Analysis of Single Nucleotide Polymorphism in *Fim H* Gene from Uropathogenic *E.coli*

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Abstract

Background: Urinary tract infection is defined as presence of microbial pathogens in the urinary tract with associated symptoms. The infection affects both lower and upper urinary tracts and is known as acute cystitis and polynephritis respectively. This study done to detect the variation of single nucleotide in FimH gene and its correlation with the pathogenicity of uropathogenic *Escherichia coli* caused the urinary tract infection.

Materials and Methods: The study group included 150 patients with Urinary tract infection. There were 120 (80%) female and 30 female (20%) their age ranges between (5-55) years, seen in Al-Hussein Teaching Hospital for the period from January 2019 to March 2019. The dominant form (48.39%) of patients with urinary tract infection caused by UPEC was found in the age group of 24-39 years old. Urine samples were collected from the patients. Bacterial isolation was identified by microscopic examination and culture characteristic on selective media MacConky agar, Eosin Methylene Blue and Chrome agar. Then the isolated were diagnosis by Vitek®2 system.

Results: Bacteriological results showed that 62(41.3%) isolates were characterized as *Escherichia coli.* Genomic DNA was extracted from the bacterial culture for further molecular study to reveal C640T and T591A fimH gene Polymorphism. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was used for this purpose and digestion of the amplified DNA products by ScarFI1390I and BsrI endonuclease respectively gave fragments with different molecular sizes which express certain genotypes. This study detected that the prevalence rate of single nucleotide polymorphism C640T FimH was (85.48%), endonuclease enzyme only affects the C allele sequence and it does not have any influence on the T allele sequence. The prevalence rate of single nucleotide polymorphism T591A fimH gene was (80.64%), endonuclease enzyme only affects the T allele sequence and it does not have any influence on the A allele sequence.

The result show only 62 isolates (41.3%) were given typical morphological characteristics and biochemical tests related to *E. coli*, while the rest 88 isolates (58.6%) belonged to pathogenic bacteria from different genera.

Keywords: *E.coli , Fim H Gene, UTIs; C640T and T591A SNPs; PCR-RFLP*

Introduction

Escherichia coli is a ubiquitous bacterial species commensal of humans and warm blooded animals. Nevertheless some strains have evolved the capability to cause both intestinal and extra intestinal illnesses (1). It is categorized into two foremost categories, enteric *E.coli* (ECEC) that mostly causes infections restricted to the mucous lining of the intestine and extra intestinal pathogens (ExPEC) which have the capability of spreading from the intestine and inflicting infections in

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other parts of the body (2)

Urinary tract infection (UTI) is one of the most widespread infections in humans which causes extreme morbidity and considerable expenses (3). UTIs have an effect on different components of the urinary tract and are classified into a number disease corporations based on infection sites. UTIs are classified into cystitis (the bladder), pyelonephritis (the kidney) and bacteriuria (the urine) (4).

Urinary tract infection are induced by means of each Gram-negative and Gram-positive bacteria, as well as by certain fungi. The most frequent causative agent for both uncomplicated and complicated UTIs is uropathogenic *Escherichia coli* (UPEC). For the agents involved in uncomplicated UTIs, UPEC is followed in occurrence by *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, group B *Streptococcus* (GBS), *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida* spp.(5). For complicated UTIs, the order of occurrence for causative agents, following UPEC as most common, is *Enterococcus* spp., *K. pneumoniae*, *Candida* spp., *S. aureus*, *P. mirabilis* and *P. aeruginosa* (6)

Uropathogenic *Escherichia coli* (UPEC) strains are regarded as a primary pathogen in about 80% of patients with UTIs (7). Successful institution of infection by UPECs wants specific cascade of events which includes adhesion to host cells colonization of tissues and in certain cases, cellular invasion. These events include different cellular and molecular pathways which rely on the presence of multiple virulence factors (8). UPEC has many virulence factors that assist its colonization, invasion, and survival within the host urinary system. These factors include adhesins, siderophores, toxins, capsule production, and proteases. In addition, several autotransporter (AT) proteins, which are typically associated with phylogeny (9), are correlated with virulence and have been identified in UPEC (10).

Methods and Materials

The current study was conducted on 150 patients (120 females, 30 males) were seen Al-Hussein Teaching Hospital from January 2019 to March 2019. The patients were diagnosed clinically by physician as having UTI infection . Urine samples were taken from both male and female using sterile containers. The samples were directly activation on Brain heart infusion broth and then streak on MacConkey agar, Eosin methylene blue agar , Chrom agar and incubated at 37° C for 24 hrs .

Primary identification of E. coli isolates was based on cultural, morphological characteristics, microscopically diagnosis , while the confirmatory identification test was based on selective Chrome agar medium and according to Vitek®2 system. After activation on Brain Heart Infusion broth at 37◦C for 24 hours Bacterial colonies developed on plate agar, were studied; on MacConkey Colonies appeared as a deep purple colour due to lactose fermentation. It is also appeared as circular, flat and moist with entire margin on this medium. MacConky agar is a selective plating medium used for the isolation of *Enterobacteriaceae* and related to gram negative rods. Lactose fermenting bacteria produce colonies that have varying shades of red due to conversion of neutral red indicator dye below pH 6.8 .

Genomic DNA from culture cell were extracted by using Favorprep Genomic DNA extraction kit (blood , culture cell) favorgen.

from 62 DNA samples of UPEC were subjected to a molecular detection by PCR amplification of the *fim*H gene using a specific primer, 53 isolates (85.48%) gave positive results of C640T fimH gene and 50 isolates (80.64%) gave positive result of T591A fimH gene. *fim*H gene were detected in 91.07% of UPEC isolates among 112 UPEC isolates obtained from outpatients suffering from UTIs in seven Iraqi hospitals. The high frequency of fimH gene in all studies relating to UTIs indicates the critical roles of this virulence factor in *E. coli* pathogenesis.

Distribution of fimH gene C640T polymorphism was detected by PCR-RFLP technique, the enzyme digested the 164bp fragment PCR product in C location and led to the creation of fragments with 122 and 42 bp lengths figure (1) , and the enzyme digested the 76bp fragment PCR product in T location and led to the creation of fragments with 50 and 26 bp lengths for the T591A fimH gene figure (2)

Result and discussion

Genomic DNA Extraction

The DNA is extaction from bacterial culture samples. Genomic DNA was extacted and submitted to agarose gel electrophoresis and estimated concentration and purity of each sample,samples with low value were re-extracted . figure(1).

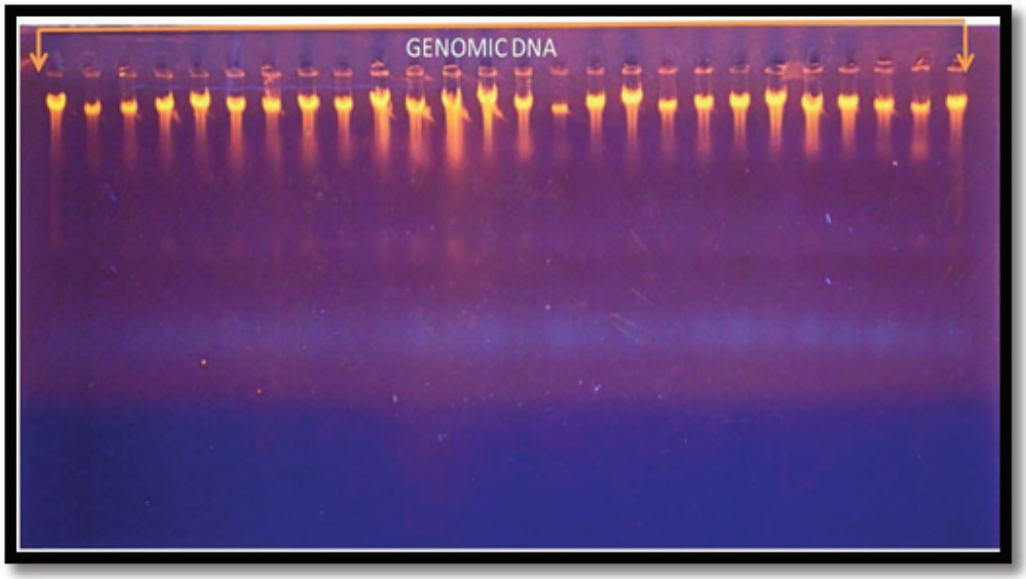

Figure (1) : Ethidium Bromide stained agarose gel electrophoresis appearance that displays Genomic DNA that was extracted from uropathogenic *E. coli*

DNA Amplification

The products of successful binding between the extracted DNA and specific primers for C640T and T591A fimH gene were detected by gel electrophoresis analysis using DNA marker and the products size was 164 bp and 76 bp respectively . Figure (2)

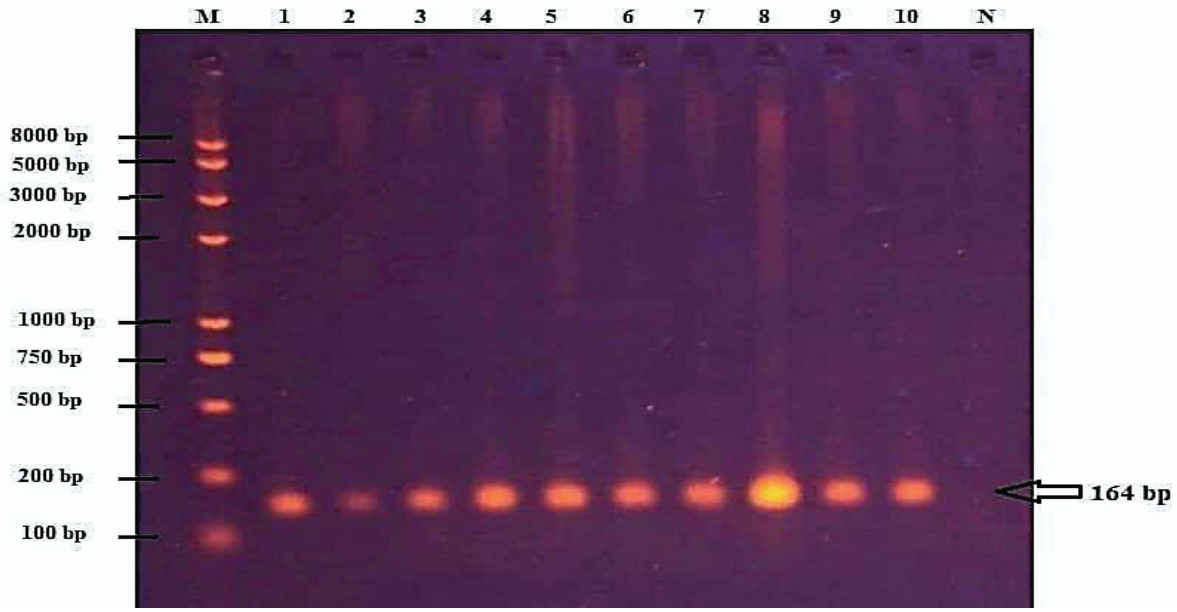

Figure (2) : Gel Electrophoresis of PCR Amplified 164bp of C640T fimH gene. Lane (M):DNA molecular size marker, Lane (N): negative controls

Detection of Genes Polymorphism

Distribution of fimH gene C640T polymorphism was detected by PCR-RFLP technique, the enzyme digested the 164bp fragment PCR product in C location and led to the creation of fragments with 122 and 42 bp lengths figure (3) , and the enzyme digested the 76bp fragment PCR product in T location and led to the creation of fragments with 50 and 26 bp lengths for the T591A fimH gene figure (4), these results agree with the reports of other studies such as (Molaie *et al.,* 2016)

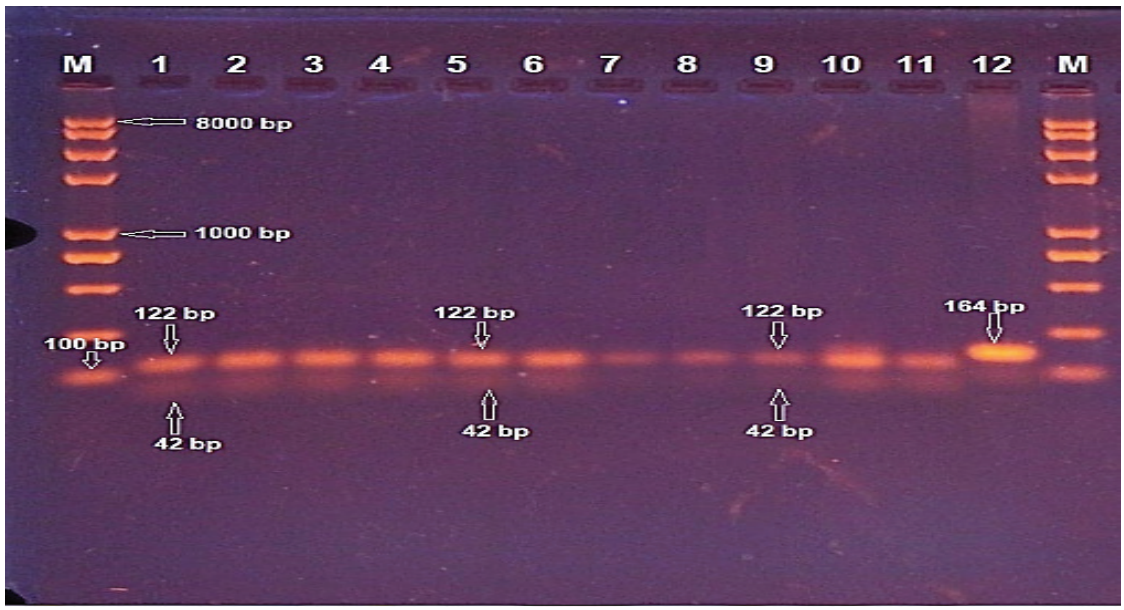

Figure (3) : Genotyping analysis of C640T SNP. ScarFI1390I enzyme digested the 164bp fragment PCR product in C location and led to the creation of fragments with 122 and 42 bp lengths. . lane M: Marker (8000-100 bp); lanes (1-11) digested PCR products, lane 12 un-digested PCR products;1% agarose gel

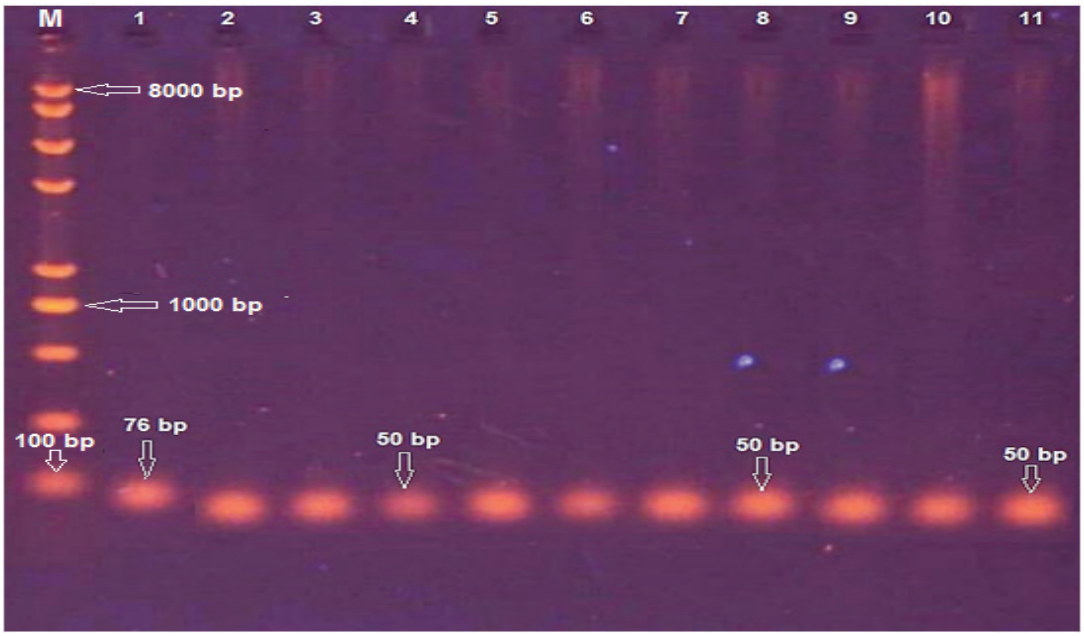

Figure (4): Genotyping analysis of T591A SNP. BsrI enzyme digested the 76bp fragment PCR product in T location and led to the creation of fragments with 50 and 26 bp lengths. The 26 bp fragment was removed from gel because of the small size of fragment . lane M: Marker (8000-100 bp);lane1 un-digested PCR products ,lanes (2-11) digested PCR products.

Distributions of FimH Genotypes and Alleles

Specific amplification of fimH gene, consist of C640T and T591A polymorphic site results in a 164 bp and a 76 bp PCR products, respectively. Genotyping was accomplished using the ScarFI1390I and BsrI restriction enzymes for C640T and T591A SNPs, respectively. ScarFI1390I enzyme only affects the C allele sequence and it does not have any influence on the T sequence. This enzyme digested the 164bp fragment PCR product in C location and led to the creation of fragments with 122 and 42 bp lengths. Table (1). Relating to T591A SNP, the T allele was affected by BsrI restriction enzymes and led to create 50 and 26 bp fragments. Table (2).

Table 1:Genotype of C640T and T591A polymorphism.

Total Number of samples	polymorphism	Genotype	n	percentage %
62	C640T	CC CT TT	53 0 0	85.48
62	T591A	TT TA AA	50 0 0	80.64

Table 2 : allelic frequency of C640T and T591A polymorphism .

Total Number of samples	polymorphism	Allelic frequency	n	percentage %
62	C640T	C T	53 0	85.48
62	T591A	T A	50 0	80.64

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Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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