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Original Article

Detection of virulence genes, phylogenetic group and antibiotic resistance of uropathogenic *Escherichia coli* in Mongolia

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Abstract

Introduction: The severity of urinary tract infection (UTI) produced by uropathogenic *Escherichia coli* (UPEC) is due to the expression of a wide spectrum of virulence genes. *E. coli* strains were divided into four phylogenetic groups (A, B1, B2 and D) based on their virulence genes. The present study aimed to assess the relationship between virulence genes, phylogenetic groups, and antibiotic resistance of UPEC.
Methodology: A total of 148 *E. coli* were tested for antimicrobial resistance against 10 drugs using the disk diffusion method. The isolates were screened by polymerase chain reaction (PCR) for detection of virulence genes and categorized into the four major phylogenetic groups.
Results: Phylogenetic group B2 was predominant (33.8%), followed by D (28.4%), A (19.6), and B1 (18.2%). A higher prevalence of *fimH* (89.9%), *fyuA* (70.3%), *traT* (66.2%), *iutA* (62.2%), *kpsMTII* (58.8%), and *aer* (56.1%) genes were found in UPEC, indicating a putative role of adhesins, iron acquisition systems, and protectins that are main cause of UTIs. The most common antibiotic resistance was to cephalotin (85.1%), ampicillin (78.4%) and the least to nitrofurantoin (5.4%) and imipenem (2%). In total, 93.9% of isolates were multidrug resistant (MDR).
Conclusions: This study showed that group B2 and D were the predominant phylogenetic groups and virulence-associated genes were mostly distributed in these groups. The virulence genes encoding components of adhesins, iron acquisition systems, and protectins were highly prevalent among antibiotic-resistant UPEC. Although the majority of strains are MDR, nitrofurantoin is the drug of choice for treatment of UTI patients in Ulaanbaatar.

Key words: uropathogenic *Escherichia coli*; virulence genes; antibiotic resistance; polymerase chain reaction; PCR; Mongolia.

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Introduction

The incidence of urinary tract infection (UTI) is estimated to be about 150–250 million cases worldwide [1]. UTIs are one of the inflammatory diseases produced by high multiplication of many pathogens in the urinary apparatus, resulting in alterations in the function of the urinary tract and kidneys [2]. UTI is particularly a major problem for females; nearly 50% of all women will experience at least one UTI in their lifetime and, of those, about 25% will have one or more recurrent infections [3]. In Mongolia, UTI status has not been described, but females between 20 and 40 years of age accounted for 60.3% of all patients with chronic pyelonephritis [4].

Strains of uropathogenic *Escherichia coli* (UPEC) are the primary cause of community-acquired UTIs (70%–95%) and a large portion of nosocomial UTIs (50%), accounting for substantial medical costs and morbidity worldwide [5,6]. The interaction between UPEC and epithelial cells is a multifactorial and complex phenomenon which involves several adhesins produced according to the stage of infection, while adherence to epithelial cells is essential for successful colonization and establishment. The expression of other genes encoding virulence factors contributes to disease severity [3,7]. The genes in the pathogenicity islands may also be virulence associated and encode a variety of different virulence factors, such as adherence factors (*e.g.*, type 1 and P fimbriae), toxins (*e.g.*, hemolysin and cytotoxic necrotizing factor), secretion systems, and siderophores (*e.g.*, aerobactin and yersinabactin) [8-10]. Some of the most important virulence genes of UPEC strains that are associated with severe UTIs are aerobactin (*aer*), P fimbriae (*pap*), type 1 fimbriae

(*fimH*), afimbrial adhesin I (*afaI*), hemolysin (*hly*), S fimbriae (*sfa*), adhesins, and fimbriae; however, other virulence genes such as *kpsMT*, *traT*, *iutA*, *cvaC*, *ibe*, *fyuA*, and *usp* are known to be involved in pathogenicity of this organism [11].

The number of multidrug-resistant strains of *E. coli* has progressively increased, causing treatment limitations [12,13]. Several studies have shown that antibiotic resistance in UPEC is increasing year by year [14-17]. Cephalosporins, fluoroquinolones, and trimethoprim-sulfamethoxazole are often used to treat community and hospital infections caused by *E. coli* and resistance to these agents is responsible for delays of inappropriate therapy with subsequently increasing morbidity and mortality [18]. Until the late 1990s, UPEC was relatively susceptible to first-line antibiotics; however, several surveillance studies during the 2000s across Europe and North and South America showed that between 20% and 45% of UPEC was resistant to first-line antibiotics including cephalosporins, fluoroquinolones, and trimethoprim-sulfamethoxazole [19]. The knowledge of drug resistance patterns in a geographical area and the formulation of an appropriate hospital antibiotic policy will go a long way in the control of these infections. Therefore, it is necessary to know the antibiotic susceptibility pattern of pathogenic *E. coli* to select the correct antibiotics for proper treatment of infections caused by it [20].

Phylogenetic analyses showed that *E. coli* strains were divided into four phylogenetic groups (A, B1, B2 and D) based on their genetic polymorphisms [21]. It is known that the expression of virulence genes and phylotypes varies with geographical location [17,22].

The present study aimed to describe the profile of UPEC from Mongolian women (or patients) with UTIs by the identification of virulence genes, phylogenetic group, and resistance to antibiotics.

Methodology

Bacterial strains

A total of 148 *E. coli* strains were isolated from the urine cultures of patients that presented to the bacteriological laboratory of the First Central Hospital and National Center for Communicable Diseases, Ulaanbaatar city, from July 2012 through April 2013. Identification of these strains was performed biochemically with the VITEK 2 Compact System and API 20E system (BioMerieux, Marcy-l'Etoile, France). Strains were stored at -20°C in skim milk with glycerol until they were used.

Antibiotic susceptibility testing

The disk diffusion method was used to determine antibiotic susceptibility of the isolates on Muller-Hinton agar (Difco, Franklin Lakes, USA). Each isolate was tested for antibiotic susceptibility using a panel of the following antibiotics: ampicillin (AMP) 10 μg, cefuroxime (CXM) 30 μg, gentamicin (GEN) 10 μg, nitrofurantoin (NIT) 300 μg, ciprofloxacin (CIP) 5 μg, imipenem (IPM) 10 μg, ceftazidime (CAZ) 30 μg, cephalotin (CEF) 30 μg, cefoxitin (FOX) 30 μg, and trimethoprim/sulfamethoxazole (SXT) 1.25/23.75 μg (BioLab, Budapest, Hungary). The plates were incubated at 37°C for 24 hours, and inhibitory zone diameters were measured. Interpretation of results followed criteria recommended by Clinical Laboratory Standard Institute (CLSI) [23].

DNA extraction

E. coli isolates were grown in Luria-Bertani agar (Difco, Franklin Lakes, USA) at 37°C overnight. Bacteria were resuspended in sterile distilled water and boiled at 95°C for 10 minutes. After centrifugation, the supernatants were stored as DNA template at -20°C until they were tested by polymerase chain reaction (PCR) [24].

PCR method to determine virulence genes

PCR was performed with the Accupower PCR Premix (Bioneer, Daejeon, South Korea) according to the manufacturer's instructions. Triplex PCR was used to identify 15 virulence genes of UPEC: *hlyA* (1177 bp), *kpsMTII* (272 bp), *ibeA* (170 bp), *cvaC* (680 bp), *traT* (290 bp), *papGII* (190 bp), *fimH* (508 bp), *iutA* (300 bp), *papC* (200 bp), *afa/draBC* (559 bp), *sfa/focDE* (410 bp), *papGIII* (258 bp), *usp* (1,000 bp), *fyuA* (880 bp) and *aer* (602 bp). PCR primer sequence for each virulence gene was taken from Johnson and Stell [25], Yun *et al*. [13], and Yamamoto *et al*. [26]. The PCR steps were as follows: initial denaturation at 95°C for 12 minutes, followed by 25 cycles of denaturation at 94°C for 30 seconds, annealing at 66°C for 30 seconds, extension at 68°C for 3 minutes, followed by a final 10-minute extension at 72°C. After amplification, the PCR products were separated by electrophoresis in a 2% agarose gel, stained in ethidium bromide solution, and visualized with a GelDoc 2000 gel documentation system (BioRad, Hercules, USA).

Phylogenetic analysis

The *E. coli* strains were categorized into the four major phylogenetic groups (A, B1, B2 and D) by triplex PCR following the protocol proposed by Clermont *et al*.

[21], using two virulence genes (*chuA*, encoding the heme transporter protein in *E. coli* O157:H7 and *yjaA*, initially identified in the genome of *E. coli* K-12) and one DNA fragment TspE4.C2 [21].

Statistical analysis

Statistical analysis was performed using Fisher's exact test and Chi-square test. The level of significance was set at $p < 0.05$.

Results

Phylogenetic analysis

Phylogenetic grouping was as follows: B2, 50 (33.8%) strains; D, 42 (28.4%) strains; A, 29 (19.6%) strains; and B1, 27 (18.2%) strains.

Virulence genes

Higher prevalence (above 50%) was observed for the *fimH*, *fyuA*, *traT*, *iutA*, *kpsMTII*, and *aer* genes (89.9%, 70.3%, 66.2%, 62.2%, 58.8%, and 56.1%, respectively). The *papC*, *papGII*, *afa/draBC*, *usp*, *sfa/focDE*, *hlyA*, *ibeA*, *papGIII*, and *cvaC* genes registered prevalence lower than 25% (20.3%, 17.6%, 15.5%, 12.8%, 8.8%, 8.1%, 4.7%, 1.4%, and 0.7%, respectively). Some isolates harbored the adhesive genes either singly or in combination. The prevalence of genes coding individually for fimbrial adhesions, *pap* and *sfa*, was 39.3% (58/148) and 8.8% (13/148), respectively. The prevalence of the *afa* gene coding for afimbrial adhesion was 15.5% (23/148). Six strains possessed both *pap* and *sfa* genes, while two isolates harbored both *pap* and *afa* genes. Table 1 shows the distribution of virulence genes with respect to the phylogenetic groups.

Most of the virulence genes were associated with the phylogenetic group B2. The *fimH*, *kpsMTII*, and *traT* genes were widely distributed among all groups (A: 93.1%, 48.2%, 62.1%; B1: 85.1%, 55.6%, 66.7%; B2: 96%, 68%, 72%; and D: 83.3%, 57.1%, 62%, respectively). The *cvaC* and *ibeA* genes were found only in the B2 group. The *papC*, *papGII*, *iutA*, *fyuA*, *aer*, *hlyA*, *ibeA*, and *usp* genes were positively associated with group B2. With respect to negative associations, *hlyA* and *sfa/focDE* were less prevalent in the D group, the *iutA* and *afa/draBC* genes were significantly negatively associated with the B group, and the *usp* gene was negatively associated with the A group.

Antibiotic resistance

The disk diffusion method indicated that antibiotic resistance was above 50% to CEF (85.1%), AMP (78.4%), and SXT (70.9%) in the UPEC strains. Sensitivity values above 50% were found to GEN (57.4%), CIP (58.1%), CAZ (66.2%), CXM (76.4%), FOX (93.9%), NIT (94.6%), and IPM (98%).

In this study 93.9% (139 cases) of isolates were considered multidrug resistant (MDR), and 45.9% (68 cases) of the investigated strains were resistant to at least seven of the examined antibiotics. Isolates susceptible to all studied antibiotics were not observed. Six isolates were resistant to all studied antibiotics. These strains were distributed among phylogenetic groups as follows: A, 2 strains; B1, 1 strain; D, 2 strains; and B2, 1 strain. Among these, five or six virulence

Table 1. Relationships among phylogenetic group and virulence genes.

Gene	Phylogenetic group (n, %)				Total
	A (n = 29)	B1 (n = 27)	B2 (n = 50)	D (n = 42)	
papC	7 (24.1%)	2 (7.4%)	16[a] (32%)	5 (11.9%)	30
papGII	6 (20.7%)	2 (7.4%)	14[a] (28%)	4 (9.5%)	26
papGIII	1 (3.4%)	0	1 (2%)	0	2
sfa/focDE	3 (10.3%)	4 (14.8%)	6 (12%)	0[b]	13
fimH	27 (93.1%)	23 (85.2%)	48 (96%)	35 (83.3%)	133
afa/draBC	7 (24.1%)	0[b]	8 (16%)	8 (19%)	23
iutA	19 (65.5%)	11[b] (40.7%)	39[a] (78%)	23 (54.8%)	92
fyuA	17 (58.6%)	16 (59.3%)	44[a] (88%)	27 (64.3%)	104
aer	13 (44.8%)	13 (48.1%)	35[a] (70%)	22 (52.4%)	83
kpsMTII	14 (48.3%)	15 (55.6%)	34 (68%)	24 (57.1%)	87
cvaC	0	0	1 (2%)	0	1
traT	18 (62.1%)	18 (66.7%)	36 (72%)	26 (61.9%)	98
hlyA	2 (6.9%)	2 (7.4%)	8[a] (16%)	0[b]	12
ibeA	0	0	7[a] (14%)	0	7
usp	0[b]	1 (3.7%)	15[a] (30%)	3 (7.1%)	19

[a]$P < 0.05$; [b] Negative association.

genes were often detected per strain. There was no significant difference in the phylogenetic group composition with respect to antibiotic resistance (Table 2).

Statistical analysis revealed the existence of the following associations: UPEC strains positive for *papC* and *papGII* genes were more resistant to AMP and SXT; for *kpsMTII* gene were more resistant to AMP; for *traT* gene were more resistant to CXM; for *usp* gene were more resistant to CIP than the isolates negative for these genes ($p < 0.05$) (Table 3).

Resistance to GEN, CIP, and CXM were significantly associated with all the genes encoding siderophore, aerobactin (*iutA*, *aer*). Furthermore, isolates positive for *sfa/focDE* genes were more sensitive to AMP than were isolates negative for *sfa/focDE* genes; isolates positive for *afa/draBC* genes were more sensitive to CIP than were isolates negative for *afa/draBC* genes.

Discussion

Cell morphology and molecular biology studies have revealed that UPEC express several surface structures and secrete protein molecules, some of which are cytotoxic, peculiar to the strains of *E. coli* causing UTIs [27]. In the present study, we aimed to detect the virulence genes, phylogenetic group, and antibiotic resistance of UPEC in Mongolia.

The distribution of virulence genes and the phylogenetic group are different among countries. For example, in Russia [28], UTI isolates belonged more often to phylogenetic group A. In Spain and the United States, lower percentages of phylogenetic group D were reported [7]. In the present study, the predominant

Table 2. Prevalence of resistance among various phylogenetic groups of uropathogenic *E. coli* isolates.

Antibiotics by glass	Antibiotics	Phylogenetic group (n, %)				Total
		A (n = 29)	B1 (n = 27)	B2 (n = 50)	D (n = 42)	
Aminoglycosides	GEN	13 (44.8%)	9 (33.3%)	22 (44%)	19(45.2%)	63
	AMP	23 (79.3%)	21 (77.8%)	36 (72%)	36(85.7%)	116
	CEF	21 (72.4%)	24 (88.9%)	45 (90%)	36 (85.7%)	126
Beta-lactams	FOX	4 (13.8%)	1 (3.7%)	2 (4%)	2(4.8%)	9
	CXM	5 (17.2%)	9 (33.3%)	6 (12%)	15 (35.7%)	35
	CAZ	7 (24.3%)	10 (37%)	16 (32%)	17 (40.5%)	50
Sulfonamides	SXT	17 (58.6%)	21 (77.8%)	34 (68%)	33 (78.6%)	105
Quinolones	CIP	10 (34.5%)	11 (40.7%)	21 (42%)	20 (47.6%)	62
Carbapenem	IPM	1 (3.4%)	1 (3.7%)	1 (2%)	0	3
Nitrofurans	NIT	4 (13.8%)	2 (7.4%)	0	2 (4.8%)	8

GEN: gentamicin; AMP: ampicillin; CEF: cephalotin; FOX: cefoxitin; CXM: cefuroxime; CAZ: ceftazidime; SXT: trimethoprim/sulfamethoxazole; CIP: ciprofloxacin; IPM: imipenem; NIT: nitrofurantoin.

Table 3. Association between virulence genes and antibiotic resistance.

Genes	Antibiotic resistance (n)									
	CEF	AMP	SXT	GEN	CIP	CAZ	CXM	FOX	NIT	IPM
papC	27	20	26[a]	12	8	8	4	2	0	2
papGII	25	16[a]	23[a]	12	7	7	4	1	0	2
papGIII	1	2	2	1	1	0	0	0	0	0
sfa/focDE	10	6[b]	8	6	4	2	1	2	0	2
fimH	114	103	93	59	57	48	33	8	8	2
afa/draBC	18	16	13	9	5[b]	6	6	0	1	0
iutA	79	72	69	46[a]	45[a]	36	27[a]	5	4	2
fyuA	91	84	76	48	48	39	28	5	5	2
aer	74	66	63	43[a]	42[a]	33	25[a]	4	4	2
kpsMTII	76	63[a]	60	32	36	27	20	6	4	2
cvaC	1	0	0	0	0	0	0	0	0	0
traT	84	77	71	44	40	36	28[a]	5	5	1
hlyA	11	7	10	4	3	4	2	1	0	1
ibeA	7	6	5	4	5	2	1	0	0	0
usp	17	16	16	12	14[a]	10	5	1	0	1

[a]$P < 0.05$; [b] Negative association; CEF: cephalotin; AMP: ampicillin; SXT: trimethoprim/sulfamethoxazole; GEN: gentamicin; CIP: ciprofloxacin; CAZ: ceftazidime; CXM: cefuroxime; FOX: cefoxitin; NIT: nitrofurantoin; IPM: imipenem.

phylogenetic group was B2 (33.8%), followed by D (28.4%), A (19.6%), and B1 (18.2%). Comparing to researchers' results from China, geographically located closest to our country, Luo *et al*. [29] and Cao *et al*. [30] reported the most common phylogenetic group in the UPEC isolates was B2 and D. Our results support these findings. Phylogenetic group A, associated with commensal strains, represented 19.6% of isolates, which was higher than in some studies [31,32], suggesting that the gastrointestinal tract is the main reservoir of strains that may be able to colonize the urinary tract, in accordance with previous observations.

UPEC strains encode a number of virulence genes that enable the bacteria to colonize the urinary tract and persist in the face of highly effective host defense [33]. Surface virulence factors of UPEC include a number of different types of adhesive factors, of which the presentation of adhesive molecules is the most important determinant of pathogenicity [33]. The percentage frequency of each gene was detected, and the *fimH* gene had the highest presence rate (89.9%), followed by *papC* (20.3%), *papGII* (17.6%), a*fa/draBC* (15.5%), *sfa/focDE* (8.8%), and *papGIII* (1.4%). Tiba *et al*. conducted a study on the genetics of virulence genes of pathogenic *E. coli* from patients with cystitis. The frequency of virulence genes *fimH, papC*, *sfa*, and *afa* was 97.5%, 32.7%, 27.8%, and 6.2%, respectively [34]. In another study by Usein *et al*., the prevalence rates of *fimH, sfa/foc, papC*, and *afa* in Romanian adults with UTI was 86%, 23%, 36%, and 14%, respectively [35]. The higher number of samples having *pap* and *sfa* genes together as compared with other combinations could be due to the localization of these genes on the same pathogenicity island of UPEC strains [36]. In our study, the combination of *pap/sfa* genes was more frequently detected than *pap/afa* genes, which is similar to previous studies [36,37].

Cellular morphology and molecular biology studies have revealed that UPEC express siderophore production peculiar to the strains of *E. coli* causing extraintestinal infections [38]. The majority of infectious *E. coli* strains possess multiple systems for ferric ion uptake, a relatively low affinity aerobactin system and two high affinity systems, yersiniabactin and enterobactin [39]. The prevalence of siderophore receptors were as follows: *iutA*, *fyuA*, and *aer* were present in 62.2%, 70.3%, and 56.1% of the samples, respectively. The expression of *iutA* and *aer* genes was significantly more prevalent in the GEN-, CIP-, and CXM-resistant *E. coli* strains than in these antibiotic-susceptible *E. coli* strains. Comparing across groups, the virulence genes were found in the strains of phylogenetic groups D and B1, and most frequently in group B2. Our results are confirmed in other studies [7,40].

In this study, we considered UPEC strains and their sensitivity patterns to different groups of antibiotics that are commonly administered to treat the infections. The studied UPEC isolates showed a high resistance to CEF (85.1%) and AMP (78.4%) and a high sensitivity to IPM (98%) and NIT (94.6%). The data from studies in India [41], Iran [42], and Mexico [43] also demonstrated the low resistance to NIT and high resistance to AMP. Considering the relative antibiotic resistance rate, NIT can be recommended for the treatment of UTIs. In Mongolia, fluoroquinolones and extended-spectrum cephalosporins are commonly used for treatment of UTIs and other infections. Therefore, AMP, CEF, and CIP resistance rates may be elevated due to the wide usage of these antibiotics. According to the findings of the current study, UPEC strains showed high sensitivity to NIT. Sensitivity to NIT may result from the lower frequency of the use of this drug. Fluoroquinolones, including CIP, are not recommended as first-line antibiotics for the treatment of UTIs, but they are generally used for empirical therapies. In the present study, 41.9% of the UPEC strains showed resistance to CIP. These results correspond to those of other studies conducted by López-Banda *et al*. [7] and Giray *et al*. [44], who reported 62.3% and 47% resistance in *E. coli* to CIP, respectively.

A high incidence of MDR strains was also detected among the present isolates. Of the UPEC strains, 93.9% demonstrated MDR phenotype and showed resistance to three or more of the tested antibiotics. Similar results were obtained from other studies [41]. The rate of MDR in UPEC isolates was 92.5% in India [41]. In another study on UTIs in Iran, the rate of MDR *E. coli* isolates was 82.1% [31]. MDR causes major consequences such as the empirical therapy of the *E. coli* infections, as well as a possible co-selection of antimicrobial resistance, which is mediated by the MDR plasmids [41].

Conclusions

In the present study, groups B2 and D were the predominant phylogenetic groups among UPEC strains in Mongolia, and virulence-associated genes were mostly distributed in these groups. The virulence genes encoding components of adhesins, iron acquisition systems, and protectins were highly prevalent among antibiotic-resistant UPEC. Although the majority of strains are MDR, nitrofurantoin is the drug of choice for treatment of UTI patients in Ulaanbaatar.

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