RESEARCH ARTICLE

Distribution of *fimH* Gene in Local Isolates of Adhesive Uropathogenic *Escherichia coli*

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ABSTRACT

Adhesion is an influential step for bacterial vigor in clinical micro-environments**;** type 1 fimbriae are essential virulence factors that help uropathogenic *E. coli* in invasion and colonization of uroepithelial cells, the first step of UTIs and biofilm formation. Type 1 fimbriae of *E. coli* contain FimH protein at the tip encoding via *the fimH* gene cluster; this study was conducted for determining the *fimH* gene distribution in uropathogenic *E. coli* isolated from UTIs patients.

The results of adhesion assay show that (83.6%) of uropathogenic *E. coli* were high adherent isolates. While the results of *E. coli fimH* gene amplification prove that, for all *E. coli* isolates, the *fimH* gene was found in (87.1%), while among high adherent isolates it was found in (92.6%), and that shows the function of type 1 fimbriae in the colonization and infection of urinary tracts in addition to other adhesions virulence agents of uropathogenic *E. coli.*

Keywords: Adhesion, *FimH* gene, Type 1 fimbriae, Uropathogenic *E. coli*.

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INTRODUCTION

Fimbriae or pili are adhesions virulence agents of uropathogenic *E. coli*, play an influential function in urinary tract infections (UTIs). Uropathogenic *E. coli* has numerous adhesive organelles; which include type1, P, S, F1C, and long polar fimbriae. Of these organelles, mannose-sensitive or common fimbriae (type 1 fimbriae) were occur in 95% of *E. coli* isolates, Uropathogenic *E. coli* directly stimulated via type 1 fimbria to invade UECs, it play a role in later re-infections when they help in forming intracellular bacterial colonies.[1-3] There are five parts forming type 1 fimbriae, beginning from the pilus tip, FimF, FimG, and FimH (tip fibrillum), FimA (pilus rod), and FimD (inserted in the outer membrane). 300 amino acids are produced for the precursor FimH protein, and they are subject to biochemical processing to become a mature protein of two hundred seventy-nine amino acids, type 1 fimbriae adhesive sub-unit, FimH, is a fundamental determinative subunit, having noticeable affinity to urinary tract receptors; therefore, FimH adhesions are significant for invasion the different favorable sites by *E. coli*. *E. coli* pathogenicity and adhesion to suitable cells ability may increase because of fimH protein roles. The fimH protein has been appeared to be an effective receptor binding molecule which discriminates structures that contain a-D-mannose. For that, type 1 fimbriated bacterium has adhered easily to epithelial cells. The fimH adhesin has occurred on the tip of each fimbria and intermediate along the shaft of fimbria, two major domains form the fimH protein, each constituting almost one half of the molecule; the N-terminal domain include the receptor binding site, while the recognition sequences for export and bioassembly included in the domain of C-terminal.[4,5]

The *fimH* gene cluster is 903bp long; this segment encodes all fimbrial synthesis machinery and regulatory elements. The DNA variants were prevalent in commensal, intestinal, and uropathogenic *E.coli*, however, genetic variations were prevalent because stressful environment make these isolates adaptable by genotypic changes and consequently phenotypic variants were prevalent among these groups.[6-8] Mature fimH adhesin contains two domains connected by an 8-amino acid linker. These are the domain of a mannose-binding lectin, which expands from amino acids 1–150, as well as the domain of pilin consisting of amino acids 159–279. Mannose-binding part of the *fimH* gene has no variation among sequenced uropathogenic *E. coli*. In addition to that, many amino acids (27, 62, 66, and 163) will could be mutated at the positive selection in uropathogenic *E. coli* when compared with commensal isolates.[9-12]

The present study was conducted for the determination of the *fimH* gene distribution in uropathogenic *E. coli* isolated from UTIs patients.

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PATIENTS AND METHODS

Isolates of Bacteria

The study includes 225 *E. coli* isolated from UTIs patients. The bacterial collection started from June 2017 to November 2018, including out and in-patients in Alramadi Teaching Hospital, Anbar, Iraq. The samples were microscopically examined for pyuria (WBCs > 5/hpf) before cultivated on MacConkey, and blood agar, incubation conditions were 37 Celsius for 24 h., and the identification of isolates was done depending on colony morphologies, grams stain, catalase, oxidase, indole production, MR-VP broth, citrate utilization, TSI, in addition to ortho-nitrophenyl-β-galactoside (ONPG).

Adhesion Assay

To isolate human uterine epithelial cells (UECs); early morning midstream urine samples were taken from healthy females, centrifuged at 3 thousand rounds per minute, for 5-10 min.; sediments were washed four times with phosphate buffer saline (pbs:10mM KH2PO4/K2HPO4 and 150mM PH = 7.2). After that, the suspension of UECs with phosphate buffer saline was done. The UECs\hpf value for the suspension was counted.[13]

Adhesion assay was made according to Eden *et al*., 1997; the nutrient broth media inoculated with uropathogenic *E. coli* and incubated at 37 Celsius for 24 hours, the bacteria were centrifuged for 7 minutes at 5000 rounds per minute, bacterial sediments resuspended with phosphate buffer saline. One milliliter of UECs suspension and one milliliter of uropathogenic *E. coli* suspension was mixed in a sterile test tube; and incubated at 37 celsius for 60 minutes with continuous gentle shaking. After that, a smear was done from each tube and stained with Gram's stain, the number of adherent uropathogenic *E. coli* was counted by using oil immersion lens of light microscope. The adhesion assay was repeated three times for each isolate, in addition to that, isolates that gave an adhesion mean higher than 250 cells per 10 uroepithelial cells considered as high adherent isolates, while isolates that gave an adhesion mean less than 250 cells per 10 uroepithelial cells considered as low-adherent isolates.

DNA extraction from uropathogenic *E. coli*

Was based on Wright M.H. *et al.*, 2017 with slight adjustments; 5m1 of 18 hour culture transferred to a falcon test tube, precipitate the bacteria via centrifugation at 8000 rounds per minute for 10 min., and suspend the pellet with 467µL of 50mM Tris-Cl and 10mM EDTA (pH 8.0), the suspensions transferred into 1.5mL micro-tube. 30µL 10% SDS and 5µL proteinase K were added, inverted gently for 5 minutes, and incubated for one hour at fifty Celsius. Add 525µL PCI (Phenol-Chloroform-Isoamyl alcohol) solution and inverted gently for 10 minutes. They were centrifuged at 12,000rpm for 15 minutes, and the aqueous phase was transferred into sterile 1.5mL micro-tube. Taking care must not disturb the bilayer of the mixture. The same volume of -20^{o}C 100% ethanol was added and gently mixed via inversion. They were centrifuged at 13,000rpm for 20 minutes Supernatant decanted carefully and left pellet to dry at room temperature. Pellet was resuspended in 50µL Tris EDTA (TE) buffer and left it to resolved overnight at 4-8 Celsius. 260/208 ratio was used to calculate the DNA concentration, and the presence of bacterial DNA was confirmed via electrophoresis 5µL of final output on a 1% agarose.

Amplification of *E. coli fimH* gene

All bacterial isolates were subjected to screening for the presence of the *fimH* gene by specific primers:

D1F: (AATGTGGGGCAAAACCTGG)

D1R: (TAT CCG TTC TCG AAT TAT AAA).(16)

Reaction mixture consisted of 5µL of DNA template, 12.5µL of 2X Go Taq green master mix., 2.5µL of D1F primer, 2.5µL of D1R primer, 2.5µL of distilled water, while P.C.R procedure was: 94 celsius for 5minutes; 30 cycles at 94 Celsius for 30sec., 55 celsius for 30sec., and 72 Celsius for 1min., subsequent with 72 celsius for 5 minutes as final extension, before maintained at 4 Celsius. The 10µL of PCR products were run for gel electrophoresis on 2% TBE agarose gel and the gel was stained by E.B and viewed via U.V eliminator.

Statistical analysis

Results were analyzed statistically using the Chi-Square test, Mean, standard deviation (STD) and percentage to find the significance of probability level according to Statistical Package for the Social Sciences (SPSS) ver22 program. The p values < 0.05 were regarded as significant

RESULTS

Bacterial Isolates

From June 2017 to November 2018, 225 isolates of uropathogenic *E. coli* were obtained from urinary tract infections patients (pyuria; WBCs > 5/hpf).

Adhesion Assay

The mean of uropathogenic *E. coli* cells adherent on 10 uroepithelial cells ranged from 125.3 to 281, while the standard deviation ranged from 20.29 to 1.73. There were 188 (83.6%) high adherent isolates of uropathogenic *E. coli.* (Figure 1).

Amplification of *E. coli fimH* gene

FimH gene was amplified using specific primers, appeared as a band of about 217 base pairs in agarose (Figure 2).

Of all isolates, the *fimH* gene was found in 196 (87.1%), while among high adherent isolates, it was found in 174 (92.6%), and among non-adherent isolates *fimH* gene was found in 22 (59.5%) (Table 1).

Of the 196 positive isolates for the *fimH* gene, 104 (53.1%) and 92 (46.9%) belonged to hospitalized patients and outpatients, respectively.

DISCUSSION

Adhesion is the greatest importance step for bacterial vigor in clinical micro-environments, uropathogenic *E. coli* facing numerous niches during the UTIs, including urine, and cytoplasmic space of uroepithelial cells, where intracellular colonization takes place. Type 1 fimbria intermediate adhesion and invasion of human uroepithelial cells 4, because they are

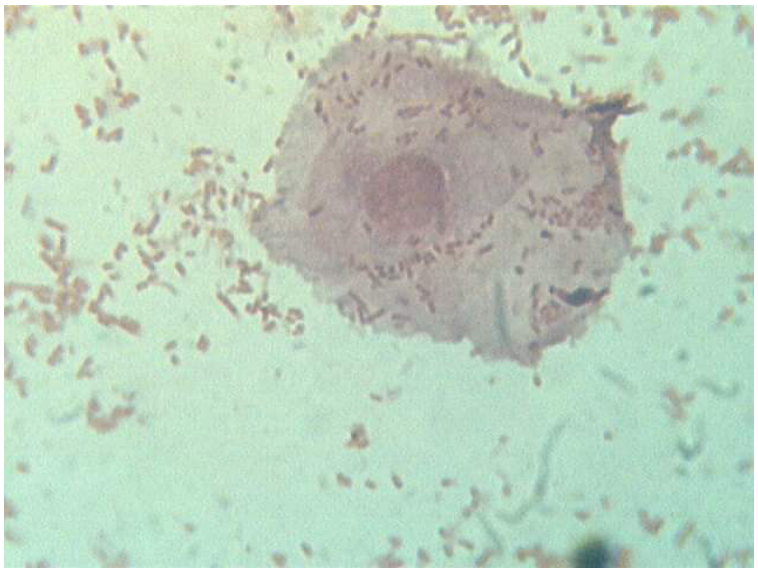

Figure 1: Adhesion of uropathogenic *E. coli* on uroepithelial cell.

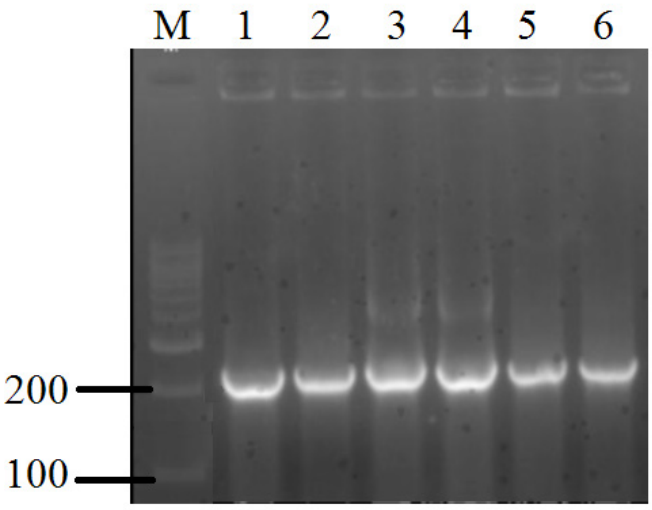

Figure 2: P.C.R amplification of the *fim*H gene

Table 1: Distribution of *fimH* Gene in Uropathogenic *Escherichia coli*

	Distribution of fimH Gene		
Isolates of E. coli	*P.C.R +ve No. : %*	*P.C.R –ve No. : %*	*Total*
High Adherent Isolates	174 : (92.6)	14 : (7.4)	188
Non-Adherent Isolates	22 : (59.5)	15 : (40.5)	37
Total	196 : (87.1)	29 : (12.9)	225

Chi-Square= 2.0, P= 0.157

imperative to the colonization of urinary tracts, as well they have a critically effects UTIs in mice.[12] FimH adhesion help's uroepithelial cell colonization, thereby, bacteria will resist removing out via urine and bacterial invasion will begin[4,], in addition, type 1 fimbriae expression increased during the early biofilm stage[17] for all that current study investigated the distribution of *fimH* gene that encodes the elements of fimbrial synthesis machinery as well as regulatory in uropathogenic *E. coli*.

Uro-epithelial cells are a main of human urine particular, transitional epithelia and squamous epithelia are dominated quantitatively. It can function as recipients for adhering uropathogens after repeated washings with phosphate buffer saline.[13] The bacterial concentration used in the *in vitro* adhesion test is 10^7 bacteria per mL., isolates that gave an adhesion mean higher than 250 cells per 10 uroepithelial cells

considered as high adherent isolates, depending on this, the results show that (83.6%) of uropathogenic *E. coli* were high adherent isolates. However, all bacterial isolates were used in *fimH* gene amplification to detect the presence of type 1 fimbria in high and low adherent isolates.

DNA primers were previously defined for the adhesin-encoding *fimH* gene, which was the most frequently found in uropathogenic *E. coli*.[16] These primers consist of sequences internal to highly conserved regions of the *fimH* gene. In the current research, PCR was used for test *fimH* gene existence. The study showed that detection of the adhesion systems by the PCR method appears highly specific and reliable method.

The results showed that majority of uropathogenic *E. coli* isolates had *fimH* gene, it was found in 196 (87.1%) isolates, 104 (53.1%) obtained from hospitalized patients and 92 (46.9%) obtained from outpatients, furthermore, 174 (92.6%) of high adherent isolates and 22 (59.5%) of low-adherent isolates were contained *fimH* gene. However, several previous studies found *fim*H genes expressed in uropathogenic *E. coli* promptly obtained from urine samples, *Watts et al. (2010)* found that most abundant virulence gene was *fimH*, it detected in 98% of *E. coli* strains obtained from UTIs patient. While *Arabi et al. (2012)* investigated adhesions genes frequency in uropathogenic *E. coli* and found that *fimH* gene frequency was 87.7%. *fimH* was found in another strains of *E. coli*, for example, *Biscola et al. (2011)* found that in enterohemorrhagic *E. coli*, *fimH* gene was existed in (97%) of non O157 : H7 *E. coli* and in all O157 : H7 *E. coli*, in addition to *Kaczmarek et al. (2012)* Who reported that; *fimH* gene existed in 100 % of K1 antigen containing *E. coli*, as well as, in 97% of K1 antigen not containing strains. Heterogeneous subpopulations of piliated uropathogenic *E. coli* and there are variations among strains belonged to these subpopulations that give as significant knowledge to understand *E. coli* adherence and their functions in UTIs pathogenesis. In this study, specific primers depending techniques were used, that let us check the type 1 fimbriae containing cells presence *in vitro*, and it's so important step, preparing to use such techniques for analyzing subpopulation dynamics *in vivo*.

FimH gene was found in 92.6% of the high adherent isolates, which refers to the distribution level of Type 1 fimbriae among uropathogenic *E. coli*. The great percentage of *fimH* lead to increase adhesion to uroepithelial cells and colonization capability of *E. coli*; because that, *fimH* could help in vaccines production to avoid infections with via preventing adhesion and colonization of microorganism, addition to this, fimH can be played as a mean to design fast detection tests. Furthermore, epidemiological studying of community and hospital-associated *E. coli* infections can been typing via *fimH* SNP analysis and can be used as genotypic analyses implement to uropathogenic *E. coli*.[12]

CONCLUSIONS

Uropathogenic *E. coli* isolates were high adherent, and the majority of them contained *fimH* gene, shows functions of type 1 fimbriae in colonization and infection of urinary tracts with the addition of other adhesions virulence agents of uropathogenic *E. coli.*

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