ORIGINAL ARTICLE

Some Virulence Genes Profile of *Escherichia coli* Stains Isolated from Urinary Tract Infection in Iraqi patients

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ABSTRACT

Background: The urinary tract infection (UTI) is the most frequent bacterial infectious illness seen in clinical practice, accounting for considerable morbidity and high medical expenses. *Escherichia coli* is the most prevalent bacterium causing UTIs. The expression of a broad range of virulence factors by *E. coli* contributes to the severity of UTI. This research investigated the function of *E. coli* virulence determinants in the pathogenesis of UTI. The aim of the present study is to identify virulence genes in Uropathogenic *E. coli* which are responsible for UTI by isolation and identification of major microorganisms (Uropathogenic *E. coli*) that cause UTI and detection of antimicrobial susceptibility of the bacterial isolates. The determination of some virulence uropathagant genes such as *cnf, hly*, and *fimH* genes by PCR.

Material and Methods: From March 2022 to June 2022, approximately 200 clinical urine samples were collected from patients suffering from UTI of both genders; (160) urine samples from females and (40) urine samples from males in age groups ranging from 15–50 years who attended Al-Sadr Teaching Hospital, Al-Zahrawi Surgical Hospital, and Misan Hospital for child and childbirth in Misan city, Iraq.

Results: In the current study, *E.coli* has some virulence factors such as *cnf, hly*, and *fimH* for (40) *E.coli* stains were reported as a positive 4(10%), 30(75%), and and 13(32.5%) respectively.

Conclusions: Our findings suggest that looking into the bacterial pathogenicity linked to UTIs might help doctors provide better care.

Keywords: *Escherichia coli*, Urinary tract infection, Virulence's genes, Antibiotic resistances

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INTRODUCTION

A urinary tract infection (UTI) is one of the most frequent infectious diseases and a serious public health issue worldwide.[1] Asymptomatic, acute, and chronic mayati are the three types of UTI prevalent in both men and women. Acute UTI is most often observed in females of all ages; all patients are treated as outpatients and are very rarely admitted to the hospital.[2] Infections may be caused by bacteria, fungi, yeasts, and viruses, which are more easily discovered. As a result, you may be unaware of a potentially fatal condition that can lead to chronic pyelonephritis and chronic renal failure.[3] The most common pathogen for uncomplicated UTIs is *Escherichia coli* (75–95%), followed by *Klebsiella pneumoniae*, *Staphylococcus saprophyticus, Enterococcus faecalis*, group B streptococci, and *Proteus mirabilis*. Both simple and complex UTIs can be caused by *E. coli*.[4]

MATERIAL AND METHOD

Samples collection: About 200 of clinical urine samples were collected from patients suffering from UTI of both gender, (160) urine samples from female and (40) from male in age groups range from (15–50) years all patients attending to Al-Sadr Teaching Hospital, Al Zahrawi Surgical Hospital, and Misan Hospital for child and childbirth in Misan city, Iraq, from March 2022 to June 2022. The clinical history of each case and full information was taking directly from the patients.

Isolation and Identification of *E. coli*

Each urine sample was cultured in a MacConkey and blood agar and incubated at 37°C for 24 hours to stimulate bacterial growth. They were then streaked on general and differential culture medium and cultured for 24 hours at 37°C. Lactose fermenting colonies streaked on MacConkey agar plates were chosen and re-cultured on new MacConkey agar plates to

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Table 1: The sequence of primers that used this study.

Name gene		*Primer sequence*	*Tm (°C)*	*GC%*	*Size of Product (bp*	*Ref.*
Cnf	F	3-TTATATAGTCGTCAAGATGGA-5	51	33	693	9
	R	5-CACTAAGCTTTACAATATTGA-3	9.47	29		
hly	F	3-AGATTCTTGGGCATGTATCCT-5	57.6	43	556	10
	R	5-TTGCTTTGCAGACTGTAGTGT-3	57.6	43		
fimH	F	3-AACAGCGATGATTTCCAGTTTGTGTG-5	5.61	42	465	11
	R	5-ATTGCGTACCAGCATTAGCAATGTCC-3	4.62	46		

Table 2: distribution of the patients according to the living location

City center No. (%)	Country side No. (%)	Total (%)
60 (30)	140 (70)	200 (100)

yield pure, well-separated colonies. To explore the appearance of green metallic sheen colonies, the colonies were streaked with eosin methylene blue and the plates were cultured for a further 24 hours at 37°C.[5] Positive growth was identified by morphological, microscopical, and biochemical assays (Vitek-2 system for bacterial growth detection).[6] Identification through microscopic examination. All smears were made from a single colony of juvenile bacterial isolate cultured on culture media for 18–24 hours, fixed on a clean and dry slide, and inspected under a light microscope with a gram stain.[7]

DNA Extraction of *E. coli*

By using DNA bacterial extraction (origin Hungarian) according to manufactured companies, Agarose gel electrophoresis according to clinical and laboratory standards institute[8] and in Table 1, the primer used in this search for all virulence factors of *E. coli*.

Polymerase Chain Reaction (PCR) Protocols for Virulence Factors Gene of *E. coli*

The PCR program for amplification of virulence genes for identification of *E. coli*. For 5 minutes at sec 45, perform all steps as initial denaturation 95ºC. Denaturation-2, 95ºC for 1 cycle. Annealing 58ºC, sec 45, extension-1, 72ºC, in sec45 during 35 cycles, finally in 1 cycle, extension-2, 72ºC in 5 minutes.

RESULTS AND DISCUSSION

Data Description of Study Population

As in Table 1, about 200 urine samples were collected from patients suffering from UTIs aged 15 to 50 years. The subjects were categorized into groups according to gender. Most patients were females at 80% (160/200), while the males represented 20% (40/200) of the total patients. In Table 1, the distribution of the patients according to the living location address showed that patients who live in the countryside had higher mean values than those who live in the city center (Table 2).

Isolation and Identification of *E. coli*

In this study, 200 urine samples were collected from patients with UTIs of both genders at different ages. The urine samples were cultured on the nutrient agar, MacConkey agar plates, blood agar plates, CCA agar and EMB agar plates for isolation

Table 3: Types of Bacterial Isolates recovered from UTI urine Sample.

Bacterial Isolates	*No.*	*%*
Escherichia coli	66	37.71%
Others	109	62.29%
Total	175	100%

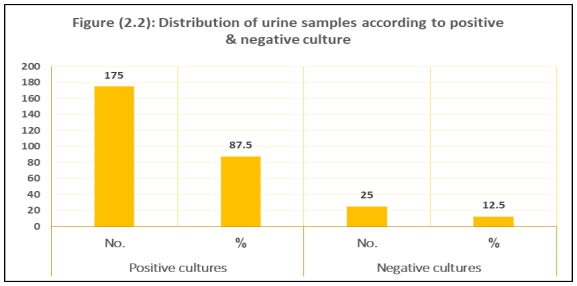

Figure 1: Distribution of urine sample according to positive and negative culture

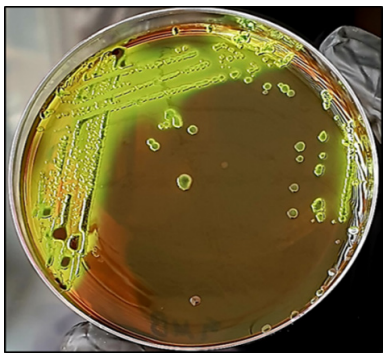

Figure 2: Colonies of *E. coli* (green metallic sheen) on EMB agar at 37°C for 24 hours.

and identification of *E. coli*, and 87.5% (175/200) samples showed positive bacterial cultures, whereas no growth was seen in the other 12.5% (25/200) samples as in Figure 1.

Among 175 positive culture samples, only 37.71% (66/175) isolates belonged to *E. coli*, and 62.29% (109/175) isolates belonged to other genera of bacteria as show in Table 3 and Figure 2.

Profile Virulence Genes of *E. coli*

In the present study, *E. coli* has some virulence factors such as *cnf, hly,* and *fimH,* for 40 *E. coli* stains as shown in Figures (3 to 8). *E. coli* is present in most UTI causes in both sexes.[12] Several virulence determinants contribute to the pathogenicity of *E. coli* in UTI, which produce different genes detected by PCR.[13] Some genes coding virulence genes like *fimH, hly,* and

cnf genes all play a role in the degree of pathogenicity of *E. coli* in UTI patients. The first virulence gene identified in this study was *fimH,* gene which had a positive rate of 30 (75)%, which agrees with other published data.[14] These virulence genes agree with 4, which recorded 80% as a positive rate in cystitis patients.[15] Wang *et al.*[16] recorded the role of pathogenic *E. coli* in the patients suffering from UTI was 92% of *FimH* gene or adhesion gene. Another results was similar to this study was recorded 86%[17] from 108 *E. coli* stain isolated from women clinically diagnosed with UTI were screening to detect virulence gene antibiotics resistance. According to different study done by Karimian *et al.*[18] was recoreded from total of 123 strain of *E. coli* isolated from hospitalized patients with UTI these results recorded *fimH* gene was 79.67%.[19] The other virulence genes in *E. coli* stains in this study were hly and *cnf* genes. These genes encoded two toxins implicated in this damage and dysfunction of local immune response.[20] The result of PCR assay was detected hemolysis gene *hly*. In a recent study, the *hly* gene was found to be positive 13(32.5%) of the time and negative 27(67.5%) of the time; however, this study disagreed with result data 4. Karimian *et al.*[18] was study a total of 123 stains of *E. coli* form hospitalized patients with UTI detected by PCR hly gene was 50.4% while about 75 patients suffering from bacteriuria caused by UIT and hemolysin elevated 45% (31) in patients with urosepsis.[21] In contrast to higher elevated rate of hemolysin in Brazile[22] and low percentage (25.3%) in cystic patients.[23] A third virulence gene was cytotoxic necrotizing factor-1 (CNF gene) was reported with 4(10) as a positive rate and 36(90%) negative results. These studies agree with the reported rate of 1(3%)[25] as in Tables 4 and 5.

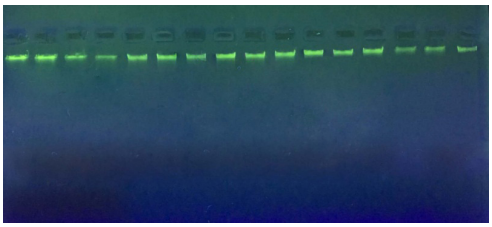

Figure 3: Gel electrophoresis of genomic DNA extraction from bacteria 1% agarose gel at 1houre

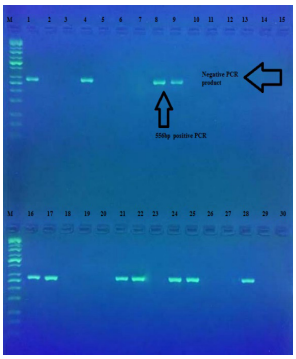

Figure 4: Gel electrophoresis of genomic DNA extraction from bacteria 1% agarose gel at 1-hour

Table 4: Virulence gene of *E. coli*

E. coli strain No	Hly	FimH	CNF
Ec 1	+	+	-
Ec2	-	+	-
3	-	+	-
4	+	+	+
5	-	+	+
6	-	+	-
7	-	+	-
8	+	+	-
9	+	-	+
10	-	-	-
11	-	+	-
12	-	+	-
13	-	+	-
14	-	-	-
15	-	+	-
16	+	+	+
17	+	+	-
18	-	+	-
19	-	+	-
20	-	+	-
21	+	+	-
22	+	-	-
23	-	-	-
24	+	+	-
25	+	+	-
26	-	+	-
27	-	-	-
28	+	-	-
29	-	-	-
30	-	+	-
31	-	+	-
32	+	+	-
33	-	+	-
34	+	-	-
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37	-	+	-
38	-	+	-
39	-	-	-
40	-	+	-

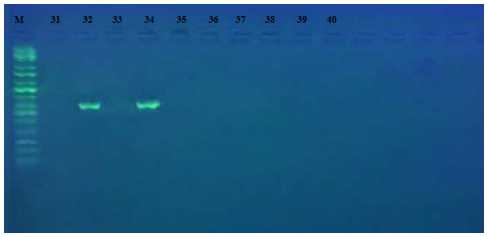

Figure 5: PCR product the band size 556bp of hly GENE . The product was electrophoresis on 1.5% agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours. M: DNA ladder (100).

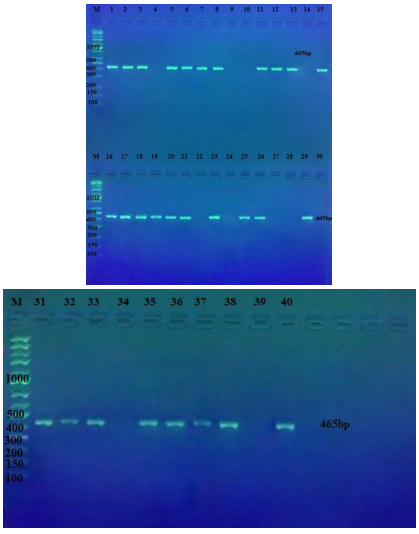

Figure 6: PCR product the band size 465bp of *FIM GENE* . The product was electrophoresis on 1.5% agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours. M: DNA ladder (100).

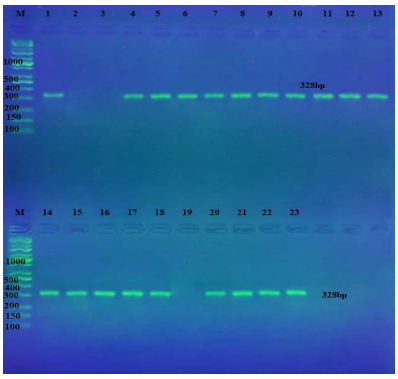

Figure 7: PCR product the band size 328 bp of *P fimbriae GENE* . The product was electrophoresis on1.5 % agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours. M: DNA ladder (100).

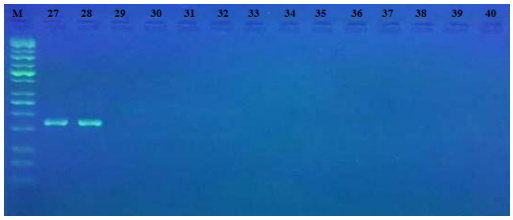

Figure 8: PCR product the band size 328 bp of *P fimbriae GENE* . The product was electrophoresis on 1.5% agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours. M: DNA ladder (100).

Table 5: A percentage of positive and negative virulence factors gene

Genes	*Positive (%)*	*Negative (%)*
hly	13(32.5)	27(67.5)
fimH	30(75)	10(25)
cnf	4(10)	36(90)

CONCLUSION

- Among the bacteria, *E. coli* is the species that is most common producing UTI .
- Males are less likely to have UTIs than females.
- The best recommended antibiotic to use in case of *E. coli* causing UTI can be the meropenem, amikacin, uropathogenic *E. coli* isolates are mostly resistant to amoxicillin-clavulanic acid.
- There is a relationship in *cnf, hly, afa, papC* and *fimH* genes with UTI, *papC*, *fimH*, genes are present simultaneously in some isolates which gives a clarified perception that *E.coli* has more than one adhesion factors
- There are variations in nucleotides sequences of *cnf, hly,* afa, papc and *fimH* genes in this study's isolates (Iraqi isolates) compared to the sequences of the same genes of foreign isolates.

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