Study Of Some Virulence Genes Of Escherichia Coli Isolated From Urinary Tract Infection Patients And Their Relationship To Biofilm Production

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Abstract

Background: Urinary system Infection (UTI) can be caused by Uropathogenic Escherichia coli (UPEC) strains that encode surface and secretory virulence factors. These strains can colonize the host urinary system and facilitate bacterial development. **Aim**: The current study was aimed to detection of some virulence genes of Escherichia coli isolated from urinary tract infection patients and their relationship to biofilm production. **Materials & methods**: 117 Clinical samples were collected from Kirkuk Hospital in Kirkuk city for the period from October 2023 to February 2024. Based on the culturing and biochemical features of E. coli colonies developing on blood agar, MacConkey agar and Eosin-methylene blue (EMB), the colonies were diagnosed after incubation for 24 hours at 37 ^{0}C. Diagnosis were confirmed by Vitek 2 system. **Results**: Forty two (35.9%) of all specimens showed positive results for the growth of E. coli. Of the total samples, 75 (64.1%) showed negative results for the growth of E. coli. The results of the current study show that the E. coli showed total resistance (100%) to Ampicillin. while Tobromycin showed high sensitive 100%. While different degrees of sensitivity were found towards the rest of the study antibiotics. The percentage of E. coli for biofilm production. Whereas, 18(42.9%) E. coli isolated was strong biofilm production, 11(26.2%) E. coli isolated was moderate biofilm production, and 8(19.0%) E. coli isolated was weak biofilm production. In the current study, 37(88.1%), 20(47.6%) E. coli isolates possessed FimH and Cnf1 genes, respectively, as virulence factor. **Conclusions**: In the present study the prevalence of FimH and Cnf1 genes in UPEC were determined and the results showed that among biofilm producers, FimH was the most prevalent Urovirulence gene followed by Cnf1.

Introduction

Throughout the world, millions of individuals of all ages and gender identities suffer from urinary tract infections (UTIs), which are among the most common infections caused by bacteria [1-2]. A urinary tract infection (UTI) is defined as having more bacteria than 10^5 microliters per milliliter in the urine. Anatomically, UTIs are divided into two categories: lower UTIs, which include the bladder and urethra, and upper UTIs, which involve the kidney, pelvis, and ureter. Above the urethra, the urinary system is normally sterile [3]. Gram-negative bacteria belonging to the Enterobacteriaceae family are the primary cause of urinary tract infections. The most common type of them is called uropathogenic Escherichia coli (UPEC). Because of the formation of biofilms and the existence of numerous virulence factors that rely on the invasion, and survival of uroepithelium cells, E. Coli strains have the capacity to cause urinary tract infections. For the majority of uropathogens, bacterial biofilm development is a significant virulence factor

that affects infection duration, resistance, and recurrence [4]. It is a representation of clusters of cells adhered to surfaces and encased in a matrix of extracellular polymeric substances (EPS) generated by the bacteria [5], shielding them from antibiotics, phagocytosis, and host defense mechanisms. Adhesion Type 1 fimbriae (fimH), a remarkably versatile UPEC virulence factor that can stabilize the attachment of the bacteria to different types of cells throughout the urinary tract, and toxins like Cytotoxic necrotizing factor 1 (cnf1) toxins, which cause cytoskeleton rearrangements in host cells, are the reasons why uropathogenic E. coli strains are pathogenic [6–9]. Therefore, the current study was aimed to detection of some virulence genes of E. coli isolated from urinary tract infection patients and their relationship to biofilm production.

Materials & methods

Specimen Collection

117 Clinical samples were collected from Kirkuk Hospital in Kirkuk city for the period from October 2023 to February 2024 from patients. The method of collecting samples included the following: midstream urine sample in sterile urine cup taken from both sexes in different age.

E. coli of identification

Based on the culturing features of the E. coli colonies developing on blood agar, EMB, and MacConkey agar, were diagnosed after incubation for 24 hours at 37 ^{0}C. biochemical tests were used to identification of E. coli and these tests including methyl red, citrate, urease, voges-proskauer, catalase, oxidase, KIA, and indole test. Vitek 2 system was carried out in order to identify and diagnose bacteria.

Antibiotic susceptibility test (AST)

According to clinical laboratory standards institute (CLSI) guidelines, the Kirby-Bauer disc diffusion technique with Muller Hinton (MH) agar was used for the AST for all isolates (CLSI, 2020). The antibiotics discs (Bioanalyse(USA)) used in this study were Ceftazidime (30ug), Tobramycin (10µg), Amikacin (30ug), Levofloxacin (5µg), Cefotaxime (30ug), Trimethoprim (5µg), Cefixime (5µg), Ciprofloxacin (30ug), Nalidixic acid (30ug), Vancomycin (30 µg), Azithromycin (30 µg), Imipenem (10ug), Clindamycin (10µg), and Gentamicin (10ug). Multidrug-resistant (MDR) organisms were defined as those that exhibited resistance to several antimicrobial agent types, classes, or subclasses.

Biofilm formation by Congo red test

Dissolve 37 g/L of brain heart infusion broth (BHI) and 50 g/L of sucrose in 500 ml of distilled water to make Congo red agar. Put in 10 grams of agar-agar. After that, the volume was adjusted to 900 ml of distilled water and the medium was autoclaved to achieve sterilization. 0.8 g of congo red stain and 100 ml of distilled water were combined to create the dye, which was then autoclaved to guarantee sterility. After autoclaving the medium and dye and allowing it to cool to 50 oC, the dye was applied to the agar. The medium was poured into Pyrex plates. Using this medium, the biofilm growth of the isolates was found [10].

Genetic Study

Isolating Genomic DNA from Gram Negative Bacteria

The purpose of the Wizard® Genomic DNA Purification Kit is to separate DNA from Gram-negative bacteria.

PCR procedure

Detecting fimH the and cnf1 sequences have been performed through the conventional PCR The polymerase chain reaction (PCR) combination of 25 µL final volume was prepared from Go Taq Green

Master Mix (2X) 12.5 µL, forward primer 1 µL, reverse primer 1 µL, DNA template 3 µL, and nuclease-free water 7.5 µL. The primers that have been utilized in the presented research have been listed in table (3-7).

Table (3-7): Uropathogenic E. coli virulence genes PCR assayprimers

Primer	Primer sequence	Length (bp)	TA (°C)
fimH-F	5- GCCAATGGTACCGCTATCCC-3	580	63.7
fimH-R	5- CGGAGAGGTAATACCCCAGG-3		63.7
cnf1-F	5-CGGCATCTACTATGAAGTGG-3	810	56.8
cnf1-R	5-GGATAGATTGCACGCTGACG-3		60

Agarose Gel Electrophoresis of DNA

In order to distinguish the bundle size of the PCR interaction on the agarose gel, electrophoresis has been used to detect DNA fragments after the procedure of extraction or to identify the result of the PCR interaction in the presence of standard DNA.

Results

Samples distribution

117 urine samples from patients with UTIs were used in the current investigation (table 1). According to the results, 42 (or 35.9%) of the total samples showed positive results for the growth of E. coli when it was cultured on blood agar, MacConkey agar, and EMB. The EMB medium gave the growth of the bacteria a green metallic sheen. Of the total samples, 75 (64.1%) showed negative results for the growth of E. coli.

Table (1): Distributed of study samples according to UTI

	No. (%) **+ve culture**	No. (%) **-ve culture**	Total No.(%)
Patients	42(35.9%)	75(64.1%)	117 (100.0%)

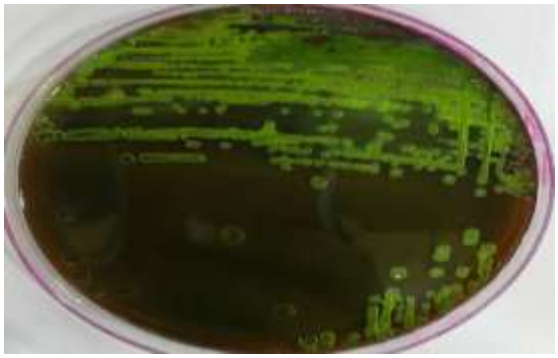


Figure (1): E. coli colonies on EMB agar.

Antibiotic susceptibility test

E. coli showed total resistance (100%) to Ampicillin. 23.1% sensitive to vancomycin and Clindamycin. Otherwise, E. coli showed 24.6%, 29.2%, 12.3%, 21.5%, 75.4%, 90.8%, 20.0%, 26.2, 24.6%, 50.8%, 89.2% and 50.8% sensitive to Trimethoprim, Ceftazidime, Cefotaxime, Cefixime, Gentamycin, Imipenem, Nalidixic acid, Ciprofloxacin, Levofloxacin, Amikacin and Azithromycin. While Tobromycin showed high sensitive 100.0% respectively, as shown in table (6).

Table (6): Antibiotic susceptibility test of E. coli

Antibiotics	Sensitive %	Intermediate %	Resistant %	P value
AMP	0.0	0.0	100.0	0.001
VN	23.1	6.2	70.7	
DA	23.1	0.0	76.9	
TMP	24.6	4.6	70.8	
CAZ	29.2	16.9	53.9	
CTX	12.3	4.6	83.1	
CFM	21.5	6.2	72.3	
CN	75.4	10.8	13.8	
IMI	90.8	4.6	4.6	
NA	20.0	0.0	80.0	
CIP	26.2	7.6	66.2	
LEV	24.6	10.8	64.6	
AZT	50.8	13.8	35.4	
AK	89.2	3.1	7.7	
TOB	100	0.0	0.0	

AMP= Ampicillin, VN= Vancomycin, DA=, Clindamycin, TMP=Trimethoprim, CAZ= Ceftazidime, CTX=, Cefotaxime, CFM=Cefepime, CN=Gentamicin, IMI=Imipenem, NA=Nalidixic acid, CIP=Ciprofloxacin, LEV= Levofloxacin, AZT = Azithromycin, AK=Amikacin, TOB = Tobramycin.

Biofilm ability of E. coli

Figure (2, 3) shows biofilm production when E. coli was grown on Congo red agar, where the black color of the colonies indicates biofilm production. The percentage of E. coli for biofilm production. Whereas, 18(42.9%) E. coli isolated was strong biofilm production, 11(26.2%) E. coli isolated was moderate biofilm production, and 8(19.0%) E. coli isolated was weak biofilm production.

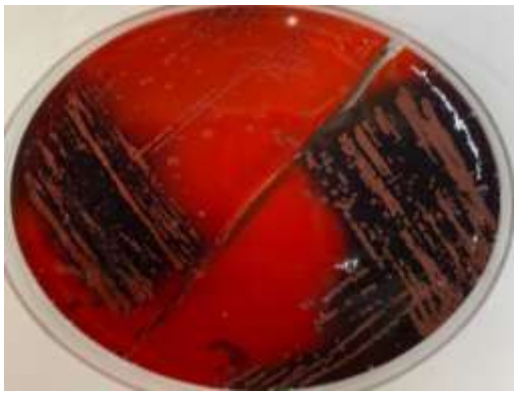
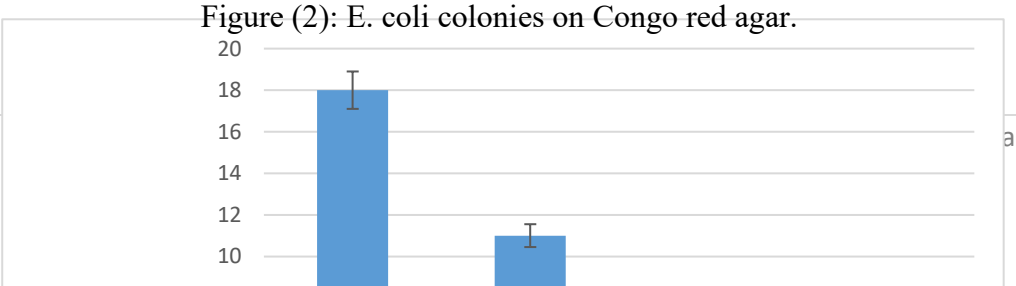


Figure (2): E. coli colonies on Congo red agar.



Genetic study

In the current study from 42 UPEC isolate, 37(88.1%) E. coli isolates possessed FimH gene and 20(47.6%) E. coli isolates possessed Cnf1 gene as virulence factor as shown in figure (4, 5).

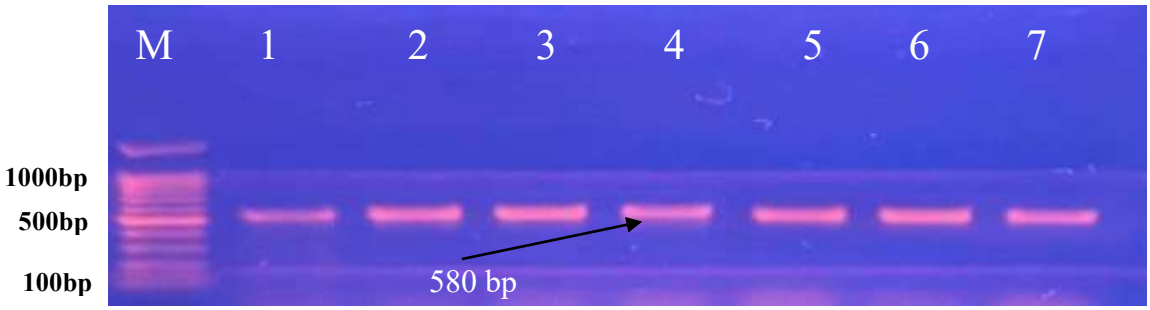


Figure (4): PCR amplification of 580bp *FimH* gene by 1.4% agarose gel electrophoresis.

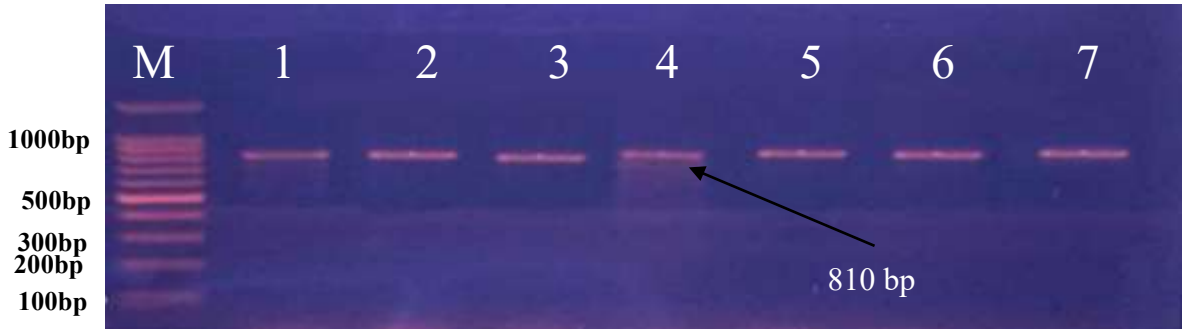
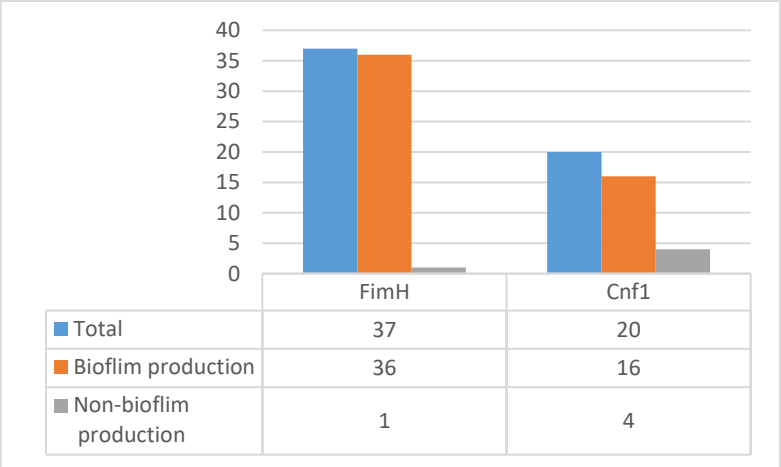


Figure (5): PCR amplification of 810bp *Cnf1* gene by 1.4% agarose gel electrophoresis. Ladder: M, Lane (1-7): PCR product of 7 E. coli isolates from urine samples

The correlation between the virulence genes and Biofilm production

The frequency of the FimH and Cnf1 genes in UPEC was assessed in the current study, and the findings indicated that among biofilm producers, FimH was the most common Urovirulence gene, followed by Cnf1 (fig: 6).



	FimH	Cnf1
Total	37	20
Bioflim production	36	16
Non-bioflim production	1	4

Figure (6): virulence genes frequency among Biofilm and None Biofilm forming of E. coli isolates.

Discussion

One of the most chronic diseases in men of all ages is UTIs [11]. The E. Coli infection rate, according to the current results, was 35.9%. This finding is consistent with a different study conducted in Iraq [12]. It has been amply demonstrated that the same bacteria is the primary global source of UTIs. An isolation rate of 45.67% for E. Coli was found, in contrast to a study conducted in a different nation [13].

The prevalence of Ampicillin resistance was determined to be 91.7%, and the percentage of resistance to Ampicillin recorded in this study was almost identical to those published by other research as [14]. Furthermore, this study supports [15]'s findings, which state that βlactamases are produced by Gram-negative bacteria, giving them the ability to demonstrate resistance to β-lactam antibiotics. (ESBLs) are a class of enzymes that can hydrolyze a variety of β-lactams, including penicillin and cephalosporins like ceftazidime, cefotaxime, and ceftriaxone, but not cefoxitin [16]. The isolates of E. Coli were shown to be very sensitive to imipenem and exceedingly resistant to ampicillin, according to the antibiotic sensitivity profile of the urine samples. This outcome is in line with past studies showing the high imipenem sensitivity of E. Coli isolates [18] and the notable ampicillin resistance of isolates from urine samples [17]. Similar results were found by [19] in a research conducted in Iraq.

In terms of biofilm formation, 37 (88.1%) of the 42 isolates that were tested were able to produce biofilms; this is less than what was reported by [20–21], who found that biofilm development was 100% common in UPEC. The prevalence of 80% [22] and 84.3% [23] reported in other studies is comparable with the findings of the current investigation. The majority of strains were able to form strong biofilms, which is in line with other research [21, 24]. These discrepancies could be the result of variations in methodology, including the source of sample isolation, study durations, geographic locations, and environmental factors that affect biofilm capacity and experimental settings [22–23].

According to [25], fimH detection was observed in 92% of the isolates in the current study, which is consistent with the findings of adhesion type 1 fimbriae found to be extremely frequent in 37 isolates. Another study [26] showed that 98% of the E. Coli strains isolated from UTI patients had the fimH gene. Furthermore, [27] examined the frequency of FimH at 87.7%. Additionally, it was discovered by [28] that

93% of the UPEC isolates have the fimH gene. The current investigation found a correlation between the presence of the fimH gene and the creation of biofilms, as seen in (fig: 4).

It was recently revealed [29] that the expression of various adhesins, including type 1 fimbriae, can be linked to the biofilm formation by extraintestinal E. coli. In addition, it was discovered by [30] that adhesive structures expressed in the early phases of biofilm growth included type 1 fimbriae (fim H). Despite reports of the existence of virulence factors (genes) that enable UPEC to form biofilms in vitro, the quantitative link between virulence factors and biofilm has produced inconsistent findings [23, 31]. Strong biofilm-producing bacteria had a greater fimH gene prevalence, which is consistent with earlier research [32–33]. Based on earlier research, strong and moderate biofilm-producing strains expressed a high frequency of type 1 fimbriae [33]. However, the results of [34], which showed that the bacteria genetic profiles fimH and Cnf1, were related with high biofilm development, are similar with the association that the current investigation revealed between the Cnf1 gene and biofilm production.

Conclusions

It was concluded from the study that Escherichia coli isolated from urinary tract infection patients possess the virulence genes (Fim and Cnf), which are related to biofilm production, which causes an increase in the virulence of Escherichia coli upon infection.

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