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Detection and Characterization of fimH gene in Escherichia coli Isolated from Urine Samples of Diabetic Adult females with Urinary Tract Infection

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Abstract

Ninety Five Escherichia coli isolates have been obtained from urine samples of unmarried diabetic adult females suffering from UTI of age ranging from (18-35) years were admitted to different hospitals and primary health centers during the interval between June to November 2020 in in Babylon Governorate, Iraq. All of these (95) urine samples were undergone bacterial culturing, biochemical tests, and then multiplex PCR assay for investigating the presence of the fimH gene in of Uropathogenic Escherichia coli (UPEC) isolates. It was found that 87 (91.6 %) among (95) isolates of the UPEC strains were contain the fimH gene. Thus, it was highly prevalent among diabetic adult females with UTIs. On the other hand, all the (95) isolates of E. coli strains were undergone to Haemagglutination Assay for determining their ability to possess virulence factor (Type 1 fimbriae). The results revealed that all the (87) isolates which contain the fimH gene were able to possess type 1 fimbriae. Some antibiotics, including (Ciprofloxacin, Ampicillin, Trimethoprim and Ceftazidime) have been used to study their effects on Type 1 fimbriae, and it has been discovered that these antibiotics at the subinhibitory concentrations do not kill bacteria, but fully inhibit Type 1 fimbriae.

Keywords

UTIs, Escherichia coli, Diabetic patients, fimH gene, Type1 fimbriae, PCR technique.

Introduction

Urinary tract infection (UTI) is an inflammatory illnesses caused by the high proliferation of a large number pathogens within the urinary tract, leading to changes in the functioning of the kidneys and urinary system. One of the major problems for women is a UTI, about (50-80%) of the women had UTI at least once during their lifetime [1]. The most important urinary bacterial pathogen is Escherichia coli strains, accounting for up to 80% of UTIs [2]. Some virulence factors such as pili or fimbriae have been shown in Uropathogenic E. coli (UPEC) strains, that facilitate the attachment to uroepithelial and increased the levels of K capsular antigen [3]. Additionally, the pathogenesis of UTIs influenced by virulence factors found in UPEC strains. The virulence determinants more frequent in UPEC are including

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adhesions "type 1 fimbriae, p fimbriae, curli fimbriae, a fimbrial adhesion and flagellum", and several virulence factors such as the production of hemolysins, aerobactin and cytotoxic necrotizing factor1. These are essential for UPEC colonization, extraintestinal survival, and cytopathic effect development [4]. E. coli strains isolated from diabetic female patients suffering from UTI were able to possess Type 1 fimbriae at a rate (81.8 %) as a result have the ability to adhere the uroepithelial cells in diabetic females more than non diabetic females [5]. The fimH gene was investigated among Uropathogenic E. coli strains isolated from hospitalized and out-patients suffering from urinary tract infection and FimH has the highest binding ability which lead to increase of E. coli pathogenicity [6].

The aims of the present study were to identify the fimH gene and Type1 fimbriae among UPEC strains isolated from urine samples of unmarried diabetic adult females suffering from UTIs, and study the effects of some antibiotics such as Ciprofloxacin, Ampicillin, Trimethoprim and Ceftazidime on virulence factor (Type 1 fimbriae).

Materials and Methods

Urine sample collection and bacterial isolates

In the present study, Ninety Five isolated E. coli strains were evaluated. These isolates were obtained from urine samples, collecting from unmarried diabetic adult females with UTIs of age ranging from (18-35) years who admitted to different hospitals and primary health centers during the interval between June to November 2020 in in Babylon Governorate, Iraq.

The urine samples were collected in the sterile containers and transferred to the laboratory through two hours. The collected urine samples were inoculated on the surface of Blood agar, MacConkey agar, and (EMB) agar. After inoculation the plates were incubated for 24 hr. at 37°C. Following that, Gram's stain and biochemical tests were performed on the recovered bacterial colonies for the conformation of E. coli strains such as " catalase, oxidase, citrate utilization, indole production, methyl red-Voges Proskauer, and triple iron sugar, ortho-nitrophenyl-β-galactoside (ONPG) test" as described in [7].

Haemagglutination Assay (HA)

Haemgglutination assay was carried out according to the procedure of [8] for determining the ability of UPEC isolates to possess Type 1 fimbriae, and it is conducted in the presence of an equivalent amount of some antibiotics only for UPEC isolates which are capable of possessing type 1 fimbriae, including (Ciprofloxacin, Ampicillin, Trimethoprim and Ceftazidime)

Molecular detection of fimH gene

The detection of fimH gene in UPEC isolates was done as previously described [9], by using specific primers with the amplicon size. A single reaction mixture containing 5 µL of PCR buffer (10X), 1.2 U Taq DNA polymerase (Fermentas), 1.25 mM MgCl2, 125 µM dNTPs (Fermentas), 0.5 µL of forward and reverse primer, and 3 µL of the DNA template. Final volume was completed to 50 µL by adding nuclease free water to the PCR mixture. The PCR amplification products were observed on (1.5%) agarose gel which by ethidium bromide staining, then photographed when visualized on UV transilluminator. The

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nucleotide sequence of the primer sets and PCR conditions are shown in Table (1).

Table (1): Sequence of primer and PCR conditions

Gene	Name and sequence primer (5'-3')	Product size (bp)	PCR conditions
fimH	**fim1:** GAGAAGAGGTTTGATTTAACTTATTG **fim2:** AGAGCCGCTGTAGAACTGAGG	559	94°C for 3 min. 1X 94°C for 60 Sec. 58°C for 70 Sec. 40 X 72°C for 70 Sec. 72ºC for 6 min. 1X

Results

Ninety Five Escherichia coli isolates were related to unmarried diabetic adult females suffering from UTI. All these isolates were investigated by culturing, biochemical tests, and then screened by a molecular PCR assay to detect and identify the fimH gene. The results revealed that 87 (91.6 %) out of 95 isolates were positive for the fimH gene as shown in fig. (1).

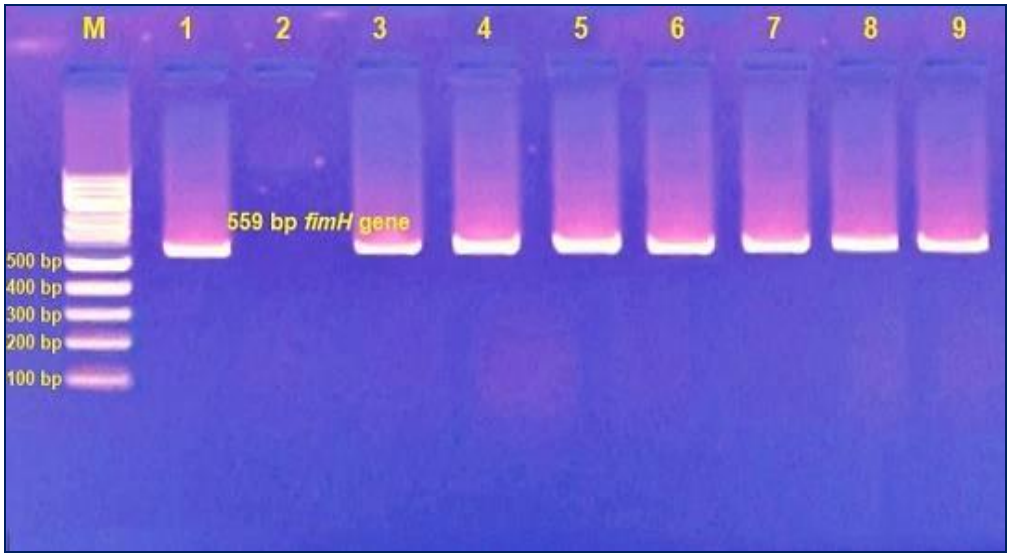

Figure (1): Agarose gel electrophoresis of E.coli fimH gene detection. Lane M: marker with 100 bp ladder, Lane 1 and 3-9: positive for fimH gene, while lanes 2: negative for fimH gene, amplicon size: 559 bp.

Also, all the (95) E. coli isolates were undergoing Haemagglutination Assay, and the results revealed that all the (87) isolates which contain fimH genes were able to possess Type 1 fimbriae. Some antibiotics, including (Ciprofloxacin, Ampicillin, Trimethoprim and Ceftazidime) have been used to study their effects on Type 1 fimbriae as shown in Table (2).

Table (2): Antibiotics' effects on Type 1 fimbriae of UPEC isolates

Agglutination UPEC isolates	Ciprofloxacin	Ampicillin	Trimethoprim	Ceftazidime

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	with	without	with	without	with	without	with	without
87	-	+	-	+	-	+	-	+

Discussion

A number of virulence factors found in UPEC strains that assist them colonize the urogenital tract. At the tip of the type 1 fimbriae, FimH adhesion mediates binding of bacteria to the urothelial cell membrane, avoiding the wash of bacteria by the flow of urine and initiate bacterial invasion [10]. The presence of the fimH gene was determined because the FimH virulence factor linked to the cases of UTIs was not commonly established from the UPEC strains which extracted from unmarried diabetic adult females with UTIs in Babylon, Iraq.

Multiplex PCR assay was used to investigate the presence of the fimH gene in isolates of Uropathogenic Escherichia coli (UPEC). It was found that 87 (91.6 %) among (95) isolates of the UPEC strains were contain the fimH gene. Thus, it was highly prevalent among diabetic adult females with UTIs and the results of our study were corresponded to the previous study results published by [11], who studied eighteen isolates of UPEC were collected from females suffering from UTIs and stated that the (100%) of the isolates had the fimH gene. Moreover, the most prevalent virulence gene in the UPEC strains is fimH gene, being found in 61 out of 90 isolates at a rate of (68%) of the urinary tract infections isolates studied [12].

Beside, The findings of the current study revealed that E. coli isolates with Type 1 fimbriae have heights adhering ability of the uroepithelial cells of diabetic females suffering from UTIs. Another study demonstrated that females with diabetes have more bacteriuria than non-diabetic females, thus, fimbriated E. coli isolates are capable of adhering of the uroepithelial cells of diabetic females suffering from UTIs more than non-diabetic females [13]. Furthermore, different substances (e.g., albumin, glucose) in diabetic patients' urine or differences in uroepithelial cells can cause E. coli with type 1 fimbriae to bind more diabetic female's uroepithelial cells than non-diabetic female's uroepithelial cells. This may be due to the fact that diabetic uroepithelial cells have various glycoprotein receptors (uroplakins that line the bladder mucosa) on their cells, resulting in a higher attach ability [14].

On the other hand, some antibiotics, including (Ciprofloxacin, Ampicillin, Trimethoprim and Ceftazidime) have been used to study their effects on Type 1 fimbriae. It was discovered that all these antibiotics at the subinhibitory concentrations do not kill bacteria, but fully inhibit Type 1 fimbriae. A previous similar result study was recorded by [8], were stated that while certain antibiotics' sub inhibitory concentrations do not destroy bacteria, but they are able to alter the certain essential aspects of bacterial cell function, resulting the interfere with numerous pathogenecity factors of bacteria. According to the results study recorded by [15], the antibiotics can inhibit the expression of adhesions on the surface of bacteria, or change the bacterial form such that microorganisms can't attach to receptors on the surface of animal cells.

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Conclusions

The study concluded that fimH gene was highly prevalent in E. coli isolates among diabetic adult females with UTIs and presence of Type 1 fimbriae in UPEC strains which mediated the adherence ability of E. coli strains to the uroepithelial cells that result in increase of E. coil pathogenicity. Additionally, several antibiotics at the subinhibitory concentrations do not kill bacteria, but can alter the molecular architecture of bacteria's exterior surface as well as a variety of bacterial functions, resulting in affecting bacterial virulence.

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