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ORIGINAL ARTICLE

Phylogenetic analysis, biofilm formation, antimicrobial resistance and relationship between these characteristics in Uropathogenic *Escherichia coli*

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Abstract

Background In the present study, we examine the prevalence of phylogenetic groups, O-serogroups, adhesin genes, antimicrobial resistance, the level of gene expression associated with biofilm formation, and the presence of extended-spectrum beta-lactamase (ESBL) in UPEC strains isolated from both pediatric and adult patients.

Methods In this cross-sectional study, 156 UPEC isolates were collected from UTI patients. ESBL-producing isolates were detected using the double-disc synergy (DDS) method, and biofilm formation was assessed through a microplate assay. The presence of O-serogroups, adhesion factors and resistance genes, including *ESBLs* and *PMQR* genes, was detected by PCR, and isolates were categorized into phylogenetic groups using multiplex PCR. Additionally, the quantitative real-time PCR method was also used to determine the expression level of genes related to biofilm.

Results During the study period, 50.6% (79/156) of the samples were obtained from children, and 49.4% (77/156) were from adults. The highest rate of resistance was to NA (91.7%), while FM (10.9%) had the lowest rate of antibiotic resistance. In addition, 67.9% (106/156) of UPEC isolates were ESBL producers. Most of UPEC isolates belonged to phylogenetic group B_2 (37.1%). This study revealed that $bla_{CTX\text{-}M}$ and *qnrS* are widely distributed among UPEC isolates. The mean expression levels of *fimA* genes were significantly higher in non-biofilm producers than in biofilm producers ($p < 0.01$).

Conclusions The high antibiotic resistance rates in this study highlight the significance of local resistance monitoring and investigating underlying mechanisms. Our findings indicate the dominance of phylogroup B_2 and group D as the prevailing phylogenetic groups. Consequently, it is imperative to investigate the epidemiological aspects and characterize UPEC isolates across diverse regions and time frames.

Keywords Uropathogenic *Escherichia coli* · Antimicrobial resistance gene · Phylogenetic group · Virulence gene · Biofilm formation · Real Time PCR

Abbreviations

KTPs	Kidney transplant patients
SD	Standard deviation
UPEC	Uropathogenic *Escherichia coli*
UTIs	Urinary tract infections
VFs	Virulence factors
MDR	Multidrug resistant
EMB	Eosin Methylene Blue
PCR	Polymerase Chain Reaction
BHI	Brain Heart Infusion

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AST	Antimicrobial susceptibility testing
CLSI	Clinical Laboratory Standards Institute
OD	Optical density
ESBL	Extended-spectrum beta-lactamase
DDS	Double-disc synergy
PMQR	Plasmid-Mediated Quinolone Resistance
ARGs	Antimicrobial resistance genes

Introduction

Uropathogenic *Escherichia coli* (UPEC) is the predominant cause of urinary tract infections (UTIs) and a common hospital opportunistic pathogen. UPEC has become one of the most challenging therapeutic problems due to the acquisition of plasmids encoding antibiotic resistance genes to β-lactams and quinolones [1, 2]. β-lactams and fluoroquinolones are extensively used to treat UTIs due to their efficacy, oral bioavailability, wide spectrum of action, and patient compliance.

However, the prevalence of multidrug-resistant (MDR) microorganisms has hindered the use of these antibiotics in recent decades [1, 3]. *ESBL* genes have also been discovered on a pathogen isolate's special plasmids, which encode plasmid-mediated quinolone resistance (PMQR) factors, including *qnr*, *aac (6')-Ib-cr*, and *qepA*. Qnr proteins bind to bacterial DNA gyrase and topoisomerase IV to protect them from quinolones. Bacteria have *qnr* genes A, B, C, D, S, E, and VC. *aac (6')-Ib-cr* and drug effluxes such *qepA* and *oqxAB*, are other PMQR determinants [3]. Mobile genetic elements such as conjugative plasmids, transposons, insertion sequences, and genomic islands can transfer antimicrobial resistance genes (ARGs) across *E. coli* strains. Recombination in *E. coli* strains is moderately caused by these components. However, *E. coli* populations are clonal and can be categorized into various phylogenetic groups [4, 5]. Phylotypes A, B1, B2, D, C, E, F, G, and the *Escherichia* cryptic clade I can be assigned to *E. coli* based on the presence or absence of four genetic sequences: *chuA*, *yjaA*, *TspE4*, and *arpA* [6]. *E. coli* strains are also classified by serological typing of their H (flagellar), O (lipopolysaccharide), and sometimes K (capsular) surface antigens. O1, O2, O4, O6, O7, O8, O15, O16, O18, O25, and O75 O-antigen types are typically expressed in UPEC clones [7].

Biofilm formation is a hallmark of opportunistic pathogens like UPEC. Bacterial cells adhered to biotic or abiotic surfaces form a biofilm. UPEC's biofilm mode produces many virulence factors (VFs) that aid biofilm cell adhesion to biotic or abiotic surfaces [8]. UPEC strains have evolved multiple fimbrial adhesins that assist them in colonizing the urinary tract [9]. Type 1 fimbriae consist of a major protein, *FimA*, and ancillary proteins, *FimF*, *FimG*, and the adhesin *FimH*, all of which are encoded by the *fim* gene cluster [10]. S and P fimbriae are expressed by the *sfa* operon and the *pap* gene cluster, which includes 11 genes (*papA* to *papK*). In conclusion, type 1 fimbriae cause UTIs by invading host cells, while P and S fimbriae facilitate infection [11]. Overall, the existence and expression levels of genes associated with biofilm formation determine the potential for biofilm formation.

Several studies in Iran and other countries have examined different characteristics in UPEC strains. Nevertheless, there has been a lack of comprehensive research investigating bacterial characteristics, including antibiotic resistance patterns, the distribution of *β-lactamase* and *PMQR* genes, phylogenetic groups, adhesion genes, O-serogroups, biofilm formation capacity, and biofilm-related gene expression levels in UPEC isolates from both children and adults with UTIs in northern Iran. This study fills a critical gap in research and illuminates UPEC-induced UTIs in this region and population.

Materials and methods

Study design and bacterial isolation

The study included 156 UPEC strains obtained from urine samples from 77 adults and 79 children who were admitted to hospitals affiliated with Babol University of Medical Sciences, north of Iran, during the period 2021–2022 [12].

The identification of the strains was accomplished by observing their colony morphology on eosin methylene blue (EMB) agar plates (Merck, Germany). The verification of lactose-fermenting colonies was performed through standard microbiological and biochemical tests, such as MR-VP, citrate, urease, nitrate reduction, motility at 37 °C, indole production, and gas production. Subsequently, a polymerase chain reaction (PCR) detection based on the *uid* gene for β-glucuronidase of *E. coli* was conducted to confirm the species [13]. Confirmed *E. coli* isolates were inoculated into brain heart infusion (BHI) broth containing 20% glycerol at 37 °C for 24 h, then stored at − 80 °C until further use.

Antimicrobial susceptibility testing (AST)

Antimicrobial susceptibility testing was conducted using the Kirby-Bauer disc diffusion method on Mueller–Hinton agar (Merck, Germany) following the guidelines established by the Clinical Laboratory Standards Institute (CLSI) [14]. The 16 antimicrobial drugs tested were: amoxicillin/clavulanic acid (AMC, 20/10 µg), ceftazidime (CAZ, 30 µg), cefixime (CFM, 5 µg), meropenem (MEN, 10 µg), trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 µg), ciprofloxacin (CP, 5 µg), ofloxacin (OFX, 5 µg), nalidixic acid (NA, 30 µg), norfloxacin (NOR, 10 µg), nitrofurantoin (FM, 300 µg),

gentamicin (GM, 10 µg), amikacin (AN, 30 µg), cefazolin (CZ, 30 µg), ceftriaxone (CRO, 30 µg), cefotaxim (CTX, 30 µg), and ampicillin-sulbactam (SAM, 20 µg) (Padtan Teb, Iran). The resistance score (RS) was determined based on the number of antibiotics to which an isolate demonstrated resistance [15]. Additionally, isolates demonstrating resistance to at least one agent in at least three antimicrobial categories were classified as MDR [16].

Detection of ESBL-producing *E. coli*

To detect ESBL production, ceftazidime (30 µg/mL) and cefotaxime (30 µg/mL) were used both with and without clavulanic acid (10 µg/mL). A positive result for ESBL production was identified as an increase of 5 mm or more in the zone diameter when clavulanic acid was present [9]. *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as negative and positive control strains, respectively.

Biofilm formation assay

Biofilm formation by UPEC strains was tested using the microtiter plate assay as previously described [17]. The optical density (OD) of stained adherent biofilm was measured using an ELISA autoreader (Bio-Rad, USA) at a wavelength of 570 nm. The experiment was performed in triplicate and repeated three times. The data were interpreted based on the criteria proposed by Stepanović et al. [17].

Detection of UPEC serogroups, VFs, and antibiotic resistance genes

Genomic DNA was extracted from fresh colonies as described previously [18]. The purity and concentration of the isolated DNA were determined using a NanoDrop spectrophotometer (Thermo Scientific Wilmington, USA). Isolates were screened for the presence of O-serogroups (*O1*, *O15*, *O16*, and *O25*) and VFs, including *fimH, fimA, pap,* and *sfa* genes. Moreover, isolates harboring resistance determinants, including *ESBLs* (bla_{TEM}, bla_{SHV}, and $bla_{\text{CTX-M}}$) and *PMQR* genes (*qnrA, qnrB*, and *qnrS*) were detected by PCR.

PCR amplification was carried out using a 12 µL mixture, which included 5 µL of pre-made MasterMix (AMPLIQON, Denmark), 5.2 µL of DNase-free distilled water, 0.3 µL of each primer (at a concentration of 10 pmol/µL), and 1.2 µL of the DNA template. The selection of target genes and the nucleotide sequences of the oligonucleotide primers adhered to previously described protocols [19]. The PCR thermal conditions are detailed in Table S1. The resulting PCR products were resolved on a 1.5% agarose gel prepared in 1X TBE (Tris/Borate/EDTA) buffer, and then visualized under ultraviolet light following staining with SafeStain loading dye (CinnaGen Co., Iran). Moreover, sequences of the *O1*, *O15*, *O16*, *O25* serotypes, and the *qnrB* and *qnrS* genes were deposited in the NCBI database with the accession numbers OQ469818, OR826277, OQ469820, OQ469819, and OQ469817, along with OR826276 (https://www.ncbi.nlm.nih.gov/nuccore/OQ469818.1, https://www.ncbi.nlm.nih.gov/nuccore/OR826277.1, https://www.ncbi.nlm.nih.gov/nuccore/OQ469820 , https://www.ncbi.nlm.nih.gov/nuccore/OQ469819, and https://www.ncbi.nlm.nih.gov/nuccore/OQ469817, as well as https://www.ncbi.nlm.nih.gov/nuccore/OR826276.1).

Phylotyping of UPEC by quadruplex PCR

All UPEC isolates were assigned to phylogenetic groups according to the new method of Clermont et al. [6]. Multiplex PCR was performed using 12 µl reactions containing 5 µL of MasterMix (AMPLIQON, Denmark), 4.2 µL of DNase-free distilled water, 0.2 µL of each primer (10 pmol/µL), and 1.2 µL of DNA template. Gene amplification was achieved using the following thermal cycling protocol: an initial denaturation step of 5 min at 94 °C, followed by 30 cycles, each comprising 30 s at 94 °C, 30 s at 57 °C, and 30 s at 72 °C. The process was concluded with a final extension step of 5 min at 72 °C. Subsequently, the PCR products were subjected to electrophoresis on a 1.5% agarose gel that was stained with SafeStain loading dye (CinnaGen Co., Iran). Visualization was achieved by exposing the gel to ultraviolet light.

Quantitative real-time PCR (qRT-PCR) assay

QRT-PCR was used to examine the transcriptional expression of genes associated with type I fimbriae (*fimA* and *fimH)* in UPEC isolates positive for both ESBL and bla_{CTX-M}, with or without biofilm formation. Therefore, these isolates underwent RNA extraction and genomic DNA removal using an RNeasy Mini kit (Viragene Akam Co.). Each RNA extract was adjusted to a final concentration of 150 ng/µL. Following the manufacturer's instructions, the RNA was reverse-transcribed into cDNA using a High Capacity cDNA Reverse Transcription Kit for single-stranded cDNA synthesis (Parstous Co.), and all the cDNAs were stored at -20 °C. The real-time PCR amplification reaction was prepared in a total volume of 25 µL, comprising 2.5 µL of cDNA, 12.5 µL of RealQ Plus Master Mix Green from Ampliqon in Denmark, 1 µL each of forward and reverse primers (each at a concentration of 10 nM), and RNase- and DNase-free water adjusted to a final volume of 25 µL. The primer sequences used for this analysis can be found in Table S2. To normalize gene expression, the D-glyceraldehyde-3-phosphate

dehydrogenase A (*gapA*) gene was used as an internal control. Real-Time PCR was performed as follows: one cycle of initial denaturation at 95 °C for 15 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 56, 51, and 49 °C for *fimA*, *fimH*, and *gapA*, respectively, for 30 s, and extension at 72 °C for 30 s. Expression levels of each gene in the clinical strains are presented as fold changes compared to transcript levels in *E. coli* ATCC 25922. These changes were determined using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

Statistical analysis was conducted using IBM Corp.'s SPSS™ software, version 16, located in the United States. Categorical variables were presented as frequencies and percentages. To assess significant differences, Fisher's exact test or the Chi-square (χ^2) test was employed. A P value less than 0.05 was considered indicative of statistical significance. Continuous variables, such as antibiotic resistance scores, were compared between groups using one-way analysis of variance (ANOVA), followed by pairwise comparisons conducted using the Gabriel post hoc test.

Results

Demographic information of the participants

During the study period, 50.6% (79/156) and 49.4% (77/156) of UPEC isolates were obtained from children and adults, respectively. Moreover, 74.4% (116/156) and 25.6% (40/156) of UPEC isolates were obtained from female and male patients, respectively. All UPEC strains were isolated from inpatients diagnosed with UTI. The age range of the study group was from less than 1 year to 80 years old.

Phenotypic antimicrobial resistance of UPEC isolates

The antibiotic susceptibility pattern revealed varying frequencies of antibiotic resistance among the 156 UPEC isolates. Specifically, 99.4% (155/156) of the isolates exhibited resistance to at least one or more antibiotics. Notably, the highest resistance rate was observed against NA (91.7%), followed by SXT (74.4%). In contrast, FM (10.9%) and MEN (14.1%) exhibited the lowest rates of antibiotic resistance. Based on the study by Magiorakos et al. in 2012, the prevalence of MDR isolates was 88.5% (138/156). The total resistance rates of UPEC strains to quinolone antibiotics were as follows: NA (91.7%, 143/156), CP (66%, 103/156), OFX (48.7%, 76/156) and NOR (59%, 92/156). Based on ESBL screening test, 67.9% (106/156) of UPEC isolates were ESBL producers.

Further analysis revealed that all ESBL-positive strains (106 isolates) were resistant to CAZ and CTX, followed by NA (91.5%, 97/106), while MEN (4.7%, 5/106) and FM (7.5%, 8/106) were the most effective antibiotics against ESBL-positive isolates.

Out of non-ESBL isolates (50 isolates), the highest resistance rate was to NA (92%, 46/50), followed by SXT (78%, 39/50), while the most effective antibiotic was CAZ (12%, 6/50) and CTX (12%, 6/50). Furthermore, ESBL-producing isolates exhibited a higher resistance rate than non-ESBL-producing isolates against CTX (100% vs. 12%, $P<0.001$), CAZ (100% vs. 12%, $P<0.001$), and CRO (56.6% vs. 38%, $P<0.001$). While, among non-ESBL-producing isolates, the resistance rates to MEN (34% vs. 4.7%, $P<0.001$), FM (18% vs. 7.5%, $P=0.05$), and GM (34% vs. 13.2%, $P=0.002$) were significantly higher compared to ESBL-producing isolates, which is a rather strange finding. The complete antibiotic resistance patterns for both ESBL-negative and positive isolates are shown in Fig. 1.

Presence of *β-lactamase* genes

Out of 156 UPEC isolates, $bla_{\text{CTX-M}}$ (72.4%) was the most predominant gene, followed by bla_{TEM} (43.6%) and bla_{SHV} (7%). Additionally, 2.6% (4/156) of isolates had three *ESBL* genes, while 18.6% (29/156) had both $bla_{\text{CTX-M}}$ and bla_{TEM}, 11.5% (18/156) had both $bla_{\text{CTX-M}}$ and bla_{SHV}, and 2.6% (4/156) had both bla_{TEM} and bla_{SHV} simultaneously. Moreover, among non-ESBL isolates, the frequency of the bla_{SHV} gene was significantly higher than in ESBL isolates (7% vs 4%, $P<0.02$).

Presence of *PMQR* genes

PCR amplification of the *PMQR* genes revealed that 42.9% (67/156) of the isolates harbored at least one *PMQR* gene, with the following distributions: 37.2% (58/156) for the *qnrS* gene (38.7%; 41/106 ESBL-positive and 34%; 17/50 ESBL-negative), and 10.3% (16/156) for *qnrB* (9.4%; 10/106 ESBL-positive and 12%; 6/50 ESBL-negative). However, none of the isolates harbored the *qnrA* gene. Also, 3.8% (n = 6/156) of isolates harbored both *qnrB* and *qnrS* simultaneously.

There was no significant relationship between the distribution of *qnrS* and *qnrB* genes in ESBL-negative and positive isolates ($P=0.57$ and $P=0.62$). However, among ESBL-positive isolates, the *qnrS* gene was found to be relatively more prevalent than in ESBL-negative isolates, while compared to ESBL-positive isolates, the *qnrB* gene was detected to be relatively more common in ESBL-negative isolates (Fig. 1). moreover, the distribution of *qnr* genes among

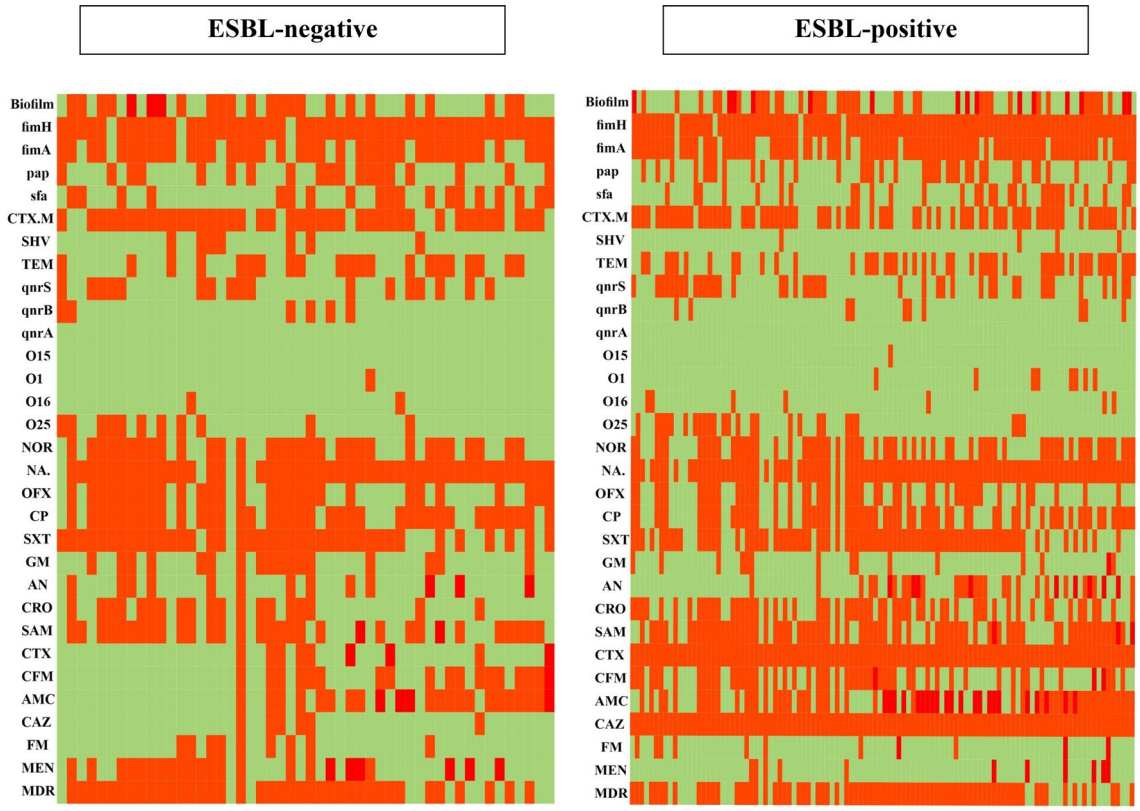

Fig. 1 Antibiotic resistance profile, resistance genes, O-serogroups, biofilm formation of *uropathogenic Escherichia coli* isolates, sorted based on ESBL phenotype. *AMC* amoxicillin/clavulanic acid, *CAZ* ceftazidime, *CFM* cefixime, *MEN* meropenem, *SXT* trimethoprim-sulfamethoxazole, *CP* ciprofloxacin, *OFX* ofloxacin, *NA* nalidixic acid, *NOR* norfloxacin, *FM* nitrofurantoin, *GM* gentamicin, *AN* Amikacin, *CRO* ceftriaxone, *CTX* cefotaxim, *SAM* ampicillin-sulbactam

UPEC isolates obtained from both adults and children is presented in Table 1. The proportion of *qnrS* and *qnrB* genes among adults compared to children was relatively high, but the differences were not statistically significant. Additionally, Table 2 presents the relationship between resistance to quinolone antibiotics and the distribution of *qnr* genes.

Phylogenetic analysis

Based on the quadruplex PCR assay findings, a total of 137 out of 156 UPEC isolates were assigned to 7 of the 8 phylogenetic groups, and the results are presented in Table 3. The majority of UPEC isolates belonged to phylogenetic group B_2, accounting for 37.1% of the total sample, followed by groups D, A, and E (12.8%), group F (6.4%), and B_1 (5.1%). Notably, none of the isolates tested positive for phylogenetic group C. Among the remaining isolates, 12.1% were found to be untypeable.

Following group B_2, phylogenetic groups A and D exhibited a higher prevalence among the isolates from children, whereas in adults, following group B_2, groups E, A, and D were more commonly observed. However, no significant difference was noted between the two groups regarding phylogenetic distribution.

Moreover, except for phylogenetic group A and F, the proportion of B_2, B_1, D and E were more prevalent in ESBL-producing isolates. However, the statistical analyses did not reveal any significant correlation between ESBL-producing isolates and phylogenetic groups.

Antimicrobials resistance according to phylogenetic group

Phylogenetic groups showed variations in terms of antibiotic susceptibility patterns. The most resistant phylogroup was phylogroup A (RS median 9; range 5–15), followed by phylogroup B_2 (RS median 8; range 2–14) and D (RS median 9; range 2–11), while the most susceptible phylogroup was phylogroup B_1 (RS median = 6; range 0–8). Resistance to MEN and FM was not reported in phylogroup B_1. Moreover, the statistical analyses reveal a significance association between the phylogenetic groups and antibiotic resistance score ($P = 0.032$). The median RS decreased progressively from a high of 9 (RS mean: 8.9, range 5–15) for phylogroup

Table 1 Comparison of the adults and children isolates according to their resistance genes, O-serogroups, and phylogenetic groups

Variables	Total	Children N = 79 No (%)	Adults N = 77 No (%)	P value
Virulence factor genes				
pap	57 (36.5)	36 (45.6)	21 (27.3)	0.01
sfa	49 (31.4)	32 (40.5)	17 (22.1)	0.01
O-serogroups				
O25	37 (23.7)	4 (5.1)	33 (42.9)	<0.001
O16	9 (5.7)	4 (5.1)	5 (6.5)	0.74
O1	9 (5.7)	9 (11.4)	0	0.003
O15	1 (0.6)	1 (1.3)	0	1
ESBL genes				
$bla_{CTX\text{-}M}$	113 (72.4)	53 (67.1)	60 (77.9)	0.13
bla_{SHV}	11 (7)	4 (5.1)	7 (9.1)	0.32
bla_{TEM}	68 (43.5)	45 (57)	23 (29.9)	0.001
ESBL phenotypic	106 (67.9)	56 (70.9)	50 (64.9)	0.42
Plasmid-mediated quinolone resistance (*PMQR*) genes				
qnrA	0	0	0	–
qnrB	16 (10.2)	7 (8.9)	9 (11.7)	0.56
qnrS	58 (37.1)	24 (30.4)	34 (44.2)	0.07
Phylogenetic groups				
A	20 (12.8)	13 (16.5)	7 (9.1)	0.12
B1	8 (5.1)	3 (3.8)	5 (6.5)	
B2	8 (37.1)	25 (31.6)	33 (42.9)	
D	20 (12.8)	13 (16.5)	7 (9.1)	
E	20 (12.8)	7 (8.9)	13 (16.9)	
F	1 (0.6)	4 (5.1)	6 (7.8)	
Unknown	10 (6.4)	13 (16.5)	6 (7.8)	

B_2 isolates to a low of 6 (RS mean: 5.37, range, 2–9) for phylogroup B1 isolates. Also, based on Tables 4 and 5, among the adult isolates, phylogenetic groups A and B_2 had the highest RS, while in the children's isolates, the highest RS was associated with phylogenetic groups A and D.

B-lactamase, *PMQR*, adhesion, and O-serogroups genes according to phylogenetic group

The $bla_{CTX\text{-}M}$ gene was found to be more prevalent in phylogroups B_2 (75.9%) and E (75%). The presence of the bla_{TEM} gene was significantly associated with phylogroup D compared to other phylogroups (P = 0.007). In this regard, both adult and children isolates follow this pattern as well. *qnrS and qnrB* were more frequently found in phylogroups D and F, respectively. The carriage of *qnrS and qnrB* was not significantly associated with different phylogroups.

Regarding the phylogroups, as shown in Table 3, the prevalence of *pap* was higher in phylogroups D (60%), followed by B_2 (37.9%), whereas the frequency of *sfa* was more prominent in phylogroup B_2 (48.3%) and F (30%). Moreover, isolates belonging to phylogroup B_2 carried the *sfa* gene more frequently than strains belonging to the other phylogroups (P = 0.031). Regarding the distribution of O-serogroups in different phylogroups, a significantly higher frequency of *O25* was detected among members of B_2 than in other phylogroups (P < 0.001). Furthermore, *O16*, *O1*, and *O15* were more frequent in B_1, B_2, and D phylogroups, respectively. However, Tables 4 and 5 show the serogroup distribution within the phylogroups of the UPEC strains isolated from adults and children.

Association between biofilm formation with phylogenetic groups, O-serogroups and resistance genes

The biofilm-producing strains were predominantly clustered in phylogenetic groups B_1 and B_2. On the other hand, the majority of the weak biofilm-formers and moderate biofilm-formers strains belonged to phylogroup B_2 (Table 3). On the other hand, no statistically significant difference was observed between biofilm formation capacity and both phylogenetic groups and ESBL production (Fig. 2). Additionally, Table 6 displays the relationship between antibiotic resistance patterns and biofilm formation, and Table 7 illustrates the connection of biofilm with VFs, O-serogroups, and resistance genes.

Expression levels of the genes involved in biofilm formation

To determine the expression levels of the genes involved in biofilm formation, we analyzed the expression level of each of the genes involved in biofilm formation in UPEC strains

Table 2 Distribution of *qnr* genes in relation with quinolone resistance

Antibiotic	Pattern	*qnrS*-positive No. (%)	*qnrS*-negative No. (%)	P value	*qnrB*-positive No. (%)	*qnrB*- negative No. (%)	P value
CP	103 (66)	42 (40.8)	61 (59.2)	0.22	12 (11.7)	91 (88.3)	0.58
OFX	76 (48.7)	27 (35.5)	49 (64.5)	0.74	8 (10.5)	68 (89.5)	1
NA	143 (91.7)	50 (35)	93 (65)	0.07	15 (10.5)	128 (89.5)	1
NOR	92 (59)	38 (41.3)	54 (58.7)	0.24	11 (12)	81 (88)	0.43

CP Ciprofloxacin, *OFX* Ofloxacin, *NA* Nalidixic acid, *NOR* Norfloxacin

Table 3 Distribution of resistance genes, O-serogroup and antibiotic resistance pattern with respect to the phylogenetic group in all patients

Variables	Unknown n = 19 No (%)	F n = 10 No (%)	E n = 20 No (%)	A n = 20 No (%)	D n = 20 No (%)	B1 n = 8 No (%)	B2 n = 58 No (%)	P value
Virulence factor genes								
pap	8 (42.1)	3 (30)	7 (35)	5 (25)	12 (60)	0	22 (37.9)	0.08
sfa	6 (31.6)	3 (30)	3 (15)	4 (20)	4 (20)	1 (12.5)	28 (48.3)	0.03
fimA	17 (89.5)	8 (80)	14 (70)	17 (85)	16 (80)	6 (75)	47 (81)	0.87
fimH	18 (94.7)	10 (100)	19 (95)	20 (100)	19 (95)	8 (100)	54 (93.1)	0.9
O-serogroups								
O25	2 (10.5)	0	4 (20)	0	3 (15)	1 (12.5)	27 (46.6)	<0.001
O16	3 (15.8)	1 (10)	0	0	0	1 (13.5)	4 (6.9)	0.15
O1	4 (21.1)	0	0	0	0	0	4 (6.9)	0.08
O15	0	0	0	0	1 (5)	0	0	0.62
ESBL genes								
$bla_{\text{CTX-M}}$	17 (89.5)	7 (70)	15 (75)	14 (70)	10 (50)	5 (62.5)	44 (75.9)	0.19
bla_{SHV}	0	0	2 (10)	2 (10)	2 (10)	1 (12.5)	4 (6.9)	0.7
bla_{TEM}	12 (63.2)	3 (30)	8 (40)	12 (60)	14 (70)	2 (25)	17 (29.3)	0.007
ESBL phenotypic	12 (63.2)	5 (50)	15 (75)	12 (60)	15 (75)	6 (75)	40 (69)	0.76
Biofilm production								
Non adherent	5 (26.3)	4 (40)	10 (50)	9 (45)	6 (30)	2 (25)	19 (32.8)	0.8
Weak	12 (63.2)	5 (50)	6 (30)	10 (50)	12 (60)	5 (62.5)	32 (55.2)	
Moderate	2 (10.5)	1 (10)	3 (15)	1 (5)	2 (10)	1 (12.5)	7 (12.1)	
Strong	0	0	1 (5)	0	0	0	0	
Plasmid-mediated quinolone resistance (*PMQR*) genes								
qnrA	0	0	0	0	0	0	0	–
qnrB	3 (15.8)	2 (20)	1 (5)	3 (15)	1 (5)	0	6 (10.3)	0.670
qnrS	9 (47.4)	3 (30)	8 (40)	6 (30)	11 (55)	4 (50)	17 (29.3)	0.383
Antibiotic resistance								
Penicillins + b-lactamase inhibitors								
AMC	8 (42.1)	8 (80)	6 (30)	8 (40)	9 (45)	3 (37.5)	26 (44.8)	0.47
SAM	6 (31.6)	5 (50)	14 (70)	16 (80)	11 (55)	2 (25)	46 (79.3)	0.001
3rd and 4th generation cephalosporins								
CAZ	13 (68.4)	6 (60)	15 (75)	15 (75)	15 (75)	6 (75)	41 (70.7)	0.97
CFM	6 (31.6)	5 (50)	5 (25)	11 (55)	8 (40)	3 (37.5)	31 (53.4)	0.27
CTX	13 (68.4)	6 (60)	15 (75)	15 (75)	15 (75)	6 (75)	41 (70.7)	0.97
CRO	8 (42.1)	3 (30)	9 (45)	11 (55)	12 (60)	1 (12.5)	34 (58.6)	0.14
Carbapenem								
MEN	2 (10.5)	1 (10)	4 (20)	2 (10)	2 (10)	0	11 (19)	0.32
Sulfonamides								
SXT	14 (73.7)	8 (80)	14 (70)	13 (65)	14 (70)	7 (87.5)	45 (77.6)	0.88
Quinolones								
CP	12 (63.2)	8 (80)	6 (30)	16 (80)	19 (95)	3 (37.5)	39 (67.2)	<0.001
OFX	8 (42.1)	5 (50)	5 (25)	9 (45)	9 (45)	2 (25)	38 (65.5)	0.03
NA	17 (89.5)	9 (90)	17 (85)	20 (100)	17 (85)	6 (75)	56 (96.6)	0.07
NOR	11 (57.9)	7 (70)	7 (35)	14 (70)	15 (75)	2 (25)	36 (62.1)	0.059
Nitrofurans								
FM	2 (10.5)	1 (10)	2 (10)	4 (20)	2 (10)	0	6 (10.3)	0.97
Amino-glycosides								
GM	5 (26.3)	2 (20)	3 (15)	7 (35)	2 (10)	1 (12.5)	11 (19)	0.77
AN	5 (26.3)	1 (10)	4 (20)	6 (30)	2 (10)	1 (12.5)	14 (24.1)	0.81
Resistance score (mean, median, range)	7.57, 8, 2–13	7.70, 8, 2–11	6.75, 6.5, 0–12	8.9, 9, 5–15	8.05, 9, 2–11	5.37, 6, 2–9	8.15, 8, 2–14	0.03

AMC amoxicillin/clavulanic acid, *CAZ* ceftazidime, *CFM* cefixime, *MEN* meropenem, *SXT* trimethoprim-sulfamethoxazole, *CP* ciprofloxacin, *OFX* ofloxacin, *NA* nalidixic acid, *NOR* norfloxacin, *FM* nitrofurantoin, *GM* gentamicin, *AN* amikacin, *CRO* ceftriaxone, *CTX* cefotaxim, *SAM* ampicillin-sulbactam

Table 4 Distribution of resistance genes, O-serogroup and antibiotic resistance pattern with respect to the phylogenetic group in adults

Variables	Unknown n = 6 No (%)	F n = 6 No (%)	E n = 13 No (%)	A n = 7 No (%)	D n = 7 No (%)	B1 n = 5 No (%)	B2 n = 33 No (%)	P value
Virulence factor genes								
pap	2 (33.3)	1 (16.7)	4 (30.8)	0	4 (57.1)	0	10 (30.3)	0.21
sfa	1 (16.7)	1 (16.7)	1 (7.7)	0	0	1 (20)	13 (39.4)	0.07
fimA	5 (83.3)	6 (100)	9 (69.2)	5 (71.4)	5 (71.4)	4 (80)	25 (75.8)	0.63
fimH	5 (83.3)	6 (100)	12 (92.3)	7 (100)	6 (85.7)	5 (100)	29 (87.9)	0.68
O-serogroups								
O25	2 (33.3)	0	3 (23.1)	0	3 (42.9)	1 (20)	24 (72.7)	<0.001
O16	0	1 (16.7)	0	0	0	1 (20)	3 (9.1)	0.52
O1	0	0	0	0	0	0	0	–
O15	0	0	0	0	0	0	0	–
ESBL genes								
$bla_{\text{CTX-M}}$	6 (100)	4 (66.7)	10 (76.9)	5 (71.4)	5 (71.4)	3 (60)	27 (81.8)	0.71
bla_{SHV}	0	0	2 (15.4)	1 (14.3)	2 (28.6)	0	2 (6.1)	0.40
bla_{TEM}	4 (66.7)	0	3 (23.1)	3 (42.9)	3 (42.9)	1 (20)	9 (27.3)	0.22
ESBL phenotypic	3 (50)	4 (66.7)	10 (76.9)	2 (28.6)	4 (57.1)	4 (80)	23 (69.7)	0.37
Biofilm production								
Non adherent	1 (16.7)	2 (33.3)	7 (53.8)	4 (57.1)	4 (57.1)	1 (20)	14 (42.4)	0.73
Weak	5 (83.3)	3 (50)	4 (30.8)	3 (42.9)	3 (42.9)	3 (60)	15 (45.5)	
Moderate	0	1 (16.7)	2 (15.4)	0	0	1 (20)	4 (12.1)	
Strong	0	0	0	0	0	0	0	
Plasmid-mediated quinolone resistance (*PMQR*) genes								
qnrA	0	0	0	0	0	0	0	–
qnrB	2 (33.3)	0	1 (7.7)	1 (14.3)	0	0	5 (15.2)	0.45
qnrS	4 (66.7)	1 (16.7)	5 (38.5)	3 (42.9)	6 (85.7)	3 (60)	12 (36.4)	0.14
Antibiotic resistance								
Penicillins + b-lactamase inhibitors								
AMC	2 (33.3)	4 (66.7)	5 (38.5)	3 (42.9)	3 (42.9)	1 (20)	13 (39.4)	0.83
SAM	2 (33.3)	5 (83.3)	9 (69.2)	7 (100)	4 (57.1)	2 (40)	27 (81.8)	0.04
3rd and 4th generation cephalosporins								
CAZ	4 (66.7)	5 (83.3)	10 (76.9)	5 (71.4)	4 (57.1)	4 (80)	24 (72.7)	0.95
CFM	3 (50)	4 (66.7)	3 (23.1)	4 (57.1)	3 (42.9)	2 (40)	18 (54.5)	0.53
CTX	4 (66.7)	5 (83.3)	10 (76.9)	5 (71.4)	4 (57.1)	4 (80)	24 (72.7)	0.95
CRO	3 (50)	3 (50)	5 (38.5)	6 (85.7)	4 (57.1)	1 (20)	27 (81.8)	0.02
Carbapenem								
MEN	1 (16.7)	1 (16.7)	4 (30.8)	2 (28.6)	2 (28.6)	0	11 (33.3)	0.77
Sulfonamides								
SXT	5 (83.3)	5 (83.3)	9 (69.2)	6 (85.7)	6 (85.7)	4 (80)	26 (78.8)	0.97
Quinolones								
CP	3 (50)	4 (66.7)	3 (23.1)	6 (85.7)	6 (85.7)	1 (20)	27 (81.8)	0.001
OFX	2 (33.3)	4 (66.7)	3 (23.1)	5 (71.4)	5 (71.4)	1 (20)	26 (78.8)	0.006
NA	4 (66.7)	5 (83.3)	10 (76.9)	7 (100)	4 (57.1)	3 (60)	31 (93.9)	0.09
NOR	3 (50)	4 (66.7)	3 (23.1)	5 (71.4)	4 (57.1)	1 (20)	27 (81.8)	0.006
Nitrofurans								
FM	2 (33.3)	1 (16.7)	1 (7.7)	3 (42.9)	1 (14.3)	0	6 (18.2)	0.42
Aminoglycosides								
GM	2 (33.3)	1 (16.7)	3 (23.1)	5 (71.4)	1 (14.3)	1 (20)	9 (27.3)	0.24
AN	0	0	2 (15.4)	3 (42.9)	0	0	6 (18.2)	0.16
Resistance score (mean, median, range)	7.16, 8, 2–13	7.83, 9, 2–11	6.30, 6, 0–12	10, 11, 5–15	7, 9, 2–11	4.80, 3, 2–9	8.87, 9, 2–14	0.039

AMC amoxicillin/clavulanic acid, *CAZ* ceftazidime, *CFM* cefixime, *MEN* meropenem, *SXT* trimethoprim-sulfamethoxazole, *CP* ciprofloxacin, *OFX* ofloxacin, *NA* nalidixic acid, *NOR* norfloxacin, *FM* nitrofurantoin, *GM* gentamicin, *AN* amikacin, *CRO* ceftriaxone, *CTX* cefotaxim, *SAM* ampicillin-sulbactam

Table 5 Distribution of resistance genes, O-serogroup and antibiotic resistance pattern with respect to the phylogenetic group in Children

Variables	Unknown n = 13 No (%)	F n = 4 No (%)	E n = 7 No (%)	A n = 13 No (%)	D n = 13 No (%)	B1 n = 3 No (%)	B2 n = 25 No (%)	P value
Virulence factor genes								
pap	6 (46.2)	2 (50)	3 (42.9)	5 (38.5)	8 (61.5)	0	12 (48)	0.64
sfa	5 (38.5)	2 (50)	2 (28.6)	4 (30.8)	4 (30.8)	0	15 (60)	0.64
fimA	12 (92.3)	2 (50)	5 (71.4)	12 (92.3)	11 (84.6)	2 (66.7)	22 (88)	0.82
fimH	13 (100)	4 (100)	7 (100)	13 (100)	13 (100)	3 (100)	25 (100)	0.85
O-serogroups								
O25	0	0	1 (14.3)	0	0	0	3 (12)	0.41
O16	3 (23.1)	0	0	0	0	0	1 (4)	0.09
O1	4 (30.8)	0	0	0	0	0	4 (16)	0.07
O15	0	0	0	0	1 (7.7)	0	0	0.53
ESBL genes								
$bla_{\text{CTX-M}}$	11 (84.6)	3 (75)	5 (71.4)	9 (69.2)	5 (38.5)	2 (66.7)	17 (68)	0.34
bla_{SHV}	0	0	0	1 (7.7)	0	1 (33.3)	2 (8)	0.27
bla_{TEM}	8 (61.5)	3 (75)	5 (71.4)	9 (69.2)	11 (84.6)	1 (33.3)	8 (32)	0.04
ESBL phenotypic	9 (69.2)	1 (25)	5 (71.4)	10 (76.9)	11 (84.6)	2 (66.7)	17 (68)	0.47
Biofilm production								
Non adherent	4 (30.8)	2 (50)	3 (42.9)	5 (38.5)	2 (15.4)	1 (33.3)	5 (20)	0.56
Weak	7 (53.8)	2 (50)	2 (28.6)	7 (53.8)	9 (69.2)	2 (66.7)	17 (68)	
Moderate	2	0	1 (14.3)	1 (7.7)	2 (15.4)	0	3 (12)	
Strong	0	0	1 (14.3)	0	0	0	0	
Plasmid-mediated quinolone resistance (*PMQR*) genes								
qnrA	0	0	0	0	0	0	0	–
qnrB	1 (7.7)	2 (50)	0	2 (15.4)	1 (7.7)	0	1 (4)	0.09
qnrS	5 (38.5)	2 (50)	3 (42.9)	3 (23.1)	5 (38.5)	1 (33.3)	5 (20)	0.72
Antibiotic resistance								
Penicillins + b-lactamase inhibitors								
AMC	6 (46.2)	4 (100)	1 (14.3)	5 (38.5)	6 (46.2)	2 (66.7)	13 (52)	0.19
SAM	4 (30.8)	0	5 (71.4)	9 (69.2)	7 (53.8)	0	19 (76)	0.006
3rd and 4th generation cephalosporins								
CAZ	9 (69.2)	1 (25)	5 (71.4)	10 (76.9)	11 (84.6)	2 (62.7)	17 (68)	0.47
CFM	3 (23.1)	1 (25)	2 (28.6)	7 (53.8)	5 (38.5)	1 (33.3)	13 (52)	0.56
CTX	9 (69.2)	1 (25)	5 (71.4)	10 (76.9)	11 (84.6)	2 (66.7)	17 (68)	0.47
CRO	5 (38.5)	0	4 (57.1)	5 (38.5)	8 (61.5)	0	7 (28)	0.14
Carbapenem								
MEN	1 (7.7)	0	0	0	0	0	0	0.53
Sulfonamides								
SXT	9 (69.2)	3 (75)	5 (71.4)	7 (53.8)	8 (61.5)	3 (100)	19 (76)	0.71
Quinolones								
CP	9 (69.2)	4 (100)	3 (42.9)	10 (76.9)	13 (100)	2 (66.7)	12 (48)	0.01
OFX	6 (46.2)	1 (25)	2 (28.6)	4 (30.8)	4 (30.8)	1 (33.3)	12 (48)	0.86
NA	13 (100)	4 (100)	7 (100)	13 (100)	13 (100)	3 (100)	25 (100)	-
NOR	8 (61.5)	3 (75)	4 (57.1)	9 (69.2)	11 (84.6)	1 (33.3)	9 (36)	0.09
Nitrofurans								
FM	0	0	1 (14.3)	1 (7.7)	1 (7.7)	0	0	0.55
Amino-glycosides								
GM	3 (23.1)	1 (25)	0	2 (15.4)	1 (7.7)	0	2 (8)	0.62
AN	5 (38.5)	1 (25)	2 (28.6)	3 (23.1)	2 (15.4)	1 (33.3)	8 (32)	0.90
Resistance score (mean, median, range)	7.76, 8, 4–11	7.50, 7.5, 6–9	7.57, 7, 5–10	8.30, 8, 5–11	8.61, 9, 6–11	6.33, 6, 6–7	7.20, 7, 3–11	0.24

AMC amoxicillin/clavulanic acid, *CAZ* ceftazidime, *CFM* cefixime, *MEN* meropenem, *SXT* trimethoprim-sulfamethoxazole, *CP* ciprofloxacin, *OFX* ofloxacin, *NA* nalidixic acid, *NOR* norfloxacin, *FM* nitrofurantoin, *GM* gentamicin, *AN* amikacin, *CRO* ceftriaxone, *CTX* cefotaxim, *SAM* ampicillin-sulbactam

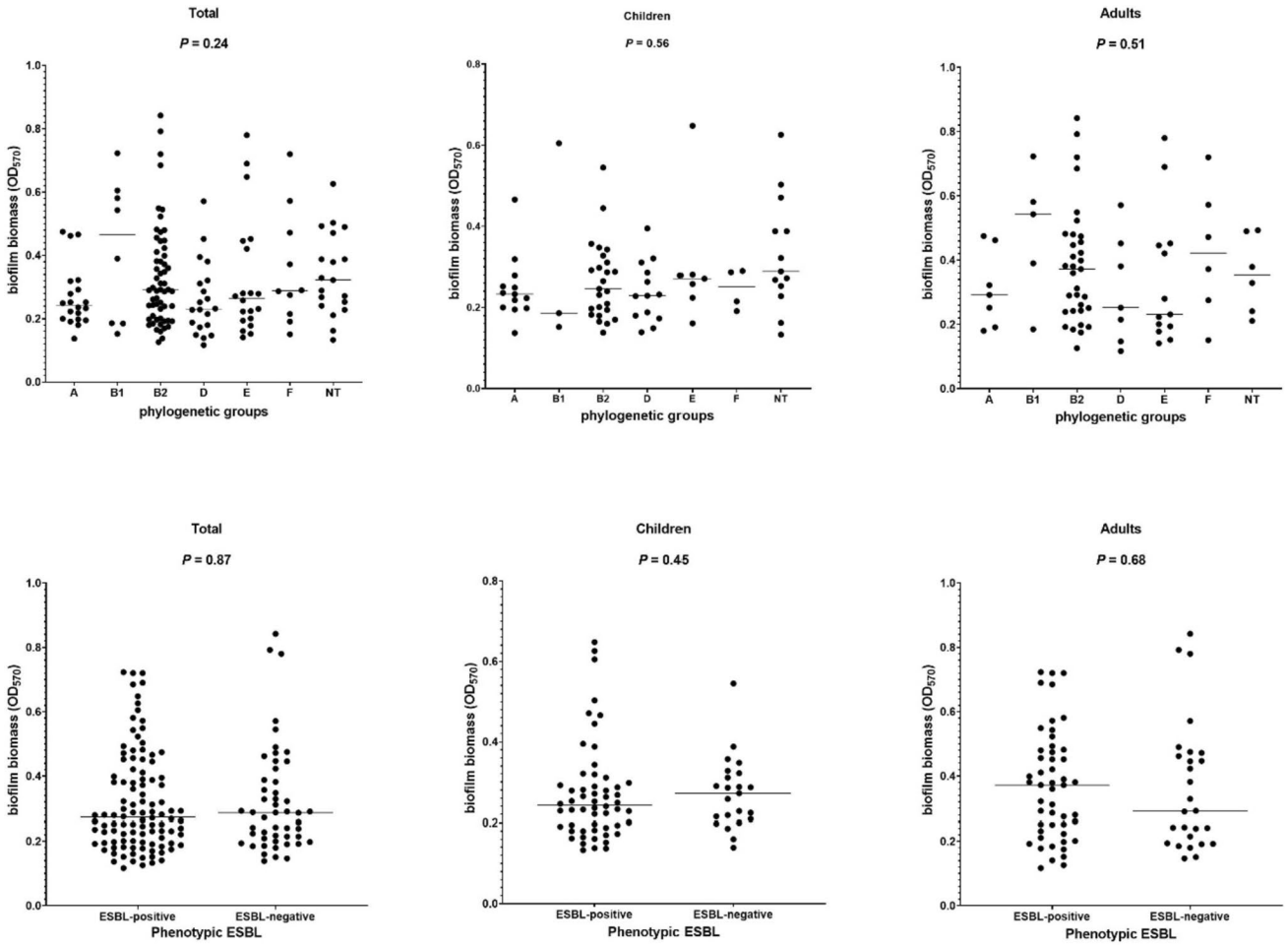

Fig. 2 Biofilm formation, according to phylogenetic group and ESBL production. Results are expressed as scatter plots, where bars indicate median values with interquartile range. Each dot is the average from two independent experiments with eight replicates of each strain per experiment. $P < 0.05$, ANOVA + Tukey's multiple comparison post-test

compared with those in *E. coli* ATCC25922 strains by real-time PCR. According to these results, the mean expression levels of *fimA* genes were significantly higher in non-biofilm producers than in biofilm producers ($P < 0.01$). A significant correlation was observed between the increased expression of the *fimH* gene and the ability to form biofilms (p=0.004) (Fig. 3).

Discussion

UPEC cause UTIs in both children and adults, but significant differences exist [20]. In children, particularly in infants and young children, UTIs are more prevalent due to anatomical and physiological factors. On the other hand, UTIs in adults, especially the elderly, may have different risk factors and clinical presentations, including underlying medical conditions and compromised immune system [21].

This is the first study comparing UPEC isolates from children and adults with UTI in northern Iran to examine factors such as antibiotic resistance patterns, distribution of *β-lactamase* and *PMQR* genes, phylogenetic groups, adhesion genes, O-serogroups, biofilm formation capacity, and gene expression levels (genes associated with biofilm formation). In this study, a total of 156 confirmed UPEC isolates were analyzed, with an approximately equal distribution between children (50.6%) and adults (49.4%).

UPEC isolates were more prevalent in women, accounting for a total of 116 cases, representing 74.4%. This finding is consistent with several previous studies [22–24] and supports the notion that sex plays a crucial role in the prevalence of UTIs, as reported by Rahn [25]. Furthermore, the prevalence of UPEC isolates in hospitalized patients with UTIs corresponds to the well-established understanding that UTIs are a frequent nosocomial infection [26].

Table 6 Antibiotic resistance pattern based on biofilm formation

Antibiotics	Non-biofilm n = 56 No (%)	Weak n = 82 No (%)	Moderate n = 17 No (%)	P value
Penicillins + b-lactamase inhibitors				
AMC	31 (55.4)	33 (40.2)	5 (29.4)	0.089
SAM	40 (71.4)	53 (64.6)	13 (76.5)	0.525
3rd and 4th generation cephalosporins				
CTX	38 (67.9)	59 (72)	14 (82.4)	0.507
CAZ	38 (67.9)	59 (72)	14 (82.4)	0.507
CFM	25 (44.6)	37 (45.1)	5 (29.4)	0.475
CRO	33 (58.9)	34 (41.5)	11 (64.7)	0.060
Carbapenem				
MEN	10 (17.9)	8 (9.8)	4 (23.5)	0.206
Sulfonamides				
SXT	47 (83.9)	57 (69.5)	11 (64.7)	0.105
Quinolones				
CP	37 (66.1)	53 (64.6)	12 (70.6)	0.894
OFX	19 (33.9)	34 (41.5)	6 (35.3)	0.649
NA	52 (92.9)	73 (89)	17 (100)	0.304
NOR	39 (69.6)	43 (52.4)	10 (58.8)	0.130
Nitrofurans				
FM	8 (14.3)	8 (9.8)	1 (5.9)	0.547
Aminoglycosides				
GM	11 (19.6)	16 (19.5)	4 (23.5)	0.928
AN	14 (25)	11 (13.4)	8 (47.1)	0.006
MDR	46 (82.1)	58 (70.7)	11 (64.7)	0.31
Resistance score (mean, median, range)	8.17, 8, 0–12	7.39, 8, 2–15	8.52, 9, 4–14	0.131

AMC amoxicillin/clavulanic acid, *CAZ* ceftazidime, *CFM* cefixime, MEN meropenem, *SXT* trimethoprim-sulfamethoxazole, *CP* Ciprofloxacin, *OFX* ofloxacin, *NA* nalidixic acid, *NOR* norfloxacin, *FM* nitrofurantoin, *GM* gentamicin, *AN* amikacin, *CRO* ceftriaxone, *CTX* cefotaxim, *SAM* ampicillin-sulbactam

The high prevalence of antibiotic resistance among UPEC isolates is a major concern in clinical practice, particularly in developing countries. This issue arises from factors such as the widespread availability of antibiotic drugs, overprescribing, and indiscriminate use of antibiotics [27]. In this study, the majority of the isolates exhibited significant resistance to a wide range of tested antibiotics, with 88.5% of strains demonstrating an MDR phenotype. This high prevalence of MDR strains underscores their role as a leading causative agent of a significant health issue in our area, which aligns with findings reported in previous studies conducted in various regions [28–30]. Additionally, this highlights the immediate requirement for the careful utilization of antibiotics and the development of alternative strategies to address the treatment of UTIs caused by MDR-UPEC strains.

Our study revealed that the isolates displayed the highest resistance to NA (91.7%), followed by SXT (74.4%), and the lowest resistance to FM (10.9%) and MEN (14.1%). These findings are consistent with previous reports on UPEC resistance worldwide [12, 31–33]. For example, in a comprehensive study conducted in Iran, Gharavi et al. demonstrated that UPEC had low resistance rates against MEN, AN, and FM [34]. Therefore, the use of NA for the treatment of UPEC in this particular region may not be effective. Conversely, FM and carbapenem drugs such as MEN are considered more favorable choices for the initial treatment of UTIs. Additionally, the study's discovery of low resistance to FM may be attributed to its infrequent use in standard UTI treatments.

Previous studies have reported a high prevalence of quinolone resistance in Iran. In this regard, most of the isolates were resistant to all the fluoroquinolone-containing antibiotics, a finding consistent with prior research conducted in Iran [1, 23]. However, notably, resistance to NA exceeded that observed for CP, OFX, and NOR. This discrepancy could potentially be attributed to the prolonged utilization of NA in the studied region, spanning a period of over five decades.

In a meta-analysis study conducted by Moghaddam et al., the resistance rates of quinolone agents, NA and CP, among UPEC strains were reported to be 68.4% and 61%, respectively [35].

Jomehzadeh et al. [1] demonstrated that among UPEC strains, resistance rates to NA, CP, OFX, gatifloxacin, levofloxacin, and moxifloxacin were 71.9%, 61.4%, 50%, 34.2%, 34.2%, and 29.8%, respectively, in a teaching hospital located in southwest Iran. Our study's findings were in agreement with these results [1]. In light of the high fluoroquinolone resistance rates observed in children, Akgoz et al. recommended avoiding the prescription of these antibiotics in pediatric populations and uncomplicated cases [36].

Furthermore, several observations have indicated that the emergence of quinolone resistance and the spread of resistance genes could potentially be attributed to the transmission

Table 7 Distribution of VFs, O-serogroups, *PMQR* genes in relation to biofilm formation

Biofilm formation	*pap*	*sfa*	*O25*	*O16*	*O1*	*O15*	*qnrB*	*qnrS*
Non-biofilm	33.9	30.4	26.8	0	7.1	0	14.3	35.7
Weak	36.6	32.9	22	9.8	3.7	1.2	7.3	37.8
Moderate	41.2	23.5	23.5	5.9	11.8	0	11.8	41.2

PMQR plasmid-mediated quinolone resistance, VFs virulence factors

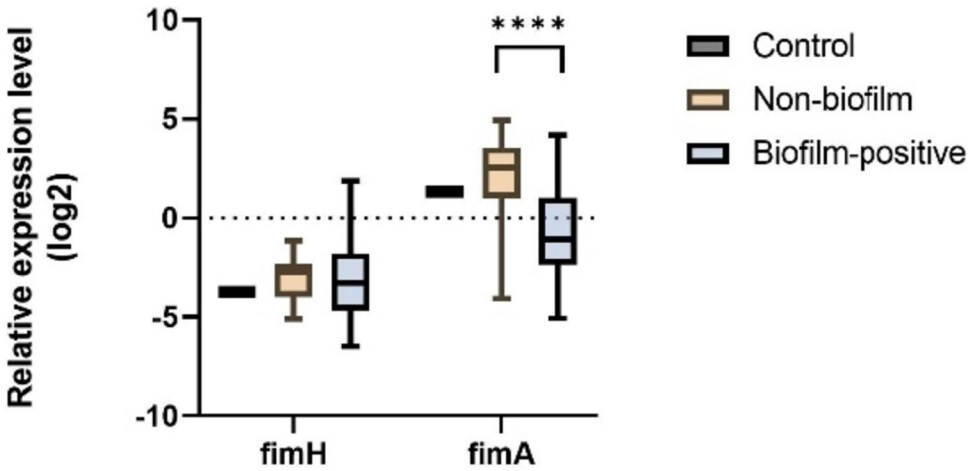

Fig. 3 Relative expression of genes involved in bacterial biofilm formation in uropathogenic *Escherichia coli*. For each gene, * indicate a statistically significant ($P < 0.05$) difference in expression levels, calculated by ANOVA and Tukey's test

of bacteria from animals to humans. This transmission pathway involves the consumption of poultry and meat products, which, in turn, can be associated with the improper use of antibiotics within the livestock industry [37].

According to our findings, 67.9% of the isolates exhibited the production of ESBLs. A meta-analysis conducted in Iran from 2008 to 2018 reported a substantial proportion of UPEC isolates (43.2%) being phenotypically identified as ESBL producers [38]. Moreover, higher prevalence rates of ESBL-producing UPEC strains have been documented in other regions, such as Shiraz [39], Rasht [40, 41], and Isfahan [42].

This heightened frequency may indicate an increasing trend of ESBL-producing strains over time. The elevated prevalence of ESBL-producing *E. coli* in Iran and other developing countries contrasts with the rates observed in developed countries such as Denmark (1.5%) [43], Germany (8.0%) [44], and the United States (7% to 15%) [45]. This difference can potentially be attributed to the extensive utilization of β-lactam drugs as the primary treatment for UTIs and the absence of stringent policies regarding antibiotic consumption and prescription in these regions. Regrettably, in Iran, there has been insufficient focus on antimicrobial screening programs, resulting in infections caused by bacterial strains that are resistant to multiple drugs being one of the leading factors contributing to illness and death among patients. In Iran, there are currently no clear protocols for controlling ESBLs.

Moreover, there was variation in the susceptibility patterns of drugs between ESBL-positive and ESBL-negative isolates. ESBL-positive strains exhibited a high level of susceptibility to MEN and FM, whereas ESBL-negative isolates showed a more favorable response to CAZ and CTX. This unexpected result suggests the presence of other resistance mechanisms or genetic factors contributing to the higher resistance rates in non-ESBL-producing isolates. The prevalence of antibiotic resistance, particularly ESBL, is higher in developing countries compared to developed nations due to the socioeconomic status of the society and the availability of antibiotics, which leads to self-medication, consumption of counterfeit drugs, improper dosage, and no adherence to antibiotic therapy. Therefore, the findings of this investigation highlight the urgent need for proactive surveillance systems to monitor the emergence and dissemination of ESBL in our region, given the high prevalence observed.

PMQR genes, including *qnr*, have been studied extensively in Iran. However, there is a lack of well-known reports that specifically address the molecular detection of PMQR determinants in UPEC, particularly in northern Iran. Regarding *PMQR* genes, our study identified a significant proportion of isolates carrying at least one *PMQR* gene, primarily *qnrS* followed by *qnrB*. This finding is consistent with previous reports that emphasize the involvement of *qnrS* and *qnrB* in quinolone resistance among UPEC isolates. In line with our findings, Sadeghi et al. [7] and Rezazadeh et al. [46] have also reported the absence of *qnrA* genes in their isolates. However, variations in the prevalence of the *qnrA* gene have been observed across different studies, indicating the need for further research to better understand the regional and temporal dynamics of these resistance determinants. Additionally, our results reported that 3.8% of isolates harbored both *qnrB* and *qnrS* simultaneously.

The findings revealed a high prevalence of $bla_{\text{CTX-M}}$ and bla_{TEM} genes, while bla_{SHV} exhibited a lower frequency. Importantly, meta-analytic studies conducted in diverse regions of Iran have consistently reported a relatively high prevalence of *ESBL* genes, which aligns with our results [38, 47]. Comparable to our investigation, other studies have found a high occurrence of $bla_{\text{CTX-M}}$ and bla_{TEM}-positive isolates in various countries and regions, including northern and eastern Europe [48]. However, our study showed a higher prevalence of these genes compared to studies conducted in South Africa and Nigeria [49, 50]. Interestingly, a higher frequency of the bla_{SHV} gene was

observed among non-ESBL isolates in comparison to ESBL isolates. This finding contradicts certain previous studies [51, 52] that reported a higher prevalence of bla_{SHV} in ESBL isolates. The variation in the distribution of genes highlights the dynamic nature of *β-lactamase* genes among different UPEC populations, emphasizing the necessity for ongoing monitoring and surveillance.

Furthermore, the distribution of *qnrS* and *qnrB* genes was analyzed in relation to ESBL status and age group, but no significant correlation was observed. This suggests that the mechanisms responsible for *qnr* genes and ESBL production may act independently. Additionally, the age group did not demonstrate a significant impact on the distribution of these genes.

Phylogenetic analysis indicated that the most frequently observed phylotype was B_2, followed by $D = A = E > F > B_1 > C$, respectively. These findings are consistent with previous studies conducted in Iran [53, 54] as well as other countries, including Ethiopia [55], Pakistan [56], China [57], and India [58], which also reported B_2 as the predominant phylogroup, followed by D. However, an intriguing contrast was observed in a study conducted by Mohsin et al. [59] in Iraq, where phylotype F emerged as the most frequent, followed by C, B_2, E, A, D, and B_1, respectively [59]. While there is some variation, the prevailing trend across multiple studies supports the predominance of the B_2 and D phylotypes in UPEC populations.

The differences in the prevalence of phylogenetic groups may be due to various factors such as genetic traits of the host, the location of the infection, the geographical area, the methodology used, the origin of the isolates, and differences in the sample size. In contrast, this study identified significant variations in antibiotic resistance patterns among different phylogenetic groups. Phylogroup A displayed the highest RS, followed by B_2 and D, whereas phylogroup B_1 exhibited the lowest RS. These findings suggest a correlation between phylogenetic group and antibiotic resistance, supporting the notion that phylogenetic group contributes to the development of antimicrobial resistance [60]. The post hoc analysis indicated a significant difference in RS between phylogenetic groups A and B_1, with RS significantly higher in phylogroup A compared to B_1 ($P = 0.032$). The elevated RS in phylogroups A, B_2, and D further underscores the challenges in treating infections caused by strains belonging to these groups.

Our findings revealed that 64.1% of UPEC isolates exhibited biofilm-producing ability, with a majority of them belonging to phylogenetic groups B_1 and B_2. However, no significant association was found between biofilm formation and phylogenetic groups. The previous literature has reported an association between biofilm-forming ability and phylogenetic groups B_2 and B_1 among UPEC isolates [61, 62]. Moreover, there were no notable distinctions noted in the ratio of biofilm-positive strains between ESBL-positive and ESBL-negative isolates.

On the other hand, $bla_{CTX\text{-}M}$ was found to be more prevalent in phylogroups B_2 (75.9%) and E (75%), while bla_{TEM} was more frequently detected in phylogroups D (70%) and A (60%). Additionally, the presence of the bla_{TEM} gene was significantly associated with phylogroup D compared to other phylogroups ($P = 0.007$), which is in line with previous reports [63]. Regarding *PMQR* genes, our results did not reveal any significant associations between *qnrS* or *qnrB* and different phylogroups.

The findings of this study showed that serogroup *O25* had the highest frequency, as determined by O antigen. In this regard, serogroup *O25* was predominant among adults, while serogroup *O1* was predominant among children. Serogroup *O1* is primarily associated with capsular K antigens and is linked to extraintestinal infections in humans. It is a major causative factor for UTIs and sepsis in pediatric patients. Regarding the distribution of O-serogroups among different phylogroups, a significantly higher frequency of serogroup *O25* was detected among members of phylogroup B_2 compared to other phylogroups ($P < 0.001$). Additionally, serogroups *O16*, *O1*, and *O15* were more frequently found in phylogroups B_1, B_2, and D, respectively. These results emphasize the need for action against isolates belonging to phylogroups B_2 and D, not only in adults but also in children.

Our findings suggest that non-biofilm-producing *E. coli* strains exhibit significantly higher levels of *fimA* gene expression when compared to their biofilm-producing counterparts. Previous studies have also demonstrated that the *fimA* gene and operon are highly expressed in certain *E. coli* isolates, whereas the type 1 fimbrial adhesin, *fimH*, which plays a crucial role in virulence and invasion during murine infection, was found to be expressed in only a limited number of patients. *FimH* is vital for connecting *E. coli* with host cell receptors during catheter-associated biofilm formation, and mutations affecting its expression can result in reduced virulence in UPEC [64, 65].

Conclusion

In conclusion, the high fluoroquinolone resistance rates in this study underscore the importance of local resistance monitoring and investigation of underlying mechanisms. Our results reveal the dominance of phylogroups B_2 and D as the predominant phylogenetic groups in both adults and children. Additionally, our results indicate that members of phylogroup A exhibit the highest RS. This study further confirms the widespread presence of $bla_{CTX\text{-}M}$, bla_{TEM}, and *qnrS* among UPEC isolates, which also harbor essential adhesion-associated virulence genes, particularly *papA* and

fimH. Moreover, phylogenetic groups B_1 and B_2 demonstrate a greater capacity for biofilm production compared to other phylogroups. These study outcomes affirm the heterogeneity and complex genetic backgrounds of UPEC isolates. Consequently, it is imperative to investigate the epidemiological aspects and characterize UPEC isolates across diverse regions and time frames.

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

Declarations

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical approval The study was approved by the Research Ethics Committee of Babol University of Medical Sciences (No. IR.MUBABOL.HRI.REC.1401.142.), Babol, Iran. In this study, bacteria isolated from clinical samples in the clinical microbiology laboratory were used. However, all enrolled patients provided informed written consent before entering the study. The parents/legally authorized representatives of the minor UTI patients in the study gave their written informed consent.

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