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ORIGINAL ARTICLE

Typing of the uropathogenic E.coli strains using O-serotyping and detection of pap adhesion-encoding operon by polymerase chain reaction

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ABSTRACT

Background: Urinary tract infection (UTI) is a frequently diagnosed renal and urologic disease. Escherichia coli is by far the most common etiologic agent of this disease. This study was aimed to type the E.coli strains isolated from the patients with urinary tract infection using sero-grouping. Detection of pap adhesion-encoding operon was also targeted.

Patients and methods: A total of 130 E. coli strains isolated from patients with UTI were investigated for O-serotyping. The presence of pap adhesion-encoding operon was detected using polymerase chain reaction (PCR).

Results: In serogrouping with 13 antisera, 86 strains (66.14%) were O-serogroupable and belonging to O1, O6, O15, O18 & O20 serogroups, while 44 strains (33.86%) were O-non typeable. Predominant serogroups were O6 and O18. The PCR results showed that 61% of strains exhibited the pap genotype. Serogroups O1, O6, O15 and O18 possessed pap operon. There was an obvious correlation between the pap operon and the O-serogroups of the strains.

Conclusion: Our results showed that obtained protein patterns of the isolated strains were more reliable than serotyping results for typing purposes. Our findings indicated that pap adhesion–encoding operon has an important role in the development and severity of UTI. Many cases of serious urogenital diseases are caused by a limited number of uropathogenic E.coli strains that generally possess special virulence factors such as pap operon.

Keywords: *Urinary tract infection, E. coli, Polymerase chain reaction, O-Serotyping, pap adhesion-encoding operon.*

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INTRODUCTION

Urinary tract infections (UTIs) are among the most common infectious diseases encountered in the clinical practice, mainly being associated with different members of the Enterobacteriaceae family (1-4). In fact, Escherichia coli (E. coli) are the most predominant pathogen causing UTIs (3). E.coli, the most prevalent facultative gram-negative bacillus in the human fecal flora, usually inhabits the colon as an innocuous commensal (5). In general, rates of UTIs are higher among women than men (3,6). Urinary infection most commonly occurs in patients with anatomically and functionally normal urinary tracts and involves spontaneous ascent of bacteria from the urethra to the bladder and to the

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kidneys and bloodstream (1,3,7). Frequent sexual intercourse may cause trauma as well as move vaginal or bowel bacteria to the urethral opening, facilitating ascent to the bladder. Sexual activity may also transmit uropathogens (7). Among the factors of pathogenicity that commonly expressed by these ascending uropathogenic E.coli strains, adherence to uroepithelial cells seems to be very important (1-5). Adhesion to the uroepithelium may protect the bacteria from urinary lavage, increasing their ability to multiply and invade renal tissue (3-5,8,9). Specific adhesion is mediated by bacterial proteins designated adhesins which may or may not be associated with fimbriae, they can be differentiated on the basis of their binding receptor specificity (1,10).

Some of the recognized E.coli virulence factors are adherence to uroepithelial cells, certain O and K serotypes, hemolysin production, resistance to the bactericidal effect of serum, the ability to adhere to human uroepithelial cells, and aerobactin production (3,5,8,11-15). A specific family of glycolipids, the globoseries, was shown to act as receptors on human uroepithelial cells and erythrocytes for the majority of uropathogenic E. coli strains attaching to or hemagglutinating those cells (16). P fimbriae are the most important mannose–resistant adhesion expressed by E.coli causing extra-intestinal infections. The pap (pyelonephritis–associated pilus) operon encodes P fimbriae, which mediate galactosylgalactose (α-D-galactose -(β 1-4) β-D-galactose) specific adherence to host epithelial surfaces in the intestine, vagina, urinary tract and moiety of the P blood group antigens via their tip adhesion molecule PapG. PapG occurs in three molecular variants, encoded by alleles I-III of the corresponding gene papG (3-5,12,13,17,18).

P-fimbriae are known to occur in association with a limited number of E.coli serotypes (4,5,9,12,19,20). The most frequent serogroups of the P-fimbriated strains belonged to one of the six serogroups O1,O2,O4,O6,O7,O18 (20). E.coli strains that cause UTI belong to a limited number of serogroups mainly O1, O2, O4, O6, O7, O14, O15, O18, O22 and O75 (5,8,19-22).

In this study, we examined the presence of pap adhesion-encoding operon by polymerase chain reaction (PCR) in a significant number of E.coli strains isolated from patients with UTI. The correlation between the presence of pap operon and O-serogroups was also analyzed.

PATIENTS and METHODS

A total of 130 E.coli strains were isolated from patients with UTIs symptoms (97 women and 33 men) who referred to Sina and Ekbatan hospitals in Hamadan (West of Iran). They aged 1-70 years. Bacterial count was more than 105 per ml in freshly voided midstream urine samples.

Serogrouping: All of the isolates that were identified as E.coli by biochemical tests were stored in BHI agar at -20°C and subcultured on Tryptic Soy Agar (TSA). Isolated E.coli were serotyped using 13 different monovalent O-antisera (Eurobio, France). The antisera were selected on the basis of their reported association with pathogenic E.coli that causes infection in human. The O-antisera (13 types) used in our study were: O1, O6, O8, O15, O18, O20, O25, O55, O78, O86, O111, O119 and O125. Five O-antisera were belonged to EPEC strains (O20, O55, O111, O119 and O125), however, the remaining belonged to UPEC strains. Serotyping was performed on glass slide and confirmed. Strains that did not show agglutination with any of the 13 used O-antisera was defined as O non- typed (Nt) (5,8,23).

Preparation of bacterial DNA: Bacteria were grown in Luria broth medium without glucose (10g of tryptone, 5g of yeast extract and 5g NaCl per lit, pH=7) for 18 hours at 37°C (1,9). DNA to be amplified was released from whole organisms by boiling. Bacteria were harvested from 1 ml of an overnight broth cultures, suspended in 200µl of

sterile water and incubated at 100°C for 10 minutes. Following centrifugation of the lysate, a 150µl aliquot of the supernatant was stored at -20°C as a stock template DNA (1,9). E.coli strain HB101 was used as a negative control (absence of adhesion) and E.coli J96 carrying at least three separate adhesion-encoding operons (pap- prs and foc) was used as a positive control (adhesion presence) (1).

Primer sequence determination for PCR: Primer was derived from the published sequence of papC gene. Oligonucleotiedes pap1 and pap2 flanked the 313 bp pstI internal fragment of pap C and targeted a 328 bp DNA segment (1,3,9). The presence of the papC gene was established using the conditions described by Yamamoto et al (24). Their sequences were as follows:

pap1: 5`- GACGGCTGTACTGCAGGGTGTGGCG – 3`

pap2: 5`- ATATCCTTTCTGCAGGGATGCAATA – 3`

Amplification procedure: PCR was carried out in a total volume of 50µl containing 10µl of the template DNA ,each of the primers at 0.5µM , the four deoxynucleoside triphosphates (each at 200µM) , 10mM Tris hydrochloride (pH=8.3) ,1.5mM $MgCl_2$, 50mM KCl, 0.01% gelatin and 1.2U of Ampli Taq DNA polymerase (1,9). The reaction mixture was overlaid with 3 drops of mineral oil. PCR amplifications consisted of 25 cycles of denaturation at 94°C for 2 minutes, annealing at 57°C for 1 minute and extension at 72°C for 2 minutes in a thermal cycler (Ependroph). Ten microliters of the reaction mixture was then analyzed by electrophoresis on 2% agarose gel and the reaction products were visualized by staining with ethidium bromide (1,9).

RESULTS

The distribution of E.coli serogroups among 130 studied strains is summarized in Table 1. Altogether, 86 strains (66.14%) were grouped with 13 O-antisera, and 44 strains (33.86%) were non-typable. Serogroup O6 was the most frequently identified (23.8%), followed by O18 serogroup (12.3%) and O15 (6.9%). Totally, these three predominant serogroups comprised 43.0% (n=56) of all E.coli strains.

In this study, most of the uropathogenic O1 (6/6), O15 (8/9), O6 (26/31) and O18 (13/16) E.coli strains possessed pap operon. Table 2 shows distribution of E.coli pap adhesion among 130 strains. P fimbriae were expressed in 79 (61%) of E.coli strains isolated from the urine of patients with UTI (Fig 1).

Table 1. Frequency of E.coli serogroups in the patients with urinary tract infection

Serogroup	Number	Percent
O1	6	4.61
O6	31	23.84
O8	7	5.40
O15	9	6.92
O18	16	12.30
O20	8	6.15
O25	6	4.61
O55	1	0.77
O78	0	0
O86	1	0.77
O111	0	0
O119	1	0.77
O125	0	0
Non- typed	44	33.86
Total	130	100

Table 2. Relationship between the presence of pap adhesion–encoding operon and the O-serogroups in E.coli isolated from patients with urinary tract infection

Serogroup	Number	Pap+	Percent
O1	6	6	100
O6	31	26	84
O8	7	1	14
O15	9	8	89
O18	16	13	81
O20	8	-	-
O25	6	2	33
O55	1	-	-
O78	-	-	-
O86	1	-	-
O111	-	-	-
O119	1	-	-
O125	-	-	-
Non- typed	44	23	52
Total	130	79	-

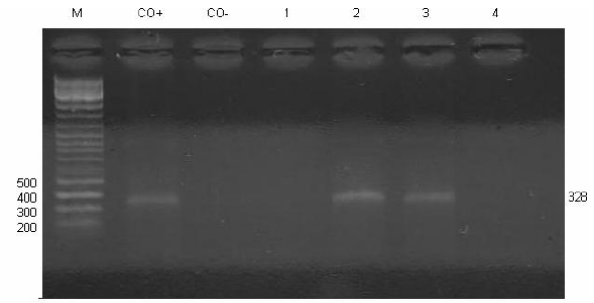

Figure 1. Agarose gel electrophoresis of amplified DNA products in PCR. Lane M: Molecular size markers, Lane Co+ : positive control (presence of adhesion), Lane Co^-: negative control (absence of adhesion), Lanes 1&4: pap negative, Lanes 2 & 3: pap positive.

DISCUSSION

E.coli is the most common cause of acute pyelonephritis which is the most serious form of UTI, particularly harmful to newborns and small children (1). In E.coli strains, the P fimbriae is considered as an essential virulence factor causing pyelonephritis (1,4,5,12,17,20,22). The pap operon encodes for the p fimbriae adhesion which has been shown to mediate attachment to specific cell surface glycopeptides present throughout the urinary tract. They facilitate colonization and invasion of the renal parenchyma (3,5,12,18).

The ability to adhere to epithelial surface has been shown to be a prerequisite for E.coli strains to colonize the urinary tract and cause UTI in the absence of urological abnormalities (9).

E.coli strains from a relatively small number of O serogroups, mainly O1, O2, O4, O6, O7, O18 and O75 have been reported to account for a major part of O-groupable UTI strains from different parts of the world (9). The majority of uropathogenic E.coli strains examined in our study were one of 3 serogroups (O6, O15 and O18). It has repeatedly been reported that strains belonging to these serogroups possess specific virulence factors which confer on their special invasive ability (9). Our results almost support this theory. The pap operon investigated in this study was particularly present in strains belonging to 4 serogroups most frequently detected in uropathogenic E.coli.

Furthermore, there was an apparent correlation between the pap operon and the O-serogroups of the strains. In this study, pap was found in 61% of investigated uropathogenic E.coli strains. Sero-groups O6 and O18 were predominant serogroups that isolated from patients. Serogroup O20 from EPEC strains might be the cause of UTI in 8 patients.

Among the virulence factors of uropathogenic E.coli, the adhesive properties are easy to test and our study detected the adhesion systems by the PCR method. The development of the PCR assay should facilitate detection of the adhesion determinants, which may be of use to clinicians as an indirect means of diagnosing underlying urinary disease. Furthermore, identification of the virulence factors of E.coli isolates may be helpful in epidemiological studies.

In conclusions, our findings indicated that pap adhesion–encoding operon has an important role in the development and severity of UTI. Many cases of serious urogenital disease are caused by a limited number of uropathogenic E.coli strains that generally possess special virulence factors such pap operon.

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