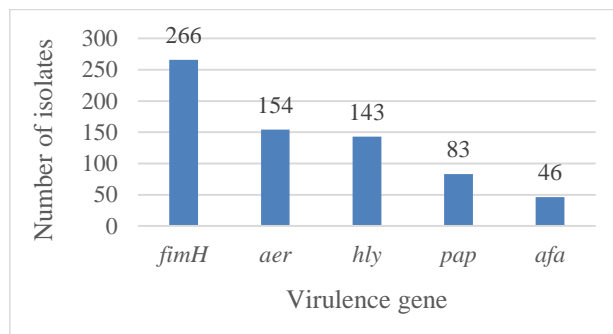


Figure 1. Virulence genes of uropathogenic *Escherichia coli*.

hospital documentation (Figure 1).

Antimicrobial susceptibility: Table 2 displays the RIS (Resistance, Intermediate, and Susceptible) statuses; the drug intermediate rate was left out even though it could be computed from the rates of susceptible and

Table 2. Primers used for phylogenetic of uropathogenic *Escherichia coli*.

Gene	Sequence (5'-3')	Size (bp)
<i>chuA</i>	F ATGGTACCGGACGAACCAAC	288
	R TGCCGCCACTACCAAAGACA	
<i>yjaA</i>	F CAAACGTGAAGTGTCCAGGAG	211
	R AATGCGTTCCTAACCTGTG	
<i>TspE4.C2</i>	F CACTATTTCGTAAGGTCATCC	152
	R AGTTTATCGTCTCGGGTTCGC	
<i>arpA</i>	F AACGCTATTCCGACGCTTGC	400
	R TCTCCCATACCGTACGCTA	

resistant infections.

The most susceptible strains to MEM (95.7%) were the UPEC strains, followed by TZP (90%). Meanwhile, resistance percentages for CIP (51.5%), SXT (74%), and AMP (100%) were discovered. Antimicrobial susceptibility testing revealed that 120 isolates (38.7%) produced ESBL. As shown in Table 1, the proportion of sensitive isolates for AMP, SXT, CAZ, GEN, TZP,

and MEM was lower in the ESBL-producing isolates than non-ESBL-producing isolates. Table 2 indicates that there was no significant difference in the high incidence of sensitivity to MEM between the two groups (9% for ESBL-producing isolates and 6.1% for non-ESBL-producing isolates).

Characterization of resistance genes: Genes of the ESBL type (*blaTEM*, *blaSHV*, and *blaCTX-M*) were identified in this investigation. 90 of the 120 ESBL-producing isolates were positive for *blaTEM* by PCR. *blaCTX-M* was detected in 64 isolates, while *blaSHV* was detected in 14. These isolates that produce CTX-M exhibited high resistance to AMP, CIP, and CRO. The isolates that were positive for *blaTEM* exhibited elevated resistance to AMP, SXT, GEN, and CAZ. High resistance rates to AMP, CIP, and SXT were observed in *blaSHV*-positive isolates. At the same time, fourteen isolates were positive for *blaCTX-M* and *blaSHV*. These isolates exhibited high resistance to AMP, CIP, and CRO. A high resistance rate to AMP, CIP, CAZ, and CRO was observed in thirty-four isolates simultaneously positive for *blaCTX-M* and *blaTEM*. Five isolates were simultaneously positive for *blaCTX-M*, *blaTEM*, and *blaSHV* and exhibited resistance to CAZ and CRO.

Prevalence of virulence genes among UPEC isolates: The virulence genes that were the subject of the study were identified in 287 (92.5%) isolates, while the remaining isolates were negative for virulence genes. Among the 310 *E. coli* isolates, *fimH* 247 (86.0%) was the most prevalent, followed by air 145 (50.5%), only 133 (46.3%), and pap 111 (38.6%) isolates, as indicated by the adhesion gene analyses. The correlation between the virulence and antimicrobial resistance genes was analyzed, and it was determined that AFA was associated with *blaCTX-M* in 25 (8.7%) isolates. Among the 310 *E. coli* isolates, *blaTEM* was detected in 29.0%, *blaCTX-M* in 20.7%, and *blaSHV* in 4.5% of isolates.

Pulsed-field gel electrophoresis: PFGE was employed to investigate the genetic relationships among the 310 ESBL-producing isolates. A threshold of 80% was used to identify 57 PFGE patterns and three clusters (A–C). (Figure 1). This study applied a similarity cutoff value of 80% to define clonal clusters in the PFGE dendrogram. This threshold is widely accepted in molecular epidemiological studies of

Escherichia coli to indicate genetic relatedness, as it reflects a balance between genetic similarity and epidemiological significance. Previous research has commonly used the 80% cutoff for *E. coli* PFGE clustering^{27, 28}, making it a standard criterion for defining closely related strains in hospital and community settings.

For clarity, a scale bar indicating genetic similarity percentage was added to the dendrogram. Clustering was performed using the unweighted pair group method with arithmetic mean (UPGMA) based on Dice similarity coefficients, with an 80% similarity cutoff used to define clusters.

The association of the antimicrobial susceptibility, virulence factors, and clusters is visually illustrated in Figure 2 by combining PFGE with previous results of ESBL-producing isolates. The resistance profile of resistance genes is reported in Table 2, which is based on PFGE patterns. According to our dendrogram analysis, most cluster A isolates were non-ESBL and susceptible to all antibiotics employed.

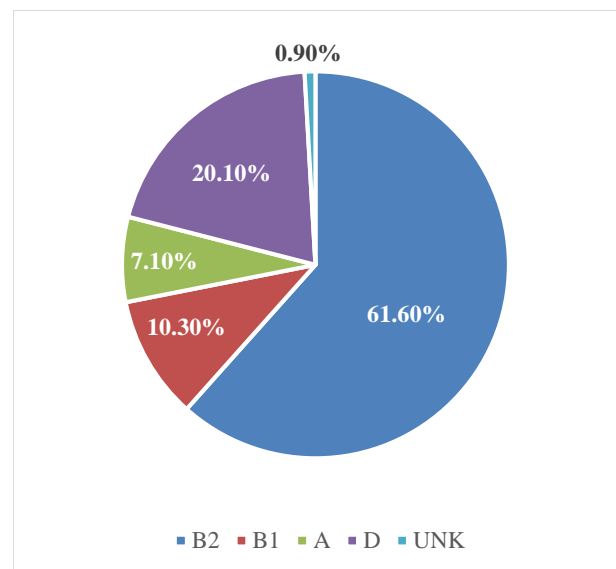


Figure 2. Phylogroup.

Conversely, most cluster B members were positive for TEM, CTX-M, or both as resistance genes. Cluster C members resisted ceftriaxone and ciprofloxacin and possessed the CTX-M gene. The same distribution was observed in all clusters concerning all virulence genes. Based on our data, it is evident that cluster B had the highest concentration of ESBL, while cluster C had the lowest. There is a correlation between virulence and

antimicrobial resistance. Numerous studies have reported that UPECs resistant to ampicillin, sulfonamides, tetracycline, streptomycin, and fluoroquinolones are typically less virulent than their susceptible counterparts.

Additionally, it has been reported that *E. coli* that form biofilms are more susceptible to nalidixic acids than damaging acids in biofilm formation. Similar results were observed in this study, indicating that a lower prevalence of virulence genes was associated with resistance to AMP, TZP, CIP, SXT, and GEN. Nevertheless, the mechanism by which the relationship between drug resistance and reduced severity is elucidated remains obscure (Table 3).

Table 3. Antibiotic resistance comparison between ESBL and non-ESBL isolates.

Antibiotic Name	Resistance in ESBL (n/total)	Resistance in non-ESBL (n/total)	p-value
AMP	120 / 120	180 / 190	0.02
SXT	100 / 120	120 / 190	0.00
CIP	70 / 120	80 / 190	0.00
GEN	60 / 120	70 / 190	0.03
CRO	80 / 120	60 / 190	0.00
CAZ	85 / 120	55 / 190	0.00
TZP	10 / 120	5 / 190	0.04
MEM	4 / 120	2 / 190	0.31

Discussion

Uropathogenic *E. coli* isolates are the primary cause of urinary tract infections and may be accountable for nearly 90% of UTIs. This investigation aimed to evaluate local isolates' clonal relationship and gene distribution regarding antimicrobial resistance and virulence. Our research offers critical insights into the antimicrobial susceptibility, virulence factor distribution, and clonal relationship of UPEC from this region. The data indicated that the resistance rates for several antimicrobial agents typically employed to treat urinary tract infections (UTIs) are elevated in this facility. Fluoroquinolone is one of the first-line agents recommended for the empirical treatment of UTIs, and it is essential to emphasize this.

Our research demonstrates that urinary tract infections necessitate additional antimicrobial agents. Only MEN and TZP exhibited a resistance rate of less than 10%, suggesting they may be promising options for

the empiric treatment of UTIs. This study's antimicrobial resistance (AMR) rates are consistent with national and international trends. For instance, the 100% resistance rate to ampicillin and high resistance to trimethoprim-sulfamethoxazole (74%) align with previous reports from Iran. A study by Hojabri et al. reported that 90.8% of *E. coli* isolates collected from various clinical samples in Iran were multidrug-resistant (MDR)²⁹. Additionally, a study conducted in Tehran in 2021 found that 75% of *E. coli* isolates were ESBL producers, with high resistance rates to ciprofloxacin (85%) and ceftriaxone (80%)³⁰.

Globally, the World Health Organization's Global Antimicrobial Resistance Surveillance System (GLASS) has reported that *E. coli* isolates exhibit ampicillin resistance rates exceeding 80% and fluoroquinolone resistance above 50% in several countries, including Iran³¹.

Moreover, data from the EMBARK study, a global surveillance project on uropathogens, indicated that resistance to ciprofloxacin among *E. coli* UTI isolates ranged from 30% to 60%, depending on the region, which aligns with our finding of 51.5%³².

These high resistance rates underscore the urgent need for continued local AMR surveillance and the implementation of effective antibiotic stewardship strategies, particularly in hospital settings.

The ESBL-producing isolates should not be reported as susceptible to cephalosporins³³, as per a reference guideline (M100-S29). A high incidence of ESBL production (50%) was observed in uropathogenic *E. coli* in this study.

The presence of *aer*, *fimH*, and *hlyA* genes in UPEC isolates has been demonstrated in this study. The *aer* gene responsible for iron acquisition was present in nearly all isolates^{20, 34}. The frequency of the *aer* gene in our study was higher than that of recent studies. *hlyA* is one of the secreted toxin factors in *E. coli* isolates, and it is implicated in the dysfunction of local immune responses and tissue injury. In contrast to certain studies, this investigation demonstrated that the prevalence of *hlyA* among UPEC isolates is elevated²⁰. *fimH* promotes the adhesion of UPEC to the urothelium cells and aids in forming bacterial biofilms³⁵. The distribution of the *fimH* gene in our isolates is consistent with that of other studies^{1, 34}.

Recently, PFGE has been effectively employed in DNA

fingerprinting and clonal relationship studies as a gold standard method in recent studies. PFGE is a reproducible typing method employed in molecular epidemiology with a high level of discriminatory power. This investigation offers additional details regarding the distribution of urinary isolates of *E. coli* strains. The XbaI restriction enzyme could be employed to separate fragments of the digested DNA chromosome using the PFGE technique. The XbaI enzyme, which is more potent^{36, 37}, was effectively employed in this study to differentiate the isolates from the UTI patients in the hospital. The analyzed isolates produced an exclusive profile with DNA bands ranging from 15 to 20 and a molecular weight of 33.3kbp to 1135kbp using the XbaI enzyme.

In contrast, other studies have demonstrated variations in the number of bands and molecular weights. In the molecular characterization of *E. coli* isolates from patients with cystitis and pyelonephritis, Anvarinejad et al. indicated various products with a molecular size of 2 to 660 kbp. In the genotyping of *Shiga* toxin produced by *E. coli* isolates³⁸, Dong et al. reported 15–20 distinct bands. By categorizing UPEC strains²⁸, Ejrnaes et al. reported 15–20 bands with molecular diameters ranging from 50kbp to 1200kbp³⁹. In the present investigation, three clusters of *E. coli* isolates with 80% similarity were identified using a deep dendrogram. Strains with 17 and 20 bands had the most significant proportion, whereas those with 15 bands had the lowest rate.

Dolatyar et al. explored PFGE's effectiveness in researching pathogen-produced extended-spectrum beta-lactamase (ESBL) enzymes^{26, 40}. Watabe and colleagues employed PFGE-CHEF to conduct an epidemiological investigation on *E. coli* O157: H7 isolates collected in Northern Ireland between 1997 and 2000, and the findings were compared to phage typing analysis³⁶. They observed that PFGE-CHEF typing was more effective in distinguishing *E. coli* O157 isolates than phage typing²⁰. Jones et al. employed PFGE to determine the presence of *E. coli* O157 and *Salmonella* isolates in an epidemic and found that 20% of clusters were from the same source⁴¹. Xiaoli et al. in China investigated the genotypic characteristics of multidrug-resistant UTIs *E. coli* using PFGE. At an 85% cutoff value, 40 PFGE types were identified in 51 MDR *E. coli* isolates.

Given the substantial genetic variety among our MDR isolates, there was a strong relationship between antibiotic resistance patterns⁴².

The resistance profile of resistance genes, which was generated using PFGE patterns, was depicted in our dendrogram in Table 2. The dendrogram analysis revealed that most isolates in cluster A were non-ESBL and pan-susceptible to all antimicrobials being used. The most prevalent cluster, cluster B, was characterized by TEM, CTX-M, or both resistance genes. The CTX-M gene was present in the members of cluster C, which exhibited resistance to ciprofloxacin and ceftriaxone. The distribution of all virulence genes was consistent across all clusters.

It is evident from our data that the frequencies of ESBLs were highest in cluster B and lowest in cluster C. Additionally, we discovered that a substantial number of the isolates from phylogenetic cluster B contained virulence genes. It seems that there is a correlation between virulence and antimicrobial resistance. Evidence displayed a high prevalence of MDR-UPEC⁴³. Additionally, it has been reported that *E. coli* that form biofilms are more susceptible to nalidixic acids than those that do not⁴⁴. However, the mechanism of the correlation between reduced virulence and drug resistance remains obscure.

This study has several limitations. First, it was conducted in a single hospital in Tehran, which may limit the generalizability of the results to other healthcare settings or geographic regions. Additionally, clinical data such as the specific type of urinary tract infection (e.g., cystitis, pyelonephritis) were limited. Molecular typing was performed only using the PFGE method, and whole-genome sequencing was not applied, which could have provided deeper insights into clonal relationships and resistance mechanisms.

Conclusion

Our findings indicated that UPEC isolates had a high rate of resistance to antibiotics, which could potentially pose a significant public health concern. Cluster B was associated with a high level of drug resistance, while Cluster A was correlated with a low level of drug resistance. The information derived from the similarity of the pulsotypes among isolates can assist physicians in comprehending the antibiotic-resistant patterns of

various isolates from various sources and making an informed decision when prescribing antibiotic medication.

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Conflict of interest

The authors further declare that they have no conflict of interest.

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