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Molecular Detection of Some Virulence Genes in E. coli Isolated from Urinary Tract Infections

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Abstract. General Background: Urinary tract infections (UTIs) are among the most prevalent bacterial infections worldwide, predominantly affecting women and often caused by Escherichia coli (E. coli). Specific Background: The pathogenicity of E. coli in UTIs is primarily associated with its virulence genes, which enable bacterial adhesion, toxin production, and iron acquisition, thus facilitating colonization and persistence in the urinary tract. Knowledge Gap: Despite extensive research, the prevalence and distribution of major virulence genes among E. coli isolates in specific regions, such as Karbala, Iraq, remain insufficiently characterized. Aims: This study aimed to detect five virulence genes (HlyA, papC, iutA, CNF-1, and Sfas) in E. coli isolates obtained from women with UTIs, using molecular PCR techniques. Results: Among 24 isolates, the genes HlyA, papC, CNF-1, and Sfas were detected in 45.83%, 50%, 37.5%, and 37.5% of samples respectively, while iutA was absent. Novelty: This is one of the few studies providing molecular evidence of virulence gene distribution in UTI-associated E. coli within Iraq, highlighting regional variation compared to previous international reports. Implications: The findings enhance understanding of the molecular basis of UTI pathogenicity and may inform the development of targeted diagnostic and therapeutic strategies.

Highlights:

1. The study identifies the distribution of five major virulence genes in E. coli isolated from UTI cases.
2. PCR analysis reveals variation in gene frequency, indicating differing pathogenic potentials among isolates.
3. The findings emphasize the importance of gene detection for improving therapeutic and preventive strategies against E. coli infections.

Keywords: CNF-1, HlyA, iutA, papC, Sfas, UTI

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Introduction

This takes away metabolic waste from the blood. Urinary tract infections (UTIs) are one of the most common infectious diseases in the community. according to a ban report by the Centers for Disease Control [1]. Urinary tract infection is second medical problem in most societies following the respiratory infection [2]. Women and girls of all ages can get UTIs because the female urethra is much shorter than the male urethra, which allows bacteria near the vagina to enter a woman's urinary tract more easily than in men, as well as elevates during sexual intercourse or urination. The infections above OLD women gets via hormonal changes, weakened immune system or lower immunity, Pregnancy and underlying diseases as cancer (very rare), diabetes (common cause)or HIV that leads to immunosuppression and weaker immune defense system. All these factors further add on to the risks of UTIs [3]. Women are more susceptible to UTIs than men, in part because it has a much shorter distance between their urethral opening and the bladder, as well as the inability to release bacteria via urination from the body that can settle within the vagina or rectum. Risk factors for E. coli infection include frequent sexual intercourse and use of spermicides [4]. UTIs can be categorized as complicated (cUTIs) or uncomplicated (uUTIs) [5].

*The pathogenisis is due to bacterial colonization from the urethra to the bladder. The main bacteria which lead to UTIs are derived from the gut flora, of which Escherichia coli and Klebsiella pneumoniae are the most predominant causative organisms, etc [6].

E coli is a gram negative bacillus that colonizes the human intestine. It has been heralded to cause around 80% of the uncomplicated community-acquired urinary tract infections [7]. A number of E. coli isolates are pathogenic outside the intestine. The site of infection by E. coli is varied (urinary, pulmonary, central nervouse system). The processes involved in E. coli accession to the urinary tract illustrate its ability to adapt from the completely different environment of gut [8]. E. coli strains have many virulence factors that enable them to migrate to other places in the body, reproduce, and cause infection. These factors include molecular adhesion, iron acquisition systems, invasion proteins, and toxins. These factors are controlled by several genes [9]. One of the most important secretory virulence factors of the lipoprotein of E. coli is called alpha hemolysin (HlyA), which is an alpha hemolysin (HlyA) toxin capable of lysing red blood cells and host cells, this process can aid the passage of bacteria through mucosal barriers, disrupt effector immune cells, and release host nutrients and iron reserves [10]. E. coli bacteria contain the papC gene, which is located at the door pap of the operon. This gene is considered one of the most important virulence genes associated with the bacteria's ability to adhere [11]. The iutA gene in E.coli encodes the, and facilitates iron acquisition by mediating the uptake of siderophores [12]. Cytotoxic necrotizing factor-1, CNF-1, produced by E. coli strains [13]. It is named for its ability to cause rabbit skin erosion when isolated and tested [14]. CNF-1 belongs to the family of dead toxins and the presence of the CNF-1 gene from E. coli in other Gram-negative cells is attributed to the occurrence of horizontal gene transfer [15]. Also, the S fimbriae gene, Sfas, is considered a virulence gene in E.

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coli because it promotes bacterial penetration into the tissues [16] . The currents study aimed to investigate the incidence of some genes responsible for virulence factors in E. coli isolates from urinary tract infection.

Methods and Materials

Twenty-four *E. coli* isolates were collected from women diagnosed with urinary tract infections at the Gynecology and Obstetrics Teaching Hospital in Karbala Governorate. The bacteria were activated on MacConkey agar media and purified. After that, DNA was extracted from bacteria using the kit provided by Intron Biotechnology, Inc., and purified according to the company's established instructions. The extracted DNA was used in PCR to detect the *HlyA* , *papC* , *iutA* , *CNF-1* , and *Sfas* .

The PCR machine was programmed according to the specific conditions for the *HlyA* , *papC* , *iutA* , *CNF-1*, and *Sfas* gene as shown in the Table 1.

Three microliters of forward and reverse primers with 5 µl of DNA extracted from bacteria were added to the master mixture and the volume used was completed with distilled water to 20 µl and introduced into the PCR machine after programming the machine to the appropriate conditions for each. The replication products were detected by electrophoresis of the product in an agar gel and detection of bands using a UV device.

Table 1: sequence of target primer and appropriate conditions for PCR

Gene	Sequence	Conditions	
HlyA *1177 bp*	F-AACAAGGATAAGCACTGTTCTGGCT R-ACCATATAAGCGGTCATTCCCGTCA	**Primary denaturation:** 94 c for 10 min **Denaturation:** 94 c for 1min. **Annealing:** 64-68 c for 1-3 min **Extension:** 72 cfor 7 min. **Final extenuation:** 72 c for 7 min. **No. of cycle:** 36	
papC -200 bp	F-GTGGCAGTATGAGTAATGACCGTTA R: ATATCCTTTCTGCAGGGATGCAATA	**Primary denaturation:** 95 c for 10 min. **Denaturation:** 95 c for 45 sec. **Annealing:** 58 c for 52 sec. **Extension:** 63 c for 25 sec.	

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		Final extenuation: 72 c for 10 min**.** **No. of cycle:** 42	
iutA-302 bp	F-GGCTGGACATCATGGGAACTGG R- CGTCGGGAACGGGTAGAATCG	**Primary denaturation:** 95 c for 10 min. **Denaturation:** 95 c for 45 sec. **Annealing:** 58 c for 52 sec. **Extension:** 72 c for 25 sec. **Final extenuation:** 72 c for 10 min. **No. of cycle:** 42	
CNF-1-498 bp	F-AAGATGGAGTTTCCTATGCAGGAG R-CATTCAGAGTCCTGCCCTCATTATT	**Primary denaturation:** 95 c for 5 min **Denaturation:** 95 c for 30 sec. **Annealing:** (42-45-47-49-52) c for 45 sec. **Extension:** 72 c for 45 sec. **Final extenuation:** 72 c for 7 min**.** **No. of cycle:** 35	
Sfas-240bp	**F- GTGGATACGACGATTACTGTG** **R- CCGCCAGCATTCCCTGTATTC**	**Primary denaturation:** 94 c for 5 min **Denaturation:** 94 c for 30 sec. **Annealing:** 63c for 30 sec. **Extension:** 68 c for 3min. **Final extenuation:** 72 c for 10 min**.** **No. of cycle:** 35	

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Results

The frequency of the target genes in the current study was not widely distributed among the isolates tested as shown in Table 2. PCR results demonstrated that *HlyA* was present only in 11 isolates, **whereas** *papC* gene was present in 12 isolates out of 24. Also*, CNF-1* demonstrated a positive result in 9 isolates while *iutA* did not show any positive results. Lastly, *Sfas* gene displayed a positive result in 9 isolates as shown in Table 2. Figure 1 describes a bar chart with the positive rates for each tested gene [17].

Table (2): The distribution of the target genes in *E.coli* isolated.

NO.	***HlyA*- 1177 bp**	***papC* - 200 bp**	***CNF-1* - 498 bp**	***iutA*-302 bp**	***Sfas*- 240bp**
1	**Positive**	**Negative**	**positive**	**Negative**	**Positive**
2	**Positive**	**positive**	**Negative**	**Negative**	**Negative**
3	**Negative**	**Negative**	**positive**	**Negative**	**Positive**
4	**Positive**	**positive**	**Negative**	**Negative**	**Positive**
5	**Negative**	**Negative**	**positive**	**Negative**	**Positive**
6	**Positive**	**Negative**	**Negative**	**Negative**	**Negative**
7	**Negative**	**positive**	**positive**	**Negative**	**Positive**
8	**Negative**	**positive**	**positive**	**Negative**	**Positive**
9	**Positive**	**positive**	**Negative**	**Negative**	**Positive**
10	**Negative**	**Negative**	**Negative**	**Negative**	**Negative**
11	**Negative**	**positive**	**Negative**	**Negative**	**Negative**
12	**Positive**	**positive**	**Negative**	**Negative**	**Negative**
13	**Negative**	**Negative**	**Negative**	**Negative**	**Negative**
14	**Negative**	**positive**	**Negative**	**Negative**	**Negative**
15	**Positive**	**Negative**	**positive**	**Negative**	**Negative**
16	**Positive**	**Negative**	**Negative**	**Negative**	**Positive**
17	**Negative**	**positive**	**positive**	**Negative**	**Negative**
18	**Positive**	**positive**	**Negative**	**Negative**	**Negative**
19	**Negative**	**Negative**	**positive**	**Negative**	**Negative**
20	**Negative**	**Negative**	**Negative**	**Negative**	**Negative**
21	**Positive**	**Negative**	**Negative**	**Negative**	**Negative**
22	**Negative**	**positive**	**Negative**	**Negative**	**Positive**
23	**Positive**	**Negative**	**positive**	**Negative**	**Negative**
24	**Negative**	**positive**	**Negative**	**Negative**	**Negative**

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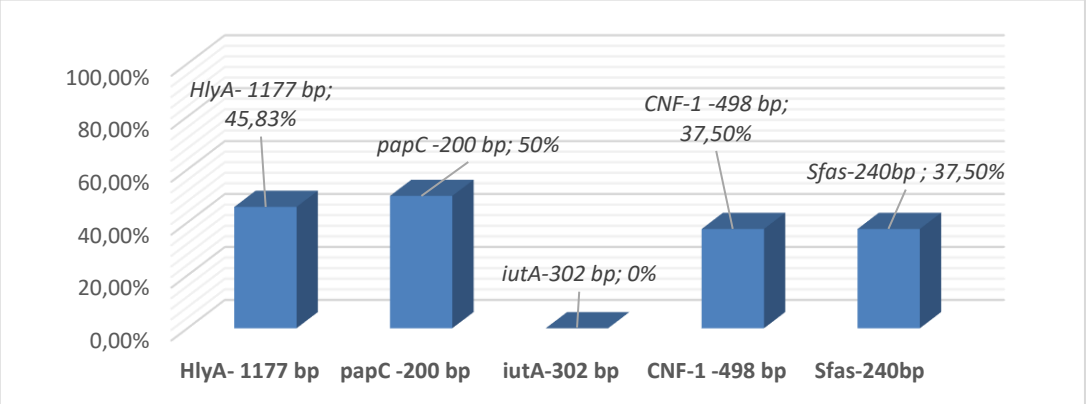

Figure (1): - Some gene frequencies in *E. coli* isolates in percentage. The data is presented on two axes: x (genes) and y (percentage frequency). Each bar shows the frequency of a single gene, with the height proportional to the percentage [18]. The frequency of the gene was calculated using the following formula: Frequency % = number of positive samples/ total number of samples *100

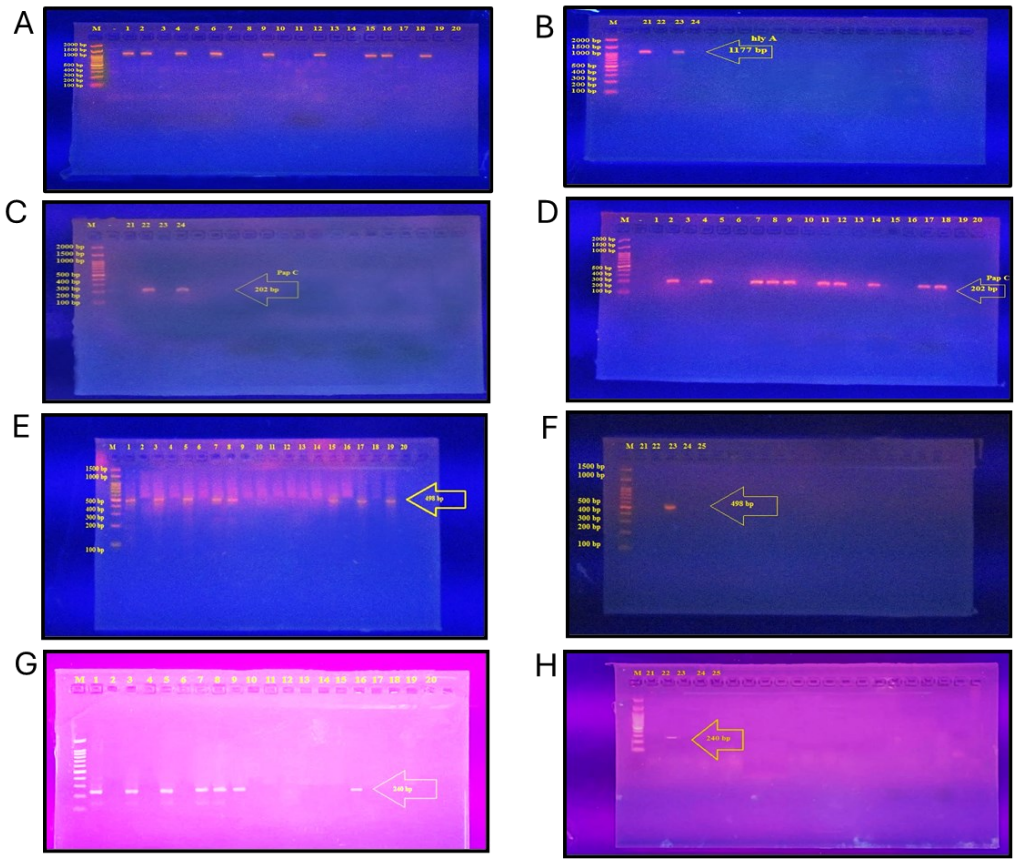

Figure 2: Distribution of the five tested genes in *E. coli* isolates. The results represent the PCR results on agarose gel (5%) using specifically designed primers for the five tested genes. The A and B represents the distribution of *HlyA* gene in *E. coli* isolates and C and D show the Distribution of the *Pap C* 202 bp gene in *E. coli* isolates. The distribution of the *CNF-1* -498 bp gene in *E. coli* isolates is represented in E and F, While the distribution of the *Sfas*-240bp gene in *E. coli* isolates is shown in G and H [19].

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Discussion

One well known and widely used prokaryotic bacteria is E. coli. In our study 5 genes one of them gene called hemolytic gene (hly A 1177 bp) which encode for a virulent factor increasing by this way the spreading nature, in our work we noticed that % hly Agene Distribution was very high (45.83%) when it compare with study done in IRAQ-baghdad they found also out of 63 E coli isolates there were10 (15.9%) haveorgenethe hly A[20], and also in another local Iraq- tikrit is was35 mentioned than 75% from20isolates have HLYA [21]. However, in the third study (in Kerman, Iran) the rate of this gene was 28.8 which is much lower than that found in our study percentage. And the second gene in this work was pap C- 200 bp,percentage of this gene is 50% and it is distributed across half of the isolates, while its percentage in the other studies showed a slight variation such as The frequency of pap C (38.8 %) [22] comparing to our study. In another study at Assiut University Hospital Egypt, PapC was the most frequent virulence gene detected in 55% [23]. In the present study, we further explored the virulence gene responsible for iron uptake in bacteria. All the isolates in this work were negative for the polymerase chain reaction of that gene. This is an indication that these bacteria are weakly virulent, as opposed to the report of [24] where the gene was moderately frequent at 62%. Investigation of cytotoxic necrosis factor (CNF-1) is one of the most critical toxins in E. coli species, and its prevalence among isolates was 37.5%; it was reported to be about 19% from different reports in Turkey [25]. Finally, 37.5% carriage of biofilm-forming bacterial resistance to antibiotics gene sfaS was observed. Although 8.9% is a low frequency, but this percentage was higher than that in the other study carried out in Egypt [26].

Conclusion

Detecting virulence genes in E. coli bacteria that cause UTI in women is a crucial step in understanding the nature of the infection and the progression of the disease. Identifying these genes helps to reveal the mechanisms by which the bacteria attack host cells and resist the immune system, enabling the development of effective therapeutic strategies that precisely target these mechanisms. This investigation also contributes to improving the ability to predict the severity of infection and the likelihood of recurrence, which helps in improving preventive and therapeutic measures and reducing the risk of chronic infection or complications.

Interests Disclosure

The authors report no potential conflicts of interest

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