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Research Article

Uoropathogenic *Escherichia coli* chromosomal study of *cnf1* and *fyuA* genes among patients of urinary tract infection in Kirkuk city

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Keywords
UPEC, cnf1, fyuA, Cystitis, Pyelonephritis.

Abstract
This research has taken place at Kirkuk university/college of science/department of biology, Kirkuk hospital, pediatric hospital, and Azadi teaching hospital, in Kirkuk city, Iraq. Around 150 urine samples were taken from different ages and Patients of both genders with infections of the urinary tract according to symptoms and manifestations diagnosed by the examining physician then, these samples were investigated by a bacterial cell and an optical microscope detection next to heavy pus formation was clear evidence of harmful bacteria's existence. Positive samples were cultivated and stored for genetic research by conventional PCR. A total of 93 urine samples contained positive pathogenic bacteria cultures, with 51 of them showing *E. coli* growth, out of 51 patients, 37 (72.5%) had Cystitis, whereas 14 (27.4%) had Pyelonephritis. The isolates were identified using API 20E, and this investigation revealed that females were infected at a higher rate than men, with 39 isolates (76.4%) infected compared to 12 isolates (23.5%). *E. coli* chromosomal DNA was extracted using a gene-aid DNA isolation kit. Genetic pattern occurrence of *fyuA* and *cnf1* genes were investigated using conventional PCR, *fyuA* gene was found in all 51 (100%) isolates and also this study yielded that only 13 (25.4) isolates have carried *cnf1* gene of which 12 isolates from Cystitis and only 1 isolate from Pyelonephritis. We conclude that iron uptake is crucial for UPEC which makes a relationship between the gene *fyuA* and UTI, The strains isolated from Cystitis show the greatest diversity of genes pattern than strains isolated from pyelonephritis, cytotoxic necrotizing factor 1 toxin encoded by *cnf1* gene occur more likely in Cystitis.

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1. Introduction

Urinary Tract Infections (UTIs) are one of the most frequent disorders caused by a variety of pathogens. This infection affects women more than men. because of various anatomical and physiological abnormalities in the urinary system (Tan and Chlebicki, 2016). If the lower urinary tract is affected then It's known as a bladder infection (cystitis), and if It's a problem with the upper urinary tract then It's known as kidney infection (pyelonephritis) (Lane and Takhar, 2011).

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Uropathogenic *Escherichia coli* (UPEC) is referred to as the UTIs most common reason. Even though UPEC lacks any distinguishing characteristics, certain serotypes are more common (Langermann *et al*., 1997; Johnson and Stamm, 1989). Although UPEC is the most common reason for UTIs, other bacteria or fungi might occasionally be the reason. Sexual intercourse, female anatomy, obesity, diabetes, and family history are all risk factors. (Flores-Mireles *et al.,* 2015).

While uropathogenic virulence factors are less common in commensal strains of any fecal E. coli than in UPEC strains, they frequently exist together on pathogenicity islands (PAIs). There are no known uropathogenic virulence factors that can be used to identify UPEC isolates separately or even in combination (Hacker *et al.,* 1997). In recent years, the majority of scientists have supported the theory that "UPEC developed from nonpathogenic strains". The cause for the transformation of non-pathogenic strains to pathogenic strains is the acquisition of new virulence factors through accessory DNA horizontal transfer positioned at the chromosomal or plasmid level (Johnson *et al.,* 2005).

E. coli strains isolated from humans or animals with diarrhea or extraintestinal infection produce cytotoxic necrotizing factor1 (cnf1). (Tavechio *et al*., 2004). A gene called (Cytotoxic necrotizing factor1 gene) cnf1 gene encodes this toxin (Caprioli *et al*., 1983). CNF1 toxin is a protein toxin that causes multinucleation (cytotoxicity) in cultured cells and necrosis (necrotizing) in rabbit skin. It was first characterized as a toxin that induced dermonecrosis in rabbits when CNF-producing *E. coli* strains were injected intradermally. The development of multinucleated cells in culture was the first cytotoxic activity identified (Caprioli *et al*., 1983).

CNF1 synthesis, like *E. coli* hemolysin, is more frequently associated with UPEC strains that cause more severe UTIs (Welch, 2017), When cnf1 is found in UPEC strains, it is invariably connected to the holy CABD operon, which is a strange and intriguing observation (Landraud *et al*., 2003).

Uptake of ferric yersiniabactin, the 71 kDa outer membrane protein encoded by the *fyu* gene serves as a receptor for Fe-Ybt siderophore absorption. (Hancock *et al*., 2008). The bacteria can grow in the host and produce systemic infection because iron is readily available (Ratledge .,2007). In some ExPEC strains, yersiniabactin was discovered along with other components of a gene cluster known as the high pathogenicity island (HPI), which encodes proteins for the manufacture of the yersiniabactin siderophore and its absorption mechanism (Johnson and Stell 2000).

The study aims to unveil the pattern of occurrence of both genes *cnf1* and *fyuA* among well-characterized *E. coli* urine isolates from patients with urinary tract infections.

2. Materials and methods

2.1. Assay for strain identification and collection of samples

A total of 150 midstream urine samples were obtained from hospitalized urinary tract infection patients in Kirkuk/Iraq hospitals. During October 2018 and

February 2019, MacConkey agar, Blood agar, and Eosin methylene blue agar were used to culture all 150 specimens.

The following biochemical tests were used to confirm E. coli's differentiation from other lactose fermenters in the Enterobacteriaceae: negative in the Voges-Proskauer, Simmons citrate, urease production, methyl red positive, indole positive, and acid/acid with gas generating in the TSI agar. Biochemical tests for final E. coli identification were based on growth morphology on EMB agar and the API 20 E test.

2.2. Method of DNA extraction and polymerase chain reaction

Deoxyribonucleic acid extraction

Bacterial chromosomal DNA was collected using a gene-aid DNA isolation kit and screened using an electrophoresis apparatus in a 0.8 percent agarose gel stained with ethidium bromide, then imaged using an ultraviolet trans-illuminator.

Nanodrop

The Deoxyribonucleic acid was tested using a nanodrop device adjusted to 260/280nm, and the DNA was then stored at -20°C until further usage.

Analyses of polymerase chain reactions

Based on predefined primers, this molecular method has been used to detect virulence factor genes cnf1 and fyuA in Escherichia coli. The primers for the cnf1 and fyuA genes were used in PCR runs on a thermal-cycler (MyGene, model MG25+). According to table (1).

Table 1. Gene information and primers

Gene(s)	Sequences of primers (5'–3')	name of primer	length of the gene (nt)	Product size (bp)	Primer's source	Genebank ID for reference
cnf1	AAGATGGAGTTTCCTATGCAGGAG	Forward	3045	498	(Johnso and Stell, 2000)	X70670
	CATTCAGAGTCCTGCCCTCATTATT	Reverse				
fyuA	TGATTAACCCCGCGACGGGAA	Forward	2022	787		Z38064.1
	CGCAGTAGGCACGATGTTGTA	Reverse				

According **to AccuPower® PCR PreMix from Bioneer (Korea)** technique, PCR was carried out in 20 µl volume reaction mixtures including 1 µl of each primer, 3 µl of crude template DNA to be added to the ready to use PreMix, then were completed to 20 µl by adding deionized water. For both genes, the appropriate annealing temperature was 65°C.

In the thermal cycler, PCR amplifications consisted of 25 cycles of denaturation at 94°C for 2 minutes, annealing at 65°C for 1 minute, and extension at 72°C for 2 minutes, with minor tuning.

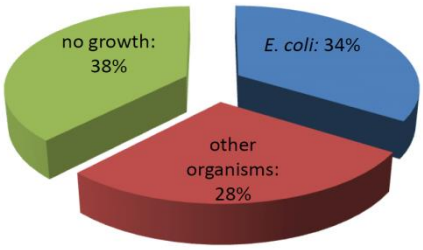
2.3. Analyze the product

The PCR product was evaluated using an electrophoresis apparatus in a 1.5 percent agarose gel with TBE buffer stained with Ethidium Bromide, and then viewed and documented using an ultra-violet trans-illuminator.

4. Results

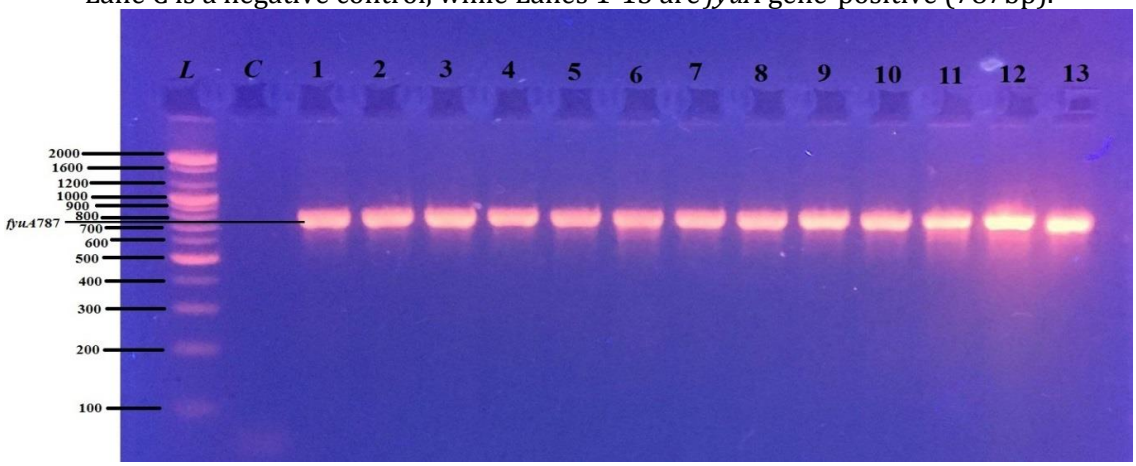
A total of 150 urine samples were obtained from patients suspected of having a urinary tract infection, 93 (62%) of them had positive culture of pathogenic bacteria, 51 (34%) of which showed growth of *E. coli* and the other 42 (28%) were positive for other bacteria and the rest of the samples 57 (38%) showed no bacterial growth and it is demonstrated in figure (1).

Figure 1. Bacterial profile obtained from a patient with UTI.



Furthermore, 37 (72.5%) of the 51 samples were from patients with Cystitis, while the remaining 14 (27.4%) came from patients with Pyelonephritis. this study yielded that females with E. coli infection had 39 isolates (76.4%) more than males 12 isolates (23.5 %). Genetic pattern occurrence of *fyuA* and *cnf1* genes were investigated using conventional PCR, *fyuA* genes were found in all 51 (100%) isolates, and electrophoresis run of PCR product for *fyuA* genes is shown in figure (2).

Figure 2. PCR product electrophoresis in 1.5 percent agarose for the inspection of the *fyuA* gene (787bp) at 70 volts for 60 minutes, stained with ethidium bromide, L: 100-2000bp Lane C is a negative control, while Lanes 1-13 are *fyuA* gene-positive (787bp).



This study yielded that only 13 (25.4) isolates have carried *cnf1* gene of which 12 isolates from Cystitis and only 1 isolate from Pyelonephritis, an electrophoresis run of PCR product for *cnf1* genes is shown in figure (3). The results of gene occurrence *E. coli* isolated from Cystitis and Pyelonephritis patients are shown in table (2).

Figure 4-18. Electrophoresis of PCR products in 1.5 percent agarose for the inspection of the cnf1 gene (498bp) at 70 volts for 60 minutes, stained with ethidium bromide, L: 100-1500bp Ladder, Lane C is a negative control, whereas lanes 1, 3, 4, 7, 8, and 13 are positive for the *cnf1* gene (498bp) and lanes 2, 5, 6, 9, 10, 11, and 12 are negative for the *cnf1* gene.

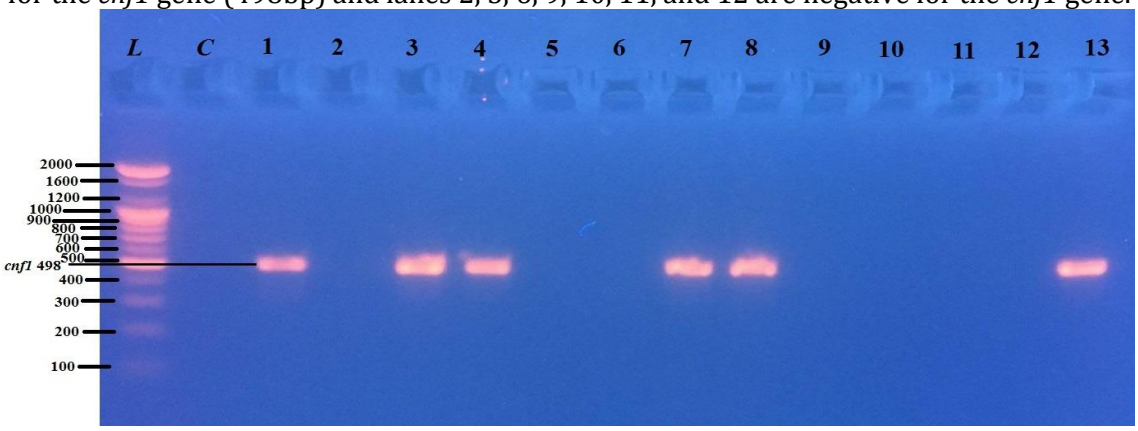


Table 2. Gene distribution according to illness type, pyelonephritis, and cystitis

Gene / Case	N.	*fimH*	*cnf1*
Cystitis	37	37 (100%)	12 (32.4%)
Pyelonephritis	14	14 (100%)	1(7.1%)
Total	51	51 (100%)	13 (25.4%)

5. Discussion

The fyuA gene, which codes for iron uptake (ferric yersiniabactin uptake), was found in all 51 UPEC isolates (100 %). This has to do with the receptors they have in the urinary system. Iron deficiency in the human urinary tract is a very serious possibility (Hancock *et al*., 2008). By horizontal gene transfer, the high pathogenicity island encoding the yersiniabactin iron absorption mechanism has spread throughout the enteric group (Schubert *et al*., 1998), Iron is required by both human and bacterial cells. Competes for the host's attention. Because the content of iron in the urinary system is very low in a normal state, it is considered that germs do not exist there.

Hancock *et al*. (2008) found similar results. The findings revealed a strong link between this gene and urinary tract infections, Vigil *et al.,* (2011) found that (89.2 %) of UPEC isolated from cystitis were *fyuA* positive, while (93.5 percent) of UPEC isolated from Pyelonephritis were harboring the *fyuA* gene, De Souza da-Silva *et al*., (2017) discovered the *fyuA* gene in 100 % of male isolates and 82 % of female isolates, respectively, Yun *et al.*, (2014) found the *fyuA* gene in 80.0 % of *E. coli* isolated from urinary tract infection, which is lower than this study's result.

The results revealed that 13 isolates (25.4%) carried the *cnf1* gene., UPEC has been the subject of various investigations involving this gene, Landraud *et al*, (2000) found that 30% of UPEC were cnf1 positive, which is consistent with the findings of this investigation, and Abe *et al*., (2008) reported that 23.6 % of their isolates tested positive for *cnf*. Farshad et al. (2010) found that the *cnf1* gene was detected in 22.91 % of 96 uropathogenic *E. coli* isolates. Johnson & Stell, (2000) articulated that (16%) of uropathogenic *E. coli* were carrying *cnf1* gene which considered less than this study's result, Firoozeh *et al*. (2014) disagreed with the findings of this investigation, claiming that no UPEC isolates (0.0%) harbored the *cnf* gene. CNF1 toxin is produced by E. coli strains that cause uropathogenic and neonatal meningitis. The CNF1 toxin disrupts the actin cytoskeleton of the host cell, resulting in dermonecrosis. (Wang & Kim, 2013).

6. Conclusion

We conclude that iron uptake is crucial for UPEC which makes a relationship between the gene *fyuA* and UTI, the strains isolated from Cystitis show the greatest diversity of genes pattern than strains isolated from pyelonephritis, cytotoxic necrotizing factor 1 toxin encoded by *cnf1* gene occur more likely in Cystitis.

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