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Molecular Characterization and Antimicrobial Resistance of Uropathogenic *Escherichia coli*

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Background: Urinary Tract Infections (UTIs) are the most common infectious diseases in childhood. The Uropathogenic *Escherichia coli* (UPEC) strains account for as much as 80% of UTIs.

Objective: From a clinical perspective, it is important to know which virulence factors and antibiotic resistance properties are present in UPEC strains in pediatrics. Therefore, this study was carried out to investigate the molecular characterization and antimicrobial resistance of UPEC strains isolated from hospitalized patients in pediatric ward of Baqiyatallah Hospital in Tehran.

Patients and Methods: One hundred and twenty-one urine specimens were collected from the patients infected with UTIs (51 boys and 70 girls). The urine samples were cultured immediately, and those with *E. coli*-positive were analyzed for the presence of antibiotic resistance genes and bacterial virulence factors using Polymerase Chain Reaction (PCR). Also, antimicrobial susceptibility testing was performed using disk diffusion methodology with Mueller–Hinton agar according to the instruction of Clinical Laboratory and Standard Institute.

Results: Nineteen out of 51 (37.25%) urine samples from boys and 47 out of 70 (67.14%) urine samples from girls harbored *E. coli*. A significant difference was found between the frequency of UPEC strains in boys and girls (P <.05). High resistance levels to tetracycline (69.6%), ampicillin (69.6%) and norfloxacin (63.6%) were also observed. Totally, 1.66% of tested strains were resistant to more than 8 antibiotics. The incidence of genes encoding resistance against gentamicin (*aac (3)-IV*), sulfonamide (*sul1*), beta-lactams (*blaSHV* and *CITM)*, tetracycline (*tetA* and *tetB*), trimethoprim (*dfrA1*), and quinolones (*qnr*) were 25.7%, 22.7%, 83.2%, 71.1%, 19.6% and 21.2%, respectively. The most commonly detected virulence factors were *fim* (71.2%), *set-1* (66.6%), *iha* (62.1), *papG*I (59%), *usp* (56%) and *sen* (22.7%).

Conclusion: Resistant strains of uropathogenic *E. coli* had the lower incidence of uropathogenic virulence factors. We suggested prescription of imipenem and amikacin to treat pediatric patients infected with UTIs.

*Keywords:*Uropathogenic *Escherichia coli*; Virulence Factors; Antimicrobial; Pediatrics

1. Background

In the past 20–50 years, specifications of Urinary Tract Infections (UTIs) in pediatrics have changed as a result of the novel antibiotic prescription, and improvements in medicine. Therefore, management of diseases requires further investigations to provide certain information about the epidemiology and prevalence of UTIs.

UTIs are common bacterial infections in the majority of infants and children. It is difficult to diagnose UTIs in children younger than 3 years because of non-specific presentation of its symptoms.

The uropathogenic *Escherichia coli* (UPEC) is the most prevalent cause of UTIs in children (1, 2). It has been estimated that the UPEC is responsible for 70-90% of cases of UTIs in children (1, 2). The pathogenesis of UPEC is related to several bacterial virulence factors (3). Some of the most important uropathogenic virulence factors are aerobactin (*aer*), *iroN*, *kpsMT*, type 1 fimbriae, *usp*, fimbriae, o*mpT*, *set 1*, *astA*, *traT*, F1C fimbriae, *iha*, *iutA*, and group II capsule synthesis; *sfa/foc*, S and serum resistance, *fimH* and adhesins (3-5).

Diseases caused by *E. coli* often require antimicrobial therapy; however, antimicrobial prescription causes turbulence in the ecological balance between host and pathogens, which might lead to antibiotic resistance in pathogenic bacteria and finally emergence of more severe infections.

Implication for health policy/practice/research/medical education:
Uropathogenic Escherichia coli is responsible for the majority of the cases of Urinary Tract Infections (UTIs) in pediatrics. Several bacterial virulence factors play major roles in diseases like pyelonephritis, cystitis, and urethral infections in children. Therefore, detection, characterization, and study of the antibiotic resistance of Uropathogenic Escherichia coli isolated from pediatric wards in Iran hospitals are important from a clinical and epidemiological perspective.

Several studies have shown that antibiotic resistance in *E. coli* has increased over time (6, 7). Similarly, an epidemiological investigation in Iran revealed that UPEC strains were the most commonly detected pathogen in pediatric patients infected with UTIs, which showed a high incidence of resistance (20–90%) to commonly used antibiotics (8-10). Antibiotic resistance genes are known to cause antibiotic resistance in UPEC strains isolated from pediatric patients suffered from UTIs (3).

2. Objectives

Data on the incidence of virulence genes and the antimicrobial resistance of UPEC strains isolated from pediatric patients is scarce in Iran. Therefore, the present study aimed to characterize UPEC strains isolated from Iranian pediatric patients infected with UTIs at the molecular level and to investigate their susceptibility to 15 commonly used antibiotics.

3. Patients and Methods

3.1. Samples Collection and Bacterial Identification

From July to September 2012, a total of 121 urine samples were collected from boys (n = 51) and girls (n = 70) suffered from UTIs. The patients were 1-3 years old. Diagnosis of UTIs in patients was confirmed using the ultrasound technique (11). All samples were collected from the hospitalized patients in pediatric ward of Baqiyatallah Hospital in Tehran, Iran. Most of the patients had urine catheter for a week before they got UTI.

Urinary urgengy, frequency, dysuria, cloudy, bloody, and odorous urine, as well as fever chills and nausea in some cases, are the most commonly detected symptoms in pediatric patients. In order to decrease the likelihood of bacterial, cellular, or artifactual contamination, all urine samples were collected from midstream. Urine samples were collected using the Suprapubic Aspiration (SPA) method based on the standard technique of NICE (12).

All samples were immediately transferred to the Microbiology Research Center of the Islamic Azad University while kept at 4°C. Totally, 3 mL of each sample was blended with 225 mL of nutrient broth (Merck, Germany) for 2 minutes at normal speed, using a Stomacher lab blender and then incubated at 37°C for 24 hours. Next, 1 mL sample of the nutrient broth culture was mixed with 9 mL of MacConkey broth (Merck, Germany) and further incubated at 37°C for another 24 hours.

One loop of each tube was streaked on MacConkey agar (Merck, Germany). A typical purple colony of *E. coli* was streaked on Eosin-Methylene Blue agar (EMB agar; Merck, Germany) plate and incubated at 37°C for 24 hours. Green colonies with a metallic luster were considered as typical *E. coli* colonies. Such colonies were confirmed as *E. coli* using standard biochemical tests (e.g., Methyl red, Voges-Proskauer, Indole, and Citrate utilization tests). *E. coli* isolates were stored in Tryptic soy broth (TSB, Merck, Germany) containing 20% glycerol at -70°C for further characterization.

Antimicrobial susceptibility testing pattern of antimicrobial resistance was examined using the simple disk diffusion technique. The Mueller–Hinton agar (HiMedia Laboratories, Mumbai, India, MV1084) medium was used for this purpose. Antibiotic resistance of UPEC strains against 15 commonly used antibiotics was determined using the instruction of Clinical and Laboratory Standards Institute guidelines (CLSI 2012) (13).

Susceptibility of *E. coli* isolates were tested against ampicillin (10 u.disk $^{-1}$), tetracycline (30 μg.disk $^{-1}$), gentamycin (10 μg.disk $^{-1}$), amikacin (30 u.disk $^{-1}$), imipenem (30 u.disk $^{-1}$), mezlocillin (30 u.disk $^{-1}$), piperacillin (30 μg.disk $^{-1}$), cefotaxime (30 μg.disk $^{-1}$), ciprofloxacin (5 μg.disk $^{-1}$), norfloxacin (30 μg.disk $^{-1}$), cotrimoxazole (30 μg.disk $^{-1}$), ceftazidime (30 μg.disk $^{-1}$), ofloxacin (5 μg.disk $^{-1}$), nitrofurantoin (300 μg.disk $^{-1}$) and cephalothin (30 μg.disk $^{-1}$) antibiotic agents (Oxoid).

All the inoculated plates were aerobically incubated at 37°C for 18-24 h. Results were interpreted based on the instruction provided by CLSI (2012) (13). In all reactions, the *E. coli* ATCC 25922 was used as quality control organism.

3.2. DNA Extraction and Bacterial Confirmation Using Polymerase Chain Reaction

Bacteria were cultured overnight on Luria-Bertani broth (Merck, Germany) and genomic DNA was extracted from typical colonies using the DNA extraction kit (DNPTM, CinnaGen, Iran) according to the manufacturer's instruction. All *E. coli* colonies were also confirmed using the Polymerase Chain Reaction (PCR) technique (14).

The PCR method was done with a total volume of 50 μL, including 2 mM $MgCl_2$, 1 μM of forward primer, 1 μM of reverse primer (specified for the 16S rRNA gene of the *E. coli*) (Table 1), 5 μL PCR buffer 10X, 200 μM dNTP (Fermentas), 1 U Taq DNA polymerase (Fermentas) and 2.5 μL DNA template.

Then, the DNA was amplified by 31 successive cycles of denaturation at 95°C for 45 s, primer annealing at 59°C for 60 s, and DNA chain extension at 72°C for 60 s. The programmable thermal cycler (Eppendorf, Mastercycler® 5330, Eppendorf-Netheler-Hinz GmbH, Hamburg,Germany) PCR device was used in all PCR reaction.

3.3. Detection of UPEC Virulence Factors and Antibiotic Resistance Genes

Several PCR reactions were used for detecting virulence factors and antimicrobial resistance genes in UPEC isolates. List of primers used for detection of the virulence genes, and antibiotic resistance genes are shown in Table 1 (4, 5, 15-24). Table 2 presents PCR conditions. PCR products were electrophoresed using 2% agarose gels, which were stained with ethidium bromide at 90 V for 6 h, using 1×TBE (0.89 M Tris-Borate, 0.02 M EDTA, pH 8.3) as the running buffer. All products were examined under ultraviolet illumination. A set of molecular weight standards (Fermentas, GmbH, Germany) ranging from 100 bp to 2000 bp was included on each gel.

Table 1. Oligonucleotide Primers for Detection of Various Putative Virulence Genes and 16S rRNA Gene of Uropathogenic *Escherichia coli* Isolated from Pediatric Patients

Target Genes	Sequence	Size (bp)
aac(3)-IV	CTTCAGGATGGCAAGTTGGT	286
	TCATCTCGTTCTCCGCTCAT	
sul1	TTCGGCATTCTGAATCTCAC	822
	ATGATCTAACCCTCGGTCTC	
blaSHV	TCGCCTGTGTATTATCTCCC	768
	CGCAGATAAATCACCACAATG	
CITM	TGGCCAGAACTGACAGGCAAA	462
	TTTCTCCTGAACGTGGCTGGC	
tet(A)	GGTTCACTCGAACGACGTCA	577
	CTGTCCGACAAGTTGCATGA	
tet(B)	CCTCAGCTTCTCAACGCGTG	634
	GCACCTTGCTGATGACTCTT	
dfrA1	GGAGTGCCAAAGGTGAACAGC	367
	GAGGCGAAGTCTTGGGTAAAAAC	
qnr	GGGTATGGATATTATTGATAAAG	670
	CTAATCCGGCAGCACTATTTA	
set-1	GTGAACCTGCTGCCGATATC	147
	ATTTGTGGATAAAAATGACG	
sen	ATGTGCCTGCTATTATTTAT	799
	CATAATAATAAGCGGTCAGC	
astA	ATGCCATCAACACAGTATAT	110
	GCGAGTGACGGCTTTGTAGT	
sigA	TCCTCGGTATTATTTTATCC	408
	CGTAACCCCTGTTGTTTCCAC	
sap	TACCCTCCACAACAGAGAATG	832
	TACCCTCCACAACAGAGAATG	
***papG*I**	TCGTGCTGAGGTCCGGAATTT	461
	TGGCATCCCCCAACATTATCG	
***papG*II**	GGGATGAGCGGGCCTTTGAT	190
	CGGGCCCCCAAGTAACTCG	
***papG*III**	GGCCTGCAATGGATTTACCTGG	258
	CCACCAAATGACCATGCCAGAC	
kpsMT	CCATCGATACGATCATTGCACG	400
	ATTGCAAGGTAGTTCAGACTCA	
fim	GAGAAGAGGTTTGATTTAACTTATTG	559
	AGAGCCGCTGTAGAACTGAGG	
iha	CTGGCGGAGGCTCTGAGATCA	827
	TCCTTAAGCTCCCGCGGCTGA	
iroN	AAGTCAAAGCAGGGGTTGCCCG	665
	GACGCCGACATTAAGACGCAG	
ompT	ATCTAGCCGAAGAAGGAGGC	559
	CCCGGGTCATAGTGTTCATC	
usp	ACATTCACGGCAAGCCTCAG	440
	AGCGAGTTCCTGGTGAAAGC	
iss	ATCACATAGGATTCTGCCG	309
	CAGCGGAGTATAGATGCCA	
irp2	AAGGATTCGCTGTTACCGGAC	413
	AACTCCTGATACAGGTGGC	
tsh	ACTATTCTCTGCAGGAAGTC	824
	CTTCCGATGTTCTGAACGT	
vat	TCCTGGGACATAATGGTCAG	981
	GTGTCAGAACGGAATTGT	
***E. coli* 16S rRNA**	AGAGTTTGATCMTGGCTCAG	919
	CCGTCAATTCATTTGAGTTT	

Table 2. PCR Conditions for Detection of Virulence Genes and Antimicrobial Resistance Genes in Uropathogenic *Escherichia coli* Isolated from Pediatric Patients

Gene	PCR Program
tetA, tetB, dfrA1, qnr, aac(3)-IV, sul1, blaSHV, CITM	1 cycle:
	94 0C; 8 min
	32 cycle:
	95 0C; 60 s
	55 0C; 70 s
	72 0C; 2 min
	1 cycle:
	72 0C; 8 min
iss, irp2, tsh, vat	1 cycle:
	95 0C; 4 min
	32 cycle:
	94 0C; 60 s
	56 0C; 60 s
	72 0C; 2 min
	1 cycle:
	72 0C; 6 min
usp	1 cycle:
	94 0C; 2 min
	30 cycle:
	94 0C; 30 s
	58 0C; 30 s
	73 0C; 30 s
	1 cycle:
	72 0C; 10 min
iha, iroN, ompT	1 cycle:
	94 0C; 6 min
	30 cycle:
	94 0C; 45 s
	58 0C; 60 s
	72 0C; 75 s
	1 cycle:
	72 0C; 8 min
kpsMT	1 cycle:
	94 0C; 10 min
	30 cycle:
	94 0C; 60 s
	60 0C; 60 s
	72 0C; 60 s
	1 cycle:
	72 0C; 5 min
papGI, papGII, papGIII	1 cycle:
	95 0C; 2 min
	30 cycle:
	94 0C; 60 s
	69 0C; 30 s
	72 0C; 2 min
	1 cycle:
	72 0C; 10 min
fim	1 cycle:
	94 0C; 3 min
	40 cycle:
	94 0C; 60 s
	58 0C; 70 s
	72 0C; 70 s
	1 cycle:
	72 0C; 6 min
sen, set1, astA, sigA, sap	1 cycle:
	94 0C; 3 min
	30 cycle:
	94 0C; 30 s
	55 0C; 60 s
	72 0C; 60 s
	1 cycle:
	72 0C; 5 min

In order to confirm the PCR results, the sequencing method was used. For this reason, PCR products of some positive samples were purified with high pure PCR product purification kit (Roche Applied Science, Germany) according to manufacturer's recommendations. Single DNA strands were sequenced with ABI 3730 XL device and Sanger sequencing method (Macrogen, Korea). The result of the sequence of each gene was aligned with the gene sequences recorded in the GenBank database located at NCBI (National Center for Biotechnology Information).

3.4. Statistical Analysis

The data were analyzed using SPSS (Statistical Package for the Social Sciences) software and P values were calculated using chi-square and Fisher's exact tests to identify statistically significant relationships between the distribution of virulence genes and antibiotic resistance properties of the UPEC strains isolated from pediatric patients. A P value <.05 was considered statistically significant.

3.5. Ethical Issues

The present study was authorized by the Ethical Committee of the Baqiyatallah hospital of Tehran, Iran, AJA University of Medical Science, Tehran Iran and the Microbiology and Infectious Diseases Center of the Islamic Azad University of Shahrekord Branch, Iran. All patients or their parents signed the written informed consent.

4. Results

Out of 121 urine samples, 66 (54.54%) samples were positive for *E. coli* (Table 3). In addition, 19 out of 51 boys' urine samples (37.25%) and 47 out of 70 girls' urine samples (67.14%) were positive for *E. coli* (Table 3). We also found that 24 samples were positive for *Kelebsiella* bacterium (19.83%).

Antimicrobial resistance in the UPEC strains isolated from the pediatric patients is shown in Table 4. UPEC strains exhibited the highest level of resistance to ampicillin (69.6%), followed by tetracycline (69.6%), norfloxacin (63.6%), and ofloxacin (37.8%). Distribution of multi-drug resistant UPEC strains isolated from pediatric patients is shown in Table 5. We found that 1.66% of tested strains were resistant to more than eight antibiotics, while all the tested strains were resistant to three antibiotics.

The distribution of virulence genes within the UPEC strains isolated from pediatric patients is shown in Table 6. Generally, fim (71.2%), set-1 (66.6%), iha (62.1%), papGI (59%), and usp (56%) were the most common virulence genes in pediatric patients (Figure 1). The distribution of antibiotic resistance genes within the UPEC strains isolated from pediatric patients is shown in Table 7. Genes that encode resistance to beta-lactams, tetracycline and cephalothin antibiotics, i.e., blaSHV (45.4%), tetA (43.9%) and CITM (37.87%) were the most common antibiotic resistance genes in the pediatric patients (Figure 2).

Table 3. Distribution of Uropathogenic *Escherichia coli* in Pediatric Patients in Iran

Samples	No. Samples	No. Positive Samples (%)
Boys	51	19 (37.25)
Girls	70	47 (67.14)
Total	121	66 (54.54)

Table 4. Antibiotic Resistance Patterns of Uropathogenic *Escherichia coli* Isolated from Pediatric Patients

Antibiotic resistance (%)															
No. Positive Samples	**AM10** [a]	**TE30**	**GM10**	**AMK30**	**IMP30**	**MEZ30**	**PIP30**	**CefTx30**	**CIP5**	**Norf30**	**Cotr30**	**CFTZ30**	**OFLX5**	**F/M300**	**CF30**
Boy (19)	12 (63.1)	13 (68.4)	3 (15.7)	1 (5.2)	-	2 (10.5)	3 (15.7)	3 (15.7)	3 (15.7)	11 (57.8)	6 (31.5)	4 (21)	6 (31.5)	3 (15.7)	8 (42.1)
Girl (47)	34 (72.3)	33 (70.2)	9 (19.1)	2 (4.25)	1 (2.1)	5 (10.6)	6 (12.7)	6 (12.7)	7 (14.8)	31 (65.9)	15 (31.9)	11 (23.4)	19 (40.4)	9	
(19.1)	9 (19.1)														
Total (66)	46 (69.6)	46 (69.6)	11 (16.6)	3 (4.5)	1 (1.5)	7 (10.6)	9 (13.6)	9 (13.6)	10 (15.1)	42 (63.6)	21 (31.8)	15 (22.7)	25 (37.8)	12 (18.1)	17 (25.7)

[a] Abbreviation: AM10 = ampicillin (10 u.disk $^{-1}$); TE30 = tetracycline (30 μg.disk $^{-1}$); GM10 = gentamycin (10 μg.disk $^{-1}$); AMK30 = amikacin (30 u.disk $^{-1}$); IMP30 = imipenem (30 u.disk $^{-1}$); MEZ30 = mezlocillin (30 u.disk $^{-1}$); pip30 = piperacillin (30 μg/disk); ceftx30 cefotaxime (30 μg.disk $^{-1}$); CIP5 = ciprofloxacin (5 μg.disk $^{-1}$); norf30 = norfloxacin (30 μg.disk $^{-1}$); cotr30 = cotrimoxazole (30 μg.disk $^{-1}$); CFTZ30 = ceftazidime (30 μg.disk $^{-1}$); OFLX5= ofloxacin (5 μg.disk $^{-1}$); F/M300 = nitrofurantoin (300 μg.disk $^{-1}$); CF30 = cephalothin (30 μg.disk $^{-1}$).

Table 5. Incidence of Multi-drug Resistant Uropathogenic *Escherichia coli* Isolated from Pediatric Patients

Multi-Drug Resistant	Incidence Rate (%)
Resistance to two antibiotics	60 (100)
Resistance to three antibiotics	60 (100)
Resistance to four antibiotics	42 (70)
Resistance to five antibiotics	30 (50)
Resistance to six antibiotics	15 (25)
Resistance to seven antibiotics	5 (8.33)
Resistance to eight antibiotics	2 (3.33)
Resistance to more than eight antibiotics	1 (1.66)

Table 6. Distribution of Virulence Genes in Uropathogenic *Escherichia coli* Isolated from Pediatric Patients

Virulence Genes (%)																	
No. Positive Samples	*set-1*	*sen*	*astA*	*sigA*	*sap*	*PapG1*	*papG***II**	*papG***III**	*kpsMT*	*fim*	*iha*	*iroN*	*ompT*	*usp*	*iss*	*irp2*	*tsh*	*vat*
Boy (19)	9 (47.3)	6 (31.5)	-	2 (10.5)	2 (10.5)	8 (42.1)	3 (15.7)	2 (10.5)	1 (5.2)	10 (52.6)	9 (47.3)	1 (5.2)	2 (10.5)	7 (36.8)	1 (5.2)	-	1 (5.2)	-
Girl (47)	35 (74.4)	9 (19.1)	4 (8.5)	3 (6.3)	4 (8.5)	31 (65.9)	9 (19.1)	5 (10.6)	5 (10.6)	37 (78.7)	32 (68)	6 (12.7)	7 (14.8)	30 (63.8)	5 (10.6)	3 (6.3)	2 (4.25)	2 (4.25)
Total (66)	44 (66.6)	15 (22.7)	4 (6)	5 (7.5)	6 (9)	39 (59)	12 (18.1)	7 (10.6)	6 (9)	47 (71.2)	41 (62.1)	7 (10.6)	9 (13.6)	37 (56)	6 (9)	3 (4.5)	3 (4.5)	2 (3)

Table 7. Distribution of Antibiotic Resistance Genes in Uropathogenic *Escherichia coli* Isolated from Pediatric Patients

Antibiotic Resistance Genes (%)								
No. Positive samples	*aac(3)-IV*	*sul1*	*blaSHV*	*CITM*	*tet(A)*	*tet(B)*	*dfrA1*	*qnr*
Boy (19)	4 (21)	4 (21)	8 (42.1)	5 (26.3)	8 (42.1)	7 (36.8)	4 (21)	4 (21)
Girl (47)	13 (27.6)	11 (23.4)	22 (46.8)	20 (42.5)	21 (44.6)	11 (23.4)	9 (19.1)	10 (21.2)
Total (66)	17 (25.7)	15 (22.7)	30 (45.4)	25 (37.8)	29 (43.9)	18 (27.2)	13 (19.6)	14 (21.2)

Table 8. Relationship Between the Presence of Antibiotic Resistance Genes and Distribution of Virulence Factors in Uropathogenic *Escherichia Coli* Isolated From Pediatric Patients.

Antibiotic resistance genes (+)	Distribution of virulence factors (%)					
	fim	*set-1*	*iha*	*papGI*	*usp*	*sen*
***aac(3)-IV*+(17)**	3 (17.64)	1 (5.88)	1 (5.88)	4 (23.52)	-	1 (5.88)
***sul1*+ (15)**	2 (13.33)	1 (6.66)	1 (6.66)	1 (6.66)	1 (6.66)	-
***blaSHV*+ (30)**	3 (10)	2 (6.66)	3 (10)	5 (16.66)	2 (6.66)	2 (6.66)
***CITM*+ (25)**	3 (12)	2 (8)	2 (8)	3 (12)	2 (8)	1 (4)
***tetA*+ (29)**	1 (3.44)	2 (6.89)	-	3 (10.34)	3 (10.34)	1 (3.44)
***tetB*+ (18)**	1 (5.55)	-	1 (5.55)	1 (5.55)	-	-
***dfrA1*+ (13)**	1 (7.69)	-	-	-	1 (7.69)	-

Relationship between the presence of antibiotic resistance genes and distribution of virulence factors in UPEC strains isolated from pediatric patients is shown in Table 8. We found that virulence factors had low distribution in samples with positive results for antibiotic resistance genes. The most prevalent virulence genes in *aac(3)-IV*+, *sul1*+, *blaSHV*+, *CITM*+, *tetA*+, *tetB*+ and *dfrA1*+ strains were *fim* (17.64%), *fim* (13.33%), *papG*I (16.66%), fim and *papG*I (12%), *papG*I and *usp* (10.34%), *fim*, *iha* and *papG*i (5.55%) and *fim* and *usp* (7.69%), respectively.

5. Discussion

To the best of our knowledge, this is the most comprehensive report on virulence genes and antimicrobial resistance properties in UPEC strains isolated from pediatric patients in Iran. In the present study, all isolates that had UPEC virulence factors were identified as UPEC strains. This principle has been confirmed in previous studies (25, 26). Our work has identified the high presence of UPEC strains in the urine samples of pediatric patients in Iran. Totally, 37.25% of boys and 67.14% of girls were infected with UPEC strains ($P < .05$).

One possible explanation for the high prevalence of UPEC strains in girls is that they have a relatively short and wide urethra. Also, host factors such as changes in normal vaginal flora may put girls at higher risk for UTIs. The effects of genetic factors, including expression of Lewis blood group Le (a+b-) and Le (a-b-) and HLA-A3 should not be overlooked. Our results are in agreement with the results of Jadhav et al. (27) and Vollmerhausen et al. (28) which were conducted in India and Australia, respectively.

One possible explanation for the low prevalence of UPEC strains in boys of our study is that all of them were circumcised. Also, they were 1-3 years old. During the first year of life, boys have a higher incidence of UTIs; in all other age groups, girls are more prone to develop UTIs (29). Higher incidence of UTIs in uncircumcised boys has been reported previously (30).

Another important finding of our investigation relates to the distribution of antibiotic resistance pattern in UPEC strains. Totally, bacterial strains of our study had the lowest resistance against imipenem (1.5%) and amikacin (4.5%), while resistance to ampicillin (69.6%), tetracycline (69.6%) and norfloxacin (63.6%) were high. We found statistically significant ($P < .05$) association between the incidence of antibiotic resistance to ampicillin, tetracycline and norfloxacin and imipenem and amikacin. All the studies that have been conducted in this field (7, 31, 32), have shown a high distribution of antibiotic resistance against ampicillin, tetracycline and norfloxacin. High efficacy of imipenem and amikacin for treatment of the cases of UTIs due to UPEC strains has been reported previously from Iran (31, 33, 34).

The results of our study showed that considerable numbers of isolates were resistant to more than three antibiotics. Similar investigation had been reported previously (3, 31-34). Pyelonephritis, urethritis, cystitis, urethral infections and other clinical complications of infection with UPEC strains are serious among children, compelling clinicians to consider the provision of early, and empirical antibiotic therapy.

However, current recommendations and the available data (although limited in scope and only formally studied for UPEC-related infections in children) suggest that antibiotics should be withheld if UPEC infection is *suspected*, given the concerns that antibiotics may trigger the release of virulence factors, antibiotic resistance

genes and progression to pyelonephritis, urethritis and cystitis, resulting in worse clinical outcomes.

Our results indicated that UPEC strains with positive antibiotic resistance genes had lower incidence of virulence factors. It seems that the *aac(3)-IV*, *sul1*, *CITM*, *dfrA1* and *tetB* induced the higher presence of *fim* gene; *blaSHV*, *CITM* and *tetA* induced the higher presence of *papG*I gene; *tetB* induced the higher presence of *iha* gene; and finally *dfrA1* induced the higher presence of *usp* gene.

The effect of antibiotic therapy on the release of bacterial genes has been also reported by Farshad et al. (35) and Rijavec et al. (36). Farshad et al. (35) reported that the prevalence of all virulent genes was lower in resistant groups of UPEC strains but not statistically significant except for *pap* and *cnf1* with nalidixic acid

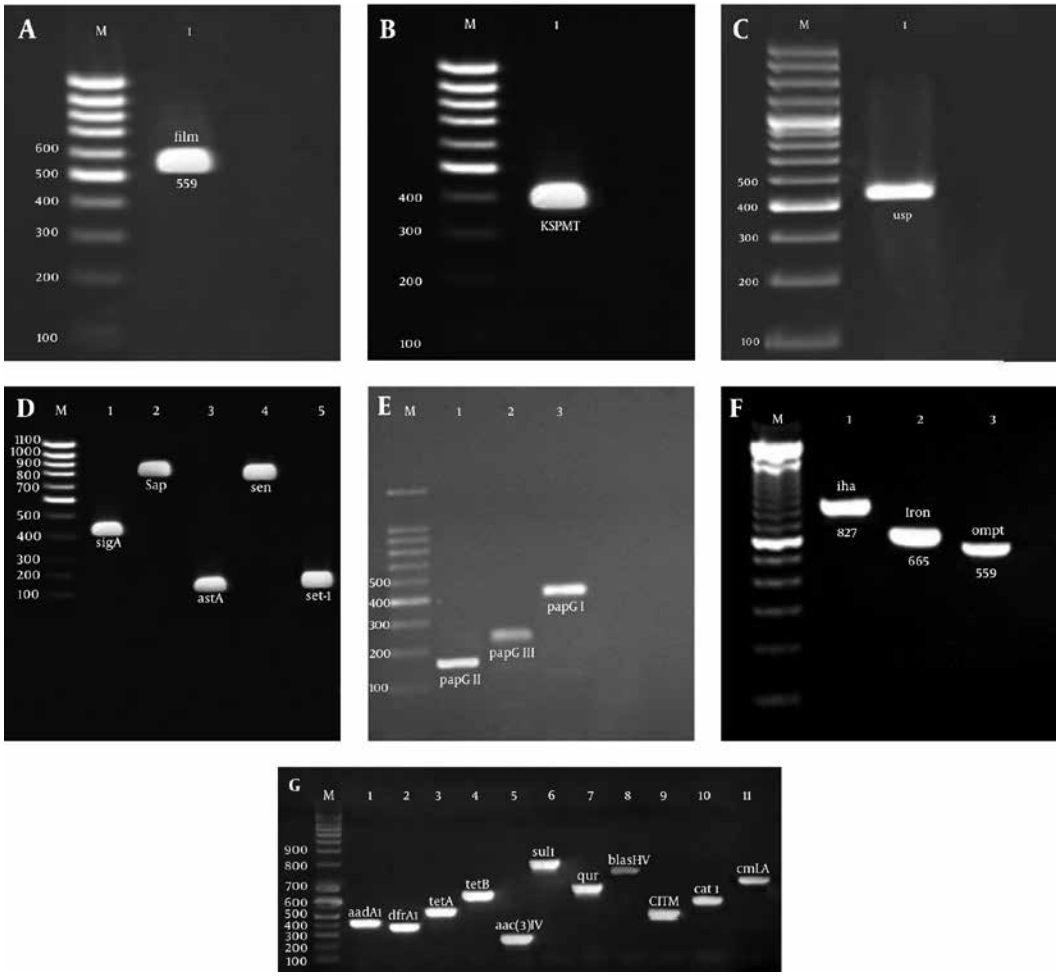

Figure 1. Application of PCR method for detection of virulence factors in Uropathogenic *Escherichia coli* isolated from pediatrics. M is 100 bp ladder and positive samples show typical light bands.

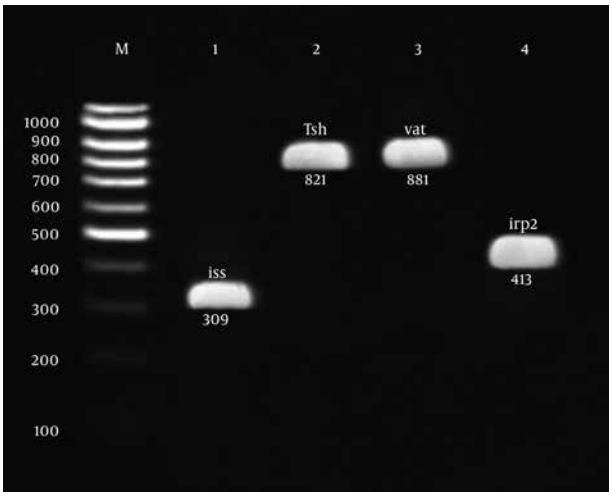

Figure 2. Application of PCR Method for Detection of Antibiotic Resistance Genes in Uropathogenic *Escherichia Coli* Isolated from pediatrics. M is 100 bp ladder and 1-11 are positive samples for various virulence factors.

(8% and 4.5% positive in resistant vs. 29.4% and 29.6% positive in susceptible groups, respectively, P < .05) was observed. Rijavec et al. (2008) showed that susceptible strains of uropathogenic *E. coli* exhibited a higher virulence factor score. The results of both Rijavec et al. (2008) (36) and Farshad et al. (2010) (35) were similar to our findings, which showed that susceptible UPEC strains had the highest distribution of *papG*, *usp* and *fim* virulence factors. Both quinolone and fluroquinolone resistant strains of previous study harbored lower distribution of *pap*, *hly*, *cnf1* and *sfa* virulence genes (37).

It seems that the susceptible strains of uropathogenic *E. coli* have higher incidence of uropathogenic virulence factors. Furthermore, because inappropriate prescriptions of antibiotics causes antibiotic resistance, it is not surprising that our study found that the distribution of *blaSHV*, *tetA*, *CITM*, *tetB* and *aac(3)-IV* antibiotic resistance genes were 45.4%, 43.9%, 37.8%, 27.2%, and 25.7%, respectively. There were statistically significant differences (P < .05) amongst the incidences of *tetA* and *tetB* and *blaSHV* and *dfrA1* genes. Incidence of *sul1* gene which encodes resistance against sulfonamides was 22.7% in our study while, its prevalence in the study of Gundogdu et al. (38) and Momtaz et al. (3) was 72% and 36.58%, respectively. Mom-

taz et al. (3) reported that *aadA1* (52.84%) and *qnr* (46.34%) had the highest incidence of antibiotic resistance genes while, *cat1* (15.44%), *cmlA* (15.44%) and *dfrA1* (21.95%) had the lowest incidence.

All of the UPEC strains harbored various virulence factors. The most commonly detected virulence factors in UPEC strains of our study were *fim* (71.2%), *set-1* (66.6%), *iha* (62.1%), *papG1* (59%), and *usp* (56%). Significant statistical differences ($P < .05$) were seen between the incidence of virulence factors in girls and boys and also between the incidence of *fim*, *set-1* and *iha* and finally between *astA*, *sigA*, *sap*, *kpsMT*, *iss*, *irp2*, *tsh* and *vat* genes. High presence of *fim*, *set-1* and *iha* genes have been reported previously by Momtaz et al. (3) (86.17% for *fim*, 79.67% for *set-1* and 17.88% for *iha*) and Momtaz et al. (3) (69.67% for *fim* and 17.88% for *iha*) (both from Iran). The distribution of *fim* gene in UPEC strains of the Arabi et al. (Iran) (39), Ananias and Yano, (Brazil) (40) and Qin et al. (China) (41) were 87.7%, 75% and 86%, respectively. *Fim* gene is involved in adhesion, invasion, and apoptosis of urothelial cells and initiates bladder pathology by binding to the uroplakin receptor complex (25).

The above-mentioned data highlight large differences in the prevalence of UPEC strains in the different studies, as well as differences in virulence genes and antibiotic resistance properties in the clinical samples. This could be related to differences in the type of sample (stool, blood, urine and other clinical samples) tested, number of samples, method of sampling, experimental methodology, geographical area, antibiotic prescription preference among clinicians, antibiotic availability, and climate differences in the areas where the samples were collected, in each study.

In conclusion, we identified a large number of virulence factors and antibiotic resistance genes and resistance to more than one antibiotic in the UPEC strains isolated from Iranian patients. Our data indicate that resistance against tetracycline and ampicillin, *fim* virulence factors and *blaSHV* antibiotic resistance gene were the most commonly detected characteristics of the UPEC strains isolated from Iranian pediatric patients with UTIs. Hence, judicious use of antibiotics is required by clinicians. It is compulsory to evaluate the prevalence of virulence factors, antibiotic resistance genes and pattern of antibiotic resistance among clinical isolates of UPEC strains.

Furthermore, because of the variation of resistance pattern in each hospital, it is important for each region and even hospital to formulate their own antibiotic policy according to their local resistance pattern. We recommended managing the children affected with community-acquired UTIs initially with imipenem and amikacin. It seems that various antibiotic resistance genes can induce the presence of certain virulence factors, but further studies must be done to prove this finding.

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Authors' contribution

All authors participated equally in the present study.

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