Antibiotic Resistance, Phylogenetic Typing and Virulence Genes Profile Analysis of Uropathogenic *Escherichia Coli* Isolated From Patients in Southern Iraq

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Abstract

Of the most common infectious diseases that occur mainly by uropathogenic *Escherichia coli* (UPEC) is urinary tract infections (UTIs). The purpose of this study was to investigate virulence factors, antibiotic resistance, and phylogenetic groups among UPEC strains isolated from patients with UTI in southern Iraq. A total of 100 UPEC isolates were collected from urine samples of UTI patients from various hospitals in southern Iraq, and confirmed by morphological and biochemical tests. Antimicrobial susceptibility testing on isolates was performed by disk diffusion method. Multiplex PCR technique was used to evaluate the phylogenetic groups and the presence of six virulence factor genes; type 1 fimbria *(fim*H*)*, A-fimbrial adhesion (*afa)*, hemolysin *(hly)*, fimbrial adhesins P (*pap*C*)*, cytotoxic necrosis factor 1 (*cnf*1), and aerobactin *(aer*). The majority of isolates belonged to the phylogenetic groups of B2 (55%) and D (32%). The most prevalent virulence factors were *fim*H (96%), followed by *aer* (47%), *pap*C (36%), *cnf*1 (17%), *hly* (15%), and *afa* (8%). Phenotypic testing showed that the isolates were most resistant to piperacillin, ticarcillin, amoxicillin/clavulanic acid (92%, 91%, and 88%, respectively) and most sensitive to amikacin and imipenem, respectively. The maximum antibiotic resistance and virulence factors were observed in the phylogenetic group B2. The results showed that the UPEC isolates had all six virulence factors with high frequency and the highest drug resistance. Besides, the results showed a direct relationship between virulence factors, gene diversity, phylogenetic background, and antimicrobial resistance in the UPEC isolates.

Introduction

Urinary Tract Infections (UTIs), after respiratory infections, are one of the most common infections among hospitalized patients and referrals to laboratories (Demirci et al., 2019).

Escherichia coli accounts for over 80–90% of community-acquired UTIs and 30–50% of hospital-acquired UTIs and is one of the major contributors to hospitalization with severe complications and high healthcare costs. The infection is more prevalent in women, and half of women experience this condition at least once in their lifetime; recurrence of infection is common. There are two pathogenic groups of *E.coli*, intestinal pathogenic *E. coli* (InPEC) and extraintestinal pathogenic *E. coli* (ExPEC) (Kaper et al., 2004; Xia et al., 2015), which include meningitis-associated *E. coli*, sepsis-associated *E.coli* and uropathogenic *E. coli* (Ørskov & Ørskov, 1985). The ExPEC strains, compared with commensal *E. coli* strains, have bigger genomes and express more virulence factors (Rasko et al., 2008).

Vital virulence factors in the UPEC developing resistance to the host defense system and contributing to adhesion, invasion, and damage to the host cell include adhesins, toxins, siderophores, polysaccharide-based protective coatings, inosines, and serum resistance-related proteins (Johnson et al., 2005). The genes expressing virulence factors are located on bacterial chromosomes, plasmids, and even bacteriophages and can be transferred horizontally or vertically between bacteria (Piatti et al., 2008). The UPEC binds to the epithelial cell lining of the urinary tract with the help of adhesins such as type 1 fimbriae, A-fimbrial (*afa*), fimbrial adhesins P (*pap*), and S-fimbrial adhesins (*sfa*) (Dobrindt et al., 2001; Mulvey et al., 1998). Cytotoxic necrosis factor 1 (*cnf1*) is produced by 40% of UPECs, which is involved in bacterial spread and survival in urinary tracts. Aerobactin, an iron-chelating agent, confers bacteria the ability of colonizing in iron-deficient environments such as urinary tract (Wiles et al., 2008). The activity of the cytolytic factor of α-hemolysin encoded by *hly*A gene contributes to bacterial invasion into the epithelial barrier (Trifillis et al., 1994).

Phylogenetic studies are of particular importance in evaluating the genetic evolution of *E. coli* and can be investigated by polymerase chain reaction (PCR), multi-locus enzyme electrophoresis, or ribotyping. According to phylogenetic studies, there are four major groups A, B1, B2, and D of the *E. coli* strains (Bonacorsi et al., 2000), which are different in environmental niches, life history characteristics, and tendency to develop disease (Gordon et al., 2008). Most of the

extraintestinal *E. coli* strains that producing UTIs belong to the B2 phylogenetic group, and a few falls into group D, with the majority of commensal strains being in group B1 and A. Phylogenetic studies of *E.coli* using multiplex PCR technique, is based on the presence of two genes, *Chu*A and *Yja*A, and anonymous DNA fragment *TspE4.C2* (Clermont et al., 2000).

The emergence of antibiotic resistance in pathogenic bacteria is one of the global treatment problems. Currently, reports show that the rate of resistance in UPEC bacteria is increasing. This is especially important in countries with misuse and overuse of antibiotics. Determination of antibiotic resistance patterns in common pathogenic bacteria is important to guide experimental and specific therapies against specific pathogens, including UPEC strains (Al-Naqshbandi et al., 2019).

The purpose of this study was to investigate the relationship between virulence factors (involved in adhesion and toxin production) and the antibiotic resistance profile with phylogenetic groups in clinical UPEC strains isolated from outpatient in some hospitals in southern Iraq.

Material And Methods

Isolation of *Escherichia coli* strains

In this study, 385 urine samples were collected from patients with UTI symptoms from May 2017 to January 2018 from Qalat Saleh, Al-Sadr, Al-Zahrawi, and Children Hospital in Maysan Governorate in southern Iraq. In total, 100 clinical isolates (62 females and 38 males) of UPEC were collected. The strains were purified, identified by API 20E Kit (bioMerieux, France), and stored at -20°C.

Antibiotic susceptibility

The antibiogram testing was performed using the Kirby-Bauer disk diffusion method in the Müller-Hinton Agar medium according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (Reller et al., 2009). The commercial antibiotic discs (Bioanalyse, Turkey) used in this study were ticarcillin (75 µg), Amoxicillin/clavulanic acid (20/10 µg), Piperacillin (30 µg), Trimethoprim-Sulfamethoxazole (1.25/23.75 µg), Doxycycline (30 µg), Ceftazidime (30 µg), Cefixime (5 µg), Ceftriaxone (10 µg), Nitrofurantoin (300 µg), Aztreonam (30 µg), Cefoxitin (30 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Norfloxacin (10 µg), Amikacin (30 µg), and Imipenem (10 µg).

Phylotyping and detection of virulence genes

The DNA was extracted by boiling and stored at -20°C until testing. Two *Chu*A and *Yja*A genes and an unidentified DNA fragment *TspE4.C2* were used to type isolates based on phylogenetic properties. Also, the prevalence of virolance genes, *fim*H, *afa*, *hly*, *pap*C, *cnf*1 and *aer* were analyzed. The phylotype and prevalence of virulence genes were demonstrated using three sets of experiments: group A comprising primers of *fim*H*, afa*, and *hly* genes; group B containing primers of *pap*C*, cnf*1, and *aer* genes; and group C includes primers of *chu*A*, Yja*A, and *TspE4.C2* genes. Table 1 shows the sequences of primers used for each gene and the reaction conditions of Multiplex PCR (Bio-Red, Germany) for each gene cluster.

Table 1 Sequences of primers used, the amount of master mix used in each run, and the temperature program of PCR reaction for each primer set (Lee et al., 2016; Sawma-Aouad et al., 2009; Miranda-Estrada et al., 2017).

Set	Primer name	Primer sequence (5'-3')	Size of product (bp)	PCR programs		M-PCR Volume (25µL)
A	*fim*H	F: TGCAGAACGGATAAGCCGTGG R: GCAGTCACCTGCCCTCCGGTA	508	1 cycle: 95 °C ------- 5 min 30 cycle: 94 °C ------- 60 s 62 °C ------- 30 s 72 °C ------- 60 s 1 cycle: 72 °C ------ 10 min	1	12.5 µL 2X PCR Master Mix Red 1 µL of each primers F & R 2 µL DNA template 8.5 µL water
	afa	F: GCTGGGCAGCAAACTGATAACTCTC R: CATCAAGCTGTTTGTTCGTCCGCCG	750			
	*hly*A	F: AACAAGGATAAGCACTGTTCTGGCT R: ACCATATAAGCGGTCATTCCCGTCA	1177			
B	*pap*C	F: GTGGCAGTATGAGTAATGACCGTTA R: ATATCCTTTCTGCAGGGATGCAATA	200	1 cycle: 95 °C ------- 5 min 30 cycle: 94 °C ------- 60 s 60 °C ------- 30 s 72 °C ------- 45 s 1 cycle: 72 °C ------- 5 min		12.5 µL 2X PCR Master Mix Red 1 µL of each primers F & R 2 µL DNA template 8.5 µL water
	*cnf*1	F: AAGATGGAGTTTCCTATGCAGGAG R: CATTCAGAGTCCTGCCCTCATTATT	498			
	aer	F: TACCGGATTGTCATATGCAGACCGT R: AATATCTTCCTCCAGTCCGGAGAAG	602			
C	*chu*A	F: GAC GAA CCA ACG GTC AGG AT R: TGC CGC CAG TAC CAA AGA CA	279	1 cycle: 94 °C ------- 4 min 30 cycle: 94 °C ------- 30 s 55 °C ------- 30 s 72 °C ------- 30 s 1 cycle: 72 °C ------- 7 min		12.5 µL 2X PCR Master Mix Red 1 µL of each ChuA and YjaA primers F & R 1.5 µL of each TspE4.C2 primers F & R 2 µL DNA template 3.5 µL water
	*yja*A	F: TGAAGTGTCAGGAGACGCTG R: ATG GAG AAT GCG TTC CTC AAC	211			
	TspE4.C2	F: GAG TAA TGT CGG GGC ATT CA R: CGC GCC AAC AAA GTA TTA CG	152			

bp = base pairs; F = forward; R = reverse

Statistical analysis

To compare the occurrence of phenotypic markers in UPEC, Chi-square and two-tailed Fisher's exact tests were used. These tests also were used to describe the association of probable virulence factors with other factors. P <0.05 was

considered statistically significant.

Results

In total, 100 isolates, of 385 urine specimens from UTI patients, were identified as UPEC and of which, 62% and 38% were related to females and males, respectively. The age range of patients in this study was between 4 months and 78 years. The highest prevalence of the disease in all isolates was observed in the age range of 0-5 years (29 samples), followed by 18 patients in the range age of 30-39 years in women and 17 patients in the range age of 20-29 years in men (Fig. 1). There was a statistically significant relationship between the frequency of bacteria and gender (P = 0.006).

Antibiotic resistance profiles

Among the UPEC isolates tested in this study, resistance rates were as follow: Piperacillin (92%), Ticarcillin (91%), Amoxicillin/Clavulanic acid (88%), Trimethoprim-sulfamethoxazole and Doxycycline (87%), Cefixime (65%), Aztreonam (59%), Ceftriaxone (58%), Ceftazidime (56%), Nitrofurantoin (45%), Cefoxitin (44%), Norfloxacin and Gentamicin (28%), Ciprofloxacin (27%), Amikacin (11%), and Imipenem (5%). Also, 94% of the isolates were resistant to at least three classes of antibiotics (multidrug-resistant, MDR). Statistical results showed that the overall antibiotic resistance rate in male patients was higher than in female patients (P <0.038), and there was a significant relationship between antibiotic resistance and patient age (Fig. 2). The rate of antibiotic resistance has increased in elderly patients (P <0.05).

Prevalence of virulence genes

The results showed that 96% of the isolates had at least one variant of the genes encoding virulence factors (Fig. 3). The highest and lowest prevalence were related to the *fim*H gene and the *afa* gene, respectively. The results also showed a high prevalence of virulence genes among the isolates. The distribution of virulence genes in this study were *fim*H (96%), *aer* (47%), *pap*C (36%), *cnf*1 (16%), *hly* (15%), and *afa* (8%) respectively. According to the results, the most frequent gene, *fim*H, was found in 33% of isolates; the most two frequent genes, *fim*H and *aer,* which were identified in 13% of isolates; the most three frequent genes were *fim*H*, aer*, and *pap*C*,* which were identified in 16% of the isolates; the most four frequent genes were *fim*H*, aer, pap*C, and *cnf*1, which were identified in 5% of isolates; and finally, the most five frequent genes were *fim*H*, aer, pap*C*, cnf*1, and *hly* genes which were identified in 4% of isolates. A total of six genes together was not detected in any of the isolates.

Prevalence of phylogenetic groups

The classification of the phylogenetic groups of *E. coli* strains was done by the triplex PCR technique using two genes, *Chu*A and *Yja*A, and a DNA fragment of *TSPE4.*C2 (Fig. 3). The highest prevalence was related to phylogenetic group B2, and there was no observation of phylogenetic group B1 between the studied isolates. In this study, 55% of isolates belonged to group B2, 32% to group D, and 13% to group A.

Relationship between the phylogenetic group and patient profile

Among phylogenetic groups, group B2 had the highest prevalence in female (59.6%) and male (50%) patients. The phylogenetic group D was more prevalent in females (33.8%) than males (28.9%). In contrast, group A had the highest prevalence in men (21%) than women (6.4%). The highest and lowest frequencies of phylogenetic groups were in the range of 0-5 years and 50-78 years, respectively (Fig. 4). No significant difference between the distribution of phylogenetic groups in isolates of male and female patients was shown by statistical analysis. Comparing of the

prevalence of phylotypes in both sexes showed that phylotype A is more common in isolates of male patients than females (P = 0.047).

Virulence gene patterns

All the studied strains exhibited 17 virulence gene patterns, referred to as Ec. Ec2 was characterized only by the *fim*H gene's presence and was the most noted pattern found in 33 isolates. Four isolates belonged to the Ec1 pattern that didn't have any virulence genes (Table 2). The B2 group had the highest frequency (15 patterns) among the obtained patterns. Group D and A were present in 9 and 7 patterns, respectively.

Table 2 Virulence gene patterns identified among the studied isolates

pattern	*fim*H	*aer*	*pap*C	*cnf*1	*hly*A	*afa*	No. of Strains	Phylogenetic groups
Ec1	-	-	-	-	-	-	4	B2, D, A
Ec2	+	-	-	-	-	-	33	B2, D, A
Ec3	+	+	-	-	-	-	13	B2, D
Ec4	+	+	+	-	-	-	16	B2
Ec5	+	+	+	+	-	-	5	B2, D, A
Ec6	+	+	+	+	+	-	4	B2, D, A
Ec7	+	+	-	-	-	+	6	B2, D
Ec8	+	-	-	-	+	-	1	B2, A
Ec9	+	-	-	+	+	-	3	B2
Ec10	+	-	+	-	-	-	3	B2, A
Ec11	+	-	-	-	-	+	2	D
Ec12	+	+	+	-	+	-	2	B2, D
Ec13	+	+	-	+	+	-	2	B2
Ec14	+	-	-	+	-	-	1	B2
Ec15	-	+	+	+	-	-	1	A
Ec16	+	-	+	-	+	-	3	B2, D
Ec17	+	-	+	+	-	-	1	B2
Total	96	47	36	16	15	8	100	

Distribution of antibiotic resistance among phylogenetic groups

The highest rates of resistance among phylogenetic groups were group B2 (56.47%), followed by group A (54.32%) and group D (42.18%). Of the 16 antibiotics studied, the most prevalent antibiotic resistance was observed in phylogenetic group B2 with nine antibiotics, including Amoxicillin/Clavulanic acid, Trimethoprim, Sulfamethoxazole,

Doxycycline, Cefixime, Ceftriaxone, Ceftazidime, Cefoxitin, Norfloxacin, and Gentamicin. Phylogenetic group A showed the highest resistance rate to 5 antibiotics of Aztreonam, Nitrofurantoin, Ciprofloxacin, Amikacin, and Imipenem. Phylogenetic group D showed the highest resistance rate to 2 antibiotics of Piperacillin and Ticarcillin. The distribution of antibiotic resistance among phylogenetic groups is shown in Table 3. Significant differences were seen between phylogenetic groups and resistance to all anti-infection agents studied, except for Piperacillin, Amikacin, and Ciprofloxacin ($P < 0.05$).

In the present study, the rate of multidrug resistance among the isolates ranged from 3 to 8 classes of antibiotics of which, 94% of isolates showed MDR resistance to at least three classes of antibiotics. The frequency of samples among the classes of antibiotics showed that most isolates belong to five classes (31.91%), four classes (25.53%), and six classes (22.34%), respectively. The highest prevalence of MDR strains was observed in phylogenetic group B2. The MDR distribution among phylogenetic groups ranks between groups B2 (56.3%), D (30.8%), and A (12.7%), respectively.

Table 3 Distribution of antibiotic resistance among phylogenetic groups

Drugs	Total Resistance	B2 (55)	D (32)	A (13%)
Piperacillin	92%	50(90.9%)	32(100%)	10(76.92%)
Ticarcillin	91%	50(90.9%)	31(96.87%)	10(76.92%)
Amoxicillin clavulanic acid	88%	49(89.09%)	28(87.5%)	11(84.61%)
Trimethoprim sulfamethoxazole	87%	50(90.9%)	27(84.37%)	10(76.92%)
Doxycycline	87%	49(89.09%)	27(84.37%)	11(84.61%)
Cefixime	65%	40(72.72%)	18(56.25%)	7(53.84%)
Aztreonam	59%	34(61.81%)	16(50%)	9(69.23%)
Ceftriaxone	58%	34(61.81%)	18(56.25%)	6(46.15%)
Ceftazidime	56%	31(56.36%)	18(56.25%)	7(53.84%)
Nitrofurantoin	45%	21(38.18%)	15(46.87%)	9(69.23%)
Cefoxitin	44%	29(52.72%)	10(31.35%)	5(38.46%)
Norfloxacin	28%	17(30.90%)	7(21.87%)	4(30.76%)
Gentamicin	28%	17(30.90%)	8(25%)	3(23.07%)
Ciprofloxacin	27%	17(30.90%)	5(15.62%)	5(38.46%)
Amikacin	11%	8(14.54%)	0(0%)	3(23.07%)
Imipenem	5%	1(1.81%)	1(3.12%)	3(23.07%)
Total	100%	497(56.47%)	261(42.18%)	113(54.32%)

Prevalence of virulence genes between the phylogenetic groups

Of the 219 virulence genes observed among the strains studied, the highest frequency was seen in phylogenetic group B2 (58.9%), and the lowest gene prevalence was observed in group D (31.96%) and group A (9.25%), respectively.

Exceptionally, the *afa* gene had the highest prevalence in groups D and B2, A, respectively. Table 4 shows the prevalence rates of each gene between phylogenetic groups.

Table 4 Prevalence of virulence genes among phylogenetic groups B2, D and A

Phylogenetic groups	*fim*H (96)	*aer* (47)	*pap*C (36)	*cnf*-1 (17)	*hly*A (15)	*afa* (8)	Total (219)
B2 (55)	53(55.2%)	29(61.7%)	23(63.88%)	11(68.75%)	10(66.66%)	3(37.5%)	129(58.9%)
D (32)	32(33.33%)	15(31.91%)	10(27.77%)	4(25%)	5(33.33%)	4(50%)	70(31.965)
A (13)	11(11.45%)	3(6.38%)	3(8.335)	2(12.5%)	0	1(12.5%)	20(9.25%)

Discussion

Urinary tract infections, one of the most common human infections, have become a serious risk to public health due to the unexpected increase in antibiotic resistance. The use of molecular phylogenetic classification and the determination of resistance and sensitivity patterns of *E. coli* in patients today can prevent the spread of many resistant infections, and help the economics and health of different communities with appropriate antibiotic treatment (Abd ALameer, 2015).

Our outcomes showed the presence of UTIs in all age groups and an approximately two-fold prevalence in females compared to males (62 females versus 38 males). The anatomical differences in the urinary system between men and women increases the frequency of disease in women; *E.coli* is part of the normal flora of the gastrointestinal system and urethra is shorter and wider in women increasing the chances of bacterial entry and colonization, resulting in ascending UTI (Dadi et al., 2020). The highest prevalence of the disease was in the age range of 0–5 years, which is not matching with the results of Shah et al. (2019) in Nepal. In a study by Tabasi et al. (2015) in Iran, the most prevalent disease was in the age range of 31–40 years in women and 51–60 years in men, which is consistent with the present results in terms of prevalence of disease in women but not in terms of age. Previous studies have shown that the prevalence of UTI is highly correlated with socioeconomic status, educational level, and sexual activity.

The highest resistance rate among *E. coli* isolates was related to Piperacillin (92%) and the lowest resistance rate to Amikacin (11%) and Imipenem (5%), which is consistent with the studies performed by others (Ahmed et al., 2019; Salehzadeh and Zamani, 2018). López-Banda et al. (2014) in Mexico showed that resistance to Imipenem (1.9%) and Amikacin (6.5%) was lower than other antibiotics. Katongole et al. (2019) in Kampala showed a low rate of resistance to Imipenem (4.5%). Given these similarities to the lowest antibiotic resistance to Imipenem and Amikacin, it can be suggested that the pattern of antibiotic administration in different parts of the world is the same and that these two drugs may be proposed as the first line of experimental treatment of UTIs.

Physicians usually treat the UTIs empirically, so awareness of epidemiological data in the area is essential to prevent unnecessary use of antibiotics in the treatment of infections and reduce unpleasant outcomes (Flores-Mireles et al., 2015). The outcomes indicated that the majority of isolates were resistant to most antimicrobials tried, and 94% of the isolates were resistant to more than three classes of antibiotics that were classified in the MDR group (Hadifar et al., 2016). The results showed that the prevalence rate of MDR strains in southern Iraq was higher than in other countries. Compared to others, while North America and Europe have the lowest rates, Asian and African countries appear to experience higher levels of MDR-UPEC, which could be due to inappropriate and overuse of antibiotics in previous years (Ventola, 2015).

Phylogenetic identification and analysis are a way to study the diversity and characteristics of *E. coli* in order to resolve alternative treatment options and establish control programs (Müştak et al., 2015). The phylogenetic studies revealed that the ExPEC strains are generally positioned in the phylogenetic group B2 and at a lower rate in group D (Ejrnæs, 2011). In the present study, the evaluation of phylogenetic groups for UPEC strains showed that most isolates belonged to phylogenetic group B2 with 55% prevalence, followed by group D and group A with 32% and 13% prevalence, respectively. The results of various studies indicated that the two phylogenetic groups B2 and D, carry more virulence factors. The results and pattern obtained from other studies suggest that most ExPEC strains fall into group B2 and then into group D, which is in agreement with the present study (Lee et al., 2016; Malekzadegan et al., 2018). Unlike some studies, the group B1 was not observed among our isolates, isolates studied in South Korea (Lee et al., 2016). One of the reasons for the differences in the present study results with the above studies can be attributed to differences in the geographical area studied and differences in the number of isolates. Some studies have shown that the highest microbial resistance rates are in phylogenetic group B2 and the lowest resistance rates in phylogenetic group A, which is in line with the present results (Noie Oskouie et al., 2019).

UPEC strains have different types of pathogenic genes that, depending on the presence or absence of some of these factors, lead to the appearance or absence of clinical manifestations. Bacteria can acquire these genes through horizontal transfer and provide flexibility in the size of the bacterial genome (4.5 to 5.5 Mb.), so strains with larger genomes will be more pathogenic (Hozzari et al., 2020). Identifying virulence factors, not only indicates the pathogenic process of bacteria, but also plays an important role in the development of vaccines and drugs (Dadi et al., 2020).

The present results also showed that 96% of the isolates had at least one variant of the genes encoding virulence factors, and the distribution of virulence genes were as follows: *fim*H (96%), *aer* (47%), *pap*C (36%), *cnf*1 (16%), *hly* (15%), and *afa* (8%), respectively. *fimH*, due to its high percentage among UPEC strains and its role in bacterial binding to urinary tract cells and colonization, is considered as a potential candidate to develop vaccine preventing urinary tract infections (Dadi et al., 2020). Bacterial access to iron plays an important role in urinary tract infections (Wiles et al., 2008), and many strains in this study encode the *aer* gene, which is necessary for iron uptake.

The highest and lowest prevalence of these genes was observed in phylogenetic group B2 and in phylogenetic group A, respectively. The results also exhibited a high prevalence of genes encoding virulence factors among UPEC isolates, which is in agreement with other studies. Lee et al. (2016) and Tarchouna et al. (2013) reported the highest and lowest prevalence of *fim*H and *afa* genes, respectively, among UPEC isolates, which is consistent with the present study. Also, our results were consistent with the results of Yilmaz and Aslantas (2020) that showed the genes, *fim*H, *pap*C, *afa, aer*, *hly*, and *cnf*1 highly distributed among all isolates.

In this study, the isolates were divided into 17 patterns (Ec1-Ec17) based on the distribution of virulence genes; the pattern Ec2 containing only the *fim*H gene was the most prevalent pattern with 33 samples. The lowest prevalence of virulence genes was observed in Ec14, Ec17, Ec8, and Ec15 patterns with only one isolate. Also, none of the six genes were detected in the four isolates belonging to the Ec1 pattern. Out of 17 different patterns, the phylogenetic group B2 was present in 15 patterns, phylogenetic group D in 9 patterns, and phylogenetic group A in 7 patterns. These results are consistent with the findings by Jalali et al. (2015) in Iran. Ali Abdi et al. (2015) detected 31 different patterns and reported that phylogenetic group B2 was present in most patterns (15 patterns). However, phylogenetic groups D and A each were present in 9 patterns, roughly in agreement with the results of the present study in terms of the prevalence of virulence genes in phylogenetic groups. Tarchouna et al. (2013) in Tunisia positioned the UPEC isolates in 23 patterns that were slightly different from the present study in the distribution of virulence genes, and reported the highest prevalence belonged to *fim*H gene.

Conclusion

In this study, the polymerase chain reaction technique was used to classify the uropathogenic *Escherichia coli* isolates into phylogenetic groups. The results showed that the highest prevalence rates in the studied isolates were related to phylogenetic groups B2 and D. Therefore, it can be stated that the isolates have high pathogenicity having virulence genes highly, as the results of different studies indicate that the two phylogenetic groups, B2 and D, carry more virulence factors than the phylogenetic groups B1 and A. The high resistance of the isolates to antibiotics indicates that it is necessary to detect resistant strains rapidly and timely in order to select appropriate treatment options and to prevent the spread of resistance. Also, it is recommended that urinary tract infections should be treated according to the regional pattern of sensitivity and resistance in order to prevent the spread of drug-resistant strains. The results of the present study showed that the urine of patients with urinary tract infection could be a potential source for the spread of *E. coli* strains with different virulence and resistance factors. Therefore, according to the high prevalence of urinary tract infection, the distribution of resistance and virulence factors, and the therapeutic failure, consequently, pyelonephritis, cystitis, and prostatitis, it is necessary to detect these strains promptly and accurately.

Declarations

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Competing interests

The authors declare that they have no competing interests.

Ethics approval

This study was granted approval by the Ferdowsi University of Mashhad [IR.UM.REC.1399.046] for studies on participants giving informed consent.

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Authors' contributions

MRS and MB participated in the study design and experiments. MA conducted experiments. MRS, MB and MA contributed to the data analysis and wrote the primary draft of the manuscript, reviewed and revised the paper. All authors have read and approved the final manuscript.

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Figures

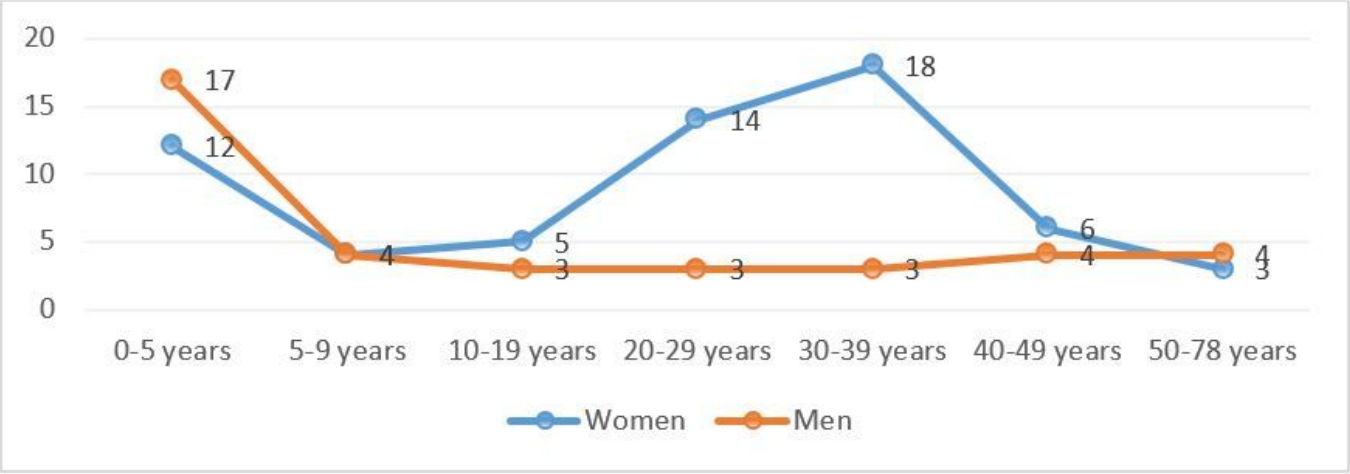

Figure 1

Disease distribution among different age groups

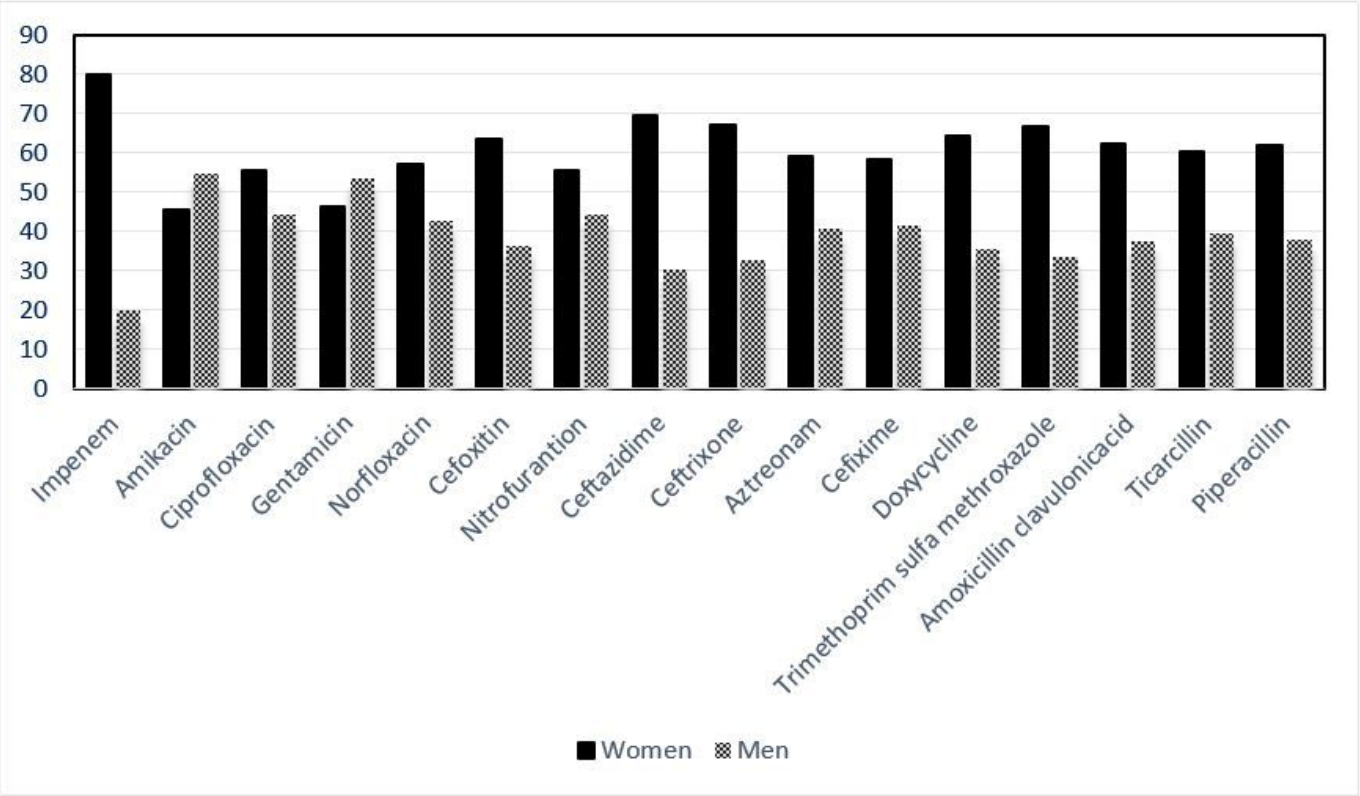

Figure 2

Antibiotic resistance profile

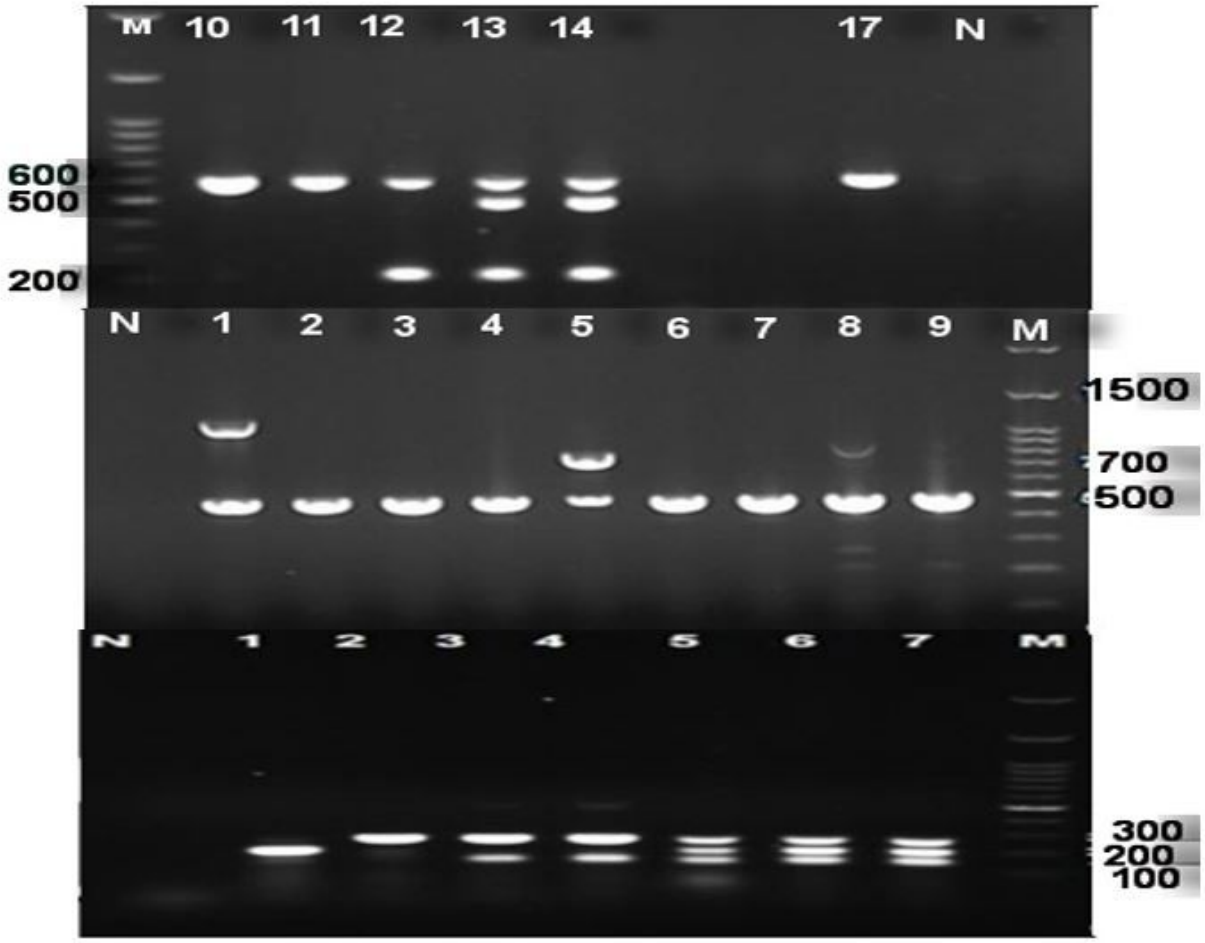

Figure 3

Results of multiplex PCR specific for virulence factors. M (DNA ladder 100 bp, Fermentase co.), 1 to 9 positive for fimH(508 bp), 5 and 8 positive for afa (750 bp), 1 positive for hly (1177 bp), 10-14 and 17 positives for aer (602 bp), 13 and 14 positive for cnf1 (498 bp), 12,13 and 14 positive for papC (200 bp), N (Negative control). Triplex PCR profiles specific for E. coli phylogenetic groups. M, marker; N, negative control; 1, group A; 2,3 and 4, group D; well 5, 6 and 7 group B2.

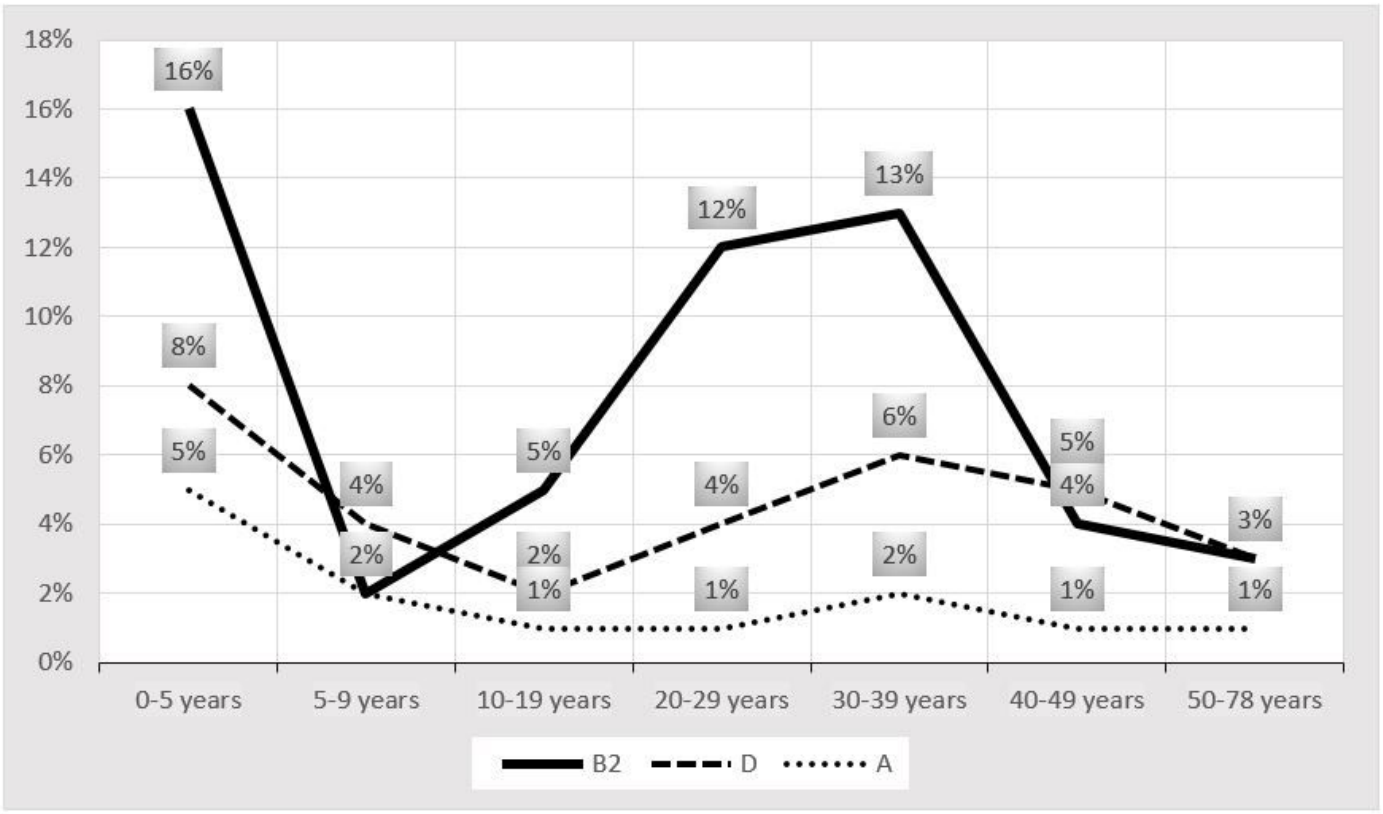

Figure 4

Relationship between phylogenetic groups and patient age