

Detection of *fimH* virulence gene in uropathogenic *Escherichia coli* Using PCR

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ABSTRACT

Urinary tract infections (UTIs) are one of main health problems caused by many microorganisms, including uropathogenic *Escherichia coli* (UPEC). The virulence factors are important in colonization of UPEC, extra-intestinal survival, and creation of cytopathic effects. In addition, the expression of special virulence factors of UPEC can contribute to uropathogenicity, as well as worsening of UTIs. In this study, 134 isolates of uropathogenic *E. coli* were collected from Baghdad/Iraq through March 2018 to April 2019. UPEC were identified by culturing on Hardy chromo UTI agar and confirmed at molecular level by using *uidA* housekeeping gene using real time polymerase chain reaction. The prevalence of *fimH* gene was studied among 134 uropathogenic *Escherichia coli* of patients attended Using polymerase chain reaction assay, Visualization of *fimH* gene (bp) by 1.5% agarose gel analysis. By comparing the bands in a sample to the DNA ladder, their approximate sizes can be determining. For instance, the bright band on the gel above 1000 base pairs (bp) in size for *fimH*. The intended gene was detected in 94.8% of the isolates and absence in 5.2%.of them.

1. INTRODUCTION

Escherichia coli is considered as a highly diverse bacterial species found naturally in the intestinal tract of humans and many animal species [1]. Although *E. coli* is known to be part of the normal gut micro biota, there are some strains that are pathogenic causing wide variety of intestinal and extra intestinal diseases [2]. Extra intestinal infections due to *E. coli* are common in all age groups and can involve almost any organ or anatomical site. Among Extra Intestinal Pathogenic *E. coli* (ExPEC), strains of Uropathogenic *E. coli* (UPEC) are most commonly associated with human disease [3]. The vast majority of *E. coli* strains encode type 1 fimbriae. Type 1 fimbriae, one of the most important proteins is the bacterial adhesins, which mediate receptor-specific interactions with target cells or tissues [4]. The fimbrial body is built from the major pilin subunit, FimA (15 kDa), while the adhesive subunit, mannose-binding protein FimH (30 kDa), is located at the fimbrial tip. Type 1 fimbriae play an important role. For intestinal *E. coli*, FimH is thought to mediate transient colonization of the oropharyngeal epithelium and, in this way, contribute to fecal/oral transmission between hosts [5,6].

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2. METHODOLOGY

2.1. Bacterial isolation and identification

In the current descriptive (cross-sectional) study, a total of 134 isolates were obtained as swabs from *E. coli* cultured bacteria isolated from urine that collected from three Hospitals in Baghdad, Iraq (Al-Emammian AL-Kadhmain Teaching Hospital, Baghdad Medical City and AL-Kindy Hospital) in period between March 2018 to April 2019. For identification of *E. coli*, 134 collected isolates were cultured on Hardy chrom UTI agar by streak plate method and incubated at 37°C for 24 hours. The identification done depend on its color on chromo UTI agar. If the isolates give rose to magenta colonies with darker pink centers, that's mean it's belong to uropathogenic *E.coli*.

Mixed infections with other bacteria were noticed on chromo UTI agar, especially with Enterococcus spp. Which gave teal to turquoise colonies as shown in appendix 1. The positive UTI agar isolates confirmed at molecular level by detection of *E. coli* species-specific *uidA* using primer set: forward primer of the gene *uidA* sequence (TCTTGCCGTTTTTCGTCCGTA), and the reverse primer sequence (CACGCCGATGTTATTGCCG) using real-time quantitative PCR on a Rotor-Gene Q MDx instrument (QIAGEN) using the SYBR-Green PCR Kit luna. Real

time thermal cycler was programmed as the following: 30 cycles of 95oC for 5secs, 57oCfor 10 secs and 72oC for10secs [7].

2.2. DNA Extraction

Escherichia coli isolates were grown in Hardychrom UTI agar at 37°C overnight. Bacteria were re-suspended in 100 µL sterile distilled water in PCR plate and boiled at 100°C for 12 minutes using a thermocycler. After centrifugation at 13000 rpm for 3 minutes, the supernatants were stored as DNA template at -20°C (for up to one week) until they were tested by PCR [8].

2.3. Polymerase Chain Reaction Amplification of *fimH* Gene:

The bacterial isolates (134) were subjected to screening for the presence of the *fimH* gene by PCR. Regarding the screening of *fimH* gene, the amplification reaction consisted of 18µl PCR-grade water along with 1 µl of each forward and reverse primers (10 pmol/µl) of *fimH* (*FimH*-F 5'-TCAGGGAACCATTCAGGCA -3') and *FimH*-R 5'-ACAAAGGGCTAACGTGCAG -3') and 2 µl DNA template (50-100ng/µl). The amplification condition included an initialization (94°C for three minutes), 35 cycles (94°C for 30 seconds, 58°C for 30 seconds and 72°C for 1 minute) and a final extension (68°C for seven minutes). The PCR amplifications were performed on The Applied biosystemthermocycler. The PCR products were electro-phoresed by running through 1.5% (w/v) agarose in TAEBuffer. A molecular marker (Biolabs # N3232S, 1kb) was used to assess the PCR products sizes [9].

3. RESULTS AND DISCUSSION

Using PCR assay, *fimH* gene was detected in 127/134 isolates accounting for 94.8% of the studied *E. coli* isolates. These isolates were successfully amplified above 1000 bp fragment of *fimH* gene while 7 isolates (5.2%) lacked it (Figure 1).

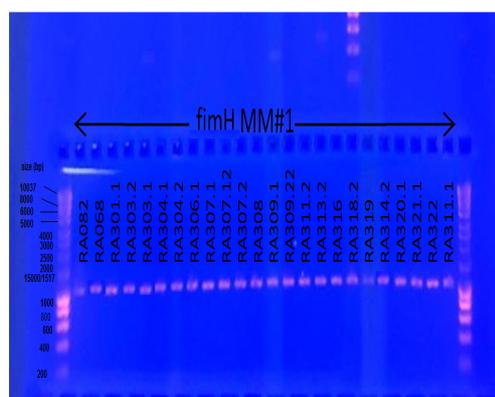


Fig : 1: Visualization of *fimH*gene (bp) by 1.5% agarose gel analysis. Power was5V/Cm, for 2 hours. By comparing

the bands in a sample to the DNA ladder, their approximate sizes can be determine. For instance, the bright band on the gel above 1000 base pairs (bp) in size for *fimH* as shown in picture.

In Pakistan, Ali *et al*, 2019, the occurrence of *fimH* gene was higher among uropathogenic *E. coli* with 100% [10].

Another study by Narmin Saeed Merza, 2018 in Iraq, the intended gene was detected in 94.3% of the isolates of uropathogenic *E. coli* [11].

The importance of the *fimH* virulence gene in uropathogenic *E. coli* assimilate in its association with worsening of UTIs. since,*FimH* is a critical determinant of tropism for the urinary tract [2]and vaginal epithelium[3]. It was shown previously that naturally occurring amino acid replacements in *FimH* can modify its tropism towards uroepithelium and various components of basement membranes. These replacements increase the monomannose (1M)-binding capability of *FimH* by affecting shear-dependent conformational properties of the protein [12].and its applicable features, that's because, only minor sequence variation within the *fimH* genes, renders the *fimH* alleles feasible for use in high-resolution typing method of *E. coli* [13] . *fimH* gene is frequently associated with UPEC strains and it is more likely to be altered or modified due to selective pressure, the phenotypic variants of *fimH* gene is earnestly associated with genetic variations thus, SNPs may contribute to the ability of organisms to cause illness conferring epidemic distribution or long term evolution of virulence [14]. SNP *fimH* analysis has discriminating power for this locus and it may be accurate enough for investigating UTI caused by UPEC that occurs over limited time periods or in confined geographical settings [15]. However, The apparent absence of an intact *fimH* locus in 5.2% of the uropathogenic *E. coli* might appear to limit this applicability [9].

4. CONCLUSION

94.8% of uropathogenic *E. coli* isolated from urinary tract infections are *fimH* producer and 5.2% of them non producer.

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Appendix 1:



الكشف عن جين الضراوه *fimH* في بكتيريا ايشيريشيا القولون الممرضة للمسالك البولية باستخدام تقنية تفاعل البلمرة المتسلسل

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الخلاصة:

التهاب المجاري البولية واحده من اهم المشاكل الصحيه التي تسببها الاحياء المجهرية ومن ضمنها بكتريا ايشيريشيا القولون . عوامل الضراوه مهمه في استعمار بكتريا ايشيريشيا القولون الممرضة للمسالك البولية والبقاء خارج الامعاء وخلق تأثيرات الاعتلال الخلوي بالاضافه الى ذلك انتاج عوامل ضراوة خاصه من قبل بكتريا ايشيريشيا القولون الممرضة للمسالك البولية ممكن يساهم باصابات الجهاز البولي وتفاقمات التهابات المسالك البولية . في هذه الدراسه تم جمع 134 عزله من بكتريا ايشيريشيا القولون المسببه للامراض البولية من بغداد /العراق من مارس 2018 الى ابريل 2019 . تم زرع العزلات على وسط UTI وتم التأكد على المستوى الجزيئي باستخدام جين *uidA* عن طريق تفاعل البلمره المتسلسل . تم دراسة انتشار جين *fimH* بين 134 عزلة من بكتريا ايشيريشيا القولون الممرضة للمسالك البولية ب استخدام تفاعل البلمره المتسلسل وتم تصوير جين *fimH* bp عن طريق تحليل هلام الاكاروز بنسبة 5% و بالمقارنة مع عينة الحامض النووي (Ladder) تم تحديد احجامها التقريبيه فعلى سبيل المثال الشريط اللامع في الهلام اعلى من 1000 زوج قاعدي لجين *fimH* وقد تبين ان 94.8% من هذه العينات تمتلك هذا الجين و 2.5% لا تمتلكه.