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Molecular Detection of Some Adhesion Genes of Escherichia Coli Isolated from Patients with Urinary Tract Infection

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Abstract

In current study, 150 samples were collected from patients with urinary tract infection of both genders and all ages starting from October 2021 to January 2022 in Diyala and Baghdad governorates, Iraq. They were tested by phenotypic methods and the results showed that 73 isolates belonged to E. coli.

In addtion of phenotypic method, all isolates diagnosed by genotypic method was done by Polymerase Chain Reaction (PCR) technique to detection papE gene, Results showed that 22 isolates (30.1%) contain this gene belonging to UPEC.

Molecular detection results also showed some adhesion genes of UPEC which included (fimH, papC, sfa, and afa) its presence among the isolates was (100%), (31.81%), (100%), and (40.9%) respectively.

Keywords

E. coli, Adhesion Genes, papE gene, UPEC.

Urinary tract infections(UTI) are of interest to researchers and medical professionals , It is one of the most common and widespread diseases and the second most common type of injury after respiratory infections (1) . Among the injuries related to society while it ranks first in terms of hospital-related injuries, It constitutes about 40% of hospital-acquired infections It affects a large percentage of the community(2).

E. coli is one of the most important members of the intestinal family, Bacilli, gram-negative, positive for the catalase test and negative for the oxidase test, positive for methyl red test, producing indole [3]. It is found within the natural flora within the human body at the same time, it is an Opportunistic pathogen thus it causes many diseases such as diarrhea , Sepsis , Meningitis [4] .

Uropathogenic Escherichia coli (UPEC) the main cause of urinary tract infections , It is responsible for 80% of cases whether communal 70-95% or acquired from the hospital 50% [5] and it is called Uropathogenic Escherichia coli because it is associated with diseases of the urinary system [6].

E.coli that cause urinary tract infections have many different virulence factors to overcome the host's defense mechanisms that allow it to cause disease [7] One of these factors fimbria which enables bacteria to that enable bacteria to attach to the lining of the epithelial cells of the urinary tract , this is bacteria have the ability to aggregate and remain in the urinary tract of the host for a long time , thus these clusters enable the bacteria to avoid the body's immune response and antibiotic resistance and make it able to cause frequent infections of the urinary tract [8].

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Materials and Methods

Isolation and Phenotypic Detection of E.coli

A total of 150 urine samples were collected from patients suffering from urinary tract infections of different ages and for both genders from October 2021 to January 2022 from private laboratories in Diyala and Baghdad governorates , Iraq. The isolates were identified as E.coli by culture tests and it was planted on MacConky Agar medium and Eosin methylene blue medium ,the dishes were incubated at a temperature of 37°C for 24 hours,Microscopy with a gram stain was used [9] in addition to biochemical tests : Catalase test, oxidase test, indole test, methyl red test, voges-proskauer test, citrate test[10].

DNA extraction and Genotypic Detection of E.coli

DNA was extracted from E.coli bacteria using laboratory kits Presto™ Mini Gdna Bacteria Kit processed by the company Geneaid . The concentration of DNA extracted from the bacterial isolates was measured using a quantitative fluorometer. Special primers were used to detect the genes under study in table (1).

PCR mixture was prepared by adding 12.5µl of master Mix , 3µl template DNA, 1.5µl from each forward and reverse primers , finally volume was completed to 7.5µl by adding nuclease free water.

PCR condition illustrated in Table (2) and PCR products were detected in 1.5 % agarose gel for 1 hr. at 100 V, stained with ethidium bromide and visualized by UV transilluminator.

Table 1: Primers and amplified PCR products used in study

Gene	Primer sequences (5' →3')	Size(bp)	Origin	Reference
papE	F: GCAACAGCAACGCTGGTTGCATCAT R: AGAGAGAGCCACTCTTATACGGACA	326	Macrogen	11
afa	F: CGGCTTTTCTGCTGAACTGGCAGGC R: CCGTCAGCCCCCACGGCAGACC	672	Macrogen	12
fimH	F: AACAGCGATGATTTCCAGTTTGTGTG R: ATTGCGTACCAGCATTAGCAATGTCC	465	Macrogen	12
papC	F: GACGGCTGTACTGCAGGGTGTGGCG R: ATATCCTTTCTGCAGGGATGCAATA	328	Macrogen	12
sfa	F: CTCCGGAGAACTGGGTGCATCTTAC R: CGGAGGAGTAATTACAAACCTGGCA	410	Macrogen	12

Table 2: PCR condition to genes used in study

Amplified gene	Initial denaturation	No. of cycles	Denaturation	Annealing	Elongation	Final extension
papE	95°C/5min	30X	95°C/20sec	64 °C/20sec	72 °C/40sec	72 °C/5min
afa	95°C/5min	30X	95°C/20sec	70 °C/20sec	72 °C/40sec	72 °C/5min
fimH	95°C/5min	30X	95°C/20sec	60 °C/20sec	72 °C/40sec	72 °C/5min
papC	95°C/5min	30X	95°C/20sec	66 °C/20sec	72 °C/40sec	72 °C/5min
sfa	95°C/5min	30X	95°C/20sec	60 °C/20sec	72 °C/40sec	72 °C/5min

Results and Discussion

Phenotypic Identification of E.coli

E.coli isolates were identified by culture tests as it was planted on the Maconky Agar medium the colonies appeared in a pink color(fermentation of lactose), Soft and shiny.while the colonies appeared on the eosin methylene blue medium with green metallic sheen(A characteristic of E.coli bacteria) . As for the microscopic diagnosis, it appeared negative for the Gram stain, a short rod.

The isolates showed that they were positive for catalase, indole and methyl red and negative for oxidase and voges-proskauer, citrate test , 73 isolates of *E.coli* were obtained.

Genotypic Identification of E.coli

All *E.coli* isolates were tested using PCR, the diagnostic gene *papE* was used and the results showed that the *papE* gene was present in only 22 of the 73 isolates from bacteria *E.coli* 30.1% (Fig 1).

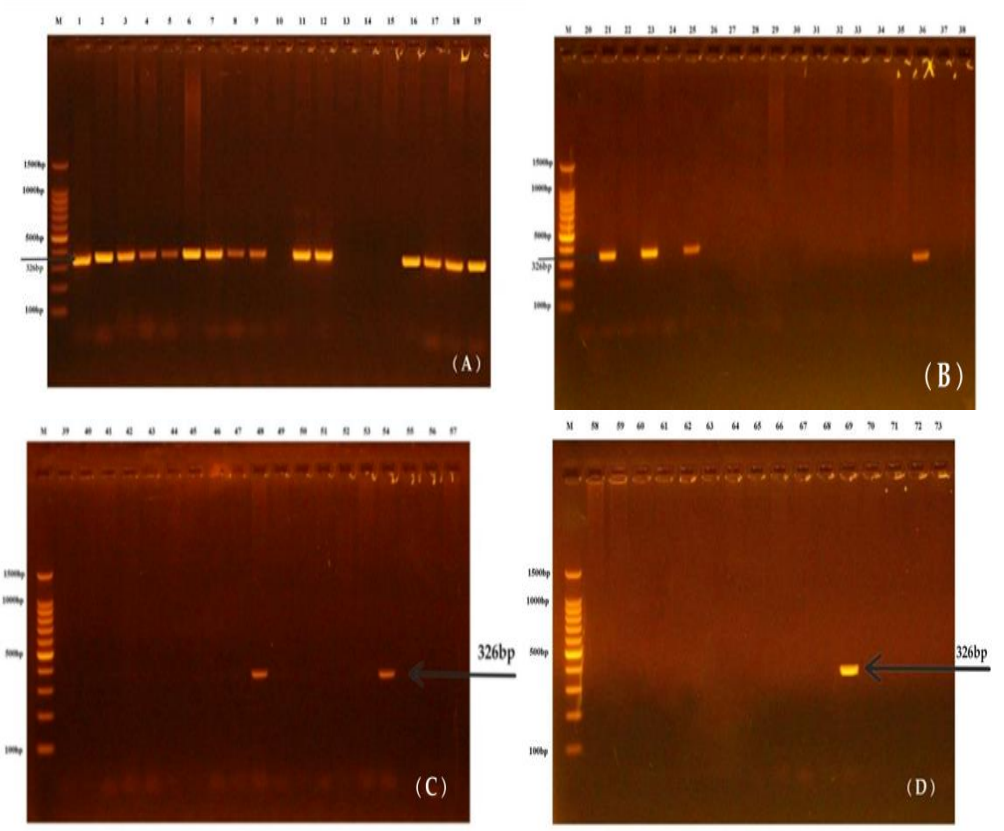


Figure1: Agaros gel electrophoresis(1.5% agarose,100v for 1hr) for *papE* gene , lane M 100bp DNA Ladder, lanes 1-9 , 11,12, 16-19 (A) 21,23,25,36(B) 48,54(C) and 69 (D) represent of *papE* bands (326bp).

The results of the current study of the *papE* gene were close to the results [13] the study included 40 urine samples for the detection of *UPEC* using the PCR technique , it was found that 37.5% were carriers of this gene. Adhesion proteins found on the surface of bacteria are among the most important virulence factors for *UPEC* these include P fimbriae and are associated with Pyelonephritis , encoded by the pap gene, which means Associated Pili-Pyelonephritis, this allows the bacteria to invade the epithelial cells and urinary tract[14].

Other adhesion genes of *UPEC* were investigated the results showed the presence of the *fimH* gene in all isolates of *UPEC* 100% (Fig 2).

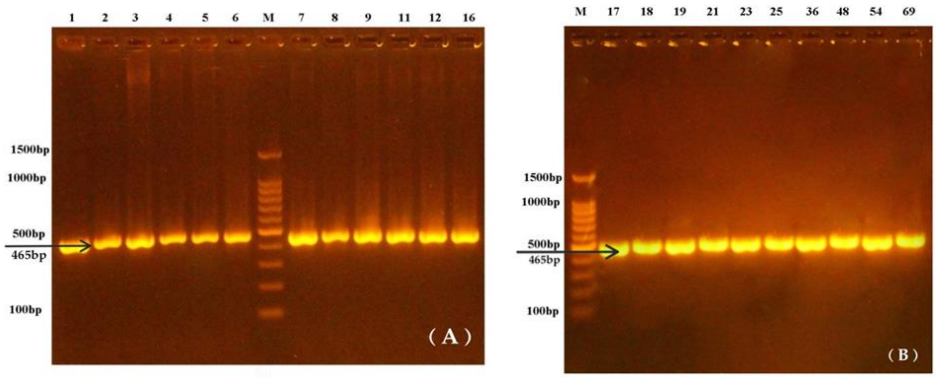


Figure2: Agaros gel electrophoresis(1.5% agarose,100v for 1hr) for *fimH* gene,lane M 100bp DNA Ladder, lanes 1-16(A) , 17-69 (B) represent of *fimH* bands (465bp).

The results of the current study agree with the findings of both [15, 16] the percentage of bacterial isolates that possess fimH gene was 100%. The fimH gene is encoded by the Type 1 Fimbriae factor, this factor is one of the most important virulence factors for E.coli that infect the urinary tract and helps bacterial cells to stick to the inner wall of the bladder and form colonies[17] .

The results of the current study showed that UPEC bacterial isolates containing the papC gene it was 31.81% (Fig 3) , the results of the current study are close to the findings Dahwash 34.2% [18].

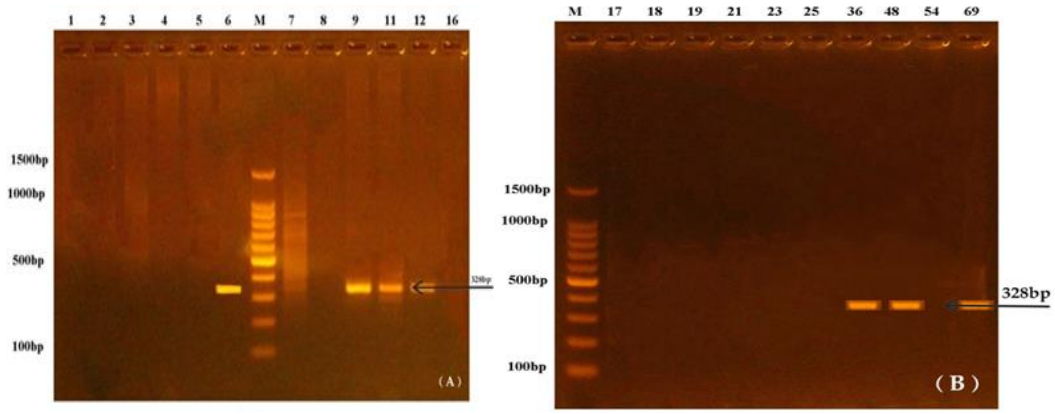
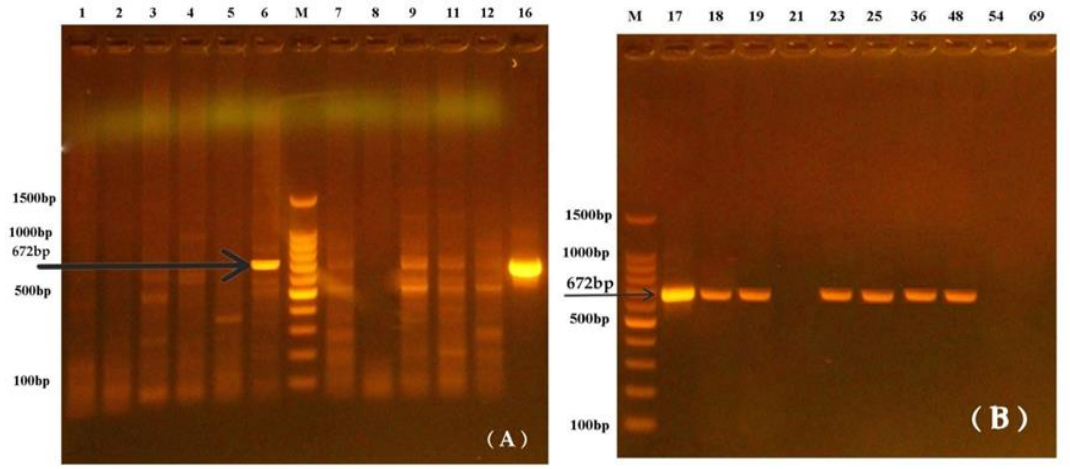


Figure (3): Agaros gel electrophoresis(1.5% agarose,100v for 1hr) for *papC* gene,lane M 100bp DNA Ladder, lanes 6,9-12(A) 36,48,69(B) represents of *papC* bands (328bp).

The papC gene is one of the genes responsible for adhesion. It binds to the outer membrane protein of the cell, as it contributes to pathogenicity by promoting bacterial colonization of host tissues and helps it stick to the host's tissues and thus the occurrence of urinary tract infection [19] .

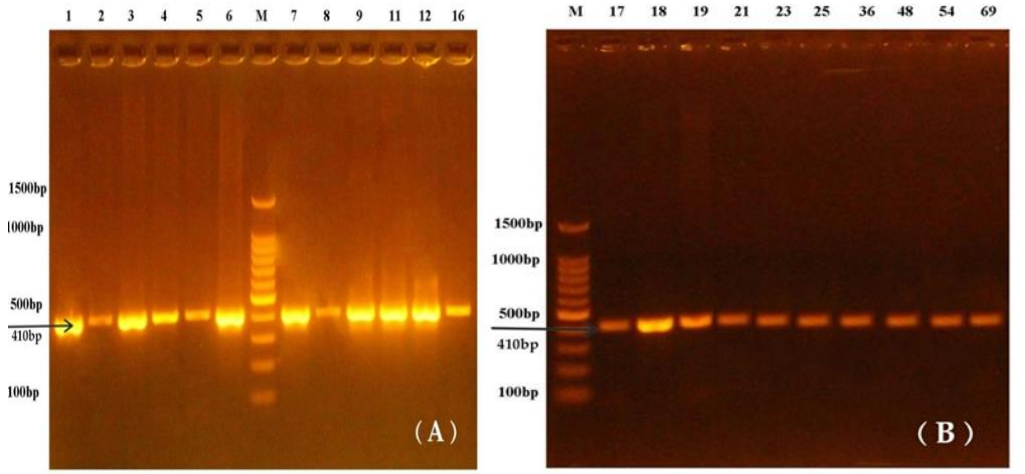
The afa gene in UPEC bacterial isolates, the results showed that 40.9% have this gene(fig 4) . In the study done in Uganda by Katongole [20] , the results showed 8% of isolates were carrying this gene .

The afa gene is instrumental in pathogenesis and is able to promote adhesion to epithelial cells and invasion of host tissues and enhances the ability of bacteria to overcome host defenses, which leads to urinary tract infection [21].



Figure(4): Agaros gel electrophoresis(1.5% agarose,100v for 1hr) for *afa* gene,lane M 100bp DNA Ladder, lanes 6,9(A) 17-19, 23-48(B) represent of *afa* bands (672bp).

As for the *sfa* gene, the results showed that the percentage of *UPEC* bacterial isolates that contain the *sfa* gene 100% (Fig 5) these results were similar to the results obtained ,the percentage of bacterial isolates that possess the *sfa* 100%[22].A gene responsible for adhesion to the epithelial cells of the kidneys and bladder and the colonization of the urinary tract and thus the occurrence of urinary tract infection[23].



Figure(5): Agaros gel electrophoresis(1.5% agarose,100v for 1hr) for *sfa* gene,lane M 100bp DNA Ladder, lanes 1-16(A) 17-69(B) represent of *sfa* bands (410bp).

Conclusion

Adhesion genes have a role in the pathogenicity of *E.coli* by promoting the colonization of bacteria and helping it adhere to host tissues and thus the occurrence of urinary tract infections . Also, these genes are related in the formation of biofilms which are considered one of the most important factors of virulence and have a role in causing pathogenicity and antibiotic resistance.

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