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Resistance investigation of uropathogenic Escherichia coli strains isolated from urinary tract infections in the north of Iran

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Abstract

Background: Urinary tract infections (UTIs) caused by uropathogenic Escherichia coli (UPEC) represent a significant global health concern. Virulence factors (VFs) expressed by UPEC strains play a crucial role in promoting bacterial pathogenicity within the urinary tract. Effective treatment of these infections is frequently complicated by the high prevalence of antimicrobial resistance exhibited by Escherichia coli. The objective of this study was to investigate the VFs and antibiotic susceptibility profiles of UPEC strains isolated in the northern region of Iran.

Methods: One hundred and five urine specimens were collected from female patients diagnosed with UTIs in Rasht, located in the north of Iran. These samples underwent culturing on both Eosin Methylene Blue (EMB) agar and MacConkey agar. Following a 24-hour incubation period at 37°C, pure bacterial isolates were identified through Gram staining and a battery of standard biochemical assays. The prevalence of six VF genes - *papC*, *sfa/foc*, *fimH*, *afa*, *ibeA*, and *neuC* - within UPEC strains was determined utilizing polymerase chain reaction (PCR) and subsequently confirmed via direct sequencing. Antibiotic susceptibility testing (AST) was conducted using the disk diffusion method, adhering to the guidelines established by the Clinical and Laboratory Standards Institute (CLSI M02).

Results: The study identified 65.71% of the isolates as Escherichia coli. Among the virulence genes examined, *fimH* exhibited the highest prevalence (100%), while *afa* was the least frequent (1.44%). Antibiotic resistance analysis revealed the highest rate against Cefazolin (66.66%) and the lowest against Gentamicin (24.63%). Notably, the prevalence of multi-drug resistance (MDR) was determined to be 73.91%.

Conclusion: This study underscored the significance of localized surveillance of UPEC isolates. This emphasis stems from the pathogen's considerable capacity for genetic mutation, coupled with the influence of environmental variables and individual patient characteristics. Understanding these dynamic factors at a local level is crucial for formulating the most effective strategies to combat UTIs.

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Introduction

Urinary tract infection (UTI) represents a prevalent global health concern due to its substantial morbidity and mortality, alongside considerable healthcare expenditures (1). Uropathogenic Escherichia coli (UPEC) serves as the primary etiological agent in the majority of UTI cases, accounting for approximately 75-95% of uncomplicated UTIs and 40-50% of complicated UTIs (2-4).

The pathogenicity of UPEC is attributed to its diverse repertoire of virulence factors (VFs), encompassing adhesins, toxins, invasins, surface polysaccharides, flagella, and iron-acquisition systems (1,4). Notably, fimbriae and afimbrial adhesins represent a prominent category of VFs. Among these, type 1 fimbriae (*fimH*), P fimbriae (*papC*), S fimbriae, F1C fimbriae (*sfa/foc*), and afimbrial adhesin (*afa*) are the most frequently identified adhesins in UPEC strains isolated from patients with UTIs (2,5).

The *FimH* protein, a mannose-specific adhesin located at the tip of type 1 fimbriae, plays a critical role in host-pathogen interactions. It facilitates bacterial adherence to uroepithelial proteins and contributes to the formation of biofilms (2,6). Similarly, P fimbriae, encoded by the pyelonephritis-associated pilus (*pap*) operon, are significant mediators of bacterial colonization. They achieve this through specific binding to digalactoside-containing receptors present on the epithelial surfaces of the intestine, vagina, and urinary tract (6,7). S fimbriae mediate the adhesion of the pathogen to the epithelial and endothelial cells lining the lower human urinary tract, thereby facilitating its dissemination within host tissues (2,4,8). Closely associated with S-type fimbriae are the F1C fimbriae, which exhibit binding affinity for β-GalNac-1,4b-Gal residues present on glycolipids. These glycolipids are expressed by the epithelial cells of the distal tubules and the collecting ducts in the kidney, as well as by the endothelial cells of both the bladder and the kidneys (2). Afimbrial adhesins represent key surface-associated VFs in UPEC strains. These adhesins mediate adherence to the decay-accelerating factor (DAF) receptor, which is expressed on the epithelial cells lining the urinary tract. Notably, strains implicated in the pathogenesis of pyelonephritis and recurrent cystitis frequently harbor the operon encoding the *afa* family of adhesins (2,5,9).

UPEC harbors a variety of virulence genes, including *ibeA* (Invasion of brain endothelium) and *neuC* (Sialic acid biosynthesis), which exhibit lower prevalence within these isolates (10). The *ibeA* protein facilitates bacterial invasion into host cells and tissues. Similarly, *neuC* is involved in the biosynthesis of the K1 capsule antigen, which confers protection against phagocytosis and promotes bacterial dissemination within the host. Notably, these two genes are observed with higher frequency in strains implicated in meningitis (5,8,10).

The cornerstone of UTI treatment lies in selecting an appropriate antibiotic characterized by broad efficacy and effectiveness. Commonly employed antibiotics worldwide include β-lactams, quinolones, and cephalosporins (11). However, research indicates a concerning trend of escalating antibiotic resistance in UPEC strains (3,9). The emergence of multi-drug resistance (MDR) strains of Escherichia coli poses a significant challenge to effective infection management. Mechanisms such as genetic mutations and the acquisition of mobile genetic elements contribute to the development of resistance against the antimicrobial activity of therapeutic agents (3,12).

Given the current absence of comprehensive strain characterization focusing on VFs within Iran, this study aimed to determine the prevalence of various previously identified VFs in UPEC strains isolated from patients diagnosed with UTIs in Rasht, a city situated in northern Iran. Furthermore, antimicrobial susceptibility testing (AST) was conducted to elucidate the susceptibility and resistance profiles of these strains against commonly prescribed antibiotics, thereby informing optimal treatment approaches.

Methods

Between August 2017 and July 2018, a total of 105 urine specimens were prospectively collected from female patients diagnosed with either hospital-acquired UTIs (HA-UTIs) or community-acquired UTIs (CA-UTIs). These patients were admitted to Razi Hospital and the Social Security Polyclinic, both located in Rasht.

Bacterial samples underwent cultivation on both Eosin Methylene Blue (EMB) agar and MacConkey agar media. Following a 24-hour incubation period at 37°C, pure bacterial isolates were obtained. Subsequent characterization and identification of these isolates were performed through Gram staining and a panel of biochemical assays. These assays included triple sugar iron (TSI) agar utilization, citrate utilization, indole production, and methyl red-Voges Proskauer (MR-VP) tests, conducted in accordance with the guidelines established by the Clinical and Laboratory Standards Institute (CLSI) (13).

The identified Escherichia coli isolates underwent cultivation in Tryptic Soy Broth (TSB) supplemented with a 10-15% glycerol solution and were subsequently cryopreserved at -20°C for subsequent analyses. DNA was extracted utilizing a commercially available DNA extraction kit (Tiangen, China), following the manufacturer's specified protocol. The integrity of the extracted DNA was assessed through electrophoresis on a 1% agarose gel.

All culture media utilized in this study, presented in both agar and broth forms, were sourced from Merck, Germany.

Detection of virulence genes

Polymerase Chain Reaction (PCR) analysis identified six virulence-associated genes: *papC*, *sfa/foc*, *fimH*, *afa*, *ibeA*, and *neuC* (8). The specific primer sequences, sourced from Takapouzist, Iran, are detailed in Table 1. The amplification reaction was performed in a final volume of 25 µL, comprising 20 µL of Master Mix (Golden Double Helix), 20 pmol/L of each primer, and 3 µL (25 ng) of extracted DNA. The PCR was conducted using a thermal cycler (Bio-Rad, Germany) under the following conditions: An initial denaturation step at 94°C for 3 minutes, followed by 30 amplification cycles. Each cycle consisted of a denaturation phase at 94°C for 1 minute, an annealing phase (With specific temperatures and durations for each primer pair detailed in Table 1), and an extension phase at 72°C for 1 minute. A final extension step was performed at 72°C for 10 minutes. Equivalent volumes of each amplified product were combined with Safe Super Stain (Golden Double Helix) and subjected to electrophoresis on a 2% agarose gel (Merck, Germany) immersed in 1X Tris-Borate-ethylenediaminetetraacetic acid (EDTA) (TBE) buffer (Cinnagen). Subsequently, the gel was visualized and documented using a UV transillumination imaging system (Labnet, USA). A 100-bp DNA ladder (Sinaclon) was employed to ascertain the molecular weight of the PCR amplicons.

Sequencing

Direct sequencing using the 3730xl DNA analyzer (Macrogen, Korea) was employed to confirm two amplified fragments per gene. Subsequently, nucleotide and protein sequence alignments were performed using the National Center for Biotechnology Information (NCBI) database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and BioEdit software version 7.2.5 to validate the presence of virulence genes via BLAST searches. Finally, phylogenetic analysis was conducted using the maximum likelihood (RAxML) model (http://www.trex.uqam.ca) (14).

Antibiotic Susceptibility Test (AST)

Antibiotic susceptibility testing of the bacterial isolates was performed using the disk diffusion method on Mueller-Hinton agar plates (Merck, Germany), adhering to the guidelines established by the CLSI (13,15). Briefly, a selection of isolated colonies was suspended in physiological saline solution and adjusted to achieve a turbidity equivalent to a 0.5 McFarland standard. Subsequently, the bacterial culture was inoculated onto a Mueller-Hinton agar plate, and antibiotic-impregnated disks were positioned at standardized intervals. The inoculated plates were then incubated at 37℃ for 24 hours. Following incubation, the diameter of the inhibition zone surrounding each antibiotic disk was meticulously measured. A total of ten antimicrobial agents from various antibiotic classes were utilized, including Ceftriaxone (30 µg), Ceftazidime (30 µg), Cefotaxime (30 µg), and Cefazolin (30 µg) of Cephalosporins, Kanamycin (30 µg), and Gentamicin (10 µg) of Aminoglycosides, Ciprofloxacin (5 µg) of Fluoroquinolones, Chloramphenicol (30 µg), and Imipenem (10 µg) of Carbapenems, Tetracycline (30 µg) of Tetracyclines and Chloramphenicol (30µg). An isolate was classified as MDR if it showed resistance to at least three different antibiotics.

Table 1. Characteristics of virulence gene amplification by polymerase chain reaction

Category	Virulence gene	Primer sequence* (5′-3′)	Amplicon size (bp)	Annealing temperature	Time (Seconds)
Fimbrial adhesins	*fimH*	TGCAGAACGGATAAGCCGTGG GTCACCTGCCCTCCGGTA	508	55	60
	papC	GACGGCTGTACTGCAGGGTGTGGCG ATATCCTTTCTGCAGGGATGCAATA	328	65	60
	sfa/foc	CTCCGGAGAACTGGGTGCATCTTAC CGGAGGAGTAATTACAAACCTGGCA	410	65	60
Afimbrial adhesins	*afa*	GCTGGGCAGCAAACTGATAACTCTC CATCAAGCTGTTTGTTCGTCCGCCG	750	65	45
Miscellaneous	*ibeA*	TTACCGCCGTTGATGTTATCA CATTAGCTCTCGGTTCACGCT	171	60	45
	neuC	AGGTGAAAAGCCTGGTAGTGTG GGTGGTACATTCCGGGATGTC	675	61	45

* Reference: (5)

Results

Analysis of 105 urine samples identified 69 (65.71%) isolates as Escherichia coli. Assessment of VF prevalence in these UPEC strains revealed that the *fimH* gene, encoding a type 1 fimbrial adhesin, was ubiquitous (100%). Among other common fimbrial adhesins, *papC* was detected in 62 (89.85%) isolates, followed by *sfa/fos* in 19 (27.53%) isolates. The *afa* gene exhibited the lowest prevalence, identified in only 1 (1.44%) isolate. Furthermore, the prevalence of *neuC* and *ibeA* was 17 (24.63%) and 7 (10.14%) isolates, respectively. The results of gel electrophoresis for the detected virulence genes are represented in Figure 1.

The alignment analysis revealed a high level of sequence identity (Ranging from 98% to 100%) between the identified VFs and the corresponding nucleotide and protein sequences deposited in the GenBank database (Table 2).

The *fimH* gene exhibited the highest level of homogeneity among the analyzed genes, demonstrating 100% coverage and sequence similarity. Moreover, genomic sequencing identified two distinct variants of the *neuC* gene within the isolated UPEC strains, designated as *neuC*-1 and *neuC*-2. These two *neuC* gene variants displayed a high degree of similarity (99.54%) to each other and showed complete identity to sequences deposited in the GenBank database via BLAST analysis. Notably, sequences homologous to *neuC*-2 were observed with greater frequency. Phylogenetic analysis of VF genes revealed three distinct clusters encompassing adhesins, *neuC*, and *ibeA* (Figure 2).

Analysis of AST data (Table 3) revealed the highest prevalence of resistance in the UPEC isolates against Cefazolin, followed in descending order by Ceftazidime, Tetracycline, Cefotaxime, Ciprofloxacin, Ceftriaxone, Imipenem, Kanamycin, Chloramphenicol, and Gentamicin (Which exhibited the lowest resistance). Notably, only 2 (2.89%) isolates displayed susceptibility to all tested antibiotics, while a substantial majority, 62 (89.85%) isolates, exhibited resistance to at least one antibiotic. The MDR rate was determined to be 51 (73.91%) (Table 4), indicating that a significant proportion of the UPEC isolates demonstrated resistance to three or more distinct classes of antibiotics.

The alignment results demonstrated a high level of sequence homology (Ranging from 98% to 100%) between the identified VF nucleotide and protein sequences and those deposited in the GenBank database (Table 2)

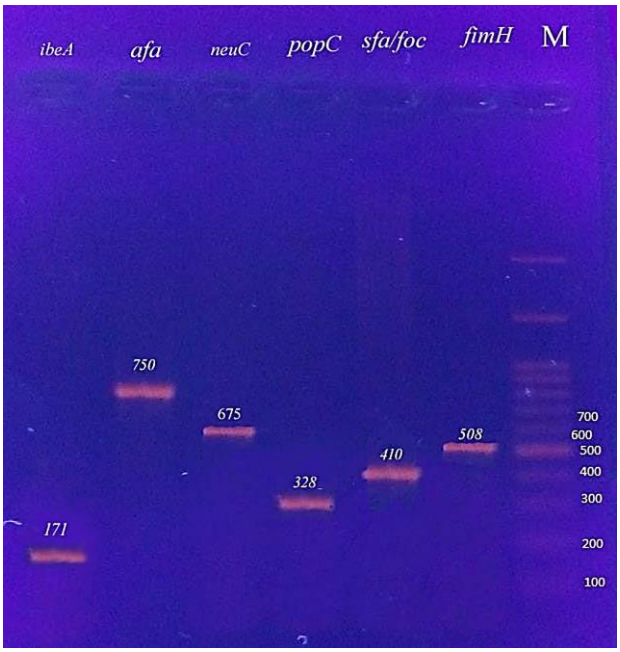

Figure 1. Gel electrophoresis of polymerase chain reaction products of virulence genes. Lane M: Ladder (100bp), lane *fim*H (508 bp), lane *sfa*/*foc* (410 bp), lane *pap*C (328 bp), lane *neu*C (675 bp), lane *afa* (750 bp) and lane *ibe*A (171 bp).

Table 2. The result of ten sequences producing significant alignments

Gene	Coverage range (%)		Identity range (%)		Accession number [c]	
	BLASTN[a]	BLASTX[b]	BLASTN	BLASTX	BLASTN	BLASTX
fimH	100	99	100	100	CP046006.1	WP_000326171.1
papC	96-100	96-99	100	99.06-100	X61239.1	QFQ50497.1
sfa/fos	100	57	100	100	CP019243.1	ADX21060.1
afa	100	58	99.86	98.53-100	CP032145.1	AEF32261.1
ibeA	100	89-91	98.84	98.08-100	CP043181.1	QDO72751.1
neuC-1	100	99	99.56-100	99.56-100	CP022730.1	GCS57713.1
neuC-2	100	99	100	99.54-100	CP043950.1	WP_087904218.1

a - Search nucleotide databases using a nucleotide query
b- Search protein databases using a translated nucleotide query
c- Accession number of the first significant alignment

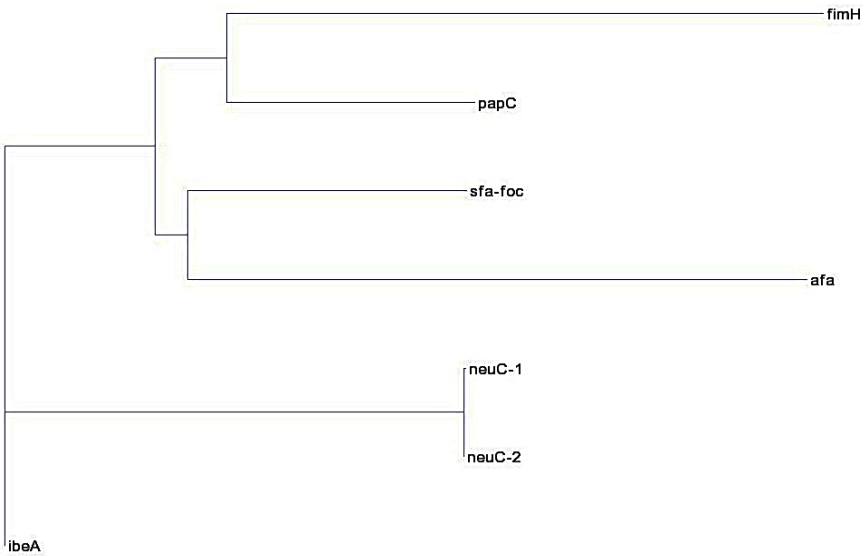

Figure 2. The dendrogram of virulence genes using the Randomized Axelerated Maximum Likelihood (RAxML) method

Table 3. Antibiotic susceptibility pattern of 69 uropathogenic Escherichia coli isolates

Antibiotic	Resistance No. (%)	Intermediate No. (%)	Susceptible No. (%)
Gentamicin	17 (24.63)	0 (0)	52 (75.36)
Kanamycin	22 (31.88)	13 (18.84)	34 (49.27)
Chloramphenicol	22 (31.88)	4 (5.79)	43 (62.31)
Imipenem	29 (42.02)	8 (11.59)	32 (46.37)
Ciprofloxacin	39 (56.52)	6 (8.69)	24 (34.78)
Tetracycline	41 (59.42)	1 (1.44)	27 (39.13)
Ceftriaxone	30 (43.47)	3 (4.34)	33(47.82)
Cefotaxime	40 (57.97)	3 (4.34)	26 (37.68)
Ceftazidime	44 (63.76)	8 (11.59)	17 (24.63)
Cefazolin	46 (66.66)	12 (17.39)	11 (15.94)

Table 4. Pattern of multi-drug resistance in uropathogenic Escherichia coli isolates

Number of multi-drug-resistant antibiotics	Incidence rate (%)
Three	5 (7.24)
Four	12 (17.39)
Five	7 (10.14)
Six	4 (5.79)
Seven	3 (4.34)
Eight	10 (14.49)
Nine	8 (11.59)
Ten	2 (2.89)
Total of MDR isolates	51 (73.91)

MDR: Multi-Drug Resistance

Discussion

Genetic analyses offer insights into pathogenic mechanisms and inform the development of rational therapeutic approaches against infection. VFs, encoded either on plasmids or within pathogenicity-associated islands (PAIs), play a crucial role in mediating bacterial colonization and persistence within the urinary tract. Current evidence suggests that these VFs are predominantly transmitted vertically, although horizontal gene transfer between bacterial lineages has also been documented (16).

The prevalence of VFs and the antibiotic resistance rates observed in the UPEC strains isolated in this research were juxtaposed with findings from comparable studies conducted in various Iranian cities over the preceding decade (Table 5).

This research focused on characterizing the UPEC population isolated from urinary specimens of female patients diagnosed with UTIs in the northern region of Iran (Rasht, Guilan Province). The analysis of virulence gene prevalence revealed that *fimH* was the most prevalent (100%), succeeded by *papC* (89.85%), *sfa/foc* (27.53%), *neuC* (24.63%), *ibeA* (10.14%), and *afa* (1.44%).

Referring to Table 5, the observed high prevalence of the *fimH* gene aligns with the majority of previously reported findings from diverse urban centers within Iran and internationally (1,17-19). While *papC*, *sfa/foc*, and *afa* represent other established common VFs present in UPEC strains, their respective prevalence rates exhibited variability. This heterogeneity likely arises from distinctions across various patient populations and the disparate geographical origins of the UPEC isolates. Limited studies have investigated the occurrence of *neuC* and *ibeA* in UPEC strains, consistently demonstrating their low prevalence within these bacterial populations.

While certain VFs, such as *fimH* and *papC*, are prevalent across multiple pathotypes of extraintestinal pathogenic Escherichia coli (ExPEC), others exhibit specificity for UPEC, including *afa* and *foc*. Conversely, specific VFs found in other ExPEC pathotypes are notably absent in UPEC strains. Examples include the K1 capsular antigens and the *neuC* gene, as well as the *ibeA* invasion gene, which are characteristic of Neonatal Meningitis Escherichia coli (NMEC) and sepsis-associated Escherichia coli (SEPEC) (2,5,8). The presence of these genes in UPEC raises pertinent questions regarding their roles in facilitating biofilm formation and host cell invasion. According to the phylogenetic classification established by Najafi et al., UPEC strains are categorized into eight distinct groups: B1, B2, F, D, E, Clade I, C, and A. Notably, a subset of strains remains unclassified. The adhesin *FimH* exhibits a widespread distribution, being detected across all phylogroups with the exception of group A. Conversely, the chaperone protein *papC* was not identified in phylogroups B1 and A. The adhesin-encoding gene *afa* was detected across multiple phylogenetic groups, specifically B2, E, D, and an unclassified group. In contrast, the *sfa* gene was exclusively identified within the unclassified group. The *neuC* gene, associated with capsule synthesis, was present in phylogroups B2, C, and the unclassified group. Furthermore, the *ibeA* gene, implicated in invasion of brain microvascular endothelial cells, was found in phylogroups B2 and the unclassified group. Collectively, these observations lend support to the occurrence of genetic recombination and the geographically influenced distribution of emergent phylogenetic groups within UPEC populations (10).

Drawing upon the data presented in Table 5, the exceptionally high prevalence of MDR phenotypes in UPEC strains isolated across various Iranian cities, as exemplified by the findings in Rasht, is evident. The elevated incidence of antibiotic resistance in UPEC isolates is a well-established observation within the global research community (11). Furthermore, the World Health Organization's (WHO) Global Antimicrobial Resistance Surveillance System (GLASS) report for the period 2016-2017 identifies Iran as one of the ten participating nations enrolled in GLASS in the Eastern Mediterranean Region. Resistance in Escherichia coli can arise through genetic mutations or the acquisition of mobile genetic elements, as observed in resistance to fluoroquinolones, penicillin, and third-generation cephalosporins. Notably, resistance to third-generation cephalosporins often confers cross-resistance to multiple other classes of antibacterial drugs in E. coli strains. This broad resistance is typically mediated by the production of enzymes known as extended-spectrum β-lactamases (ESBLs) (12). This study involved the analysis of antimicrobial resistance profiles in isolated UPEC strains. The assessment encompassed six distinct classes of antibiotics, specifically Aminoglycosides, Cephalosporins (Categorized as first- and third-generation), Chloramphenicol, Carbapenems, Fluoroquinolones, and Tetracyclines. According to the findings, when applying UTI guidelines that set a resistance threshold of >20%, the following antibiotics should not be recommended for UTI treatment due to their resistance rates: Kanamycin (22, 31.88%), Chloramphenicol (22, 31.88%), Imipenem (29, 42.02%), Ceftriaxone (30, 43.47%), Ciprofloxacin (39, 56.52%), Cefotaxime (40, 57.97%), Tetracycline (41, 59.42%), Ceftazidime (44, 63.76%), and Cephazolin (46, 66.66%). Gentamicin, however, had the lowest resistance rate at 17 (24.63%) and the highest susceptibility rate at 52 (75.36%), making it the only recommended antibiotic for treatment. The findings demonstrate a general congruence with other studies conducted within Iran and even in all countries reported in the GLASS. However, a notable exception pertains to Imipenem. While resistance rates to third-generation Cephalosporins and Ciprofloxacin are elevated, and Imipenem is typically recognized as one of the most effective antimicrobial agents against UPEC strains, the observed resistance rate to Imipenem in the cities of Rasht and Abadan (Located in the northern and southern regions of Iran, respectively) was alarmingly high. Consequently, clinicians practicing in these geographical areas should exercise heightened awareness regarding this elevated resistance. Synthesizing the aforementioned points, the variability in antibiotic susceptibility profiles across distinct populations and geographical areas necessitates the determination of the in vitro potency of isolated pathogens against currently available antibiotics. This step is critical to ensure the efficacy of therapeutic interventions.

Table 5. An epidemiological study (Comparing the prevalence of virulence factors and antibiotic resistance rate in uropathogenic Escherichia coli strains isolated in this study to similar studies carried out in different cities of Iran in the last decade)

City	Time	No.	MDR (%)	Antibiotic resistance (%)									Prevalence of VFs (%)						Ref
				CZ	CAZ	CT	CRO	TE	CP	IMP	C	GM	*ibeA*	*neuC*	*afa*	sfa/*foc*	*papCor pap*	*fimH*	
Rasht (North)	2012	33	100	-	51.51	60.6	57.57	81.81	33.33	33.33	45.45	-	-	-	-	-	-	-	(20)
	2013	110	-	-	41.8	-	-	60	43.6	-	-	50.9	-	-	-	-	-	-	(21)
	2018	69	73.91	66.66	63.76	57.97	43.47	59.42	56.52	42.02	31.88	24.63	10.14	24.63	1.440	27.53	89.85	100	*
Tehran (Capital)	2012	121	100	-	22.7	13.6	-	69.6	15.1	1.5	-	16.6	-	-	-	-	-	71.2	(22)
	2012	60	-	-	-	-	-	-	-	-	-	-	-	-	26.66	30	70	-	(23)
	2012	105	-	-	-	-	-	-	-	7.61	-	-	-	-	38.09	50.47	40	39.04	(24)
	2014	156	-	-	35.9	9.6	41	60.3	32.7	0	-	17.3	-	-	-	-	-	-	(25)
	2015	100	-	-	-	-	-	-	-	-	12	21	-	-	-	-	-	-	(26)
	2015	147	-	-	31.97	-	20.4	-	87.75	4.08	-	95.91	-	-	83.67	89.79	-	95.23	(27)
	2016	60	100	50	-	-	-	50	34	0	-	19	-	-	-	-	-	-	(28)
Shiraz (South)	2012	85	82.35	-	-	68.2	-	-	-	-	-	-	9.4	-	-	-	-	34.1	(29, 30)
	2016	126	77.8	-	65.1	-	-	-	55.6	0.8	-	19.8	-	-	46	79.4	-	99.2	(31)
	2017	121	-	-	66.9	-	-	67.8	58.7	22.3	-	35.5	-	-	-	-	-	98.3	(32)
Kerman (Southeast)	2009	137	-	71	-	-	-	-	29.18	0	-	36.45	-	-	-	35.76	18.97	-	(33)
Urmia (Northwest)	2012	25	96	-	-	-	-	65	52	-	-	65	-	-	-	-	-	-	(34)
Kashan (Central)	2013	150	74	-	49.3	-	56.7	-	61.3	0.7	-	40	-	-	0	0	16.66	-	(35, 36)
Isfahan (Central)	2013	135	63	-	55	47	-	-	45	-	-	16	-	-	-	-	-	-	(19)
Kermanshah (West)	2013	200	-	-	-	-	73.5	-	42.9	0	-	30.6	-	-	18.36	18.36	18.36	-	(37)
Bushehr (South)	2013	140	-	-	-	-	-	-	-	-	-	-	3.6	9.3	10.7	0.7	38.6	85	(10)
Zabol (Southeast)	2013	100	-	74	55	65	-	-	43	-	-	19	67	-	12	81/16	57	95	(38-40)
Ahvaz (South west)	2014	232	-	-	21.98	-	11.63	6.46	23.27	6.46	-	6.03	-	-	-	6.4	44.8	95.7	(41)
Sanandaj (West)	2015	32	-	-	40.6	65.6	-	62.5	43.7	6.2	-	37.5	-	-	15.6	-	25	-	(42)
Yasouj (Southwest)	2017	130	-	-	-	46.9	46.9	60.2	38.8	1	-	9.2	-	-	-	29	50	-	(43)
Abadan (South)	2018	100	-	-	-	70	50	91	78	59	-	-	-	-	-	-	-	-	(44)

MDR: Multi-Drug Resistance; VFs: Virulence Factors; CZ: Cefazolin; CAZ: Ceftazidime; CT: Cefotaxime; CRO: Ceftriazone; TE: Tetracycline; CP: Ciprofloxacin; IMP: Imipenem; C: Chloramphenicol; GM: Gentamicin
* This study
- Was not detected

Conclusion

Our research indicates that resistance to first and third generation Cephalosporins (Including Cephazolin, Ceftazidime, Cefotaxime, and Ceftriaxone) and Tetracycline has remained high over the years. In contrast, Imipenem is the antibiotic to which bacteria are most susceptible, although resistance has been increasing in recent years. The resistance rate for Ciprofloxacin varies across different years and locations, but there is a general trend towards increased resistance.

Conversely, Gentamicin generally displayed a trend of susceptibility across the tested isolates. Analysis of VF distribution revealed that the *fimH* gene typically exhibits dominant prevalence within UPEC populations, a pattern observed regardless of temporal and geographical origins. In contrast, the prevalence of other adhesin-encoding genes, such as *papC*, *sfa/foc*, and *afa*, demonstrated considerable variability, ranging from low to high frequencies. Furthermore, investigations suggest that the miscellaneous virulence-associated genes *neuC* and *ibeA* are present in a low proportion of UPEC isolates.

In summary, the VF profiles and antimicrobial susceptibility patterns of UPEC strains exhibit variability contingent upon the epidemiological context. Given the elevated genetic mutation rate observed in Escherichia coli, comprehensive knowledge regarding local pathogenic strains facilitates the selection of the most efficacious therapeutic interventions for these infections.

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Ethical statement

This study was approved by the Research Ethics Committee of Islamic Azad University, Rasht Branch, Rasht, Iran (IR.IAU.RASHT.REC.1402.011)

Conflicts of interest

The authors declare no conflict of interest.

Author contributions

TSh: Conceptualization; TSh, ZA, and ZM: Methodology. TSh, ZA, and ZM, and RGh: Formal analysis and investigation; TSh and RGh: Editing and writing original draft; TSh: Supervision.

Data availability statement

The data presented in the study is available on request from the corresponding author.

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