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Research Paper

Phylogenetic grouping and pathotypic comparison of urine and fecal *Escherichia coli* isolates from children with urinary tract infection

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Abstract

The aim of this study was to investigate the phylogenetic background and to assess *hlyD* (involved in the secretion of haemolysin A) and *intI1* (encoding a class 1 integrase) in *Escherichia coli* isolates derived from urinary and fecal specimens. A total of 200 *E. coli* isolates was collected from patients presenting with urinary tract infection (UTI) during September 2009 to September 2010 and screened for *hlyD* and *intI1* genes by polymerase chain reaction (PCR). Phylogenetic analysis showed that *E. coli* is composed of four main phylogenetic groups (A, B1, B2 and D) and that uropathogenic *E. coli* (UPEC) isolates mainly belong to groups B2 (54%) and D (34%) whereas group A (44%) and D (26%) are predominant among commensal *E. coli* isolates. In this study, *hlyD* was present in 26% of UPEC and 2% of commensal *E. coli* isolates. However, hemolytic activity was detected for 42% of UPEC and 6% of commensal *E. coli* isolates ($p < 0.05$). *intI1* gene was more frequently expressed in UPEC (24%) in comparison with commensal *E. coli* isolates (12%). Resistance to aztreonam, co-trimoxazole and cefpodoxime were frequently found among UPEC isolates whereas commensal *E. coli* isolates were commonly resistant to co-trimoxazole, nalidixic acid and cefotaxime. Concluding, a considerable difference between UPEC and commensal *E. coli* isolates was observed regarding their phylogenetic groups, presence of class 1 integron and *hlyD* gene, hemolysin activity and resistance pattern. The detection of class 1 integrons and *hlyD* gene was higher among UPEC compared with commensal *E. coli* isolates. These findings may contribute for a better understanding of the factors involved in the pathogenesis of UPEC.

Key words: *Escherichia coli*, urinary tract infection (UTI), phylogenetic typing groups, *hlyD*, *intI1*.

Introduction

Urinary tract infections (UTIs) currently rank among the most prevalent infectious diseases worldwide, with chronic and recurrent infections being especially problematic (Blango and Mulvey, 2010; Sabate *et al.*, 2006). The primary etiologic agents associated with UTIs are strains of uropathogenic *Escherichia coli* (UPEC) (Sivick and Mobley, 2010). Nonetheless, UPEC isolates express a wide spectrum of virulence and fitness factors that aid in successful colonization of the mammalian urinary tract (Manges *et al.*, 2004). Although often categorized as extracellular pathogens, UPEC can in fact invade a number of host cell types, including the terminally differentiated superficial facet cells and less mature intermediate and basal epithelial cells that comprise the stratified layers of the bladder urothelium. Host cell invasion is proposed to facilitate both the establishment and persistence of UPEC within the urinary tract (Johnson *et al.*, 2005; Mulvey *et al.*, 2000).

Extra-intestinal pathogenic and commensal *E. coli* typically differ in phylogenetic group and virulence attrib-

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utes. Previous studies have shown that pathogenic extraintestinal *E. coli* isolates primary belong to phylogenetic group B2 and, to a lesser extent, group D, whereas commensal *E. coli* isolates belong to groups A and B1. Moreover, pathogenic extraintestinal isolates harbour specialized virulence factors, *i.e.*, traits that confer pathogenic potential, which are infrequent among commensal isolates (Johnson *et al.*, 2001; Sabate *et al.*, 2006).

Currently, about 50 different cassettes associated with resistance genes, can be found in different classes of integrons. An integron is a two component gene capture and dissemination system, first discovered due to their rapid dissemination of antibiotic resistance, which can be found in plasmids, chromosomes and transposons.The first component consists of a gene encoding a site specific recombinase along with a specific site for recombination, while the second component comprises fragments of DNA called gene cassettes which can be incorporated or shuffled. A cassette may encode genes for antibiotic resistance, although most genes in integrons are uncharacterized. Integrons act as receptors of antibiotic resistance cassettes (Kovalevskaya, 2002).

Hemolysin is a cytolytic protein toxin secreted by most hemolytic *E. coli* isolates. In addition of lysing erythrocytes, hemolysin is a toxin for a wide range of host cells which may result in inflammation, tissue injury, and impaired host defenses. It should be mentioned that monocytes and granulocytes are highly susceptible to hemolysin cytotoxicity, whereas lymphocytes are relatively resistant. Exposure of polymorphonuclear leukocytes (PMNLs) to hemolysin stimulates degranulation and releases of leukotrienes accompanied by ATP; causes marked morphologic alterations; and impaired chemotaxis and phagocytosis (Johnson, 1991). Hemolysin production correlates closely with the toxicity of clinical *E. coli* isolates for PMNLs. Hemolysin stimulates superoxide anion and hydrogen peroxide release and oxygen consumption by renal tubular cells as well as histamine release from mast cells and basophils (Johnson, 1991).

The aim of this study was to determine the phylogenetic type of uropathogenic and commensal *E.coli*, isolated from patients with UTI in Mofid Childrens` Hospital, Tehran, Iran. In addition, the prevalence of hemolytic activity, and the assessment of *hlyD* gene (involved in hemolysin production) and of class 1 integron (a genetic element associated with antibiotic resistance) were also investigated, in order to provide additional information about *E.coli* virulence profiles.

Material and Methods

Specimens and patients

A total of 200 *E. coli* isolates were analyzed from 100 children patients of both sexes (85% female, 15% male) aged between 2-12 years with UTI (70% pyelonephritis, 30% cystitis). Of these, 100 were derived from midstream clean catch urine and 100 were from stool specimens of the patients presenting with community acquired UTI who have attended the nephrology ward of Mofid Childrens' Hospital, Tehran, Iran, during September 2009 to September 2010. The project was approved by the local Ethics Committee for Human Researches.

Samples were derived from fresh midstream urine, cultured (0.01 mL) on MacConkey agar (Sisco Research Laboratories Pvt. Ltd., USA) as well as Sheep blood agar and incubated at 37 °C for 24 h. Urine bacteria included in this study were from cultures yielding $> 10^5$ CFU/mL. Cultures with $< 10^5$ CFU/mL were further investigated only if relevant history of fever, chills, flank pain, pyuria, antibiotic intake, structural abnormalities, diabetes mellitus or any other immunocompromised state was present.

Specimens from stool samples were cultured on Trypticase soy agar (Kanto Chemical Co., Inc., Japan) with 5% sheep blood and MacConkey agar. The predominant isolate on each plate (one colony) and all morphologically distinct colonies were identified and stored for further analysis, as described by Plos (1995) and Foxman (2002). Two-three colonies, cultured on sheep blood as well as on MacConkey agar, from each stool and urine sample, were selected for molecular examination (Moreno *et al.*, 2006).

Antimicrobial susceptibility test

Susceptibility to nitrofurantoin (300 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), amoxicillin (10 µg), augmentin (30 µg), gentamicin (120µg), ceftazidime (30 µg), cefpodoxime (10 µg), aztreonam (30 µg), imipenem (10 µg), amikacin (30 µg), co-trimoxazole (25 µg) and cefotaxime (30 µg) were determined by disc diffusion assays (BBL Sensi-Disc, USA) modified by the Kirby-Bauer method using CLSI criteria (nonfastidious groupings M2-disk diffusion M100). For the purpose of analysis, intermediate susceptibility was considered as susceptible (Schlager *et al.*, 2002)

DNA extraction

DNA was extracted using the protocol described previously (Sabarinath *et al.*, 2011). The isolates were cultured on MacConkey agar plates for 24 h. One to two colonies were resuspended in 0.5 mL sterile distilled water. The cells were lysed by heating at 95 °C for 10 min and the supernatant was harvested by centrifugation at 12,000 rpm (8000 *g*) for 5 min. The supernatant was used as the source of the template DNA.

PCR amplification

Briefly, this consists of a 300 nM concentration of each oligonucleotide primer (BIO NEER, Takapouzist.co, AccuOligo^R, web: http://www.bioneer.com); 5.5 mM $MgCl_2$; 200 mM (each) deoxynucleoside triphosphates

dATP, dCTP, dGTP, and dUTP; and 0.125 U of *Taq* DNA polymerase (GENET BIO, Prime Taq ™ DNA polymerase, type:G-1002, URL:www.genetbio.com).

Phylogenetic typing group

Phylogenetic grouping of the *E. coli* isolates was determined by a simple, rapid PCR- based technique (Clermont *et al.*, 2000) that uses a combination of three DNA markers (*chuA*, *yjaA* and DNA fragment tspE4.C2), generating 279, 211 and 152-bp fragments, respectively. A triplex PCR was performed using the six primers in a single reaction. The results of these three amplifications allowed the classification of *E. coli* isolates into one of the major phylogenetic groups: A, B1, B2 or D. *E. coli* strain RS218, which belongs to phylogenetic group B2, was used as a control (Dhakal *et al.*, 2008).

Hemolytic activity and hlyD gene detection

E. coli isolates were inoculated on 5% sheep blood agar plates and incubated overnight at 37 °C. The plates were then examined for the presence of a partial or total hemolytic activity (alpha or beta) (Forbes *et al.*, 2007).

PCR was performed using the *hlyD* gene (904 bp) primers: F CTCCGGTACGTGAAAAGGAC: (Tm = 55.4 °C), R GCCCTGATTACTGAAGCCTG: (Tm = 55.7 °C) in a single reaction (Rodriguez-Siek *et al.*, 2005).

Class 1 integron detection

Isolates were analyzed by polymerase chain reaction (PCR) amplification techniques to determine whether a class 1 integron was present. Integrons were detected by PCR amplification of a class 1 integrase-specific fragment of the *intI1* gene. The primer sequences used were *intI1*-(F: GGTCAAGGATCTGGATTTGG, R: ACATGCGTGTAAATCATCGTC) in a single reaction. PCR assay was performed for cycles as follows: 1 cycle of 12 min at 94 °C; 35 cycles of 1 min at 94 °C, 1 min at 57 °C, 2 min at 72 °C; 1 cycle of 10 min at 72 °C (Lim *et al.*, 2009).

Statistical analysis

Statistical analysis was performed by using the Fisher exact and chi-square tests. The threshold for statistical significance was a p value of < 0.05.

Results

Pattern of antimicrobial resistance among *Escherichia coli* isolates

High-level resistance to azteronam (78%), co-trimoxazole (61%), cefpodoxime (48%) were found among UPEC while commensal E.coli isolates showed increased resistance to co-trimoxazole (82%), nalidixic acid (27%) and cefotaxime (27%). Resistance pattern of UPEC and commensal *E. coli* isolates were presented in Tables 1 and 2.

Table 1 - Antibiotic resistance pattern among phylogenetic groups in Uropathogenic *E.coli* isolates from children with community acquired UTI.

Antibiotic	A	B1	B2	D	Total
Augmentin	-	-	12%	2%	14%
Cefotaxime	3%	2%	39%	1%	45%
Co-trimoxazole	-	1%	50%	10%	61%
Imipenem	-	-	-	-	0%
Nitrofurantoin	-	-	2%	-	2%
Amoxicillin	-	-	16%	-	16%
Gentamicin	2%	-	19%	1%	22%
Ciprofloxacin	-	-	7%	1%	8%
Amikacin	1%	1%	5%	1%	8%
Ceftazidime	-	-	12%	-	12%
Cefpodoxime	2%	6%	38%	2%	48%
Nalidixic acid	-	-	9%	-	9%
Azteronam	1%	-	48%	29%	78%

Table 2 - Antibiotic resistance pattern among phylogenetic groups in commensal *E.coli* isolates from children with community acquired UTI.

Antibiotic	A	B1	B2	D	Total
Augmentin	-	-	7%	2%	9%
Cefotaxime	2%	-	16%	9%	27%
Co-trimoxazole	29%	14%	16%	23%	82%
Imipenem	-	-	-	-	0%
Nitrofurantoin	-	-	6%	2%	8%
Amoxicillin	9%	1%	8%	2%	20%
Gentamicin	-	-	1%	11%	12%
Ciprofloxacin	1%	-	1%	3%	5%
Amikacin	-	-	3%	-	3%
Ceftazidime	-	-	3%	4%	7%
Cefpodoxime	2%	1%	15%	1%	19%
Nalidixic acid	-	2%	16%	9%	27%
Azteronam	1%	1%	11%	6%	19%

Multi-drug resistance which was defined as resistance to 3 or more classes or sub-classes of antibiotics (Canton and Ruiz-Garbajosa, 2011), was most commonly observed in UPEC (38%) compared with commensal *E. coli* isolates (22%).

Phylogenetic typing groups

Phylogenetic groups A and D were commonly found among commensal *E. coli* isolates. However, UPEC isolates belonged to phylogenetic groups B2 and D, predomi-

nantly (Table 3). The results presented on Table 3 highlight a preliminary connection between pyelonephritis and phylogenetic group B2 ($p < 0.001$).

Distribution of hemolytic activity and hlyD in UPEC and commensal *E. coli* isolate

hlyD was detected in 26% of UPEC and 2% of commensal *E. coli* isolates, however, hemolytic activity was observed for 42% of UPEC and 6% of commensal *E. coli* isolates ($p < 0.05$) .

Distribution of intI1 in UPEC and commensal *E. coli* isolates

intI1 gene, which was significantly associated with pyelonephritis (22%) rather than cystitis (14%) ($p < 0.05$), was more frequently expressed in UPEC (24%) in comparison with commensal *E. coli* isolates (12%).

Discussion

UTI is usually treated empirically without culture but it contributes for about 10-15% prolongation of hospitalization due to the emergence of antimicrobial resistance among the causative bacteria, particularly UPEC isolates (Walter and Stamm, 2001). This may result in the spread of antibiotic resistant bacteria in the hospital and therefore, it has been suggested that more powerful antibiotics might better eliminate UPEC reservoirs and consequently reduce the incidence of chronic and recurrent UTIs among hospitalized and outpatients (Kaper *et al.*, 2004; Rodriguez-Siek *et al.*, 2005)

High incidence of co-trimoxazole resistance (61% for UPECs and 82% for commensal *E. coli* isolates) and of susceptibility to imipenem(100% for both UPEC and commensal *E. coli* isolates) were detected. These data are in agreement with the results of Farshad *et al.* (2008) for *E. coli* isolates obtained from children with community-acquired UTI. Thus, co-trimoxazole, which is a widely used for UTI treatment, has become nearly ineffective to treat UTI in this country.

In our study, different antibiotic resistance patterns were observed in UPEC compared with commensal isolates. Contrarily to the results of Alhaj *et al.* (2007), lower resistance percentages to nalidixic acid (9%), amoxicillin (16%) and gentamicin (22%) was found among UPEC compared with commensal *E.coli* isolates. Nevertheless, resistance rates to ceftazidime (12%) and augmentin (14%) among UPEC isolates were in agreement with the studies of Lim *et al.* (2009) with 47 nonrepeat *E. coli* isolates, collected from intensive care unit patients presented with UTI, in 5 public hospitals located in different areas of Malaysi. Consistent with Adegoke *et al.* (2011), our findings revealed that cefpodoxime and cefotaxime were less effective in UTI treatment than imipenem, nalidixic acid, ciprofloxacin, nitrofurantoin, augmentin and amikacin for all UPEC phylogenetic groups.

In a research by Moreno *et al.* (2006), *E. coli* isolates obtained from 150 patients presenting with acute uncomplicated cystitis, acute pyelonephritis and urinary-source bacteraemia, revealed 21% and 18% resistance to quinolones and fluoroquinolones, respectively. Recently, Shigemura *et al.* (2008) has reported the emergence of fluoroquinolone resistant *E. coli* responsible for UTI among patients attended at Kobe University Hospital, Japan. In those studies a higher resistance to quinolones (27%) than to fluoroquinolones (5%) was observed among commensal *E. coli* isolates. However, they found that resistance to the two mentioned antibiotic classes was nearly the same among UPEC (9% and 8% respectively).

It should be considered that, in our study, resistance to amikacin in UPEC (8%) and commensal *E. coli* isolates (3%) was relatively lower, considering the 27% reported in a research conducted in Colombia by Villegas *et al.* (2004) on *E. coli* isolates obtained from hospitalized patients, in a study covering 62.3% of all general hospital beds in that country.

As previously noted, class 1 integrons were more prevalent than those of class 2 (Johnson *et al.*, 1998; Muhammad *et al.*, 2011; Patti *et al.*, 2008). Similar to a research by Colgan *et al.* (2011), in our study *intI1* gene was more frequently detected among UPEC than commensal *E. coli* isolates, which may contribute for the occurrence and transmission of MDR among UPEC isolates. Our results also showed that group B2 is the most frequent *E. coli* phylogroup in UTI, as previously found (Johnson and Russo, 2002; Kovalevskaya, 2002; Mokady *et al.*, 2005). The UPEC isolates found in this studyprimarily belonged to one of two virulence groups (group B2 or D). Although a higher percentage of commensal isolates clustered into group A, a considerable proportion belonged to group D and this is

Table 3 - Phylogenetic groups distribution of UPEC and fecal *E.coli* strains in patients with UTI.

Phylogenetic groups	Number of isolates	Cystitis	Pyelonephritis
A			
Commensal	44%	20%	24%
UPEC	8%	6%	2%
B1			
Commensal	14%	4%	10%
UPEC	4%	4%	0%
B2			
Commensal	16%	0%	16%
UPEC	54%	4%	50%
D			
Commensal	26%	6%	20%
UPEC	34%	16%	18%

why a large proportion of commensal isolates were found to represent a potential human health threat, as well as the UPEC isolates (Burman *et al.*, 2003; Moulin-Schouleur *et al.*, 2006).

Thus, our data indicate that group B2 *E. coli* isolates are uncommon among commensal intestinal flora (16%); however, when present, they are highly virulent (Burman *et al.*, 2003; Moulin-Schouleur *et al.*, 2006). In this study, only 42% of UPEC isolates had hemolytic activity, 26% of which carried hlyD gene. The relatively low percentage of *hlyD* gene carriage rate, in the 100 UPEC isolates analyzed here, may be partially due to the relatively low percentage of B2 isolates (54%) detected in this study. Because B2 commensal *E. coli* isolates seem to have a privileged role in eliciting urinary tract infection, the intestinal normal flora would potentially act as a reservoir for developing UTI (Branger *et al.*, 2005). However, our findings challenge the "fecal urethral" pathway for the pathogenesis of UTI in children and instead support alternative routes of infection in this population (Johnson *et al.*, 2001a; Johnson *et al.*, 2001b).

Many studies have shown that urine isolates collectively differed dramatically from normal flora isolates with respect to phylogenetic background and virulence gene content profiles, suggesting an increased virulence potential for the urine isolates (Clermont *et al.*, 2000; Terai *et al.*, 2000; Vishalakshi, 2011). In fact, in our work, a considerable difference between UPEC and commensal *E. coli* isolates was observed regarding their phylogenetic groups, presence of class 1 integron, carriage of *hly D* gene, hemolysin activity and resistance pattern.

Thus, we can conclude that some UPEC with different phylogenetic characteristics and virulence profiles are multiple drug resistant (MDR) isolates which make them a serious, challenging health problem. However it is reasonable to suppose that UPEC and commensal *E. coli* isolates might have similar fitness properties for adapting to an extraintestinal lifestyle, which, in turns, enable commensal *E. coli* to cause extraintestinal disease in humans as well as UPEC. As previously mentioned, commensal *E. coli* may potentially serves as a source or reservoir of virulence genes for human pathogenesis. Further research will be necessary to determine if commensal *E. coli* isolates can actually overcome the hurdles necessary for human transmission through the urethral route.

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Conflict of interest

Authors have no conflict of interest.

References

Adegoke Anthony A, Okoh Anthony I (2011) Prevalence, antibiotic susceptibility profile and extended spectrum β-lactamase production among Escherichia coli from high vaginal swab (HVS). Afr J Pharm Pharacol 5:1287-1291.

Alhaj N, Mariana NS, Raha A, Ishak Z (2007) Prevalence of antibiotic resistance among *Escherichia coli* from different sources in Malaysia. Res J Pharmacolo 1:44-49.

Blango MG, Mulvey MA (2010) Persistence of uropathogenic *Escherichia coli* in the face of multiple antibiotics. Antimicrob Age Chemother 54:1855-1863.

Branger C, Zamfir O, Geoffroy S, Laurans G, Arlet G, Thien HV, Gouriou S, Picard B, Denamur E (2005) Genetic background of *Escherichia coli* and extended-spectrum beta-lactamase type. *Emerg Infect Dis* 11:54-61.

Burman WJ, Breese PE, Murray BE, Singh KY, Batal HA, Mackenzie TD, Ogle JW, Wilson ML, Reves RR, Mehler PS (2003) Conventional and molecular epidemiology of trimethoprim-sulfamethoxazole resistance among urinary *Escherichia coli* isolates. Am J Med 115:358-364.

Cantón R, Ruiz-Garbajosa P (2011) Co-resistance: an opportunity for the bacteria and resistance genes. Curr Opin Pharmacol 11:477-485.

Clermont O, Bonacorsi S, Bingen E (2000) Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl Environ Microbiol 66:4555-4558.

Colgan R, Williams M, Johnson JR (2011) Diagnosis and treatment of acute pyelonephritis in women. Am Fam Physician 84:519-526.

Dhakal BK, Kulesus RR, Mulvey MA (2008) Mechanisms and consequences of bladder cell invasion by uropathogenic *Escherichia coli*. Eur J Clin Invest 38:2-11.

Farshad SH, Japoni A, Hosseini M (2008) Low distribution of integrons among multidrug resistant *E.coli* strains isolated from children with community -acquired urinary tract infections in Shiraz, Iran. Pol j microbiol 57:193-198.

Forbes BA, Sahm DF, Weissfeld AS (2007) Bailey & Scott's Diagnostic Microbiology, Mosby Press.

Foxman B, Manning SD, Tallman P, Bauer R, Zhang L, Koopman JS, Gillespie B, Sobel JD, Marrs KF (2002) Uropathogenic *Escherichia coli* Are More Likely than commensal *E. coli* to be shared between heterosexual sex partners. Am J Epidemiol 156:1133-1140.

Johnson DE, Lockatell CV, Russell RG, Hebel JR, Island MD, Stapleton A, Stamm WE, Warren JW (1998) Comparison of *Escherichia coli* strains recovered from human cystitis and pyelonephritis infections in transurethrally challenged mice. Infect Immun 66:3059-3065.

Johnson JR (1991) Virulence Factors in *Escherichia coli* Urinary Tract Infection. Clin Microbiol Rev 4:80-128.

Johnson JR, Delavari P, Kuskowski M, Stell AL (2001) Phylogenetic distribution of extraintestinal virulence-associated traits in *Escherichia coli*. J Infect Dis 183:78-88.

Johnson JR, Stell A, Delavari P (2001) Canine feces as a reservoir of extraintestinal pathogenic *Escherichia coli*. Infect Immune 69:1306-14.

Johnson JR, Stell AL, Delavari P, Murray AC, Kuskowski M, Gaastra W (2001) Phylogenetic and pathotypic similarities between *Escherichia coli* isolates from urinary tract infec-

tions in dogs and extraintestinal infections in humans. J Infect Dis 183:897-906.

Johnson JR, O'Bryan TT, Sandberg T (2005) Phylogenetic and pathotypic comparison of concurrent urine and rectal *Escherichia coli isolates* from men with febrile urinary tract infection. J Clin Microbiol 43:3895-3900.

Johnson JR, Russo TA (2002) Uropathogenic *Escherichia coli* as agents of diverse non- urinary tract extraintestinal infections. J Infect Dis 186:859-864.

Kaper JB, Nataro JP, Mobley HL (2004) Pathogenic Escherichia coli. Nat Rev Microbiol 2:123-140.

Kovalevskaya NP (2002) Mobile Gene Cassettes and Integrons. Mol Biolo 36:196-201.

Lim KT, Yasin R, Yeo CC, Puthucheary S, Thong KL (2009) Characterization of Multidrug Resistant ESBL-Producing *Escherichia coli* Isolates from Hospitals in Malaysia. J Biomed Biotechno 2009:2009:165637.

Manges AR, Dietrich PS, Riley LW (2004) Multidrug-resistant *Escherichia coli* clonal groups causing community-acquired pyelonephritis. Clin Infec Dis 38:329-334.

Mokady D, Gophna U, Ron EZ (2005) Extensive gene diversity in septicemic *Escherichia coli* strains. J Clin Microbiol 43:66-73.

Moreno E, Prats G, Sabate M, Perez T, Johnson JR, Andreu A (2006) Quinolone, fluoroquinolone and trimethoprim sulfamethoxazole resistance in relation to virulence determinants and phylogenetic background among uropathogenic Escherichia coli. J Antimicrob Chemother 57:204-211.

Moreno E, Andreu A, Perez T, Sabatem M, Johnson JR, Prats G (2006) Relationship between *Escherichia coli* strains causing urinary tract infection in women and the dominant faecal flora of the same hosts. Epidemiol Infec 134:1015-1023.

Moulin-Schouleur M, Schouler C, Tailliez P, Kao MR, Bree A, Germon P, Oswald E, Mainil J, Blanco M, Blanco J (2006) Common virulence factors and genetic relationships between O18: K1: H7 *Escherichia coli* isolates of human and avian origin. J Clin Microbiol 44:3484-3492.

Muhammad I, Uzma M, Yasmin B, Mehmood Q, Habib B (2011) Prevalence of antimicrobial resistance and integrons in *Escherichia Coli* from Punjab, Pakistan. Braz J Microbiol 42:462-466.

Mulvey MA, Schilling JD, Martinez JJ, Hultgren SJ (2000) Bad bugs and beleaguered bladders: interplay between uropathogenic *Escherichia coli* and innate host defenses. Proc Nat Acad Sci USA 97:8829-8835.

Patti G, Mannini A, Balistreri M, Schito AM (2008) Virulence factors in urinary *Escherichia coli* strains: phylogenetic background and quinolone and fluoroquinolone resistance. J Clin Microbiol 46:480-487.

Plos K, Connel H, Jodal U (1995) Intestinal carriage of *P* fimbriated *Escherichia coli* and the susceptibility to urinary tract infection in young children. J Infect Dis 171:625-631.

Rodriguez-Siek KE, Giddings CW, Doetkott C, Johnson TJ, Fakhr MK, Nolan LK (2005) Comparison of Escherichia coli isolates implicated in human urinary tract infection and avian colibacillosis. Microbiol 151:2097-2110.

Rodriguez-Siek KE, Giddings CW, Doetkott C, Johnson TJ, Nolan LK (2005) Characterizing the APEC pathotype. Vet Res 36:241-256.

Sabarinath A, Tiwari KP, Deallie C, Belot G, Vanpee G, Matthew V, Sharma R, Hariharan H (2011) Antimicrobial Resistance and Phylogenetic Groups of Commensal *Escherichia Coli* Isolates from Healthy Pigs in Grenada. Web Med Central 42:1-10.

Sabate M, Moreno E, Perez T, Andreu A, Prats G (2006) Pathogenicity island markers in commensal and uropathogenic *Escherichia coli* isolates. Clin Microbiol Infect 12:880-886.

Schlager TA, Hendley JO, Bell AL , Whittam TS (2002) Clonal Diversity of *Escherichia coli* Colonizing Stools and Urinary Tracts of Young Girls. Infect Immun. 70:1225-9.

Shigemura K, Arakawa S, Miura T, Nakano Y, Tanaka K, Fujisawa M (2008) Significance of fluoroquinolone resistant *Escherichia coli* in urinary tract infections. Jpn J Infect Dis 61:226-228.

Sivick KE, Mobley HLT (2010) Waging war against uropathogenic *Escherichia coli*: winning back the urinary tract. Infect Immun 78:568-585.

Terai A, Ishitoya S, Mitsumori K, Ogawa O (2000) Molecular epidemiological evidence for ascending urethral infection in acute bacterial prostatitis. J Urol 164:1945-1947.

Villegas MV, Correa A, Perez F, Miranda MC, Zuluaga T, Quinn JP (2004) Prevalence and characterization of extended-spectrumβ-lactamases in *Klebsiella pneumoniae* and *Escherichia coli* isolates from Colombian hospitals. Diag Microbiol Infect Dis 49:217-222.

Vishalakshi B (2011) Detection of Virulence Markers of Uropathogenic Escherichia coli from Urinary Tract Infection, Karnataka, Banglore, 125 p (Department of Microbiology Mysore Medical College and Research Institute).

Walter E, Stamm MD (2001) An epidemic of urinary tract infections. N Eng J Med 345:1055-1057.