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Original Article

Relationships between Virulence Factors and Antimicrobial Resistance among *Escherichia coli* Isolated from Urinary Tract Infections and Commensal Isolates in Tehran, Iran

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Objectives: Uropathogenic *Escherichia coli* (UPEC) are the major cause of urinary tract infections (UTIs). Here, we determined whether sensitivity to antibiotics was related to the prevalence of iron scavenging genes, or to biofilm and hemolysis formation.

Methods: A total of 110 UPEC and 30 *E coli* isolates were collected from the urine of UTI patients and feces of healthy individuals without UTI, respectively. The presence of iron receptor genes and phenotypic properties were evaluated by polymerase chain reaction and phenotypic methods, respectively. Susceptibility to routine antibiotics was evaluated using the disc diffusion method.

Results: The prevalence of iron scavenging genes ranged from 21.8% (*ireA*) to 84.5% (*chuA*) in the UPEC. Resistance to ceftazidime and cefotaxime was significantly correlated with the presence of *fyuA* and *iutA* iron genes. Biofilm production was significantly associated with the prevalence of *fyuA* and *hma* iron genes. A higher degree of antibiotic resistance was exhibited by isolates that produced biofilms than by their non-biofilm producing counterparts.

Conclusion: Our study clearly indicates that biofilm production is associated with antibiotic resistance, and that iron receptors and hemolysin production also contribute to reduced antibiotic sensitivity. These results further our understanding of the role that these virulence factors play during UPEC pathogenesis, which in turn may be valuable for the development of novel treatment strategies against UTIs.

Introduction

Uropathogenic *Escherichia coli* (UPEC) is the most frequent cause of urinary tract infections (UTIs), and causes significant morbidity and mortality globally [1]. The UTIs include a range of disorders such as cystitis and pyelonephritis [2]. The pathogenicity of UPEC is associated with expression of several virulence factors, such as adhesion elements, flagella, toxins, capsule, serum resistance factors and iron uptake systems [3].

UPEC strains utilize different strategies to acquire iron from the host urinary tract. For example, these strains can produce iron receptors, *hma* and *chuA*, which facilitate heme uptake and transfer into the periplasm. UPEC also use iron chelators called siderophores, including salmochelin, aerobactin, enterobactin, and yersiniabactin [4,5]. Furthermore, outer membrane iron receptors confer important properties on UPEC such as colonization, biofilm production and formation of Intracellular Bacterial Communication of UPEC that are likely responsible for recurrent UTIs and increased resistance to antibiotics [6].

Other pro-virulence characteristics, such as biofilm production, also facilitate colonization of the urinary tract

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by UPEC. Biofilm formation attenuates both the activity of antimicrobial agents and the host immune response, which contributes to persistence of UPEC in the urinary tract and the consequent severe symptoms and antibiotic resistance [7].

Knowledge of the constellation of iron acquisition genes or factors correlated with biofilm production among UPEC strains is potentially valuable in developing strategies for treatment or prevention of UTIs. To date, several studies have reported the epidemiology, virulence factors and antibiotic resistance profiles associated with UPEC strains isolated from Iranian patients [8,9]. However, little is known regarding the distribution of genes encoding iron acquisition systems and their correlation with antibiotic resistance or biofilm and hemolysin formation among the UPEC strains. The objective of this study was therefore to determine the pattern of iron acquisition genes including *fyuA*, *iroN*, *iutA*, *iha*, *ireA*, *chuA*, and *hma* in UPEC isolated from Iranian patients and commensal *E coli* strains. Additionally, we investigated whether iron scavenging gene pattern was related to biofilm formation and hemolysis activity, as well as the susceptibility of the UPEC and commensal isolates to antibiotics.

Materials and Methods

1. Bacterial collection and identification

In this study, a total of 200 samples from patients with UTI symptoms were collected from different hospitals in Tehran, Iran. One hundred and ten *E coli* were isolated from these samples, which were mainly obtained from cystitis and pyelonephritis patients. All the *E coli* were isolated from women patients aged between 20 to 60 years, with a mean age of 39.5 years old. Furthermore, 30 commensal *E coli* were isolated from fecal samples of healthy women without UTI symptoms. The isolates were identified by conventional bacteriological tests. After identification, all isolates were kept frozen at −80°C in 20% (v/v) glycerol (Sigma-Aldrich, St. Louis, MO, USA) until further use. The work was done in accordance with the Ethical Principles for Medical Research Involving Human Subjects outlined in the Helsinki Declaration of 1975 (revised in 2008), and approved from the institutional review board of Pasteur Institute of Iran (No. IR.pII. REC.1394.45).

2. Biofilm production assay

The ability of the UPEC and commensal isolates to produce biofilm was evaluated according to a previous protocol [10]. Briefly, overnight cultures were diluted and cultured in 96-well microtiter plates (Greiner Bio-One GmbH, Frickenhausen, Germany). After incubation at 37°C for 48 hours, biofilms were stained using crystal violet solution (Merck, Darmstadt,

Germany). The crystal violet dye was washed, 33% acetic acid (Merck) added and absorbance was measured at optical density 590 nm with an enzyme-linked immunosorbent assay reader. For analysis of the results, isolates were classified into four categories according to their adherence capabilities, namely, non-adherent (−), weakly (+), moderately (++), and strongly (+++) adherent. *E coli* ATCC 25922 was used as a positive control.

3. Hemolysis production assay

Production of hemolysin by the isolates was evaluated using a culture method. The isolates were inoculated on 5% blood agar plates (Merck), incubated overnight at 37°C and hemolysis was detected by the presence of a zone of complete lysis of the erythrocytes (red blood cells) around the colonies.

4. Antibiotic susceptibility testing

Antimicrobial susceptibility testing was performed on Mueller-Hinton agar (Merck) using commercial antibiotic discs (MAST Group Ltd., Merseyside, United Kingdom) following the standard disc diffusion method; results were interpreted as described in the Clinical & Laboratory Standards Institute (CLSI) recommendations [11]. The antibiotics used were ceftazidime (30 µg), cefotaxime (30 µg), trimethoprim–sulfamethoxazole (SXT) (25 µg), norfloxacin (10 µg), amikacin (30 µg), imipenem (10 µg), and nitrofurantoin (300 µg). *E coli* ATCC 25922 was used as control.

5. Extraction of total DNA

For DNA extraction, the isolates were grown overnight in Luria-Bertani medium (Merck). The isolates were pelleted and genomic DNA was extracted using a phenol chloroform method according to previously published protocols [12]. The quality and quantity of the extracted genomic DNA was evaluated using a spectrophotometer and electrophoresis on an agarose gel (Sigma- Aldrich).

6. Detection of virulence iron genes by polymerase chain reaction (PCR)

All isolates were tested for the presence of *fyuA*, *iroN*, *iutA*, *iha*, *ireA*, *chu*, and *hma* genes that encode either siderophores or heme iron receptors. Table 1 shows the primers (Genfanavaran, Tehran, Iran) used for the detection of these genes. The amplification conditions were as follows: denaturation at 94°C for 3 minutes; 30 cycles of denaturation at 94°C for 1 minutes, annealing at specific temperature (Table 1) for 1 minutes, and extension at 72°C for 3 minutes; and a final extension at 72°C for 5 minutes. Finally, the PCR products were stained with ethidium bromide and analyzed

Table 1. List of primers used in this study.

Target	Primer sequence (5′ → 3′)	Annealing temperature (°C)
fyuA	F: ATGAAAATGACACGGCT R: GAAGAAATCAATTCGCG	56
iroN	F: ATGAGAATTAACAAAATC R: GAATGATGCGGTAACTC	55
iutA	F: ATGGCGCAGCGGCAG R: GAACAGCACTGAGTAGTTCA	58
iha	F: CTGGCGGAGGCTC TGAGATCA R: TCCTTAAGCTC CCGCGGCTGA	65
ireA	F: ATGAAGAACAAATATATC R: GAAGGATACTCTTACATT	53
chuA	F: ATGTCACGTCCGCAAT R: CCATTGATAACTCACGAA	57
hma	F: ATGGTTAAAGATACAATC R: CCACTGATAACGGGTAT	56

Table 2. Comparison of antibiotic resistance between UPEC and commensal isolates.

Antibiotic	UPEC (n = 110)	Commensal (n = 30)	p
Ceftazidime	65 (59.1)	7 (23.3)	0.001
Cefotaxime	54 (49.1)	6 (20.0)	0.006
Sulfamethoxazole	51 (46.4)	10 (33.3)	NS
Norfloxacin	45 (40.9)	4 (13.3)	0.005
Amikacin	21 (19.1)	2 (6.7)	NS
Nitrofurantoin	7 (6.4)	1 (3.3)	NS
Imipenem	0 (0)	0 (0)	NS

Data are presented as n(%).
UPEC = uropathogenic *Escherichia coli*; NS = not significant.

by running them on 1–2% agarose gels.

7. Statistical analysis

Comparison of gene frequencies in different groups was measured by using a two-tailed Fisher's exact test. Correlations between the presences of genes were measured by using Fisher's exact test (two-tailed). Chi-square and Fisher's exact tests were performed for the analysis of associations using Statistix 7.0 for Windows (Analytical Software, Tallahassee, FL, USA). Associations among genes were considered significant when p-values were < 0.05, in which case odds ratios and their 95% confidence intervals were calculated using the same software.

Results

1. Bacterial isolates and their epidemiological data

In total, 110 *E coli* isolates from patients with symptomatic UTIs (76.4% for cystitis and 23.6% for pyelonephritis) and 30 *E coli* isolates from stool samples of healthy women were enrolled in this study. The incidence of UTI in female patients in the age groups of 20–35, 36–50, and 51–65 years old was 44.5%, 24.5%, and 31%, respectively. Among the UPEC obtained, most of them (52.7%) were from inpatients, and were isolated from the following wards: urology 13.6%, women 11.8%, intensive care units (ICUs) 9.1%, infectious 7.3%, emergency 7.3%, and others 3.6%.

2. The prevalence of biofilm and hemolysis among the isolates

Eighty-five percent of the UPEC isolates had biofilm formation capacity. Among these isolates, 26%, 44%, and 30% of them were strong, moderate and weak biofilm producers. Half of the 30 commensal isolates could also be classed as weak biofilm producers.

Hemolysin activity was observed among 44.5% and 6.7% of the UPEC and commensal isolates, respectively; this difference was statistically significant (p < 0.001). Although hemolysin production was higher in pyelonephritis UPEC isolates than in cystitis isolates, the difference was not statistically significant (p > 0.05). In addition, UPEC isolates with biofilm-forming capacity showed significantly greater hemolysin activity (p < 0.05), while there was no significant difference between the prevalence of biofilm and hemolysin among the commensal isolates (p > 0.05).

3. Pattern of antimicrobial resistance among the isolates

Resistance patterns of UPEC and commensal isolates are presented in Table 2. The UPEC and commensal isolates showed maximum resistance to ceftazidime (59.1%), and trimethoprim-sulfamethoxazole (33.3%), respectively. Although antibiotic resistance among the UPEC isolates occurred at a higher rate than in the commensal isolates, only the resistance to ceftazidime, cefotaxime and norfloxacin was statistically significant (p < 0.05). Multi-drug resistance, which is defined as resistance to 3 or more classes or sub-classes of antibiotics [13], was most commonly observed in UPEC isolates (32.7%)

Table 3. Relationship between antimicrobial resistance and virulence factor genes in UPEC.

Virulence marker	Antibiotics (%R)					
	CAZ	CTX	SXT	NOR	AMK	NIT
Biofilm formation						
Strong	87.5	58.3	41.7	58.3	8.3	4.2
Moderate	58.5	51.2	36.6	46.3	26.8	7.3
Weak	50	39.3	42.9	32.1	14.3	3.6
Negative	41.2	47.1	82.4	17.6	23.5	11.8
p	0.01	NS	0.01	0.042	NS	NS
Hemolysin activity						
Positive	63.3	36.7	38.8	40.8	20.4	10.2
Negative	57.4	59	52.2	41	18	6.6
p	NS	0.023	NS	NS	NS	NS

UPEC = uropathogenic *Escherichia coli*; CAZ = ceftazidime; CTX = cefotaxime; SXT = trimethoprim -sulfamethoxazole; NOR = norfloxacin; AMK = amikacin; NIT = nitrofurantoin; NS = not significant.

compared with commensal isolates (6.6%).

Although the overall antibiotic resistance rate among the UPEC isolated from inpatients was higher than that observed in outpatients ($p = 0.033$), only resistance to ceftazidime and SXT was significantly more prevalent in the former group ($p = 0.001$ and 0.023, respectively). There was also a significant correlation between higher levels of resistance to amikacin ($p = 0.042$) in patients with pyelonephritis compared to those with cystitis.

4. The correlation between antibiotic resistance, biofilm and hemolysis formation

The relationship between antimicrobial resistance, biofilm and hemolysis production in UPEC is shown in Table 3. Among biofilm producer isolates, maximum resistance was observed to ceftazidime (63.4%) followed by cefotaxime (49.5%), norfloxacin (45.2%) and SXT (39.8%). There was a significant correlation between the intensity of biofilm formation and resistance to ceftazidime and norfloxacin ($p < 0.05$). Moreover, isolates that produced hemolysin were more sensitive to cefotaxime than were non-hemolysin producers. No relationship was found between resistance to antibiotics with biofilm formation and hemolysis activity in the commensal isolates ($p > 0.05$).

5. Prevalence of iron uptake genes among the tested isolates

The frequencies of the iron receptor genes are reported in Table 4, and ranged from 21.8% (*ireA*) to 84.5% (*chuA*) in the UPEC isolates. All the genes exhibited higher frequencies in the UPEC than in the commensal isolates, but no significant

Table 4. Distribution of iron acquisition genes among UPEC and commensal isolates.

Virulence gene	Prevalence of gene		p
	UPEC ($n = 110$)	Commensal ($n = 30$)	
fyuA	88 (80.0)	12 (40.0)	< 0.001
iroN	60 (54.5)	8 (26.7)	0.006
iutA	73 (66.4)	8 (26.7)	< 0.001
iha	52 (47.3)	9 (30.0)	NS
ireA	24 (21.8)	2 (6.7)	NS
chuA	93 (84.5)	10 (33.3)	< 0.001
hma	65 (59.1)	5 (16.7)	< 0.001

Data are presented as n (%) of isolates.
UPEC = uropathogenic *Escherichia coli*; NS = not significant.
p-values (by Fisher's exact test) are shown where $p < 0.05$.

difference was observed between the prevalence of iron genes *ireA* and *iha* in the UPEC and in commensal isolates.

As mentioned in Table 5, *fyuA*, *iutA* and *chuA* genes presented more frequently in cystitis and pyelonephritis isolates than in commensal isolates, while there was no significant difference in the prevalence of these genes among cystitis and pyelonephritis isolates ($p > 0.05$). The *iroN* and *iha* genes were significantly more frequent among the cystitis isolates than pyelonephritis and fecal isolates ($p < 0.05$). Furthermore, *hma* was more frequently associated with pyelonephritis than

Table 5. Comparison of the prevalence of iron genes among different groups of *Escherichia coli* strains.

VF	Prevalence of VFs				*p*		
	Total (*n* = 140)	Fecal (*n* = 30)	Cystitis (*n* = 84)	Pyelonephritis (*n* = 26)	Fecal vs. cystitis isolates	Fecal vs. pyelonephritis isolates	Cystitis vs. pyelonephritis isolates
fyuA	100 (71.4)	12 (40.0)	65 (77.4)	23 (88.5)	< 0.001	< 0.001	NS
iroN	68 (48.6)	8 (26.7)	51 (60.7)	9 (34.6)	0.002	NS	0.025
iutA	73 (52.1)	8 (26.7)	56 (66.7)	17 (65.4)	< 0.001	0.007	NS
iha	61 (43.6)	9 (30.0)	46 (54.8)	6 (23.1)	0.032	NS	0.006
ireA	26 (18.6)	2 (6.7)	19 (22.6)	5 (19.2)	NS	NS	NS
chuA	103 (73.6)	10 (33.3)	72 (85.7)	21 (80.8)	< 0.001	< 0.001	NS
hma	70 (50.0)	5 (16.7)	45 (53.6)	20 (76.9)	0.001	< 0.001	0.041

Data are presented as *n* (%) of isolates.
VF = virulence factor; NS = not significant.
p values (by Fisher's exact test) are shown where *p* < 0.05.

cystitis and fecal isolates (*p* < 0.05). *ireA* was the only iron receptor gene that was not found more frequently in cystitis or pyelonephritis cases compared with fecal isolates (*p* > 0.05) (Table 5).

6. Association between virulence iron genes in UPEC isolates

We performed pairwise comparisons of virulence iron genes against each other, and identified a wide variety of distinctive associations. We observed that *iroN* was associated positively with the presence of *iha* and *ireA*, and negatively with the presence of *fyuA*, *iutA*, *chu* and *hma* iron genes. We also found a negative association between the presence of the *iutA* gene and the *fyuA*, *iroN* and *iha* iron scavenging genes.

7. Relationship between the presence of iron genes and biofilm formation

The statistical analysis of biofilm formation and the presence of iron virulence genes are shown in Table 6. According to these results, biofilm production was significantly associated with the prevalence of *fyuA* and *hma* virulence genes (*p* = 0.006 and 0.014, respectively), but these findings were not observed for other iron genes. Our results also showed that *fyuA* and *ireA* had the highest and lowest prevalence among the strong biofilm producer isolates, respectively. In our study, there was no statistically significant correlation between the presence of iron genes and biofilm formation in commensal isolates (*p* > 0.05).

8. Association between antimicrobial resistance and virulence iron genes

Our data showed a significant correlation between resistance

Table 6. Relationship between the presence of iron genes and biofilm formation among UPEC.

Virulence gene	UPEC isolates (*n* = 110)		*p*
	Biofilm producers (*n* = 93)	Non-biofilm producers (*n* = 17)	
fyuA			0.006
Positive	89.8	10.2	
Negative	63.6	36.4	
chuA			NS
Positive	87.1	12.9	
Negative	70.6	29.4	
hma			0.014
Positive	92.3	7.7	
Negative	73.3	26.7	
iroN			NS
Positive	88.3	11.7	
Negative	80	20	
iutA			NS
Positive	84.9	15.1	
Negative	83.8	16.2	
ireA			NS
Positive	95.8	4.2	
Negative	81.4	18.6	
iha			NS
Positive	84.6	15.4	
Negative	84.5	15.5	

Data are presented as *n* (%) of isolates.
UPEC = uropathogenic *Escherichia coli*; NS = not significant.
p-values (by Fisher's exact test) are shown where *p* < 0.05.

to ceftazidime and cefotaxime with the presence of *fyuA* and *iutA* iron genes in the UPEC isolates ($p < 0.05$), while UPEC isolates harboring the *chuA* gene were more susceptible to SXT antibiotic ($p = 0.036$).

Discussion

Distinct UPEC strains use different virulence factors for pathogenicity; combined with variations in antibiotic resistance and biofilm production, this complicates the treatment of UPEC-driven UTIs [14]. Thus, development of novel therapeutic or prophylactic strategies against UPEC requires a more comprehensive understanding of virulence properties, antibiotic resistance, and their relationship with each other.

In the present study, biofilm formation was observed in the majority of UPEC isolates; this is consistent with the results of Sharma et al [15] and Fattahi et al [16]. In other studies, Soto et al [17] and Neupane et al [18] reported a lower prevalence of biofilm in the UPEC isolates. In agreement with our findings, Meshram et al [19] and Soto et al [17] demonstrated that biofilm formation was higher in the UPEC than in commensal strains. Among the UPEC, biofilm production was more frequently associated with isolates that cause cystitis when compared with isolates that cause pyelonephritis ($p = 0.002$); this suggests that biofilm formation also has a role in the adhesion and establishment of UPEC in the urinary tract of cystitis patients [20].

Our observation of higher hemolytic activity in UPEC than in fecal isolates is consistent with previous studies [21,22]. In our study, hemolysin production was more frequent in pyelonephritis than cystitis cases, indicating that hemolysin may be a virulence factor important for the pathogenesis of UPEC in pyelonephritis patients. The greater propensity for biofilm formation among hemolysin producing isolates is similar to the results of Soto et al [17] and in contrary to those in the study of Marhova et al [23].

Although the spectrum and frequency of antibiotic resistance among UPEC isolates is variable [24–26], most studies report that amikacin, nitrofurantoin and imipenem are highly efficacious against such strains [27–29]. Our study revealed co-resistance to third generation cephalosporins, quinolones, and aminoglycoside among UPEC isolates; this is similar to that of the study by Karlowsky et al [30], which also reported a high degree of co-resistance to ampicillin and SXT in UPEC isolates.

Similar to other investigations, different antibiotic resistance patterns were observed in UPEC compared with commensal isolates [31,32]. We found that the resistance rate of commensal isolates to third-generation cephalosporins and amikacin was higher than was observed in reports from other

countries [31–33]. The observed resistance in fecal *E coli* may be because of the extensive and long-term use of antibiotics [21,31,33]. Furthermore, the considerable number of MDR isolates among the UPEC found in Iran and other countries [28,34,35] may be the result of widespread use and misuse of antibiotics in hospitals and in the community [36].

In contrary to our findings and Saperston et al [37], others have reported higher antibiotic resistance in outpatients than in inpatients [25,38]. The antibiotic resistance in inpatients could be attributed to the higher rate of antibiotic therapy, which in turn creates selective pressure to evade antibiotic activity.

Similar to our results, previous studies found that biofilm-producing UPEC isolates had a higher degree of antibiotic resistance than non-biofilm producing isolates [39,40]. Insufficient antibiotic concentration and/or their delayed penetration into the deeper layers of biofilms are the major reasons for antibiotic resistance in biofilm structures [17,18]. Despite this, amikacin and nitrofurantoin showed high efficacy against biofilm producers, and could be considered as the antibiotics of choice for treatment of biofilm structures [40,41].

The prevalence of the majority of the iron receptor genes in UPEC is reportedly higher than that in commensal isolates [42,43]. Furthermore, and similar to our results, Alteri et al [42] and Bauer et al [44] found that novel non-hemagglutinin adhesion *iha* and iron-regulated element *ireA* (a siderophore receptor) were not more frequent in UPEC than in commensal isolates. In our study, yersiniabactin uptake receptor *fyuA* and heme receptor *chuA* were more frequently found in UPEC isolated from both cystitis and pyelonephritis patients. Thus, in accordance with another study [45], we suggest there is functional redundancy of *fyuA* and *chuA* in the UPEC isolates. We observed that the heme receptor, *hma*, was present more frequently in UPEC isolates from patients with pyelonephritis rather than those with cystitis; in this regard Alteri and Hagan also indicated that UPEC unable to produce *hma* have reduced fitness within the kidney during UTI [42,46]. Because the *iha* receptor confers adhesion properties, its higher prevalence in cystitis compared with pyelonephritis isolates may indicate a key role for adhesion in pathogenicity of UPEC isolates in patients with cystitis.

In accordance with our study, iron genes such as *fyuA* and *hma* (and neither aerobactin, *iutA* or *aer*, nor enterobactin genes) are reportedly upregulated in biofilm-producing UPEC [7,47]; this suggests that the iron receptors are particularly important for biofilm growth. Similar to others [48,49], we found that the presence of iron scavenger receptors is associated with antibiotic resistance. It is possible that simultaneous presence of virulence factors and resistance genes on resistance transferable elements such as integrons and conjugative plasmids increases the possibility of spreading

both virulence traits and antibiotic resistance via horizontal gene transfer [49]. Conversely, we found that susceptibility to antibiotics such as SXT was significantly associated with the presence of the *chuA* iron gene. We infer that UPEC isolates are subject to the "biological fitness cost" phenomenon, in which bacteria shut down expression of some non-essential genes in order to preserve energy [50].

In conclusion, we identified associations between different virulence factors (including the iron uptake receptors, biofilm and hemolysin production) and antibiotic resistance among the UPEC isolated from Iranian patients. These findings further our understanding of the role that these virulence factors play in the pathogenicity of UPEC isolates, which in turn could help in the development of treatment or preventive strategies against UTIs.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgments

References

[1] Brumbaugh AR, Mobley HL. Preventing urinary tract infection: progress toward an effective Escherichia coli vaccine. Expert Rev Vaccines 2012;11(6):663-76.
[2] Jan N, Meshram SU, Kulkarni A. Plasmid profile analysis of multidrug resistant E. coli isolated from UTI patients of Nagpur City, India. Rom Biotechnol Lett 2009;14(5):4635-40.
[3] Rodriguez-Siek KE, Giddings CW, Doetkott C, et al. Comparison of Escherichia coli isolates implicated in human urinary tract infection and avian colibacillosis. Microbiology 2005;151(Pt 6):2097-110.
[4] Caza M, Kronstad JW. Shared and distinct mechanisms of iron acquisition by bacterial and fungal pathogens of humans. Front Cell Infect Microbiol 2013;3:80.
[5] Garcia EC, Brumbaugh AR, Mobley HL. Redundancy and specificity of Escherichia coli iron acquisition systems during urinary tract infection. Infect Immun 2011;79(3):1225-35.
[6] Flores-Mireles AL, Walker JN, Caparon M, et al. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol 2015;13(5):269-84.
[7] Hancock V, Ferrières L, Klemm P. The ferric yersiniabactin uptake receptor FyuA is required for efficient biofilm formation by urinary tract infectious Escherichia coli in human urine. Microbiology 2008;154(Pt 1):167-75.
[8] Rashki A, Abdi HA, Shookohi M. Prevalence of genes encoding outer membrane virulence factors among fecal Escherichia coli isolates. Int J Basic Sci Med 2017;2(1):52-7.
[9] Khasheii B, Anvari S, Jamalli A. Frequency evaluation of genes encoding siderophores and the effects of different concentrations of Fe ions on growth rate of uropathogenic Escherichia coli. Iran J Microbiol 2016;8(6):359-65.
[10] O'Toole GA, Kolter R. Initiation of biofilm formation in Pseudomonas fluorescens WCS365 proceeds via multiple, convergent signalling pathways: a genetic analysis. Mol Microbiol 1998;28(3):449-61.
[11] Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; 25th informational supplement. CLSI Document M100-S25. Wayne (PA): CLSI; 2015.
[12] Asadi KM, Oloomi M, Habibi M, et al. Cloning of fimH and fliC and expression of the fusion protein FimH/FliC from Uropathogenic Escherichia coli (UPEC) isolated in Iran. Iran J Microbiol 2012;4(2):55- 62.
[13] Cantón R, Ruiz-Garbajosa P. Co-resistance: an opportunity for the bacteria and resistance genes. Curr Opin Pharmacol 2011;11(5):477-85.
[14] Osman KM, Mustafa AM, Elhariri M, Abdelhamed GS. Identification of serotypes and virulence markers of Escherichia coli isolated from human stool and urine samples in Egypt. Indian J Med Microbiol 2012;30(3):308-13.
[15] Sharma M, Aparna, Yadav S, Chaudhary U. Biofilm production in uropathogenic Escherichia coli. Indian J Pathol Microbiol 2009;52(2):294.
[16] Fattahi S, Kafil HS, Nahai MR, Asgharzadeh M, Nori R, Aghazadeh M. Relationship of biofilm formation and different virulence genes in uropathogenic Escherichia coli isolates from Northwest Iran. GMS Hyg Infect Control 2015;10:Doc11.
[17] Soto SM, Smithson A, Horcajada JP, Martinez JA, Mensa JP, Vila J. Implication of biofilm formation in the persistence of urinary tract infection caused by uropathogenic Escherichia coli. Clin Microbiol Infect 2006;12(10):1034- 6.
[18] Neupane S, Pant ND, Khatiwada S, et al. Correlation between biofilm formation and resistance toward different commonly used antibiotics along with extended spectrum beta lactamase production in uropathogenic Escherichia coli isolated from the patients suspected of urinary tract infections visiting Shree Birendra Hospital, Chhauni, Kathmandu, Nepal. Antimicrob Resist Infect Control 2016;5:5.
[19] Meshram L, Patidar RK, Khare M, et al. Comparative analysis between biofilm formation of commensal and pathogenic Escherichia coli isolates. Asiatic J Biotechnol Res 2012;3:1441-6.
[20] Rijavec M, Müller-Premru M, Zakotnik B, Zgur-Bertok D. Virulence factors and biofilm production among Escherichia coli strains causing bacteraemia of urinary tract origin. J Med Microbiol 2008;57(Pt 11):1329-34.
[21] Navidinia M, Peerayeh SN, Fallah F, Bakhshi B, Sajadinia RS. Phylogenetic grouping and pathotypic comparison of urine and fecal Escherichia coli isolates from children with urinary tract infection. Braz J Microbiol 2014;45(2):509-14.
[22] Kausar Y, Chunchanur SK, Nadagir SD, Halesh LH, Chandrasekhar MR. Virulence factors, serotypes and antimicrobial suspectibility pattern of Escherichia coli in urinary tract infections. Al Ameen J Med Sci 2009;2(1):47-51.
[23] Marhova M, Kostadinova S, Stoitsova S. Biofilm-forming capabilities of urinary Escherichia coli isolates. Biotechnol Biotechnol Equip 2010;24(suppl 1):589-93.
[24] Sedighi I, Arabestani MR, Rahimbakhsh A, Karimitabar Z, Alikhani MY. Dissemination of extended-spectrum β-lactamases and quinolone resistance genes among clinical isolates of uropathogenic Escherichia coli in children. Jundishapur J Microbiol 2015;8(7):e19184.
[25] Pourzare M, Derakhshan S, Roshani D. Distribution of uropathogenic virulence genes in Escherichia coli isolated from children with urinary tract infection in Sanandaj, Iran. Arch Pediatr Infect Dis 2017;5(3):e41995.
[26] Harwalkar A, Gupta S, Rao A, Srinivasa H. Lower prevalence of hlyD, papC and cnf-1 genes in ciprofloxacin-resistant uropathogenic Escherichia coli than their susceptible counterparts isolated from southern India. J Infect Public Health 2014;7(5):413-9.
[27] Mohajeri P, Darfarin G, Farahani A. Genotyping of ESBL producing uropathogenic Escherichia coli in west of Iran. Int J Microbiol 2014;2014:276941.
[28] Japoni A, Gudarzi M, Farshad S, et al. Assay for integrons and pattern of antibiotic resistance in clinical Escherichia coli strains by PCR-RFLP in Southern Iran. Jpn J Infect Dis 2008;61(1):85-8.
[29] Abujnah AA, Zorgani A, Sabri MA, El-Mohammady H, Khalek RA, Ghenghesh KS. Multidrug resistance and extended-spectrum beta-lactamases genes among Escherichia coli from patients with urinary tract infections in Northwestern Libya. Libyan J Med 2015;10:26412.
[30] Karlowsky JA, Kelly LJ, Thornsberry C, Jones ME, Sahm DF. Trends in antimicrobial resistance among urinary tract infection isolates of Escherichia coli from female outpatients in the United States. Antimicrob Agents Chemother 2002;46(8):2540-5.
[31] Li B, Zhao ZC, Wang MH, Huang XH, Pan YH, Cao YP. Antimicrobial resistance and integrons of commensal Escherichia coli strains from healthy humans in China. J Chemother 2014;26(3):190-2.
[32] Süzük S, Avcıküçük H, Kaşkatepe B, Aksaray S. Antibiotic susceptibility of microbiota members Escherichia coli strains isolated from stool samples

of patients attended Kırıkkale Yüksek İhtisas Hospital in ten months. Turk Hij Den Biyol Derg 2015;7(4)2:289-96.

[33] Vinué L, Sáenz Y, Somalo S, et al. Prevalence and diversity of integrons and associated resistance genes in faecal Escherichia coli isolates of healthy humans in Spain. J Antimicrob Chemother 2008;62(5): 934-7.

[34] Momtaz H, Karimian A, Madani M, et al. Uropathogenic Escherichia coli in Iran: serogroup distributions, virulence factors and antimicrobial resistance properties. Ann Clin Microbiol Antimicrob 2013;12:8.

[35] Mandal J, Acharya NS, Buddhapriya D, Parija SC. Antibiotic resistance pattern among common bacterial uropathogens with a special reference to ciprofloxacin resistant Escherichia coli. Indian J Med Res 2012;136(5):842-9.

[36] Gholipour A, Soleimani N, Shokri D, Mobasherizadeh S, Kardi M, Baradaran A. Phenotypic and molecular characterization of extended-spectrum β-lactamase produced by Escherichia coli, and Klebsiella pneumoniae isolates in an educational hospital. Jundishapur J Microbiol 2014;7(10):e11758.

[37] Saperston KN, Shapiro DJ, Hersh AL, Copp HL. A comparison of inpatient versus outpatient resistance patterns of pediatric urinary tract infection. J Urol 2014;191(5 Suppl):1608-13.

[38] Dehbanipour R, Rastaghi S, Sedighi M, Maleki N, Faghri J. High prevalence of multidrug-resistance uropathogenic Escherichia coli strains, Isfahan, Iran. J Nat Sci Biol Med 2016;7(1):22-6.

[39] Tadepalli S, Prudhivi S, Babu Myneni R, Rao S. Biofilm formation in uropathogenic Escherichia coli isolates and its association with extended spectrum betalactamase production and drug resistance. Saudi J Pathol Microbiol 2016;1(2):60-4.

[40] Makled AF, Salem EM, Elbrolosy AM. Biofilm formation and antimicrobial resistance pattern of uropathogenic E. coli: comparison of phenotypic and molecular methods. Egypt J Med Microbiol 2017;26: 37-45.

[41] Ghosh P, Saha S, Mitra G, et al. Evaluation of different virulence factors and antibiogram of uropathogenic Escherichia coli (UPEC) isolated in a tertiary care centre. Int J Med Dent Sci 2016;5(1):1027-32.

[42] Alteri CJ, Hagan EC, Sivick KE, Smith SN, Mobley HLT. Mucosal immunization with iron receptor antigens protects against urinary tract infection. PLoS Pathog 2009;5(9):e1000586.

[43] Toval F, Köhler CD, Vogel U, et al. Characterization of Escherichia coli isolates from hospital inpatients or outpatients with urinary tract infection. J Clin Microbiol 2014;52(2):407-18.

[44] Bauer RJ, Zhang L, Foxman B, et al. Molecular epidemiology of 3 putative virulence genes for Escherichia coli urinary tract infection-usp, iha, and iroN(E. coli). J Infect Dis 2002;185(10):1521-4.

[45] Spurbeck RR, Dinh PC Jr, Walk ST, et al. Escherichia coli isolates that carry vat, fyuA, chuA, and yfcV efficiently colonize the urinary tract. Infect Immun 2012;80(12):4115-22.

[46] Hagan EC, Mobley HL. Haem acquisition is facilitated by a novel receptor Hma and required by uropathogenic Escherichia coli for kidney infection. Mol Microbiol 2009;71(1):79-91.

[47] Kanamaru S, Kurazono H, Terai A, et al. Increased biofilm formation in Escherichia coli isolated from acute prostatitis. Int J Antimicrob Agents 2006;28(Suppl 1):S21-5.

[48] Lee S, Yu JK, Park K, Oh EJ, Kim SY, Park YJ. Phylogenetic groups and virulence factors in pathogenic and commensal strains of Escherichia coli and their association with blaCTX-M. Ann Clin Lab Sci 2010;40(4):361-7.

[49] Koczura R, Mokracka J, Barczak A, Krysiak N, Kaznowski A. Association between the presence of class 1 integrons, virulence genes, and phylogenetic groups of Escherichia coli isolates from river water. Microb Ecol 2013;65(1):84-90.

[50] Qin X, Hu F, Wu S, et al. Comparison of adhesin genes and antimicrobial susceptibilities between uropathogenic and intestinal commensal Escherichia coli strains. PLoS One 2013;8(4):e61169.