Infection and Drug Resistance

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ORIGINAL RESEARCH

Virulence-associated genes and drug susceptibility patterns of uropathogenic *Escherichia coli* isolated from patients with urinary tract infection

This article was published in the following Dove Press journal:
Infection and Drug Resistance

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Background: Different *Escherichia coli* phylogenetic groups, such as A, B1, B2, and D, have four functional groups – adhesins, microcins, toxins, and capsules – which can cause urinary tract infections (UTIs). A phylogenetic group with a high virulence content becomes a worldwide health concern. Resistance to antimicrobial agents increasingly complicates the management of *E. coli* extraintestinal infections, as a major source of illness, death, and increased health care costs. The aim of this study was to determine the virulence content and the antimicrobial susceptibility pattern of different uropathogenic *E. coli* (UPEC) phylogenetic groups in Ahvaz, Iran.

Methods: Phylogenetic groups, virulence-associated genes (VAGs), and antimicrobial susceptibility tests were detected by molecular and phenotypic methods in a total of 232 clinically well-characterized *E. coli* strains, isolated from two collections of patients with hospital-acquired (HA) and community-acquired (CA) UTIs.

Results: Our results revealed that among 232 UPEC strains, the most frequent phylogenetic group was phylogroup D (58%) with the greatest content in virulence factors, including *kpsM* (23%), *neuA* (76.3%, capsule), *cnf* (29.6%, toxin), and *Pap* (54.8%, adhesin). Phylogroups D and, to a lesser extent, B2 were the most drug-resistant phylogroups. In addition, phylogroup D was responsible for the majority of HA (64.7%) and CA (48.4%) infections.

Conclusion: Among UPEC strains causing UTIs, different phylogroups, through different VAGs, could cause severe infection. Knowledge about the distribution of the four functional groups and VAGs belonging to these phylogroups would significantly help to confine and prevent the development of lethal infection caused by these strains.

Keywords: uropathogenic *Escherichia coli*, drug resistance, virulence factors, Iran

Introduction

Urinary tract infection (UTI) is one of the most common bacterial infections, occurring in healthy people and immunocompromised patients.[1] Approximately, 70–95% of community-acquired (CA) cases and 50% of all nosocomial infections related to UTI are caused by *Escherichia coli*.[2] Uropathogenic *E. coli* (UPEC) strains are an economic burden for both communities and hospital resources. It has been estimated that UTIs cost about $6 billion for national health resources per year.[3] Pathogenicity is the ability of an agent to cause disease, and pathogenic bacteria possess several factors that enable them to enhance their virulence. UPEC is a member of extraintestinal pathogenic *E. coli* (ExPEC) and through a variety of virulence-associated genes (VAGs) can produce extraintestinal diseases, such as

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http://doi.org/10.2147/IDR.S199764

pyelonephritis, cystitis, sepsis, and neonatal meningitis.[1,2] This bacterium has several virulence-related functions. These virulence-related functional groups are adhesins, microcins, toxins, and capsules, which overcome the host defenses, invade host tissues, and finally trigger a local inflammatory response in the host. Based on many studies of the virulence content and phylogeny of UPEC, four phylogenetic groups have been described (A, B1, B2, and D), and also a significant heterogeneity has been shown among these strains.[4] Among these phylogroups, phylogroup B2 strains are usually the most common causing UTI. Different phylogroups have a variety of VAGs.[5,6] However, phylogroups A and B1 usually lack a distinct virulence profile.[5,6]

Antimicrobial resistance is defined as the ability of an organism to resist the action of an antimicrobial agent to which it was previously susceptible. The frequent antimicrobial resistance to bacterial infections occurs through indiscriminate use of antibiotics, default in treatment, and microbial characteristics. UTI can be controlled using an antimicrobial agent, but the increase in antimicrobial resistance has become a major concern, which promotes multiple drug resistance in UPEC, in both CA and hospital-acquired (HA) infections.[4,7] In addition, according to clinical studies, antibiotic resistance and VAGs have been induced in some *E. coli* strains in order to become virulent. Then, study of strain characterization, phylogenetic properties, and antibiotic susceptibility would improve our understanding of the epidemiology and virulence of this pathogen, and will allow the development of a rapid assay for monitoring UPEC.[7] The aim of this study was to phylotype and understand the virulence and antibiotic susceptibility pattern of *E. coli* causing UTI in Ahvaz, Iran. Thus, we studied 232 clinically well-described *E. coli* isolates from patients with UTI to determine the phylogroups, VAGs, and resistance to antimicrobial agents, and also to find the correlation of these factors to HA and CA infection.

Materials and methods

Ethics statement

The study was approved by the Research Ethics Committee of the Ahvaz Jundishapur University of Medical Sciences (No. IR.AJUMS.REC.1395.222.), Ahvaz, Iran. Written informed consent was obtained from all UTI patients.

A total of 232 UPEC strains, obtained from patients with UTI determined as HA and CA infection in Ahvaz, were collected and stored between 2013 and 2014. *E. coli* colonies were kept at −80 °C in trypticase soy broth, supplemented with 5% glycerol, until further use.[3]

Antimicrobial resistance

Antimicrobial susceptibility testing for 11 antibiotics, including ampicillin (10 μg), ceftazidime (30 μg), ceftriaxone (30 μg), ciprofloxacin (5 μg), gentamycin (10 μg), nalidixic acid (30 μg), trimethoprim–sulfamethoxazole (1.25+23.75 μg), amikacin (30 μg), nitrofurantoin (300 μg), tetracycline (30 μg), and imipenem (10 μg), was performed using the disk diffusion method, as recommended and specified by the Clinical and Laboratory Standards Institute.[8] Commercial antimicrobial disks from Roscoe Co. (Denmark) were used in this study. In addition, *E. coli* ATCC 25922 was used for quality control.[8,9] Six groups of antibiotics (MAST, CO, UK), including amino-penicillin, carbapenem, third generation of cephalosporin, tetracycline, aminoglycoside, and quinolone, were selected for susceptibility testing using the disk diffusion method. All 232 isolates were analyzed, *E. coli* ATCC 25922 was used for quality control, and the results were interpreted according to the Clinical and Laboratory Standards Institute.[8,9]

Phylotyping of UPEC by triplex PCR

ChuA, YjaA, and *TspE4*, three genetic markers, were amplified by the triplex PCR assay for classification of phylogroups of UPEC isolates.[10] Four phylogroups of *E. coli* (A, B1, B2, and D) can be distinguished by this triplex PCR method. The primer sequences used for PCR have been taken from studies by Clermont et al.[10] PCR products were run on 2% agarose gel. Based on the results obtained from the triplex PCR assay, all isolates are classified into the four phylogroups, based on the presence or absence of specific PCR-amplified fragment patterns, as follows: group B2 (*chuA*+, *yjaA*+, *TSPE4*±), group B1 (*chuA*–, *yjaA*±, *TSPE4*+), group D (*chuA*+, *yjaA*–, *TSPE4* ±), and group A (*chuA*–, *yjaA* , *TSPE4*–).[10,11]

Virulence genotyping

In this study, using gene-specific primer and PCR techniques, we analyzed four functional groups – adhesins (*draD*, type 1 fimbriae (*fimH*), P fimbriae (*papC and papG* Allele I, II and III), and S fimbriae (*sfa A*)), microcins (*mcmA, mchB, mciA, mceA*, and *cvaC*), capsules (*kpsM* and *neuA*), and toxins (*cnf* (cytotoxic necrotizing factor), *hlyA* (alpha hemolysin), and *sat* (secreted auto transporter toxin)) – in UPEC strains against wild-type *E. coli* K12. Amplification of the VAGs was carried out using primer sets selected from previous

Table 1 The primer sequences used for the PCR-based amplification of the VAGs in *Escherichia coli* isolated from UTI patients

Gene	Amplicon size (base pairs)	Primer sequence	Reference
cvaC	1181	F TGGTAGAATGTGCCAGAGCAAG R GAGCTGTTTGTAGCGAAGCC	16
fimH	419	F TTTTGCGACAGACCAACAACTAT R TTGCACATTCCCTGCAGTCAC	4
papC	347	F GGCGTGATAACGATTCTGCTTACCT R GGACATTCCCCCTGCATGTAAC	13
papG allele I	461	F TCGTGCTCAGGTCCGGAATTT R TGGCATCCCCCAACATTATCG	14,16
papG allele II	190	F GGGATGAGCGGGCCTTTGAT R CGGGCCCCCAAGTAACTCG	14,16
papG allele III	258	F GGCCTGCAATGGATTTACCTGG R CCACCAAATGACCATGCCAGAC	14,16
sfa	410	F CTCCGGAGAACTGGGTGCATCTTAC R CGGAGGAGTAATTACAAACCTGGCA	14
cnf-1	498	F AAGATGGAGTTTCCTATGCAGGAG R TGGAGTTTCCTATGCAGGAG	14
hlyA	1177	F AACAAGGATAAGCACTGTTCTGGCT R ACCATATAAGCGGTCATTCCCGTCA	4,14,15
draD	407	F ATGAACGGGAGTATAAGGAAGA R AACCGGTATTCACCAGGAGCAA	4
kpsM	454	F GCGCATTTGCTGATACTGTTGGG R GAGGGAACATGATGCAGGAGATG	4
neuA	474	F CTTCACCCTTTAGAGATTCGACCC R CCCAATAATCAAACAGCGAGTGTCC	4
sat	465	F ACAGCCTGAAACTGAAACACCGAC R ACATCACTGAAGCCAATACTCC	4,12
mcmA	278	F TAACTTCCACTCCCCGCA R ATGAGAAAACTATCTGAAAATGAAAT	13
mchB	228	F ATGCGAGAAATAACAGAATCACAG R TTAGCTACCGCCACCAGCAGAAG	13
mciA	234	ATGAGAGAAATATCAGATAACATGCTTG TTAACTGCCGCTGTTTGCACTG	13
mceA	300	ATGAGAGAAATTAGTCAAAAGGAC TTAACTACCACTACCGGAAC	13

Abbreviations: F, forward; R, reverse; UTI, urinary tract infection; VAG, virulence-associated gene.

published works (Table1).[4,12–16] The multiplex PCR assay was designed for 15 VAGs (*papG* allele III, *sfa*, and *kpsM*; *papC, papG* allele I, and *papG* allele II; *mciA, neuA*, and *draD*; *Cnf-1, mcmA, mchB*, and *fimH*; *mceA* and *cvaC*) while the uniplex PCR assay was performed for *hly*A and *sat*, and reactions were performed in a final volume of 25 µl, containing 50 ng template DNA, 10 pmol of each oligonucleotide primer pair (CinaGen, Germany), and 15 µl of master mix (CinaGen PCR master kit). Reaction mixtures were subjected to the following conditions in a thermal cycler (Eppendorf, Germany): 5 min at 94 ºC, 30 cycles of 30 s at 94 ºC, and 30 s at 60 ºC for *neuA, fimH, mchB, cnf, mcmA, mciA, draD*, and *Sat*; 67 ºC for *PapG* Allele I, *PapG* Allele II, *PapG* Allele III, *kpsM, papC, hlyA*, and *sfa*; and 52 ºC for *cvaC* and *mceA*, followed by 30 s at 72 ºC, with a final cycle of 7 min at 72 ºC, and a final hold at 10 ºC. The PCR products were run on 2% agarose gel. Amplicons were stained with ethidium bromide and photographed using the Gel Doc system, and their size was determined by comparison to a 100-bp DNA size marker (Invitrogen; Thermo Fisher Scientific, Waltham, MA, USA).

Statistical analysis

In order to identify significant associations between phylogroups of UPEC isolates and the presence of VAGs, and to detect any significant correlation among *E. coli* VAGs, Fisher's exact test (SPSS version 19.0; IBM Corporation, Armonk, NY, USA) was performed. $p<0.05$ was considered statistically significant.

Results

Antibiotic susceptibility test

Among 232 UPEC strains, 47% were completely susceptible to antimicrobial agents. Antimicrobial resistance for all antibiotics, except ceftriaxone, was less frequent among isolates from the first collection (HA isolates) than among those from the second (CA isolates). For most antibiotics, except ampicillin, ceftriaxone, trimethoprim–sulfamethoxazole, and amikacin, no significant differences in antimicrobial resistance rates were found, either among CA or HA isolates. However, resistance rates among CA isolates were greater than those for HA isolates. The results of drug resistance in different phylogenetic groups of UPEC isolates are cited in Table 2.

Phylogenetic groups

Based on the results obtained from the triplex PCR assay, the prevalence of the four main phylogroups (A, B1, B2, and D) differed in *E. coli* isolates responsible for UTI. The frequency of phylogroup D (58%) was higher than other phylogroups, followed by 16% for group A, 18% for group B2, and 7.75% for group B1. Phylogroup data were analyzed and are presented in Table 3.

Phylogenetic and VAG distribution in CA and HA isolates

The distribution of phylogeny and related VAGs, selected for the characterization of the UPEC isolates, showed different and distinctive patterns in the two collections including HA and CA infections (Table 4). The rate of phylogroup D among HA isolates was greater than for CA isolates. The type of VAGs is categorized into four groups, including adhesin/fimbria, microcin genes, cytotoxins, and protectins. Table 3 highlights the role of these virulence factors in UPEC strains. Among the aforementioned factors in Table 4, adhesins factors such as *sfa* and *draD* were detected more in the CA population, compared to the HA population.

Correlations between phylogroups and VAGs

The frequency of VAGs among UPEC ranged from 3.9% (*kpsM*) to 95.7% (*fimH*). In each UPEC strain, 17 VFGs were detected. Based on data from the multiplex and uniplex PCR assays for amplifying the total 17 VAGs in 232 UPEC samples, the distribution and correlation of the phylogenetic groups and VFsare described in detail in Table 5. Some VAGs, including *kpsM*, hlyA, and *sfaA*, were absent in phylogenetic groups A and B1, while these VAGs were present in phylogenetic groups D and B2. In addition, other VAGs with high frequency were observed more in phylogenetic groups B2 and D.

Discussion

Pathogenic and commensal strains of *E. coli* based on phylogenetic analyses are differently sorted into four major phylogenetic groups (phylogroups), named A, B1, B2, and D.[17] The VAG content (four functional groups) in UPEC is usually varied. The distribution and diversity of the VAGs are usually related to the phylogenetic groups. This work was the first study to characterize the phylogenetic group and VAG content in UPEC strains in Ahvaz, Iran. In the present study, phylogenetic groups were evaluated by a multiplex PCR technique, and so the antimicrobial susceptibility, virulence-associated traits, and correlation of these factors with their infection source were investigated in 232 UPEC strains. Based on the recent phylogenetic studies, contrary to commensal strains (phylogroups A and B1), UTIs are mostly derived from phylogroup B2 and phylogroup D of *E. coli* strains. Approximately, the distribution of some initial colonization factors, such as *fimH*, was equal in the all phylogenetic groups, but some VAGs in UPEC strains, including the genes encoding fimbria *P* (*pap*, pyelonephritis-associated pilus), *sfa*, and *draD*, play a significant role in UTI prevalence, since they promote the colonization of proximal and distal tubular cells from human kidney.[7,18–21] In the present study, phylogroup D (58%) was detected as the most common phylotype of *E. coli* strains isolated from patients with UTI, while phylogroups B2, A, and B1 were detected in 18.1%, 15.9%, and 7.8% of UPEC strains, respectively. Nevertheless, unlike our study, in the study conducted by Lara et al[22]phylogroups B2 (43%) and D (38%) were the most common phylogenetic groups responsible for UTI caused by ExPEC strains.[22] Also, our results were not consistent with the studies performed by Johnson et al and Moreno et al concerning phylogroups in UPEC, showing the highest prevalence in phylogroup B2.[6,21] Our findings are in agreement with those of previous studies performed by Poey et al[4] to detect the virulence profile of UPECin pregnant women and children with UTI, reporting that phylogroup D was responsible for the majority of UTI.[4]

Overall, in this work based on PCR analysis, the frequency of VAGs ranged from 3.9% for *mciA* to 95.7% for

Table 2 Antibiotic resistance of different UPEC phylogroups in CA and HA UTI

Antibiotic	Group (N)	Sensitive genotype to antibiotics				Antibiotic	Group (N)	Resistant genotype to antibiotics				*p*-value
		A, n (%)	B1, n (%)	B2, n (%)	D, n (%)			A, n (%)	B1, n (%)	B2, n (%)	D, n (%)	
AMP	HA (111)	15 (13.5)	7 (6.3)	12 (10.8)	77 (69.4)	AMP	HA (28)	4 (14.3)	3 (10.7)	9 (32.1)	12 (42.9)	0.751
N=139	CA (28)	10 (14.7)	5 (7.4)	13 (19.1)	40 (58.8)	N=51	CA (23)	4 (14.3)	2 (8)	4 (16)	13 (52)	**0.018**[d]
CP	HA (98)	14 (14.3)	7 (7.1)	11 (11.2)	66 (67.4)	CP	HA (31)	5 (16.1)	3 (9.7)	10 (32.3)	13 (41.9)	0.074
N=168	CA (70)	11 (15.7)	6 (8.6)	9 (12.9)	44 (62.9)	N=54	CA (23)	5 (21.7)	1 (4.3)	8 (34.8)	9 (39.1)	**0.009**[d]
TE	HA (132)	18 (13.6)	10 (7.6)	17 (12.9)	87 (65.9)	TE	HA (7)	1 (14.3)	0 (0)	4 (57.1)	2 (28.6)	0.118
N=217	CA (85)	14 (16.5)	6 (7.1)	14 (16.5)	51 (60)	N=15	CA (8)	2(25)	1 (12.5)	3 (37.5)	2 (25)	**0.029**[d]
CRO	HA (126)	17 (13.5)	9 (7.1)	16 (12.7)	84 (66.7)	CRO	HA (13)	2 (15.4)	1 (7.7)	5 (38.5)	5 (38.5)	**0.001**[e]
N=205	CA (79)	10 (12.7)	6 (7.6)	12 (15.2)	51 (64.6)	N=27	CA (14)	6 (42.9)	1 (7.1)	5 (35.7)	2 (14.3)	0.061
CAZ	HA (115)	13 (11.3)	7 (6.1)	14 (12.2)	81 (70.4)	CAZ	HA (24)	6 (25)	3 (12.5)	7 (29.2)	8 (33.3)	0.177
N=181	CA (66)	9 (13.6)	5 (7.6)	10 (15.2)	42 (63.6)	N=51	CA (27)	7 (25.9)	2 (7.4)	7 (25.9)	11 (40.7)	**0.004**[d]
SXT	HA (110)	14 (12.7)	6 (5.5)	12 (10.9)	78 (70.9)	SXT	HA (29)	5 (17.2)	4 (13.8)	9(31)	11 (37.9)	0.818
N=179	CA (69)	11 (15.9)	5 (7.2)	12 (17.4)	41 (59.4)	N=53	CA (24)	5 (20.8)	2 (8.3)	5 (20.8)	1 2(50)	**0.004**[d]
GM	HA (130)	18 (13.8)	10 (7.7)	18 (13.8)	84 (64.6)	GM	HA (9)	1 (11.1)	0 (0)	3 (33.3)	5 (55.6)	0.426)
N=218	CA (88)	16 (18.2)	7 (8)	15 (17)	50 (56.8)		CA (5)	0 (0)	0 (0)	2 (40)	3 (60)	0.691
NA	HA (124)	19 (15.3)	9 (7.3)	19 (15.3)	77 (62.1)	NA	HA (5)	0 (0)	1 (20)	2 (40)	2 (40)	0.147
N=125	CA (91)	16 (17.6)	7 (7.7)	17 (18.7)	51 (56)	N=7	CA (2)	0 (0)	0 (0)	0 (0)	2 (100)	1.000
AN	HA (112)	16 (14.3)	8 (7.1)	16 (14.3)	72 (64.3)	AN	HA (19)	3 (17.6)	2 (11.7)	5 (29.4)	7 (41.2)	0.103
N=185	CA (73)	11 (15.1)	6 (8.2)	11 (15.1)	45 (61.6)	N=39	CA (20)	5 (25)	1 (5)	6 (30)	8 (40)	0.201
FM	HA (136)	19 (14)	10 (7.4)	19 (14)	88 (64.7)	FM	HA (3)	0 (0)	0 (0)	2 (66.7)	1 (33.3)	0.213
N=217	CA (81)	15 (18.5)	7 (8.6)	16 (19.8)	43 (53.1)	N=11	CA (8)	1 (25)	1 (25)	2 (50)	4 (100)	0.864
IMP	HA (130)	18 (13.9)	9 (6.9)	19 (14.6)	84 (64.6)	IMP	HA (5)	1 (20)	1 (20)	1 (20)	2 (40)	0.261
N=212	CA (82)	15 (18.3)	5 (6.1)	14 (17.1)	48 (58.5)	N=15	CA (10)	1 (10)	1 (10)	3 (30)	5 (50)	0.542

Notes: [d]Significant relationship between drug resistance, phylogroups, and community-acquired infection ($p<0.05$). [e]Significant relationship between drug resistance, phylogroups, and hospital-acquired infection ($p<0.05$).

Abbreviations: UTI, urinary tract infection; UPEC, uropathogenic *Escherichia coli*; AMP, ampicillin; CP, ciprofloxacin; TE, tetracycline; CRO, ceftriaxone; CAZ, ceftazidime; SXT, trimethoprims–sulfamethoxazole; GM, gentamicin; NA, nalidixic acid; AN, amikacin; FM, nitrofurantoin; IMP, imipenem; HA, hospital acquired; CA, community acquired.

Table 3 Phylogeny distribution among the isolates from the urinary tract infection patients

Phylogeny (N)	Strains positive for amplification, n (%)		
	yjaA	*TSPE4*	*chuA*[a]
A (37)	37 (100)	0	0
B1 (18)	15 (83.3)	18 (100)	0
D (135)	0	105 (67)	135 (100)
B2 (42)	42 (100)	21 (50)	42 (100)

Note: [a]Presence of *chuA* is mandatory for classification into phylogroup B2 or D.

FimH in various phylogenetic groups. Our data demonstrated that some VAGs, including adhesion factors (*kpsM, sfaA, draD), hlyA*, and microcin genes (*mcmA, mciA*, and *cvaC*), were absent in phylogroup B1, while *kpsM, hlyA*, and *sfaA* were absent in phylogroup A (Table 4). Among various phylogroups, phylogroup D strains demonstrated the highest virulence scores, and consequently the most diverse virulence content. In this phylogroup, significant differences were observed in some virulence scores, such as *kpsM, neuA, cnf*, and *pap* with $p<0.001$, $p<0.001$, $p=0.022$, and $p=0.003$, respectively (Table 4). The greatest distributions of adhesion factors, *fimH*, *pap, draD*, and *sfaA*, were seen in phylogroup D strains, which may justify the highest frequency of UTI caused by this phylogroup. Similar to our study, in the study performed by Lara et al[22] the frequency of *pap* in phylogroups B2 and D was 48% and 53%, respectively.[22]

According to studies conducted by Tiba et al and Usein et al, high frequencies in some VAGs, including *sfa, hlyA*, and *papC* were observed in UPEC strains.[23,2424] In our study, in agreement with those of previous studies performed by Farshad et al and Dormanesh et al in Iran, *cnf1* with a frequency of 22.8% was more prevalent than *sat* and *hlyA*.[25–2727] In addition, in the study performed by Johnson et al in 243 ExPEC isolates from human,

Table 4 Distribution of phylogenetic groups and virulence factors among the 232 UPEC isolates in HA and CA infections

Variable	Prevalence of trait, n (%) of isolates			*p*-value
	Total N=232	HA N=139	CA N=93	
Phylogenetic group				
B2	42 (18.1)	23 (16.5)	19 (20.4)	0.489
B1	18 (7.8)	9 (6.4)	9 (9.7)	0.454
A	37 (15.9)	19 (13.7)	18 (19.3)	0.275
D	135 (58.2)	90 (64.7)	45 (48.4)	0.015[e]
Virulence factor				
Protectin				
kpsM	36 (15.5)	21 (15.1)	15 (16.1)	0.855
neuA	116 (50)	67 (48.2)	49 (52.7)	0.592
Cytotoxin				
hlyA	10 (4.3)	4 (2.8)	6 (6.4)	0.205
cnf	53 (22.8)	38 (27.3)	15 (16.1)	0.056
sat	31 (13.4)	19 (13.7)	12 (12.9)	1.00
Adhesin				
FimH	222 (95.7)	133 (95.7)	89 (95.7)	1.00
Pap (*papG* I, II, and III, *pap*C)	104 (44.8)	62 (44.6)	42 (45.2)	1.00
sfaA	15(6.4)	3 (2.1)	12 (12.9)	0.002[f]
draD	23(9.9)	8 (5.7)	15 (16.1)	0.013[f]
Microcin gene				
mcmA	17 (7.3)	14 (10)	3 (3.2)	0.070
mchB	20 (8.6)	14 (10)	6 (6.4)	0.475
mciA	9 (3.9)	8 (5.7)	1 (1)	0.089
mceA	26 (11.2)	19 (13.6)	7 (7.5)	0.202
cvaC	11 (4.7)	9 (6.4)	2 (2.1)	0.207

Notes: [e]Phylogroup D more statistically detected in the HA population, compared to the CA population ($p<0.05$). [f]Adhesin factors *sfaA*and *draD*more statistically detected in the CA population, compared to the HA population ($p<0.05$).
Abbreviations: CA, community acquired; HA, hospital acquired; UPEC, uropathogenic *Escherichia coli*.

Table 5 Distribution of virulence genes/phylogenetic groups among the UPEC strain in the UTI patients

Variable		Phylogenetic group				*p*-value
		B2, n (%)	B1, n (%)	D, n (%)	A, n (%)	
Capsule	*kpsM*[b,c]	5 (11.9)	–	31 (23)	–	<0.001
	neuA[b]	7 (16.7)	3 (16.7)	103 (76.3)	3 (8.1)	<0.001
Cytotoxin	*hlyA*[c]	3 (7.1)	–	7 (5.2)	–	0.4
	cnf[b]	7 (16.7)	3 (16.7)	40 (29.6)	3 (8.1)	0.022
	sat	7 (16.7)	3 (16.7)	19 (14.1)	2 (5.4)	0.41
Adhesin/fimbria	*FimH*	40 (95.2)	17 (94.4)	132 (97.8)	33 (89.2)	0.09
	*Pap (papG*I, II, I and II, *C)*[b]	15 (35.7)	3 (16.7)	74 (54.8)	12 (36.4)	0.003
	sfaA[c]	6 (14.3)	–	9 (6.7)	–	0.047
	draD	6 (14.3)	–	12 (8.9)	5 (13.5)	0.307
Microcin gene	*mcmA*	7 (16.7)	–	7 (5.2)	3 (8.1)	0.067
	mchB	5 (11.9)	3 (16.7)	9 (6.7)	3 (8.1)	0.342
	mciA	2 (4.8)	–	5 (3.7)	2 (5.4)	0.809
	mceA	8 (19)	3 (16.7)	11 (8.1)	4 (10.8)	0.185
	cvaC	3 (7.1)	–	7 (5.2)	1 (2.7)	0.757

Notes: [b]Virulence genes more statistically detected in phylogroup D, in comparison with phylogroups B2, A, and B1 ($p<0.05$). [c]Virulence genes exclusively detected in phylogroups B2 and D.
Abbreviation: UTI, urinary tract infection; UPEC, uropathogenic *Escherichiacoli*.

phylogroups B2 and D were responsible for most of the infection, with a frequency of 63% and 26%, respectively, and the VAG prevalence ranged from 0.4% to 98%.[2727] Our results (Table 4) demonstrated a frequency of *kpsM*, *neuA, cnf1*, and *pap* in all phylogroups and their significant association with phylogroup D, while *sfa was* more related to phylogroup B2. However, other VAGs (eg, *papC, fimH, cnf, sat, draD, mceA, papG* and *C, mcmA, mciA, mchB*, and *neuA*) had more dispersed incidence in four phylogroups with different prevalences. Then, the majority of VAGs were concentrated within phylogroup D. Then, in this work, the pathogenicity of phylogenetic groups was as follows: D>B2>A>B1.

Analysis of phylogeny and VAGs was also performed in two HA and CA collections, showing that phylogroup D and *cnf1* encoding for cytotoxin necrotizing factor are more related to HA isolates, while *sfaA* encoding for S fimbriae and *draD* encoding for adhesion factors were found to be associated with CA rather than HA isolates (Table 5). Similar to other studies, our study showed that *pap* encoding for P fimbriae was found to be related to CA rather than HA isolates.[3] Thus, overall, the adherence potency of UPEC for urinary tract cells in the CA isolates was greater than that in HA isolates, due to adhesion factors. Moreover, our findings indicate that both HA and CA infections can be due to different virulent strains. Resistance to some antibiotics, such as ampicillin, ceftazidime, trimethoprim–sulfamethoxazole, tetracycline, and ciprofloxacin, were associated with CA rather than HA isolates. Statistically significant differences were seen in relation to drug resistance and phylogroups in the CA populations. The high level of resistance among CA isolates, compared to the HA isolates, showed that antibiotic resistance in UPEC is correlated with and due to the uncontrolled use of antibiotics outside the hospital.[28] The results of this study revealed that phylogenetic groups D and B2 of UPEC isolates are resistant to antibiotics rather than other phylogenetic groups. VAGs were found to be associated with the two phylogroups D and B2. The eminent presence of VAGs associated with phylogroups B2 and D confirms that phylogroup D and, to a lesser extent, phylogroup B2 are two phylogroups that contain potent human ExPEC isolates causing UTI. Thus, the potential virulence characteristics of different phylogroups could help the bacteria to overcome host defense mechanisms and ultimately cause infection.[29] Lactobacillus products, as nonantibiotic alternatives, could be used to prevent recurrent UTIs. This could help to reduce the rising rates of antibiotic resistance.[30] An interesting and important area for future work would be to study *E. coli* isolates in women with frequent/recurrent UTIs that can be caused by adherence of vaginal isolates in different *E. coli* strains or the survival of *E. coli* in the bladder through a progression of intracellular bacterial communities.[31,32]

Conclusion

Among UPEC strains causing UTI, different phylogroups, through different VAGs, could cause severe infection. Knowledge about the distribution of the four functional groups and VAGs belonging to these phylogroups would significantly help to confine and prevent the development of lethal infection caused by these strains.

Abbreviations list

AMP, ampicillin; CP, ciprofloxacin; GM, gentamicin; NA, nalidixic acid; SXT, trimethoprim–sulfamethoxazole; TE, tetracycline; CAZ, ceftazidime; CRO, ceftriaxone; GM, gentamicin; AN, amikacin; IMP, imipenem; CA, community acquired; HA, hospital acquired; VAG, virulence-associated gene; UTI, urinary tract infection; ExPEC, extra-intestinal pathogenic *Escherichia coli*.

Acknowledgments

This work is approved by the Health Research Institute, Infectious and Tropical Diseases Research Center (No. 92106). Special thanks to the Research Affairs, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, for financial support.

Author contributions

All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to accountable for all aspects of the works.

Disclosure

The authors report no conflicts of interest in this work.

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