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Phenotypic and genotypic study associated with biofilm formation for *E. coli* isolated from UPEC

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***Abstract*---**UTI is a serious health problem with respect to antibiotic resistance and biofilms formation being the prime cause for the antibiotic resistance. In current study sixty *E.coli* isolated collected from patients with urinary tract infection. In this study, biofilm formation were detect by phenotypic and genotypic methods. The detection of biofilm formation by phenotypic method was carried out by Congo Red Agar (CRA) method and tissue culture plate (TCP) method and the genotypic method performed by detection present some gene associated with biofilm formation which include *fliC* ,*csgA* ,*fimH* and *papC*.**.** The results showed that 54(90%) from *E. coli* produced strong and moderate slime layer. The present study showed that 57(95%) from *E. coli* produced strong and moderate biofilm. Genotypic study of *E. coli* biofilm-associated genes were found in various frequencies in all isolates that included *fliC* (63.3%), *csgA* (73.3%), *fimH* (90%) and *papC* (76.6%).

***Keywords*---**Phenotypic, UPEC, UTI, *E.coli*, biofilm formation.

Introduction

Infections of the Urinary Tract are regarded as one of the most serious illnesses, ranking second among bacterial infections in the medical community, and they are prevalent, according to estimates of the number of individuals afflicted. Around 150 million individuals every year" The cost-estimation technique for identifying and treating urinary tract infections is critical since the expenses are substantial, and laboratory testing is required to achieve a state of recovery (Medina and Castillo-Pino , 2019). Urinary tract infections are dependent on the virulence of the causative bacteria and the host's sensitivity, because the infection occurs in a person whose urinary tract is anatomically and naturally normal, as

well as the possibility of bacteria ascending automatically from the urethra to the bladder and, in some cases, the kidney. Females of school age, males with an enlarged prostate the elderly and sexually active young women are four categories that are more prone to urinary tract infections than other groups." Among the key conventional diagnostic techniques for the identification of urinary tract infections are microscopic inspection and in vitro culture of mesenteric urine (Klein and Hultgren, 2020). *E. coli* bacteria urinary tract known as *E.coli* Uropathogenic (UPEC). *E. coli* bacteria is transported to the urine tract, it causes disorders of the urinary system. *E. coli* bacteria is one of the natural organisms found in the human gut and plays a vital function in balancing the intestinal environment. Bacterial invasion of host cells necessitates penetration. Alternatively, the outer cell membrane can be destroyed through physical, enzymatic, or both techniques" Many virulence factors are present in bacteria that cause urinary tract infections, which assist them bind to the surfaces of epithelial cells lining the urinary system. The bacteria attach themselves to the lipopolysaccharide receptors on the surface of the urothelial cells. Many virulence features relevant to urinary tract infection are present in these *E. coli* strains, including the development of particular adhesions and toxins (Martinson and Walk, 2020). Biofilms is an extracellular polymer structure that has a tinny sheet of microbiological societies adhering to apiece on biological or inanimate substrates (EPS). Most of bacteria's microbial lives are spent as part of a biofilm, rather than as lone individuals. Biofouling and a range of medical illnesses, including cystic fibrosis and urinary catheter cystitis, may be caused by the creation of biofilms. Over time, bacteria cling to a solid surface that is exposed to biotic and abiotic stressors, resulting in the formation of hydrated EPS and biofilms (Stabnikova, et al., 2021). The microenvironment of biofilms may be anaerobic because of the lower oxygen level within than on the surface of the film. Bacterial cell formation slows or ceases in biofilms due to the constrained nutritional environment (Guzmán-Soto, et al., 2021).

Methods

Bacteria

Urinary samples collected from diabetes patients in diabetes center in AL-Sader Hospital, after collected the samples each sample processed as the following

1- Urinary sample centrifuged in 3000rpm for 5m.
2- Discharged the supernatant layer and toke pellet layer.
3- Toke some drops from a pellet layer and cultured on blood agar, MacConkey agar and Mannitol salt agar.
4- After growth the bacteria on this media the bacteria diagnosed by biochemical test.

Biofilm formation

1-Tissue culture plate method (TCP)

Detection of biofilm formation by tissue culture plate method (TCP) assay according to (Lizcano et al., (2010).

2-Congo red Agar method (CRA)
Detection of biofilm formation by Congo red Agar method (CRA) assay according to (Freeman *et al.*,1989).

Extraction of Genomic DNA:

DNA of The isolated bacteria was prepared by boiling method. Briefly, colonies were suspended in 100 microliters of sterile distilled water and boiled at 100C in the water bath for 15 minutes than rapidly cooled at -20℃ for one hour, then centrifugation and the supernatant were preserved for the used in the amplification-processes (Shah *et al.*, 2017).

Gene amplification

The primers used in the PCRs are described in table 1.The DNA extract of bacterial isolates were subjected to primers genes as following: 8µL Master mix, 5µL DNA template, 1.5µL for each primers, 4µL Deionized water (dd water) by using PCR. The protocol was used depending on Promega Biosystem manufacturer's instruction. Single reaction (final reaction volume 20 µl).
3 min at 95°C, 30 cycles of 30 s at 94°C, 30 s at 60°C, and 1 min at 72°C, and a final extension for 5 min at 72°C.

Table 1: Primers used in this study

Gene	Oligonucleotide Sequence	PCR product size	Reference
papC	F:TGATATCACGCAGTCAGTAGC R: CCGGCCATATTCACATAAC	501	Janben, (2001)
csgA	F: GCAATCGTATTCTCCGGTAG R: GATGAGCGGTCGCGTTGTTA	418	Juliane, (2017)
fimH	F:TGCAGAACGGATAAGCCGTGG R: GCAGTCACCTGCCCTCCGGTA	506	Johnson, (2000)
fliC	F:ATGGCACAAGTCATTAATACCCAAC R: CTAACCCTGCAGCAGAGACA	1,497	Fields (1997)

Result and Discussion

Detection of biofilm formation by phenotypic method

1- Congo-Red Agar Method (CRA):

The results showed that 54(90%) from *E. coli* produced strong and moderate slime layer these isolated distribution between 8(13.3%)isolates produced strong slime layer indicated by formation of black colonies with dry crystalline consistency and 46(76.6%)isolates were moderate for slime producer indicated by formation of brown or semi black colonies, while 6(10%)isolates did not produce slime layer indicated by formation of pink colonies(Table2).The slime layer works on the

bacterial cell packaging, forming thin, living membranes known as biofilm its act as a buffer that inhibits the antibiotic influence within the bacteria cell and thus confers resistance (Al-Khafaji, 2018).

2- Micro Titer Plates (MTP) Method

The present study showed that 57(95%) from *E.coli* produced strong and moderate biofilm these isolated distribution between 22(36.6%)of *E.coli* isolates appeared high biofilm formation and35(58.3%) of *E.coli* isolates showed moderate biofilm formation, while 3(5%)isolates did not produce biofilm (Table2).Our result was agree with result of (Tessa *etal.,*2020) which found that about 94% of *E. coli* had the ability to produce the biofilm.

Table 2: Ability of bacteria on biofilm formation by Congo red Agar method and tissue culture plate method

Biofilm formation	CRA method	TCP method
Non	6(10%)	3(5%)
Moderate	46 (76.6%)	35(58.3%)
High	8 (13.3%)	22(36.6%)

Detection of biofilm formation by genotypic method

In the current study we selected 30 *E.coli* isolated and used four genes associated with biofilm formation as follows:

***papC* gene:**

The result showed that the *papC* gene was detected in 23/30 (76.6%) *E. coli* as in Figure (1).The *papC* are regarded as important virulence factors for attachment and infection initiation in the host and are previously also linked to antimicrobial resistance (Wang, *et al.*, 2016), As well as the biofilm formation, virulence, and pathogenesis of Avian pathogenic Escherichia coli (APEC) and papC genes, which enables the adherence and survival of *E. coli* in the internal organ in canines and humans (Yu, *et al.*, 2018).Our result was agreement with Gabrielle *et al.*,2019who has reported the *papC* gene was detected in (77%) *E.coli*.

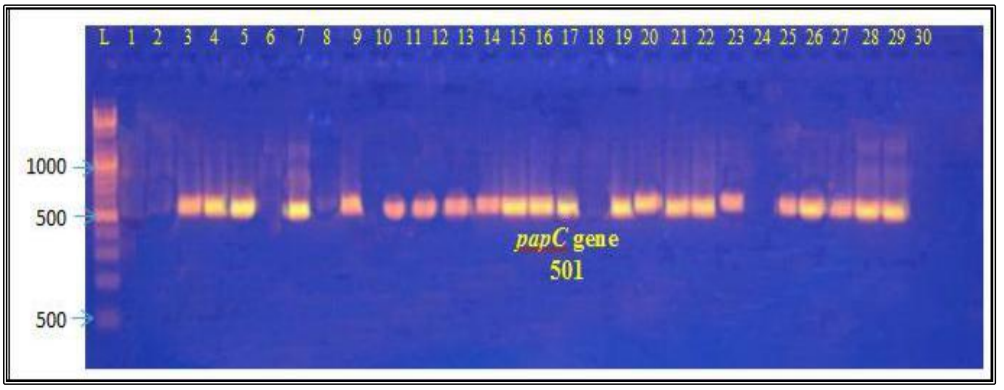

Figure 1: PCR amplification products of *E. coli* isolates that amplified with *papC* gene primers with product 501 bp.

***csgA* gene:**

The result showed that the *csgA* gene was detected in 22/30 (73.3%) *E.coli* as in Figure (2).Our result was disagreement with Juliane *et al.* 2017which showed present *csgA* gene in (100%) of *E.coli.*The extracellular matrix is one of the characteristics that separates bacteria in biofilms from planktonic bacteria, with curli fimbriae being the major ingredient of *E. coli* biofilms. Curli fimbriae's primary component, *csgA*, is involved in cell aggregation, adherence to surfaces, and biofilm formation (Frömmel *et al.*, 2013).

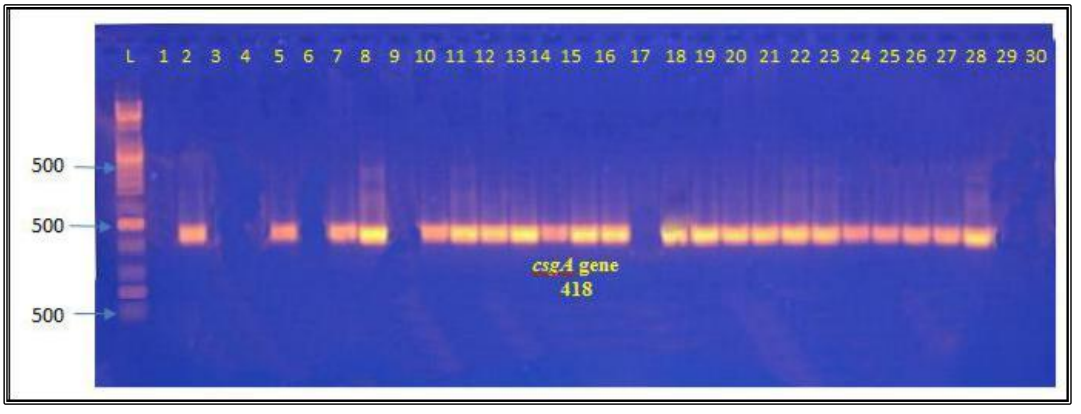

Figure 2: PCR amplification products of *E. coli* isolates that amplified with *csgA* gene primers with product 418 bp.

***fimH* gene:**

The result revealed that the *fimH* gene was detected in 27/30 (90%) *E. coli* as in Figure (3).The creation of biofilms by uropathogenic Escherichia coli is a crucial determining factor in the development of urinary tract infections (UTIs) (UPEC). Several adhesion factors play a role in bacterial cell attachment to the urinary system and biofilm formation pyelonephritis (Zamani and Salehzadeh , 2018).This

result was closed to Gabrielle et al.,2019 which reported *Fim H* gene was detected in (100%) of *E.coli*.

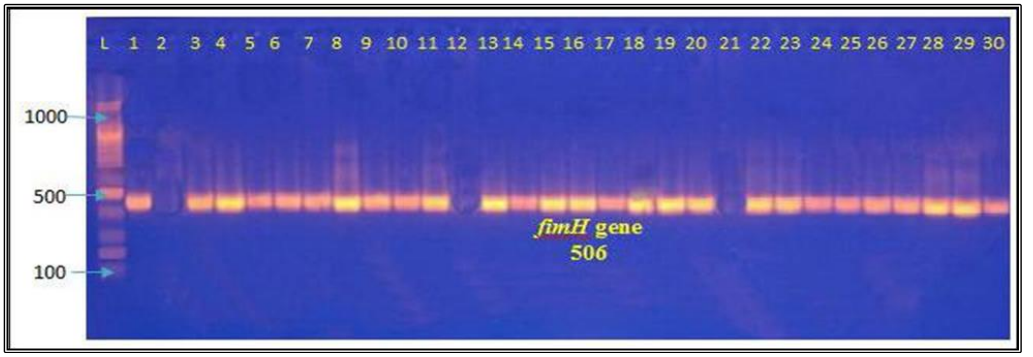

Figure 3: PCR amplification products of *E. coli* isolates that amplified with *fimH* gene primers with product 506 bp.

***fliC* gene:**

The result revealed that the *fliC* gene was detected in 19/30 (63.3%) *E. coli* as in Figure (4).This result was disagreement with Gabrielle *et al.*,2019 which showed the *fliC* gene was detected in (84%) of *E.coli*.

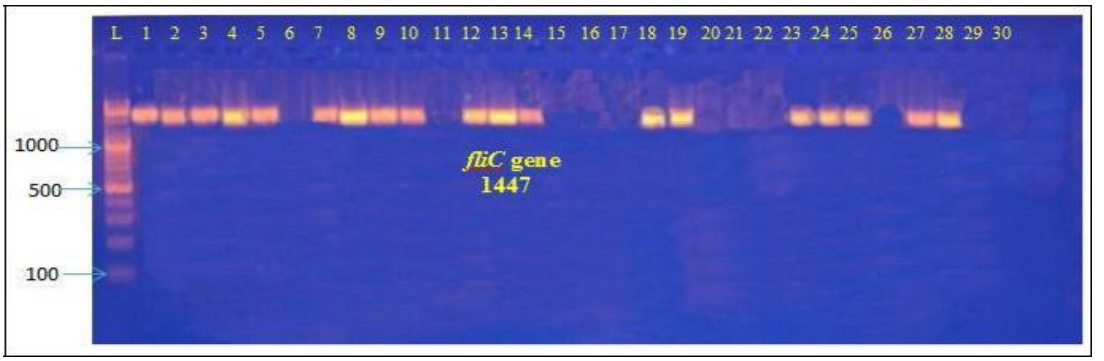

Figure 4: PCR amplification products of *E. coli* isolates that amplified with *fliC* gene primers with product 1447 bp.

Conclusions

Genotypic study of *E. coli* biofilm-associated genes were found in various frequencies in all isolates that included *fliC* (63.3%), *csgA* (73.3%), *fimH* (90%) and *papC* (76.6%).

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