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Pathogenicity determinants and epidemiology of uropathogenic *E. coli* (UPEC) strains isolated from children with urinary tract infection (UTI) to define distinct pathotypes.

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Abstract

Background/purpose: One of the most common childhood diseases is Urinary Tract Infection (UTI). Without diagnosis and treatment, it can cause irreparable effects. *Escherichia coli* cause UTI in 75% of cases. Unlike diarrheagenic *E. coli* has certain pathotypes, *E. coli* causing UTI, are not well known. For further information, we considered pathogenicity determinants and epidemiology of uropathogenic *E. coli* (UPEC) strains isolated from children with Urinary Tract Infection (UTI) to define distinct pathotypes.

Methods: One hundred *E. coli* strains (50 UPEC and 50 commensal) isolated from children with UTI were examined. Some virulence factors and specific genes were examined by PCR method. Genetic diversity was evaluated by phylogenetic typing groups.

Results: Some pathogenicity determinants were more prevalent in UPEC strains rather than commensal *E. coli* strains, significantly. There were PAI IICFT073, PAI II J96, PAI I536, PAI ICFT073, PAIII536, PAI IV, gafD, focG, vat, usp, hlyD, sat, cnf1, picU, fliC(H7), kpsMTII, kpsMTIII. UPEC were mainly found in phylogenetic typing groups B2 and D, while in commensal isolates, phylogenetic groups A and D were the most common.

Conclusion: We need a simple pathotypes screening test which can be used either as or could be beneficial to facilitate along with other experiments in establishing an UTI assessment. Unfortunately, due to the high variation in pathogenicity determinants of UPEC strains, pathotypes could not be determined using genotype and virulence factors. Knowledge of the molecular details of UPEC is mainstay of successful strategies development for treatment of UTI and prevention of its subsequent complications.

Keywords: Pathogenicity determinants, Uropathogenic *E. coli* (UPEC), Pathotypes, Children, Urinary tract infection (UTI).

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Introduction

Urinary Tract Infection (UTI) is the most prevalent infectious diseases, and very problematic worldwide [1]. Uropathogenic *E. coli* (UPEC) which can colonize successfully in urinary tract is the primary etiologic agents associated with UTI. UPEC isolates express a wide range of virulence factors and specific genes. In fact, UPEC can exceed some types of host cells that comprise the stratified layers of the bladder urothelium, such as differentiated superficial facet cells, less mature intermediate, and basal epithelial cells. Invasion of host cell is facilitated both the establishment and permanence of UPEC within the urinary tract. Pathogenic extraintestinal *E. coli* isolates or ExPEC (such as UPEC) and commensal *E. coli* typically differ in phylogenetic group and virulent characteristics. According to previous studies, pathogenic ExPEC isolates belong to phylogenetic groups B2, and D. While, commensal *E. coli* strains belong to groups A and B1. In addition, pathogenic ExPEC isolates carry specialized pathogenic factors, i.e., traits that confer pathogenic potential, which are uncommon between

commensal isolates, UPEC strains and other *E. coli* strains are involved in various extraintestinal infections. These strains are distinguished from commensal strains by particular pathogenic factors such as adherence characters, toxins genes, and iron uptake systems. Bacterial adherence depending on the assembly in fimbrial projecting or afimbrial aggregates. The toxins related to ExPEC strains mostly display cytotoxic necrotizing factor and hemolysin, contribute to destruction of eukaryotic cells.

Siderophores give to the strains the advantage of obtaining iron from the ambience to exist and reproduce. Co-expression of virulence factors contributes to the host defense system defeat and onset of infection. Pathogenicity-Associated Islands (PAI) are codifying genes localized in distinctive area on the bacterial chromosome. In addition, ExPEC strains carry some virulence factors that are rarely between commensal *E. coli* isolates. Some of these virulence factors which is encoded by PAIs, preparing a mechanism for coordinated horizontal factors, such as properties that confer the ability to transfer pathogenic virulence genes. Hacker et al. first defined PAI character as mobile genetic elements in the late 1980s. These contain short direct repeats of fragments of DNA more than >10 kb nearby to the tRNA genes and comprise insertion sequences, integrases, and have a high percent G+C content that varies from the host bacterial. Particular sets of virulence factors are associated with UTI caused by *E. coli*. A variety of virulence genes are contributed by bacterial strains operating them by an individual pathogenesis process, are named a "pathotypes." Consideration of co-occurrence of potential UTI virulence factors between different *E. coli* isolates from commensal and urine collections provides documents for defining multiple pathotypes of UPEC, but recent understanding of critical genetic discrepancy to define the pathotypes, is limited. Finding of *E. coli* extra genes involved in uropathogenesis and consideration of their distribution will further describe UPEC pathotypes and permit to a more analysis of details of how these pathotypes might vary in how they cause infection [2-6]. For further information, we considered pathogenicity determinants and epidemiology of Uropathogenic *E. coli* (UPEC) strains isolated from children with Urinary tract Infection (UTI) to define distinct pathotypes.

Methods

UTI definition

Totally, 100 *E. coli* strains isolated from children presenting symptomatic UTI of both sex and different ages (2-12 y old) were hospitalized in the nephrology ward or were visited outpatient in Mofid Children Hospital, Tehran, Iran. Isolated *E. coli* colonies were recognized by standard bacteriological procedures. Commensal *E. coli* strains, including the well-characterized strains MG1655 and HS were used as controls. All distinct colonies were recognized morphologically and were stored for molecular examination, as described previously by Plos and Foxman. Classification of phylogenetic grouping of the *E. coli* strains was performed by PCR-based method using a combination of three DNA markers (chuA, yjaA, TspE4.C2) [7,8].

Detection of E. coli virulence determinants

All isolates were tested by PCR method for the existence of 31 bacterial genes related to UTI that as following as: phylogenetic typing groups genes; *chuA, yjaA, TspE4.C2*, adhesions groups; *afa, bmaE, fimH, gafD, focG*; protectin related genes; *kpsMT* (*K1*)*, kpsMTII, kpsMTIII, rfc* (*O4 LPS*), common toxins related to UPEC; *vat, usp, cvaC, hlyD, cdtB, sat, cnf1, picU* and, different PAI; PAI *IICFT073, PAI II536, PAI III536, PAI I536, PAI IV536, PAI ICFT073, PAI I J96, PAIII J96*, Miscellaneous genes; *fliC* (*H7*) *ibeA*, *ompT*. These pathogenic factors were part of a large set of virulence genes described previously by Johnson et al. Boiled whole-cell lysates 400 ml were used as DNA template and amplification was performed in a 25 µl reaction mixture containing 4 mM $MgCl_2$, 2.5 µl reaction buffer 10X, 2.5 U iTaq TM DNA polymerase (5 U/µl), 0.5 mM each primer and 5 µl DNA template. Reactions were performed in a Gene PCR System (Eppendorff). A reaction mixture without a DNA template was used as a negative control. Isolates producing a clear zone of hemolysis around colonies on sheep-blood agar, considered to be positive for hemolysin production [2,9].

Statistics

Continuous variables were expressed as mean ± Standard Deviation (SD). Discrete variables were reported as frequency and percentage. Chi square test and Fisher's exact test were used to access the relation between quantities' variables. For all statistical analyses, a p-value of <0.05 was considered to be significant. Statistical analysis was conducted using the SPSS version 21.

Results

Characteristics of patients

Total of 100 *E. coli* isolates were analysed. Of these, 50 were collected from midstream clean catch urine and 50 isolated from stool of the same patients who were in the nephrology ward in Mofid Children Hospital in Tehran.

Phylogenetic typing group distribution

The distribution of commensal *E. coli* isolates and UPEC isolates among the four phylogenetic groups is as following: Of the 50 commensal *E. coli* isolates, 44% fell into group A, 16% into B2, 32% into D, and UPEC isolates fell into group D, 54% into B2, 8% into A.

Pathogenicity island genes distribution

Distribution *PAI* genes, such as *PAI ICFT073* (74 *vs.* 26%), *PAI IICFT073* (38 *vs.* 14%), *PAI I536* (36 *vs.* 6%), *PAI IV536* (86 *vs.* 42%), *PAI II J96* (30 *vs.* 10%) were more frequent virulence markers in UPEC isolates than commensal *E. coli* and *PAI*

II536 (22 *vs.* 4%), *PAI III536* (6 *vs.* 0%), *PAI I J96* (4 *vs.* 0%) markers was almost similar in UPEC isolates and commensal *E. coli* (Table 1).

Adhesion genes distribution

Distribution of adhesion genes, such as *bmaE* (16 *vs.* 6%), *gafD* (20 *vs.* 2%), *focG* (22 *vs.* 6%) were more frequent virulence markers in UPEC than commensal *E. coli* and *afa* (6 *vs.* 10%), *fimH* (92 *vs.* 98%) markers were almost similar in both isolates (Table 2).

Toxin related genes distribution

Distribution of toxin genes, such as *vat* (96 *vs.* 4%), *usp* (54 *vs.* 6%), *hlyD* (26 *vs.* 2%), *cdtB* (18 *vs.* 10%), *sat* (44 *vs.* 8%), *cnf1* (26 *vs.* 0%), *picU* (42 *vs.* 2%) were more frequent virulence markers in UPEC isolates than commensal *E. coli*. *cvaC* (20 *vs.* 66%) was most frequent marker in commensal *E. coli* (Table 3).

Miscellaneous genes distribution

Distribution of miscellaneous genes, such as *fliC* (H7(26 *vs.* 10%)), *ompT* (62 *vs.* 58%) were more frequent virulence factors in UPEC than commensal *E. coli*, and *ibeA* (14 *vs.* 26%) was almost similar in both of them (Table 4).

Protectins genes distribution

Distribution of protectins genes, such as *kpsMTII* (70 *vs.* 46%) was more frequent virulence gene in UPEC rather than commensal *E. coli*. The prevalence of *kpsMTI* (K1) (46 *vs.* 54%), *kpsMTIII* (14 *vs.* 2%), *rfc* (O4 LPS) (6 *vs.* 2%), were almost similar in both of them (Table 5).

Table 1. *PAI distribution between commensal E. coli and UPEC in children with UTI in Iran.*

PAI genes	Commensal *E. coli*	UPEC	p-value
PAI III 536	0 (0%)	3 (6%)	0.324
PAI IV 536	21 (42%)	43 (86%)	<0.001
PAI II CFTO73	7 (14%)	19 (38%)	<0.001
PAI I 536	3 (6%)	18 (36%)	<0.001
PAI II 536	2 (4%)	11 (22%)	<0.001
PAI I J96	0 (0%)	2 (4%)	0.329
PAI II J96	5 (10%)	15 (30%)	0.001
PAI I CFTO73	13 (26%)	37 (74%)	<0.001

Table 2. *Adhesion genes distribution between commensal E. coli and UPEC in children with UTI in Iran.*

Adhesion genes	Commensal *E. coli*	UPEC	p-value
afa	5 (10%)	3 (6%)	0.298
bma E	3 (6%)	8 (16%)	0.007
fim H	49 (98%)	46 (92%)	0.534
gaf D	1 (2%)	10 (20%)	<0.001
foc G	3 (6%)	11 (22.0)	0.006

Table 3. *Toxin related genes distribution between commensal E. coli and UPEC in children with UTI in Iran.*

Toxin genes	Commensal *E. coli*	UPEC	p-value
cdtB	5 (10%)	9 (18%)	0.194
hlyD	1 (2%)	13 (26%)	0.001
cnf1	0 (0%)	13 (26%)	<0.001
cva C	33 (66%)	10 (20%)	<0.001
usp	3 (6%)	27 (54%)	<0.001
vat	2 (4%)	48 (96%)	<0.001
sat	4 (8%)	22 (44%)	<0.001
picU	1 (2%)	21 (42%)	<0.001

Table 4. *Miscellaneous genes distribution between UPEC and commensal E. coli in children with UTI in Iran.*

Miscellaneous genes	Commensal *E. coli*	UPEC	p-value
fliC H	5 (10%)	13 (26%)	0.020
ibeA	13 (26%)	7 (14%)	0.325
ompT	29 (58%)	31 (62%)	0.895

Table 5. *Protectins genes distribution between commensal E. coli and UPEC in children with UTI in Iran.*

Protectins genes	Commensal *E. coli*	UPEC	p-value
kps MTI	27 (54%)	23 (46%)	0.707
kps MT II	23 (46%)	35 (70%)	<0.001
kps MT III	1 (2%)	7 (14%)	0.083
rfc	1 (2%)	3 (6%)	0.700

Discussion

It is thought to the pathogenic *E. coli* strains are related to the presence of virulence factors. According to products called virulence factors, *E. coli* bacteria adhere selectively to the uroepithelial mucosa, promote colonization and persist in the urinary tract, induce a local inflammatory response, and sometimes promote tissue destruction. The result of a complex combination of special attributes of the *E. coli* causes to movement of a bacterium from the intestinal tract to the kidney and bladder. Our goal in this study was to pathogenicity determinants and epidemiology of Uropathogenic *E. coli* (UPEC) strains isolated from children with UTI to define distinct pathotypes. The phylogenetic groups' distribution

varies considerably among UPEC and commensal *E. coli* isolates [10].

Group A were the most prevalent phylogenetic group (41%) between the commensal *E. coli*, and that group B2 isolates were the least whereas in UPEC isolates, group B2 being the most prevalent (54%) and group B1 being least prevalent (4%). Our results were similar with those reported by Duriez et al. who detected that group B2 was seldom *E. coli* isolates (11%), whereas groups A and B1 were the most common (40% and 34%, respectively) [11].

Also, according to studies of Duriez et al. and Picard et al. group B2 has been reported with different prevalence in commensal *E. coli* isolates between 2-19% [11-14].

Some studies detected groups A and D in enteroaggregative *E. coli*. It is proven that other pathotypes such as enteropathogenice *E. coli* (EPEC) and enterotoxigeneic *E. coli* (ETEC) belong to phylogenetic groups A and B2 [15-19].

As the same like as Johnson study, we also identified that the phylogenetic group B2 were dominant in urine isolates. One of the main reasons due to being more virulence factors in UPEC compared to other groups [20].

A significant difference on phylogenetic groups B2 and D distribution between UPEC and commensal *E. coli* due to some reasons. Being additional alternative route for causing disease and infection and being specificity of bacterial pathogenesis are the most reason on these results. ExPEC especially UPEC mainly belong to the phylogenetic group B2 and D groups while commensal *E. coli* mostly belong to group A and B1 [21-27].

As we mentioned above, the most dominant phylogenetic groups in UPEC was B2 or D. Also, we identified phylogenetic group D rather than A in commensal *E. coli*. Previous studies have demonstrated that human health are potentially affected by UPEC strains, especially group B2 with virulence genes profiles, which in the face with a high proportion of commensal isolates existence in intestinal, these genes could be transferred them [28,29].

In contrast in Duriez study, it was recognized that group B was the most prevalent between both commensal and UPEC isolates, and recommended that the detected differences with respect to previous results could be impressed by the population and age selected for study. In the present research, children were 2-12 y old; therefore, the discrepancy between the two studies cannot be illustrated by the age selected. Duriez et al. studied females aged between 18-39 y old, which is an age range with a high occurrence of the proportion of UPEC asymptomatic carriers. It was interesting that the mean number of PAIs in isolates related to groups B2 and A was alike in both UPEC and commensal *E. coli*. In comparison, isolates related to group D significantly had PAIs rather than intestinal flora, but was non-significant between isolates related to group B1. In based on Duriez et al.'s study, they compared virulence factors and phylogenetic groups between commensal strains and recognized that commensal isolates related to groups A, B1 and D. They exposed fewer virulence factors rather than ExPEC strains [11].

Actually, that commensal *E. coli* related to group B2 are less virulent than strains isolated from clinical specimens, as observed in this study and previous studies, reinforce the hypothesis that it is mainly the most virulent isolates related to these groups in intestinal tract, can produce UTI and other infections. Some of virulence factor genes locate on genetic elements called on Pathogenicity Islands (PAI) in the vicinity of tRNA genes. In UPEC these islands are detected and have proven their role [30,31].

Two multiplex PCR for detection of 8 pathogenesis- related PAI in this study were used. The prevalence of PAI in UPEC was% 89 compared to commensal *E. coli* 38%. There are evidences that the intestinal environment have *E. coli* strains, mainly belong to B2 group which contain large numbers of PAI. *PAI IV536* island pathogenesis has been seen the most common PAI in both groups. This PAI is detected a lot in the Enterobacteriaceae family [18,32-36].

We were observed *PAI IV536* (also called HPI) 19% in commensal *E. coli* isolates compared to 43% in UPEC isolates. Being high prevalence of *PAI IV536* in isolates is led to the hypothesis that HPI is a structural island to be assumed as a pathogenesis island [37,38].

However, *in-vivo* experiments showed that HPI had important role in ExPEC strains. Similar with Middendorf et al. study, the high frequency of *PAI IV536* could be explained by PAI stability in *E. coli* [39].

Based on Bingen-Bidois study in 2002 on urosepsis producing *E. coli*, the frequency of PAIs was reported as follows: *PAI IV536* (92%), *PAI IIJ96* (24%), *PAI ICFT073* (19%), *PAI I536* (1%), but neither *PAI Ij96* nor *PAI II536* was observed, and *PAI IICFT073* studied *PAI III536* were not studied. Results were showed the high frequency of *PAI IV536* but low frequency of PAI I536 (19% *vs.* 73%), and higher frequency of *PAI IIJ96* (24%) compare to PAI ICFT073 (19%) [40]. They suggested that the role of *PAI II536* and *PAI IJ96* in urosepsis pathogenesis may not be important, and no difference was identified in distribution rate of *PAI II536* among *E. coli* isolates from patients with urosepsis or pyelonephritis. Dobrindt et al. reported that 64.5% of UPEC isolates and 39.3% of non-pathogenic *E. coli* had *PAI III536* and concluded that *PAI II536* was more common than *PAI I536, PAI III536* [41]. Middendorf et al. reported that *PAI II536* and *PAI III536* were very unstable and hence were easily lost and could be explained the difference between our findings and the findings of Dobrindt [39,41].

Similar with the other studies, PAIs numbers in UPEC and commensal isolates showed that the average number of PAI in isolates belonging B2 and A groups were the same. PAIs numbers in D group were significantly higher in commensal *E. coli*, but it in the case of B1 group was not significant. Similar PAI combination was seen in many isolates that had the same number of PAIs regardless of phylogenetic groups and their origin. Although, PAI acquisition is not a random phenomenon

and is carried out in a planned mechanism. It is proven that *PAI IV536* initially gains on chromosome and establishes to stable condition.

PAI IV536 is often seen alone in strains except group B2 but sometimes there are exceptions, and it also is detected along with *PAI ICFT073*. *PAI IICFT073* may be the third island, which gained followed by *PAI IIJ96* and *PAI I536*. The *PAI II536* and *III536* in the final stage earn. This fact explains why they are so unusual and can be seen in highly virulent strains. *PAI IIJ96* and *PAI IICFT073* compete on goal to replace on tRNAphe. The results indicate that *PAI IICFT073* have more affiliation compare to *PAI IIJ96* for the target. However, when commensal populations of *E. coli* and ExPEC consider based on their phylogenetic groups reveals more differences. Because *E. coli* isolated from feces mainly belong to B1 and A groups while the population of ExPEC often belong to B2 group and has a lot number of virulence factors. Therefore, it is unclear whether all *E. coli* isolated from the intestinal tract of healthy people in a specific time, regardless of phylogenetic groups become commensal or just isolates belonging to groups A and B1 are commensal.

Similarly, we don't know in the case of *E. coli* belongs to A and B1 groups isolated from patients with UTI should be considered as natural pathogens or commensal *E. coli* in a host with healthy immune system. These findings suggest that intestinal flora may act as a reservoir and can stimulate UTI by means of commensal *E. coli* with the B2 phylogenetic group.

The prevalence of *afa* gene in UPEC and commensal *E. coli* was 6%, 10% respectively. Gene prevalence in different studies was 2-11% due to the low prevalence of this gene in the *E. coli* strains [42-46]. fimH is common in *E. coli* strains. In fact, most clinical isolates both virulent and non-virulent can produce type 1 fimbriae. In epidemiological studies, there were no evidence of the relationship between type 1 fimbriae and severity of the infection. Several studies in experimental models indicated that type 1 fimbriae could have an effective role in the stability of *E. coli* in the urinary tract. Renovation of fimH-1 adhesion greatly reduces UPEC ability to colonize the urinary tract in human volunteers and mice. In this study, fimH prevalence has been reported 92% in UPEC, and 98% in commensal *E. coli* [47-49].

It is noteworthy that fimH adhesion in UPEC plays important role to connect and invade host cells and produce intracellular bacterial apartments (biofilm formation) [48]. According to genotyping study, fimH almost more than 90% of UPEC strains and pathogenic *E. coli* in birds, was reported [50].

In Johnson et al.'s study, gafD detected more than 20% of *E. coli* isolates with other virulence factors such as sfaS, focG, afa/dra, bmaE, gafD, cnf1, cdtB, cvaC, ibeA and most was frequently in relationship with phylogenetic group B2 which was agreement with our study. In this study, focG prevalence among UPEC, commensal *E. coli* was 22%, 6%, respectively, which was agreement with Johnsons findings [29].

In this study, 20% of strains belonged to phylogenetic group B2 gene had *hlyD* gene, while only in 4% of group A and 2% of B1 and 10% of group D, *hlyD* gene was detected. Forty two percent of UPEC isolates had hemolytic activity and 26% of gene carried *hlyD*. The reason due to be the low percentage of *hlyD* gene carriers strains (10%) belonged to group B2 [24].

We identified *cvaC* 20% and 66% among UPEC and commensal *E. coli*, respectively. Low prevalence of *cvaC* in UPEC strains suggests that this gene may be placed on non-ColV plasmid or PAI such as pTJ100. In Johnson study, based on bird pathogenic *E. coli* examination, they reported *hlyA* 41%, cnf1 16%, *cdtB* 8%, but about 28% of UPEC had *cnf1*. We reported *hlyD* (26%, 2%), *cnf1* (26%, 0%), *cdtB* (18%, 10%) in UPEC and commensal *E. coli*, respectively. This disagreement is caused by differences in study population [25,41,51].

By means of PCR method, *fimH, kpsM, hlyD, usp, cnf1, afa* were detected in UPEC strains, 92%, 46%, 26%, 54%, 26%, 6%, respectively. In this study, the prevalence of fimH in UPEC strains was high. These results demonstrate that type 1 fimbriae are important virulence factor. The type 1 fimbriae have been shown to enhance inflammation and played an important role in the pathogenesis of ascending UTIs.

Connell et al. reported that infected mice by strains 01: K1: H7 with present type 1 fimbriae had more stimulation of neutrophil cells rather than infected mice by type 1 negative strains. Our results showed that fimH was associated to P and S fimbriae in UPEC strains [52-56].

In a study conducted in Japan, *usp* gene was detected in 80% of 195 strains isolated from cystitis, and 93% of the 76 strains isolated from pyelonephritis [57]. According to different studies, *usp* has often been observed associated with pyelonephritis rather than cystitis. In a study conducted by Kanamaru, *usp* was identified in 22.2% of isolates. The difference between results due to differences in studied population (women) or between clones of bacterial isolated from women in Brazil and Japan [58,59].

usp gene is homologous with zonula occludens in *Vibrio cholera*. In a study conducted in Japan, *usp* was diagnosed in 54% of *E. coli* strains isolated from healthy subjects stool samples, 80% isolated from cystitis and 93% of strains pertaining to pyelonephritis [57].

Most of UPEC strains have capsule group 2 (K1, K5) are coded by the operon kps.Capsules in UPEC associated with pyelonephritis is very common [60]. In this study, UPEC strains contained approximately 70% *kpsMTII*. All isolates was observed in relation to other virulence factors in UPEC including *kpsMTII* and *hlyA* [29,58].

In first time *cdt* producing *E. coli* were observed in relation to children with enteritis [61]. In other studies, *cdt* gene was observed in strains isolated from urosepsis *E. coli* and fecal *E. coli* and in patients with various symptoms such as diarrhea, encephalopathy [29,62].

cdt gene cause irreversible inhibition of cell cycle at the G_2/M and produce single nuclear giant cells. The results suggest that cystitis can cause *cdtB* negative strains but this *E. coli* is less

virulent compare to strains cause pyelonephritis, urosepsis and diarrhea.

Specifically, we reported 96% vat in UPEC isolates compared to 4% in commensal *E. coli* strains. In Tiba et al.'s study, 39% of the isolates in bird's pathogen (APEC) revealed vat that these mainly related in the phylogenetic group D. In commensal strains and UPEC, pic was more common in phylogenetic groups B2. *sat* (44%) was more common in related to UPEC isolates and also, *picU* (42%) was observed in UPEC more than commensal *E. coli*, too [57]. Agreement with Timothy and Germon studies, ibeA observed in *E. coli* pathogens related to UTI and along with other numerous virulence factors more naturally associated to this infection [63,64].

In another study, *ibeA* was reported in about 26% of pathogenic *E. coli* strains isolated from chickens, and was not observed in non-pathogenic strains. Also, *ibeA* in APEC, *E. coli* isolated from vagina, and infant meningitis was observed (26%), (32%), (33-40%), respectively. So, we concluded that this factor significantly associated with pathogenic strains [17,64,65].

Compared with the study of Rodriguez-Siek, we reported 26% *fliC* (*H7*) in UPEC but they reported 4.8% in APEC. The difference between results due to differences in studied population. Similar to Zhao et al. *ompT* was reported 68% in our study [50].

Based on Rodriguez-Siek study in 2005, most of UPEC causing UTI in human presented capsules *kpsMT* (*K1*), *kpsMTII* genes. These genes identified rarely in APEC. So it has been concluded that these genes closely related to ExPEC strains such as UPEC. Specifically, capsular antigen K1 often has been observed in UPEC [65-67].

However, significant relationship between these factors and producing UTI cause this hypothesis that extraintestinal movement of bacteria after acquisition of some virulence factor and ability to ascending urinary tract is very important to cause disease. It is proven that these virulence factors present in strains cause meningitis rather than other types of *E. coli* [63].

Similar to Bert, *kpsMTII* have been observed significantly more compare with the other capsular genes. Even, *kpsMT III* and *rfc* (*O4LPS*) have been reported less than 10% of the cases. In Zhao et al.'s reports, *kpsMT* (*K1*) was involved in the synthesis of capsules in 45% APEC and UPEC [50,68].

The present study provides molecular and epidemiological information about virulence factor genes found in two groups of *E. coli*. It is necessary to have a simple pathotypes screening test which can be beneficial to facilitate along with other experiments in establishing an UTI assessment. Unfortunately, due to the high variation in pathogenicity determinants of UPEC strains, pathotypes could not be determined using pathogenicity determinants. Knowledge of the molecular details of UPEC is mainstay of successful strategies development for treatment of UTI and prevention of its subsequent complications.

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