Genotypic Study of Two Virulence Factors *fimH* and *kpsMTII* in Uropathogenic *Escherichia coli* Isolates from Children Patients with Urinary Tract Infections

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Abstract:

Adhesion (type 1 fimbriae) and host defense avoidance mechanisms (capsule or lipopolysaccharide) have been shown to be prevalent in *Escherichia coli* isolates associated with urinary tract infections. In this work, 50 uropathogenic *Escherichia coli* (UPEC) isolated from children with urinary tract infections were genotypically characterized by polymerase chain reaction (PCR) assay. We used two genes; *fimH* and *kpsMTII*, both of them previously identified in uropathogenic *E.coli* (UPEC) isolates. The PCR assay results identified *fimH* (90.0)% and *kpsMTII* (**72**.0)% isolates. In the present study, was also demonstrated that these genes may be included in both or one of them within a single isolate.

Key words: Uropathogenic *Escherichia coli* (UPEC); Polymerase chain reaction (PCR); Virulence factor (VF).

Introduction:

Urinary tract infection (UTI) is one of the most common bacterial diseases in children. It is a complex event involving multiple interactions between the bacterial pathogen and host, which are difficult to separate from each other [1]. It is a common infection in children of all age groups [2]. The epidemiology of children UTI varies based on age and gender [3], the prevalence of UTI in the cumulative incidence rate only during the first 6 years of life has been calculated as high as 6.6% for girls and 1.8% for boys [4]. In many studies, *E coli* was the cause of 78% of UTI , the success of uropathogenic *E.coli* (UPEC) as pathogens can be attributed to the panoply of virulence factors that are produced by them. Several strains have acquired specific virulence attributes, which confer the ability to adapt to new niches and cause a broad spectrum of diseases [5].

The virulence factors can be broadly categorized into the adhesive factors and toxins , UPEC strains are able to produce various types of adhesions necessary for the recognition and attaching to receptors along the urinary tract including type I fimbriae (*FimH*) and capsule *(kpsMTII*) ; type I fimbriae is coded by the *fim* gene cluster ; type I pili are composite, multi-protein, hair-like appendages emanating from the surface of UPEC with its adhesive tip Fim , they aid UPEC in colonizing the kidney and bladder epithelia, *fimH* was further characterized and shown not to be necessary for the production of fimbriae, but to be involved in the adhesive property and longitudinal regulation of these structures. The receptor-binding adhesion of the type I fimbriae was identified, characterized and purified in 1988 and this protein was found to be antigenically

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conserved among strains with different pilin serotypes, and located at the pilus tip, *FimH* was later found to be the gene [6]. *FimH* functions like a typical invasin by bringing about reorganization of the actin cytoskeleton localize alterations in the host cell membrane, and uptake of the bacteria [7]. *FimH* is a mannose-binding subunit protein located at the tip of type I fimbriae. Tartof and Colleagues [8] had selected *fimH* for their analysis for several reasons. (a) FimH is a critical determinant of tropism for the urinary tract and vaginal epithelium for extra intestinal *E. coli*. An urovirulent phenotype is associated with genetic variants of this protein and hence is potentially clinically relevant; (b) The vast majority of both intestinal and extra intestinal *E. coli* express type I fimbriae; and (c) It has been reported that non-synonymous mutations accumulate at the *fimH* locus at a high rate, therefore, the level of discrimination at this locus is likely to be sufficiently high enough for studying uropathogenic *E. coli*. Capsular polysaccharides (*kpsMTII*) gene is associated with the pathogenic *E.coli*. Capsules are mainly a polysaccharide structure covering bacteria which acts to protect the bacterium from the host immune system [6].

There are no previous studies about *FimH* and *kpsMTII* genes of *E.coli* in Iraq using molecular methods in addition to the relation between the pathogenesis of microorganism and the presence of concentrations of cytokines accordingly this study has been conducted.

Materials and Methods :

Bacterial isolates: A total of 50 *E.coli* isolates were taken from urine samples of children aged (l) month to (11) years from the Welfare Teaching hospital for children in Medical city in Baghdad, from February 2012 till October 2012. The diagnosis of *E.coli* isolates were identified by standard methods include culturing on MacConkey agar, blood agar, Eosin methylene blue agar, chrome agar, and the diagnosis confirmed by using API 20 system(Bio Merieux). UTI was characterized by typical clinical symptoms of UTI and Positive urine cultures were defined by the growth of a single colony with counts > 10^5 colony forming unit/ml [9].

Preparation of bacterial DNA: Bacterial DNA was extracted from whole organisms by genomic DNA purification kit supplemented by manufactured company (Intron, Korea). The Bacteria were harvested from 1 ml of an overnight nutrient broth culture.

Polymerase Chain Reaction (PCR): The primers and PCR conditions used to amplify genes encoding virulence genes with PCR are listed in table (1). PCR assay was carried out in a total volume of 25 µL, containing 2 µL of template DNA, 2 µL of each primer, 12.5 µl of master mix, and 8.5µl of free nuclease water. The PCR amplification reaction consisted of 35 cycles of 94 ºC for one min, specific annealing temperature for each primer for one min (Table1), and a final extension at 72 ºC for one min. in a Thermal Cycler. The PCR amplification product were visualized by electrophoresis on 1.5% agarose gels for 90 min at 100 v. the size of the amplicons were determined by comparison to the 100 bp allelic ladder (promega, USA).

Table 1: Characteristics of oligonucleotide sequences used for PCR assays to detect uropathogenic *E.coli*.

Genes	Oligonucleotide sequences (5' → 3')	Size fragment (bp)	Reference	Tm (ºC)
fimH	F: TGC AGA ACG GAT AAG CCG TGG	508	11	57
	R: GCA GTC ACC TGC CCT CCG GTA			
kpsMTII	F: GCG CAT TTG CTG ATA CTG TTG	272	11	61
	R: CAT CAG ACG ATA AGC ATG AGC A			

Tm: Annealing Temperature, F: Forward ,R: Reverse.

Results:

A total of 50 uropathogenic *E.coli* (UPEC) isolates from children with UTI were genotypically characterized by the use of PCR assay. The PCR assay results identified both genes *FimH* and *KpsMTII* appeared in 31 out of 50 isolates, while gene *FimH* appeared in 45 out of 50 isolates and *KpsMTII* gene appeared in 36 out of 50 isolates lastly 4 isolates out of 50 isolates lack both of genes as shown in Table (2) and in Figures (1) and (2).

Table 2: Distribution (number and percentage) of virulence factor genes in uropathogenic *E. coli* isolates from patients with UTI

Virulence factors (genes)	Number of positive isolates	Percentage of positive isolates (%)
FimH and *KpsMTII*	31	62
FimH	45	90
KpsMTII	36	72
Absence of both genes	4	8

Type I fimbriae, adhesive subunit (*fimH*), group II capsule (*kpsMTII*).

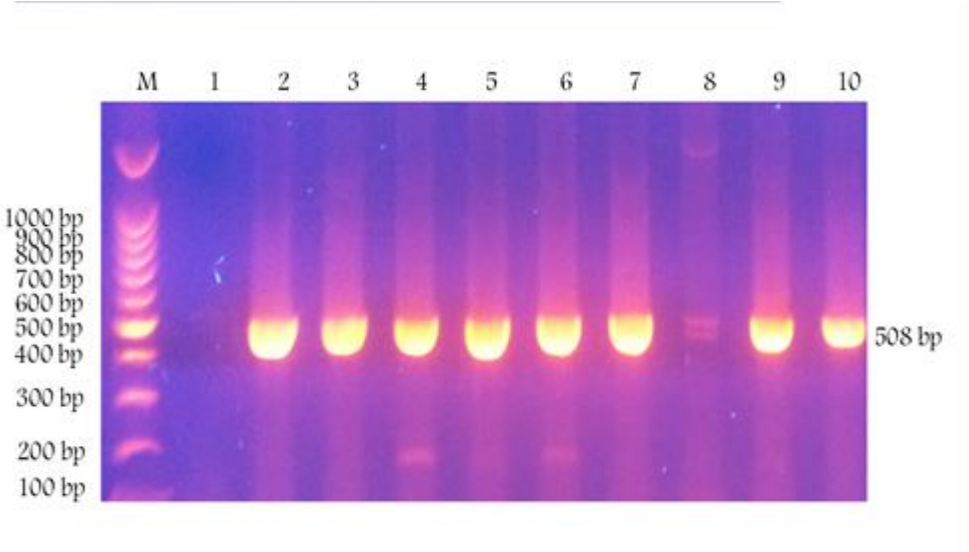

Fig. (1): Agarose gel electrophoresis of PCR amplification products of *E.coli fim*H gene (1.5% agarose, 100V, 90 min.)The amplified DNA in each lane except lanes (1 and 8) was 508 bp of *fim*H gene. Lane M: the ladder.

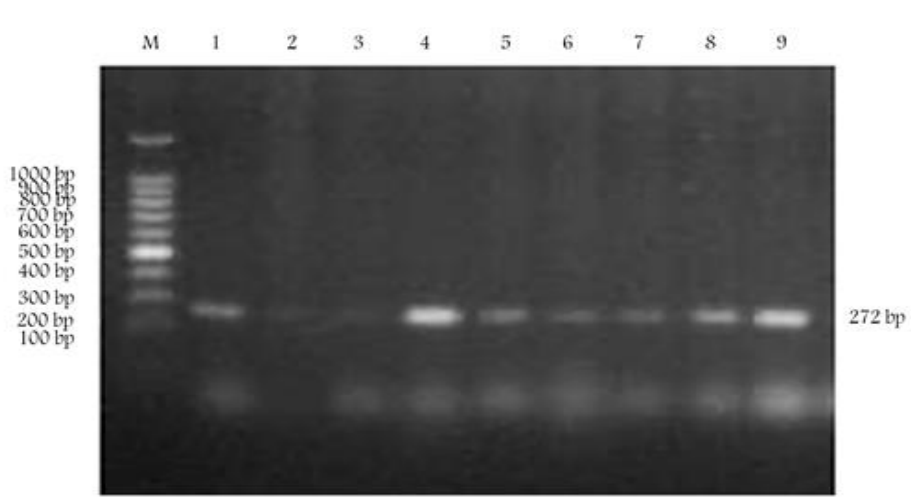

Fig. (2): Agarose gel electrophoresis of PCR amplification products of *E.coli kpsMTII* gene (1.5% agarose, 100V, 90 min.)The amplified DNA in each lane except lanes (2 and 3) were 272 pb of *kpsMTII* gene. Lane M: the ladder.

Discussion

The bacteria express a number of surface structures that enable them to interact with the environment, e.g., flagella for swimming and adhesions for attachment. These surface structures have a highly diverse size spectrum, and it must be implicit that sometimes they must interfere physically with each other in such a way that the function of some will be obstructed by the presence of other (more extended) structures [10]. Pathogenic *E. coli* are known to carry large chromosomal regions required for virulence [11]. PCR method is highly specific, informative and a powerful genotypic assay used for detection of adhesion-encoding operons and other virulence factors that can also contribute to virulence in UTI [5].

The DNA of 50 *E.coli* isolates (identified by cultural method and API 20 E) were amplified by PCR for the *fim H and kpsMTII* genes detection. The present study highlights higher frequency of *FimH* and *kpsMTII* as a virulence genes associated with the pathogenicity of uropathogenic*E.coli*. Our findings showed that *Fim*H adhesion was the most prevalent virulence factor detected, having occurred in 45 (90.0%) of isolates as seen in Table(2)and Figure(1).Similar results were obtained by previous studies [12,13,14,15]

whereas36 (72%) isolates showed a positive detection of the amplified 272 bp of *kps MTII* gene of *E.coli* by agarose gel electrophoresis as shown in table (2) and figure(2),[16] *kpsMTII* gene present group II capsules determined by kps operon Capsule is common in UPEC and is better known for contributing with urinary tract infections, bacteria need this virulence factor which helps the organisms to avoid and/or subvert host defense mechanisms[17].

While 31 (62%) of the isolates exhibited both of genes *FimH* and *KpsMTII*. Whereas 4(8%) isolates out of 50 isolates lack both of genes.

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دراسة وراثية لأثنين من عوامل الضراوة *fimH* و *kpsMTII* لبكتريا ايشريشيا القولون الممرضة للمجاري البولية والمعزولة من الأطفال المصابين بالتهاب المجاري البولية

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الخلاصة:

جمعت 50 عز لة لاشرشيا القولون من ادرار لاطفال يعانون من التهابات المجاري البولية وقد تم استخدام تقنية سلسلة تفاعل البلمرة مع استخدام باد ئات متخصصة للكشف عن جين الضراوة المشفر لبروتينات عامل الالتصاق (النوع الاول من الاهداب *fimH*) وجين الضراوة المشفر لبروتينات المحفظة او الغشاء الدهني متعدد السكر (*kpsMTII*)وكا نت نسبة و جود جين عامل الالتصاق % 90.0 بينما نسبة وجود الجين المشفر لبروتين المحفظة 72.0% .و اثبتت الدراسة امتلاك بعض العزلات لاحد الجينين بينما وجد كلا الجينين في عزلات اخرى.