



## Virulence Genes Profile of *Escherichia coli* Isolated from Urinary Catheterized and Non-Catheterized Patients

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### Abstract

The severity of UTI produced by *E. coli* is due to the expression of a wide spectrum of virulence factors. In this study the role of *E. coli* virulence determinants in the pathogenesis of UTI in urinary catheterized and non-catheterized patients has been evaluated. The isolates were recovered from 129 patients admitted to the hospital. Virulence genes of *E. coli* were detected by polymerase chain reaction analysis for the prevalence of these virulence factors. The targeted genetic determinants were those coding for Type 1 fimbriae, Pyelonephritis-Associated Pili (PAP), Antigen 43 (Ag43),  $\alpha$ -Hemolysin and Aerobactin siderophores among the studied isolates. The prevalence of genes *fimH*, *papC*, *ang43*, *hlyA* and *iutA* were 88.37%, 72.09%, 18.60%, 51.16% and 79.06% respectively. The *fimH* gene with 88.37% had a highest prevalence of virulence genes in patients with UTIs. Statistically in the present study, there was no significant difference in the prevalence of genes profiles of *E. coli* isolates causing UTI in non-catheterized and catheterized patients. However, all the studied strains exhibited 16 virulence gene patterns; among the strains isolated from patients with urinary catheter showed multitude and greatest diversity of genes patterns than strains from non-catheterized patients.

**Keywords:** Urinary Tract Infections, *Escherichia Coli*, Virulence Genes.

### الملف الشخصي لعوامل الفوعة لبكتريا *Escherichia coli* المعزولة من مرضى القثطرة البولية وغير القثطرة

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### الخلاصة

ان شدة التهاب المجاري البولية المتسببة من بكتريا *E. coli* تعود الى انتاجها لطيف واسع من عوامل الفوعة. في هذه الدراسة تم تقييم دور محددات الفوعة لبكتريا *E. coli* في مرض التهاب المجاري البولية الماخوذة من مرضى القثطرة البولية وغير القثطرة البولية. وان العزلات اخذت من 129 من المرضى الذين يرتادون المستشفى. تم تطبيق تقنية تفاعل البلمرة المتسلسل للتحري عن عوامل الفوعة لبكتريا *E. coli* ودراسة انتشار عوامل الفوعة هذه. وكان الهدف الجيني لمحددات الفوعة وكانت للجينات المشفرة لانتاج Type 1 fimbriae و Pyelonephritis-Associated Pili (PAP) و Antigen 43 (Ag43) و  $\alpha$ -

Hemolysin و Aerobactin siderophores ضمن العزلات المدروسة. وكانت نسبة انتشار الجينات *imH* و *papC* و *ang43* و *hlyA* و *lutA* هي 88,37% و 72,09% و 18,60% و 51,16% و 79,06% على التوالي. وكان الجين *imH* بنسبة وجوده 88,37% أكثر انتشاراً بين عوامل الفوعة لمرضى خمج المجاري البولية.

تبين من خلال التحليل الاحصائي لهذه الدراسة عدم وجود اي فرق معنوي في انتشار التكوين الجيني لعزلات بكتريا *E. coli* المسببة لمرض خمج المجاري البولية لكلا من مرضى القنطرة وغير القنطرة البولية. واطهرت العزلات في هذه الدراسة 16 نمطا جينيا وكانت السلالات المعزولة من مرضى القنطرة البولية اكثر واكبر تباينا في الانماط الجينية بمقارنتها بعزلات غير القنطرة البولية.

## Introduction

Urinary tract infection represents one of the most common diseases encountered in medical practice today and occurring from the neonate to the geriatric age group [1].

Uropathogenic *Escherichia coli* (UPEC) is the primary causative agent of complicated and uncomplicated UTI's. It responsible for approximately (80%) of community acquired and (50%) of nosocomial-acquired UTIs, it is isolated from the urine of about 30% of patients experiencing catheter-associated urinary tract infections (CAUTI) [2]. The risk of hospitalization, length of hospitalization and length of antibiotic therapy were three times higher in catheterized residents than in noncatheterized residents [3].

Antibiotic resistance of pathogens in the management of complicated and uncomplicated community-acquired UTIs is a serious medical problem. However widespread use of antibiotics has led to the emergence of resistant bacteria [4].

Thus, uropathogenic strains of *E. coli* are believed to display a variety of virulence determinants have been related to the development of UTIs. Among these factors; siderophores, toxins, capsules, fimbriae and others have been described [5].

Fimbriae-mediated adherence is important for the virulence of *E. coli* in the urinary tract [6]. The ability of uropathogenic *Escherichia coli* (UPEC) to adhere to host uroepithelia is an important stage in the successful colonization of the urinary tract and key events in pathogenesis of urinary tract infection (UTI) [7].

Type 1 and P fimbriae are two important adhesins found in most pathogenic *E. coli* and are involved in adhesion of UPEC to cells of the urinary tract and to catheters as well [8].

Type 1 fimbriae serve as extremely efficient adhesive organelles, it play an important role in bacterial adhesion to biotic and abiotic surfaces, invasion, persistence in the host cells and biofilm formation. It has come to be considered as a key virulence factor in UTI and CAUTI [9, 10].

P fimbriae are the second common virulence factor of UPEC, which plays an important role in the pathogenesis of ascending UTIs and pyelonephritis in humans [11]. UPEC sequentially or synergistically express of first type 1 fimbriae (mediate binding to the bladder) and then P fimbriae (mediate binding to the upper urinary tract) [12].

Like fimbriae, Ag43 protein is urovirulent help *E. coli* adhere to the epithelium and they also have a major role in aggregation, or clumping together, of the *E. coli* and form biofilm over the host urothelial cells leading to poor clearance, persistence and increases bacterial resistance to host defence mechanisms [13]. Antigen 43 (Ag43) or fluffing protein belongs to the growing family of autotransporter proteins from gram-negative bacteria [14]. Ag43 appears to be an *Escherichia*-specific most abundant phase variable surface antigen [15].

Haemolysin production is frequently associated with *E. coli* strains especially those causing more clinically severe forms of UTI [16]. It is associated with necrotoxicity, cytotoxicity and promotes microbial growth by releasing of nutrients and other factors, such as iron from lysed erythrocytes [17, 18].

Iron is an essential nutrient for the majority of bacterial species. *E. coli* uses iron for oxygen transport and storage, DNA synthesis, electron transport and metabolism of peroxides. It has also been associated with biofilm formation due to the need of the bacteria to capture iron for growth [19, 20]. But the availability of this element within the urinary tract is limited [16]. In *E. coli*, the hydroxamate siderophore aerobactin is the most effective of the several iron chelation systems [21]. Thus, the

hemolysin and heme transport systems may work in concert to take advantage of the abundant supply of heme as an iron source within the host [22].

The virulence factors function additively or synergistically in overcoming normal host defenses. The strains with a more extensive complement of virulence factors are more effective pathogens [23]. Isolates collected from urine and had greater virulence factors and more prevalent than those collected from other isolates [24]. However, there little molecular and genetic studies compare urinary catheter and non-catheter *E. coli* isolates; therefore this study was made to evaluate this issue.

## Materials and Methods

### Collection of specimens

During the period from November 2014 to February 2015, a total of 129 clinical urine specimens were collected from patients suffering from urinary tract infection of all the age groups and both sexes admitted to AL-Yarmouk Teaching Hospital in Baghdad; 92 from hospital urinary catheterized patients and 37 non-catheterized patients. Specimens were collected aseptically as follows:

- A. **Urine specimens from non-catheterized patients:** Specimens was collected from 37 patients, patients were carefully educated to collect a proper specimen by themselves; sterile dry wide necked leak proof containers were used for the urine collection, collected mid-stream urine and directly were transferred to the laboratory.
- B. **Urine specimens from catheterized patients:** 92 patients with Foley urinary catheterization, the urine specimens for culture were collected directly from the catheter or tubing, to maintain a closed drainage system using aseptic technique by puncturing the urinary catheter tubing with a sterile needle and syringe from the distal ends of the urinary catheters and they were transferred to sterile urine containers and transported immediately to the laboratory without any delay. Culture specimens were not obtained from the drainage bag [25].

### Identification of *E. coli* isolates

The specimens received were inoculated on and MacConkey, Eosin Methylene Blue and blood agar plates. Then all plates were incubated at 37°C for 24hrs.

Significant isolates were identified as species level using conventional bacteriological methods and analytical profile index (API)-20E system was employed to confirm the identification.

### DNA Extraction

The DNA of forty three *E. coli* isolates was extracted according to the instruction of the Promega kit. Nano drop spectrophotometer was used for measured the DNA concentration and purity. The extracted DNA was electrophoresed by gel electrophoresis system for proofing that the genomic DNA was intact and not sheared.

### Preparation the primers

Primers were prepared according to the instructions of manufactured company (Alpha DNA, Canada). The primers selected in this study shown in in Table- 1.

**Table 1-** Sequence of primers of virulence genes.

Gene	Primer sequence 5'→3'	Amplicon size (bp)	References
<i>fimH</i>	For: TGCAGAACGGATAAGCCGTGG	508	[26]
	Rev: GCAGTCACCTGCCCTCCGGTA		[26]
<i>papC</i>	For: GTGGCAGTATGAGTAATGACCGTTA	200	[26]
	Rev: ATATCCTTTCTGCAGGGATGCAATA		[27]
<i>agn43</i>	For: ACGACAACCATCAATAAAA	700	[28]
	Rev: CCGCCTCCGATACTGAATGC		[28]
<i>hlyA</i>	For: AACAAAGGATAAGCACTGTTCTGGCT	1177	[29]
	Rev: ACCATATAAGCGGTCATCCCGTCA		[29]
<i>iutA</i>	For: GGCTGGACATCATGGGAACTGG	300	[30]
	Rev: CGTCGGGAACGGGTAGAATCG		[30]

### PCR amplification

PCR assay was performed in a monoplex patterns. It were carried out to 43 *E. coli* isolates, 21 isolates were from hospital urinary catheterized patients and 22 isolates were from non-catheterized patients to amplify different virulence factors genes *fimH*, *papC*, *agn43*, *hlyA* and *iutA* encoding for Type 1 fimbriae, Pyelonephritis-Associated Pili (PAP), Antigen 43 (Ag43),  $\alpha$ -Hemolysin and Aerobactin siderophores respectively in *E. coli* isolates with specific primers. For optimization the primer was applied into eppendorf tube, PCR mixture was set up in a total volume of 25 $\mu$ L included 12.5 $\mu$ L of PCR green master mix, 1 $\mu$ L of each primer, and 2 $\mu$ L of template DNA have been used, the rest volume was completed to 25 $\mu$ L with sterile nuclease-free water. Negative control contained all material except template DNA; that nuclease-free water was added instead of template DNA. The amplification was carried out in a BioRad, USA model, the PCR thermocycler program described in Table- 2.

**Table 2-** PCR thermocycler program for DNA amplification of *E. coli* genes.

Stage	Temperature	Time	Number of cycles
Initial denaturation	95°C	4 min	1
Denaturation	95°C	30 sec	30
Annealing	X°C	30 sec	
Extension	72°C	1 min	
Final extension	72°C	8 min	1
Hold	4°C		

X, annealing temperature for each primer of virulence gene as fellow; *fimH*, *papC* and *hlyA*: 63°C; while *agn43* and *iutA* were 53°C and 55°C, respectively.

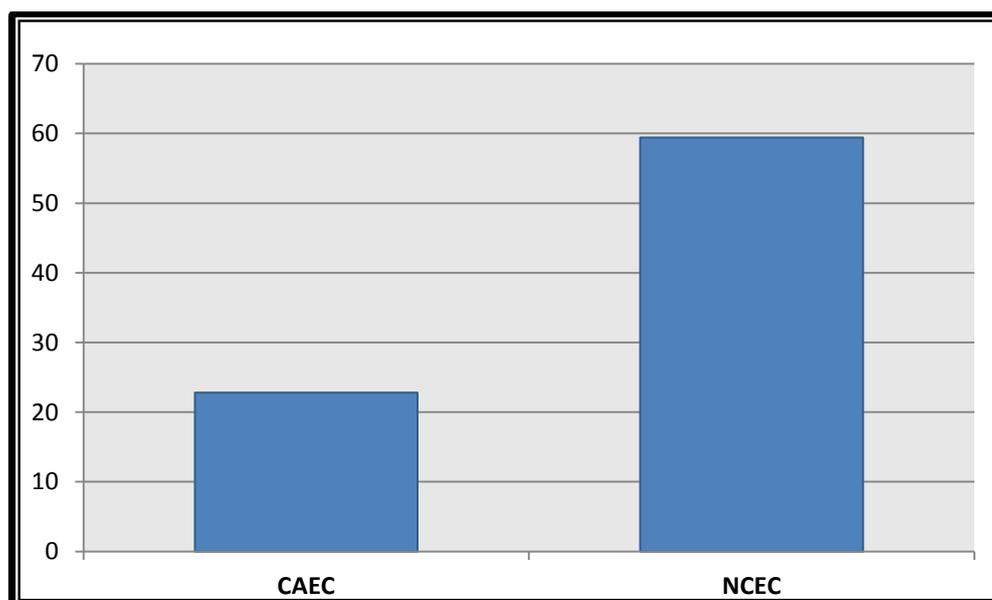
### Statistical Analysis

Comparisons of prevalence data were tested using Chi square ( $\chi^2$ ) test and Fisher's exact test with GraphPad prism (version 6) statistical software.

### Results and discussion

#### Isolation and identification of *E. coli* isolates

After performance the identification tests it was found that in a total of 43(33.3%) *E. coli* isolates, 21(22.8%) isolates were from hospital urinary catheterized patients and 22(59.4%) isolates were from non-catheterized patients of urine specimens in Figure-1.



**Figure 1-** Percentage of catheterized associated *E. coli* (CAEC) and non-catheterized associated *E. coli* (NCEC).

## Genetic study of *Escherichia coli* isolates

### DNA extraction

The DNA was extracted from all *E. coli* isolates. *E. coli* DNA was with good quantitative and qualitative states that showed one band of DNA when analyzed by the gel electrophoresis method, the purity of extracted DNA was between 1.7 and 1.9.

### PCR study

A better knowledge of the virulence characteristics of the microorganism causing the infection will allow the clinician to anticipate, up to a point, the evolution of infection in the host organism. To the best of our knowledge, this study is the first to demonstrate associations between *E. coli* virulence genes and UTI compared to CAUTI.

All of the genes were detected in different percentages. Results are presented in Figure- 2.

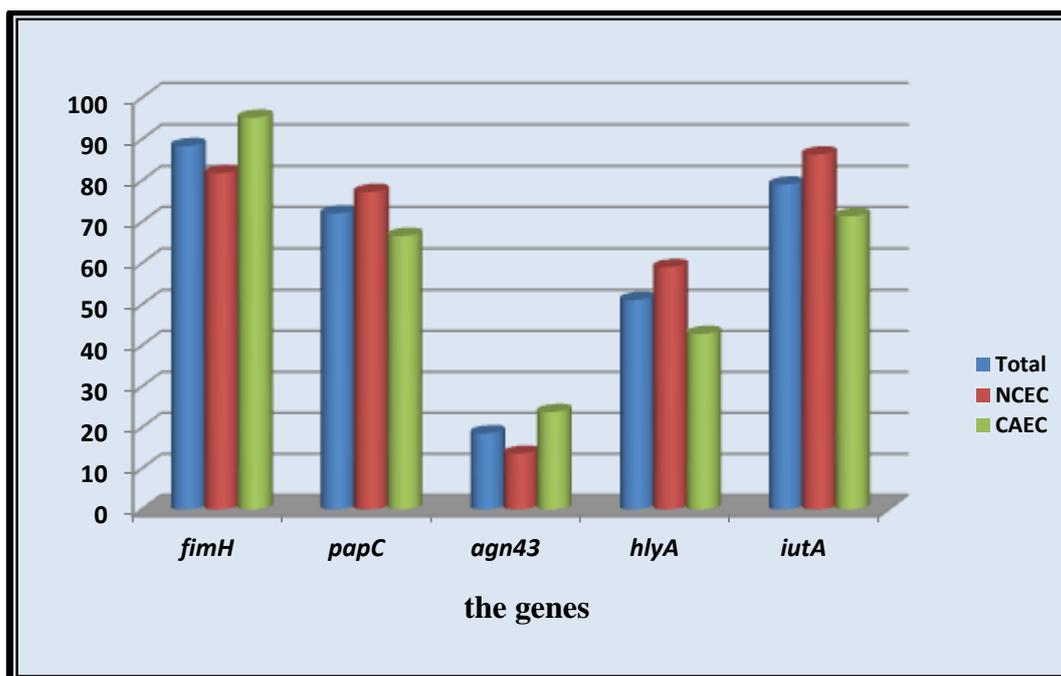
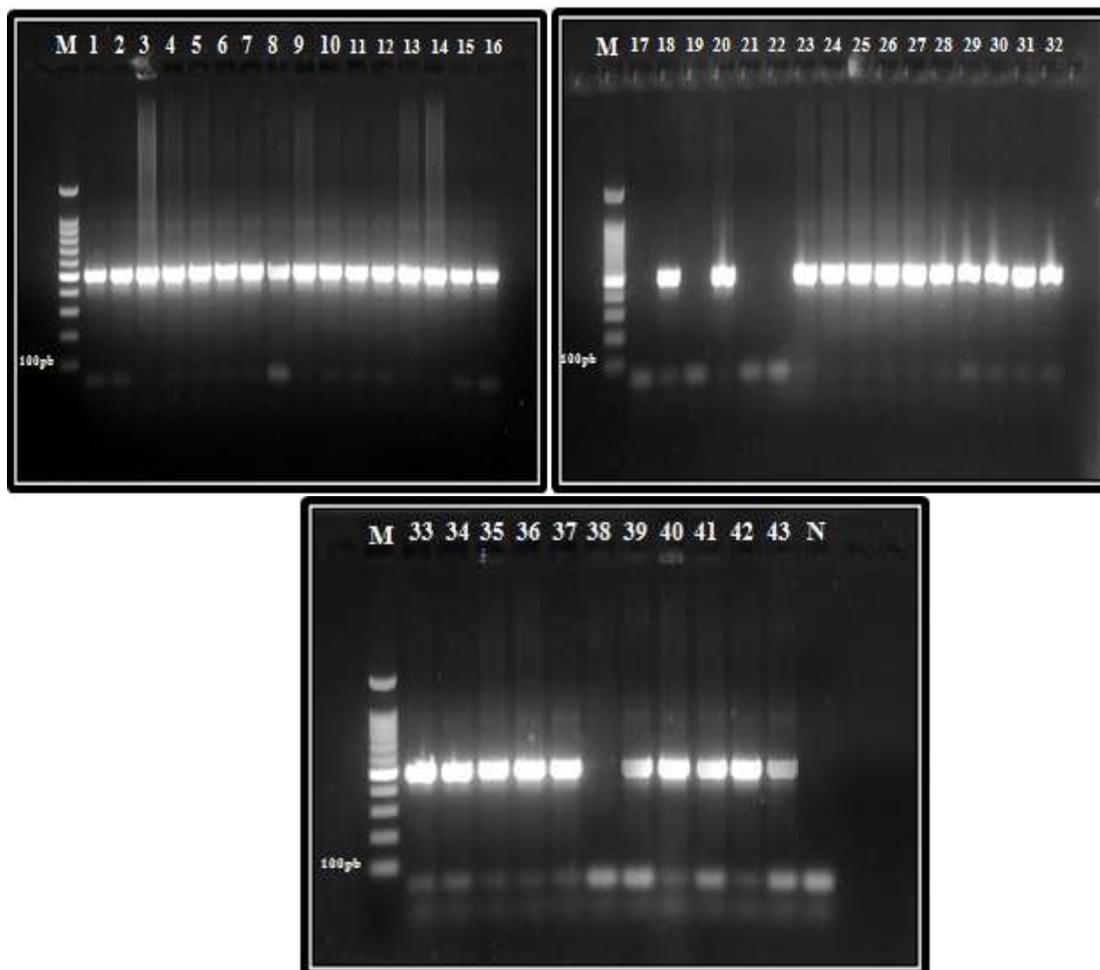


Figure 2- Prevalence of virulence factors genes in *Escherichia coli* isolates.

### Detection of *fimH* gene in *Escherichia coli* isolates

PCR were carried out to all isolates. From forty three *E. coli* isolates; the monoplex PCR assay for *fimH* gene revealed a high presence of *fimH* gene 38(88.37%) in a total, 18(81.81%) in non-catheterized patients isolates and 20(95.23%) in hospital urinary catheterized patients isolates Figure-3



**Figure 3-** Electrophoresis of amplified *fimH* (508bp). Lanes 1-22 represent non-catheterized *E. coli* isolates and Lanes 23-43 represent catheterized *E. coli* isolates, respectively. M and N lanes represented 100pb ladder and negative sample, respectively. Running conditions: Agarose gel 2%, 75 volt for 1 hrs, stained with ethidium bromide dye and visualized under UV transilluminator documentation.

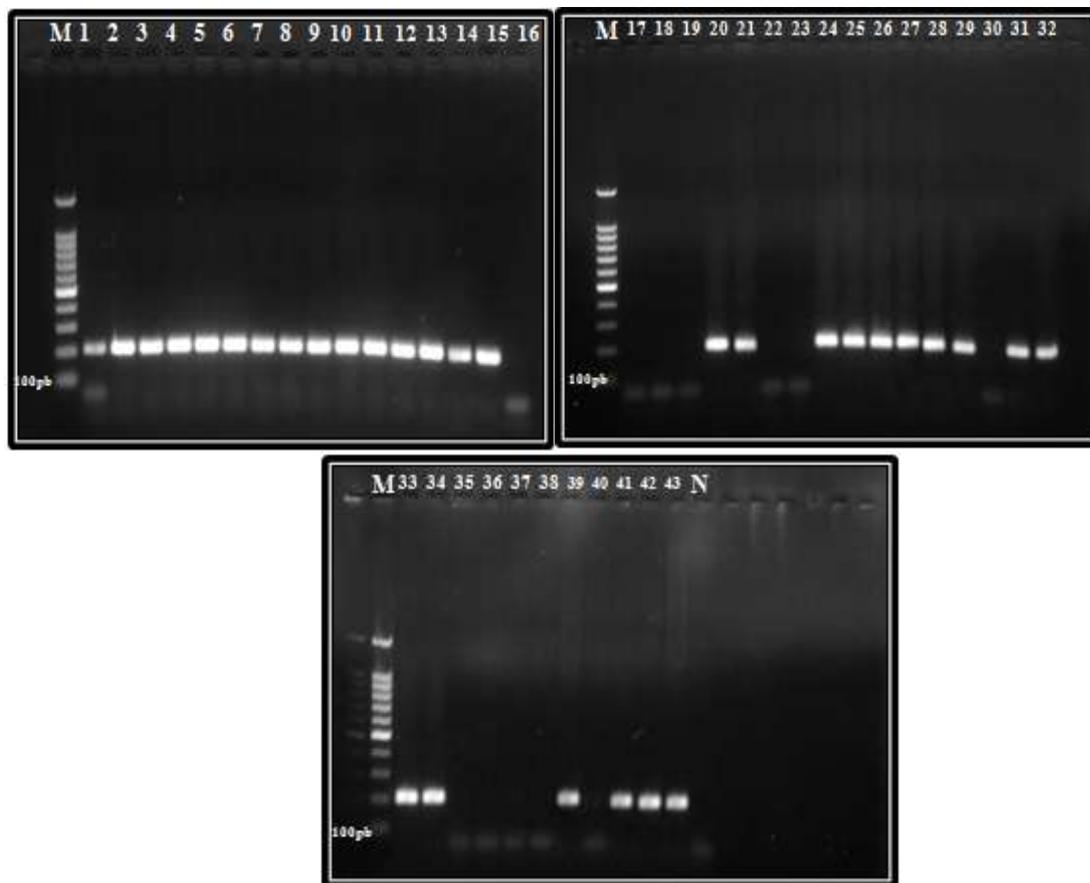
Wang *et al.* [31] studied the pathogenic role of host and *Escherichia coli* virulence factors in the development of *E. coli* bacteremia in patients with upper urinary tract infection (UTI). There was a high prevalence (92%) of the genetic determinant of type 1 fimbrial adhesin (*fimH*). While in a total of 78 *E. coli* strains isolated from adults with different types of urinary tract infections in Romani were screened for *fimH* adhesion gene was 86% [32]. A similar result 86.1% of *fimH* virulence gene was recorded by López-Banda *et al.* [33] that in 108 *E. coli* isolates from Mexican women, clinically diagnosed with urinary tract infection, were screened to identify virulence genes, phylogenetic groups, and antibiotic resistance.

But in a study done by Karimian *et al.* [34] that in a total of 123 strains of *E. coli* isolated from hospitalized patients with urinary tract infections were tested in a polymerase chain reaction for detection of *E. coli*'s virulence factors, their results showed that *fimH* gene with 79.67% had the highest presence rates of virulence genes in *E. coli* isolated from patients with urinary tract infections. A very high prevalence of *fimH* gene recorded by other studies; the prevalence of *fimH* gene among the 75 urosepsis isolates was 75 (100%) [26], 162 Uropathogenic *Escherichia coli* (UPEC) strains from patients with cystitis were genotypically characterized by polymerase chain reaction (PCR) assay and the PCR assay results identified 158 *fimH* (97.5%) [35] and among the 63 *E. coli* urine isolates from patients with UTI the prevalence of type 1 fimbriae was 95% [36].

The results of this study were corresponding to the previous studies showed that *fimH* gene with 88.37% had a highest prevalence of virulence genes in patients with UTIs.

### Detection of *papC* gene in *Escherichia coli* isolates

The adhesin *papC* fimbriae gene were showed a percentage 31(72.09%) in a total, 17(77.27 %) in non-catheterized patients isolates and 14(66.66%) in hospital urinary catheterized patients isolates Figure- 4. *PapC* gene is the second most common adhesins in UPEC.



**Figure 4-** Electrophoresis of amplified *papC* (200bp). Lanes 1-22 represent non-catheterized *E. coli* isolates and Lanes 23-43 represent catheterized *E. coli* isolates, respectively. M and N lanes represented 100pb ladder and negative sample, respectively. Running conditions: Agarose gel 2%, 75 volt for 1 hrs, stained with ethidium bromide dye and visualized under UV transilluminator documentation.

López-Banda *et al.* [33] mentioned that the prevalence of *papC* gene in his study was 62% in *E. coli* isolates from Mexican women, clinically diagnosed with urinary tract infection. Johnson *et al.* [37] studied bacterial adhesins in patients with *E. coli* urosepsis and *papC* adhesin was (82%). While *papC* in percentage 30% is the most prevalent in the *E. coli* strains of hemoculture in a study done by Koga *et al.* [38] how identified genetic features associated with virulence, and these results were compared with commensal isolates.

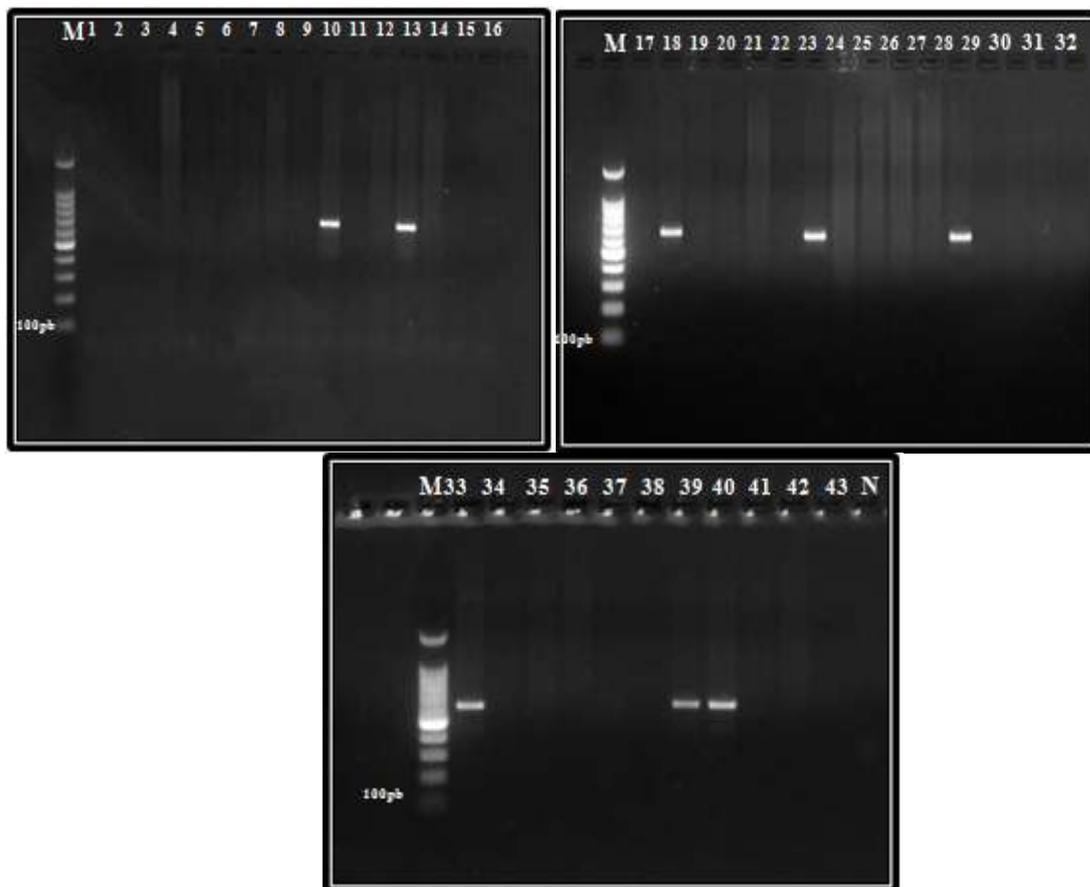
Interestingly, the *pap* adhesion encoding operon, which shows a high prevalence among uropathogenic *E. coli* strains (75%–80% of pyelonephritis strains) [39]. In a previous work Archambaud *et al.* [40] confirmed the prevalence of *pap* adhesins among uropathogenic *E. coli* strains (74.7% of the pyelonephritis-causing strains and 44.1% of the strains associated with cystitis). But in 150 *E. coli* isolates from hospitalized patients with pyelonephritis and cystitis were collected from Kashan, Iran, a higher virulence gene diversity was found among pyelonephritis UPEC isolates in comparison to cystitis UPEC isolates, showing that UPEC strains that cause pyelonephritis need more virulence factors; *pap* (27.8%) and (6.4%) respectively [41].

Le Bouguenec *et al.* [27] investigated a collection of 97 *E. coli* isolates originated from the urine of patients with pyelonephritis and *pap* operons were found in 51.5% of the isolates.

In a study done by Biroš'ova' *et al.* [42] in total, 201  $\alpha$ -haemolytic *E. coli* isolates from various clinical materials (urine samples and vaginal and rectal swabs) were examined by PCR for the presence of *pap* gene was 89%.

#### Detection of *Ang43* gene in *Escherichia coli* isolates

The frequency of *Ang43* gene encoding Antigen 43 in *E. coli* isolates evaluated and it was 8(18.60%) in a total, 3(13.63%) in non-catheterized patients isolates and 5(23.80%) in hospital urinary catheterized patients isolates Figure- 5.



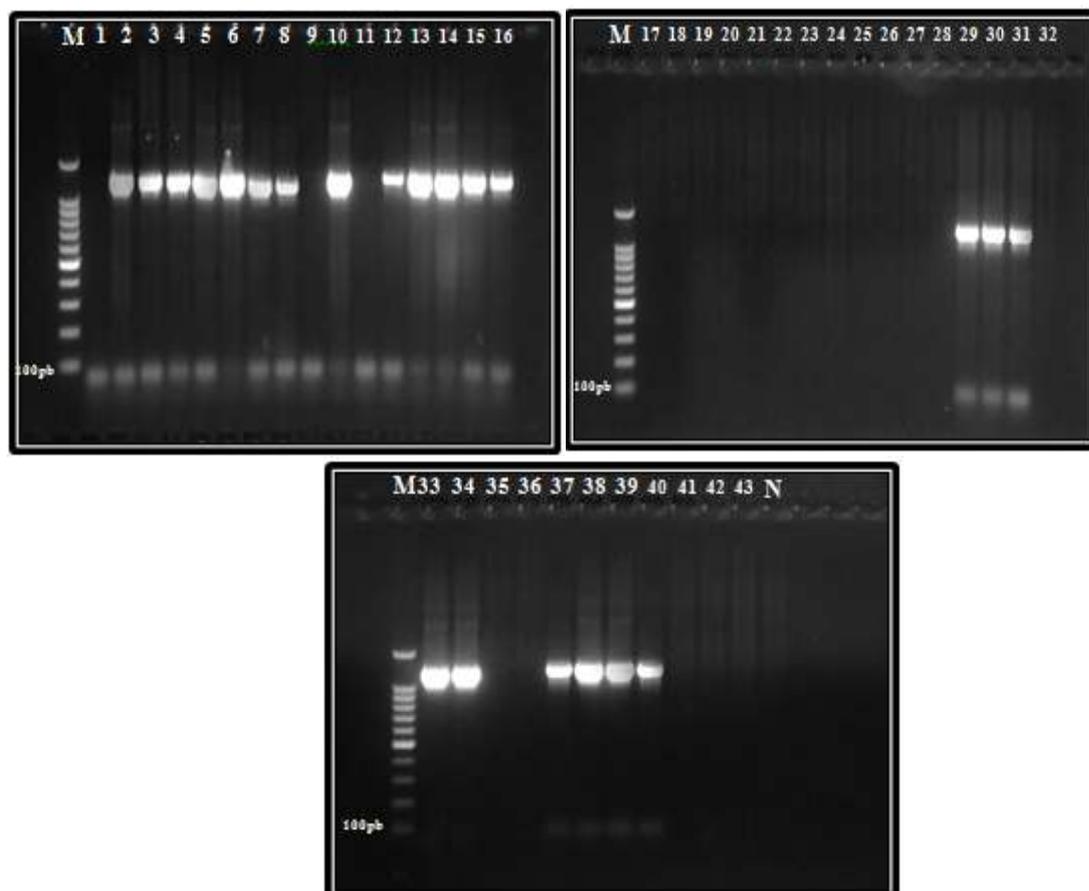
**Figure 5-** Electrophoresis of amplified *ang43* (700bp). Lanes 1-22 represent non-catheterized *E. coli* isolates and Lanes 23-43 represent catheterized *E. coli* isolates, respectively. M and N lanes represented 100bp ladder and negative sample, respectively. Running conditions: Agarose gel 2%, 75 volt for 1 hrs, stained with ethidium bromide dye and visualized under UV transilluminator documentation.

Tiba *et al.* [43] mentioned that *E. coli* samples isolated from female patients with cystitis were characterized with regard to the presence of *flu* gene coding for antigen 43 virulence factor associated with biofilm formation was amplified in 36% of strains.

In other study investigated the prevalence of 19 virulence factors and biofilm production in 86 Enteraggregative *Escherichia coli* (EAEC) isolates causing diarrhea in children less than 5 years of age from Ifakara, Tanzania and antigen 43 was presents in 33.7% of these isolates [28]. While distribution of *Ag43* virulence feature of *E. coli* isolated from patients with inflammatory bowel diseases; ulcerative colitis (UC) and crohn's disease (CD) were 47.4 % and 53.8 % respectively [44]. In other studies record the prevalence 79% of the tested *agn43* gene among the *E. coli* strains causes cystitis in women (*agn43a* 23% and *agn43b* 28%) [45]. Also in a total of 70 clinical isolates of UPEC were found a majority 77% (54/70) of the UPEC isolates and 41% (17/41) of the intestinal commensal isolates were found to be *flu* positive [46]. This finding is consistent with the results of Ulett and colleagues [47]; they reported 83% (30/36) of the UPEC isolates and 56% (35/62) of the commensal strains carried *flu*.

### Detection of *hlyA* gene in *Escherichia coli* isolates

The PCR assay results identified hemolysin producing gene (*hlyA*) this study showed 22(51.16%) in a total, 13(59.09%) in non-catheterized patients isolates and 9(42.85%) in hospital urinary catheterized patients isolates Figure- 6.



**Figure 6-** Electrophoresis of amplified *hlyA* (1177bp). Lanes 1-22 represent non-catheterized *E. coli* isolates and Lanes 23-43 represent catheterized *E. coli* isolates, respectively. M and N lanes represented 100pb ladder and negative sample, respectively. Running conditions: Agarose gel 2%, 75 volt for 1 hrs, stained with ethidium bromide dye and visualized under UV transilluminator documentation.

In study done by Karimian *et al.* [34] in Iran was found in a total of 123 strains of *E. coli* isolated from hospitalized patients with urinary tract infections were tested in a polymerase chain reaction for detection of *E. coli*'s virulence factors; and *hlyA* gene presence was 50.4% in *E. coli* isolated from patients with urinary tract infections. While similarly isolates recovered from 75 adult patients consecutively admitted to the hospital with *E. coli* bacteremia caused by upper UTI; hemolysin was evaluated 45% [31] and 43% in *E. coli* isolates from patients with urosepsis [37].

In contrast a high hemolysin percentage was recorded of study in Brazil indicated; that among the *E. coli* isolates from UTI patients, the prevalence of the hemolysin virulence factor was 96% [48] and low percentage (25.3%) recorded in UPEC from cystitis patients [35].

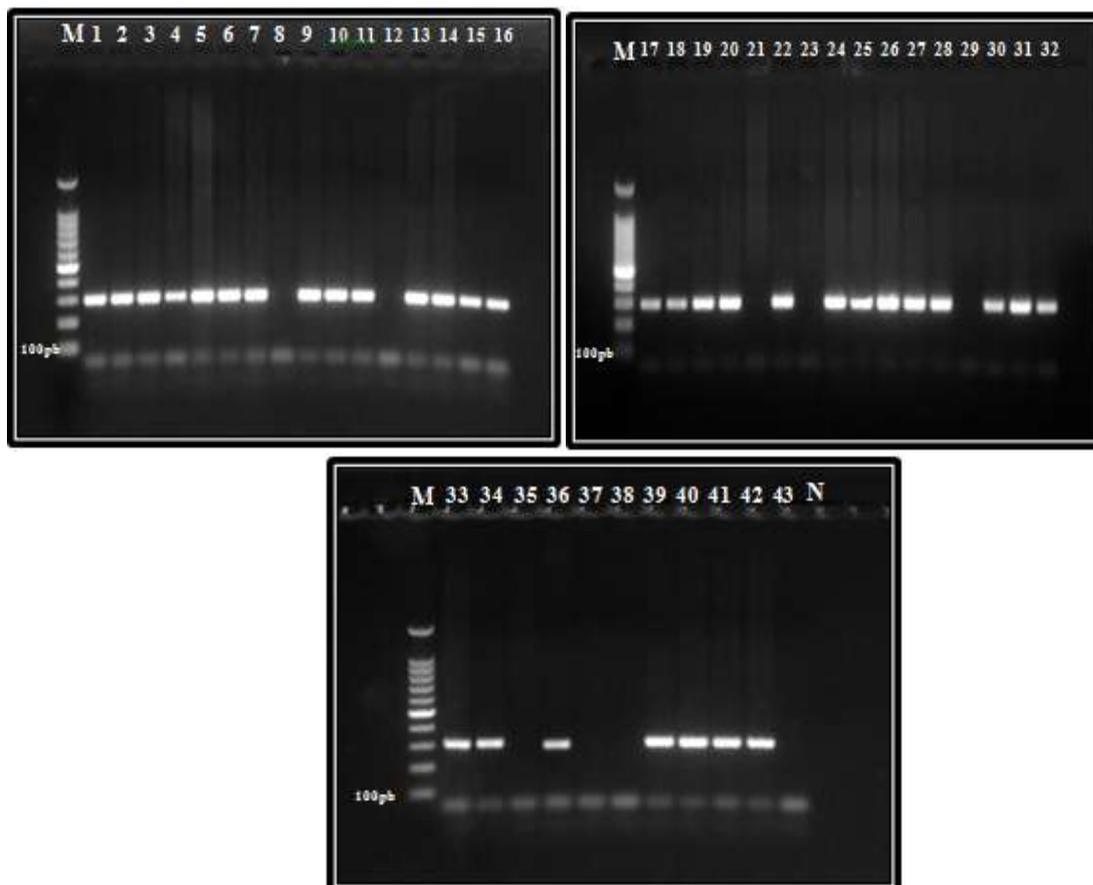
Maslow *et al.* [49] studied *E. coli* virulence factors in patients with *E. coli* bacteremia that originated in the urinary tract and 60% of the isolates were positive for *hly*. *Escherichia coli* hemolysin (*hlyA*) is a pore-forming bacterial exotoxin that may contribute to the virulence of bacteria during bloodstream infection and sepsis [38].

Ruiz *et al.* [5] mentioned differences in the presence of nine urovirulence factors among clinical isolates of *Escherichia coli* causing cystitis and pyelonephritis in women and prostatitis in men have

been studied. Hemolysin percentage in a total was 56.6%; It occurs significantly more frequently among isolates causing prostatitis 81% than among those causing cystitis 37% or pyelonephritis 52%.

#### Detection of *iutA* gene in *Escherichia coli* isolates

In this study the detection of *iutA* gene coding for aerobactin siderophores revealed 34(79.06%) in a total, 19(86.36%) in non-catheterized patients isolates and 15(71.42%) in hospital urinary catheterized patients isolates Figure- 7.



**Figure 7-** Electrophoresis of amplified *iutA* (300bp). Lanes 1-22 represent non-catheterized *E. coli* isolates and Lanes 23-43 represent catheterized *E. coli* isolates, respectively. M and N lanes represented 100pb ladder and negative sample, respectively. Running conditions: Agarose gel 2%, 75 volt for 1 hrs, stained with ethidium bromide dye and visualized under UV transilluminator documentation.

Johnson and Stell [26] recorded high prevalence of *iutA* 80% among the 75 urosepsis isolates. Also, most of *E. coli* isolates were positive for aerobactin (90.9%) [7].

Adwan *et al.* [50] confirms the prevalence 74% of *aut* gene in fifty clinical *E. coli* isolates were previously recovered from urine specimens obtained from patients suffered from urinary tract infections in Tulkarm-Palestine. Similar results recorded in the study to determine the occurrence of virulence genes expressing production of aerobactin (76.0%) among a hundred *E. coli* isolates obtained from in-and outpatients in a hospital in Brazil suffering from urinary tract infection (UTI) [48].

In contrast the study In total, 201  $\alpha$ -haemolytic *E. coli* isolates from various clinical materials were examined by PCR for the presence of the  $\alpha$ -haemolysin (*hly*), P-fimbriae (*pap*) and aerobactin (*aer*) virulence factors; The aerobactin genes were found less frequently (59%) in a total and 53% of urine samples<sup>41</sup> and previous study showed that *E. coli* strains isolated from children with UTIs 39.75% was positive for presence of *aer* [34].

While Johnson *et al.* [37] assessed 62% *iutA* gene among 182 *E. coli* blood isolates from adults with UTI-source bacteremia in comparison with fecal controls.

### Virulence gene patterns

Based on the distribution of the various targeted sequences all the studied strains exhibited 16 virulence gene patterns, referred to as P followed by a numeral in Table- 3.

**Table 3-** Virulence pattern identified among *Escherichia coli* isolates.

Patterns	Virulence genes					No. of isolates		
	<i>fimH</i>	<i>papC</i>	<i>agn43</i>	<i>hlyA</i>	<i>iutA</i>	Total	NCEC	CAEC
P <sub>1</sub>	+	+	-	-	+	11	4	7
P <sub>2</sub>	+	+	-	+	+	10	8	2
P <sub>3</sub>	+	+	+	+	+	4	2	2
P <sub>4</sub>	+	+	-	+	-	3	2	1
P <sub>5</sub>	-	-	-	-	+	3	3	0
P <sub>6</sub>	+	-	-	+	+	2	1	1
P <sub>7</sub>	+	-	+	-	+	1	1	0
P <sub>8</sub>	-	+	-	-	-	1	1	0
P <sub>9</sub>	+	-	+	-	-	1	0	1
P <sub>10</sub>	+	+	+	-	+	1	0	1
P <sub>11</sub>	+	-	-	-	-	1	0	1
P <sub>12</sub>	+	-	-	-	+	1	0	1
P <sub>13</sub>	+	-	-	+	-	1	0	1
P <sub>14</sub>	-	-	-	+	-	1	0	1
P <sub>15</sub>	+	+	-	-	-	1	0	1
P <sub>16</sub>	+	-	+	+	+	1	0	1

Four of the virulence gene patterns designated as P<sub>5</sub>, P<sub>8</sub>, P<sub>11</sub> and P<sub>14</sub> were characterized by the presence of only one gene, which was either *iutA*, *papC*, *fimH* or *hlyA* (6 strains). Four patterns (P<sub>9</sub>, P<sub>12</sub>, P<sub>13</sub> and P<sub>15</sub>) were represented by strains possessing a two gene association (4 strains). The patterns which included strains presenting three virulence genes (P<sub>1</sub>, P<sub>4</sub>, P<sub>6</sub> and P<sub>7</sub>) were the best represented (17 strains). The association of four genes was recognized in (P<sub>2</sub>, P<sub>10</sub>, P<sub>16</sub>) patterns (12 strains), followed by the P<sub>3</sub> pattern, which encompassed the five gene positive strains (4 strains).

The association of presents of the virulence factor patterns in relationship with the different source of the isolates, the strains isolated from non-catheterized patients exhibited 8 patterns. P<sub>2</sub> pattern was the most prevalence with 8 isolates followed by P<sub>1</sub> (4 isolates). Among the strains isolated from patients with urinary catheter show multitude and greatest diversity of genes patterns; it seemed to be more aggressive than strains from non-catheterized patients. 13 of the 16 genetic virulence patterns were identified, in this collection P<sub>1</sub> pattern was the most frequently detected with 7 isolates followed by P<sub>2</sub> and P<sub>3</sub> (2 isolates for each).

In comparison to this study Usein *et al.* [32] recorded in a total of 78 *E. coli* strains isolated from adults with different types of urinary tract infections all the studied strains exhibited 21 virulence gene patterns. Similar results were documented by Firoozeh *et al.* [41] nineteen different virulence patterns were found among the UPEC strains regarding the frequency of virulence determinants. Also the strains isolated from patients with pyelonephritis demonstrated more virulence gene diversity than strains isolated from patients with cystitis.

### Virulence factors by *Escherichia coli* isolates from catheterized patients

There is limited research on the genetic virulence properties of *E. coli* isolates colonizing the catheterized urinary tract makes it difficult to assess their role in a catheterized human bladder; despite the high numbers of catheterized patients and the high risk of developing bacteriuria once one is catheterized [51].

Statistically in the present study, there was no significant difference prevalence of genes profiles of *E. coli* isolates causing UTI in non-catheterized and catheterized patients as shown in the Table- 4.

**Table 4-** Prevalence of virulence factors genes in *Escherichia coli* isolates.

	<i>fimH</i>	<i>papC</i>	<i>ang43</i>	<i>hlyA</i>	<i>iutA</i>
<b>Total</b>	38(88.37%)	31(72.09%)	8(18.60%)	22(51.16%)	34(79.06%)
<b>NCEC</b>	18(81.81%)	17(77.27%)	3(13.63%)	13(59.09%)	19(86.36%)
<b>CAEC</b>	20(95.23%)	14(66.66%)	5(23.80%)	9(42.85%)	15(71.42%)
<b>P value</b>	0.0705 Ns	0.051 Ns	0.0703 Ns	0.031*	0.063 Ns

In the study done by Watts *et al.* [51], they did not observe any significant difference in the adhesin gene repertoires of 176 isolated *E. coli* strains causing asymptomatic bacteriuria ABU in non-catheterized and catheterized patients CA-ABU. They show the *fimH* gene was the most common virulence gene and was present in 98% of both ABU and CA-ABU strains. The *papG* gene was detected in 42% of ABU strains and 43% of CA-ABU strains. The gene encoding the autotransporter adhesin Ag43 (*flu*) was present in 60% of all strains, and the distributions were similar among the ABU and CA-ABU groups. Genes encoding the UPEC toxins hemolysin (*hlyA*) were also found in similar numbers in ABU strains (19% *hlyA* positive) and CA-ABU strains (27% *hlyA* positive). While the *iutA* (encoding the aerobactin receptor) and *iroN* (encoding the salmochelin receptor) genes were present in 35 to 50% of ABU and CA-ABU strains while the differences were not significant. Overall, their data suggest that nosocomial ABU and CA-ABU *E. coli* isolates possess similar virulence profiles of the UTI associated virulence genes examined, there was a similar prevalence rate for all genes.

While Schlager *et al.* [52] study the prevalence of virulence factors among *E. coli* isolated from the periurethra and urine in patients with neurogenic bladder who received intermittent catheterization in which all strains expressed type I and P adhesin.

The relative importance of potential virulence factors has been examined epidemiologically by Mobley *et al.* [53] by determining the presence or absence of the factor in strains isolated from patients with pyelonephritis, cystitis, or asymptomatic bacteriuria and urinary catheterized patients (catheter associated bacteriuria). The *E. coli* isolates from catheterized patients expressed P fimbriae was in proportions (44%) nearly half of the isolates produced P fimbriae; it is similar to those of strains isolated from cases of cystitis (40%). In addition, type 1 fimbriae were expressed by 79%, comparable to pyelonephritogenic strains. On the other hand, hemolysin production in isolates from catheter associated bacteriuria was very limited (13% of isolates). Overall, the *E. coli* isolates expressed traits similar to those of strains that cause cystitis.

In contrast a study done by Jacobsen *et al.* [3] that among 70 urinary isolates *E. coli* isolated from patients with spinal injuries undergoing long-term bladder catheterization, the prevalences of virulence factors were P fimbriae, 17%; hemolysin, 27%; and aerobactin, 33% by bioassay and 39% by gene probe. These findings indicate that the presence of a urinary catheter and a neuropathic bladder increases susceptibility to colonization of the urinary tract and identified that these strains rarely possess a complete arsenal of virulence factors possessed by strains isolated from cases of uncomplicated UTI.

In this regard Venier *et al.* [54] thought that lessvirulent organisms are capable of causing complicated UTIs such as CAUTIs; it is thought that urinary catheter bypasses the normal host defences and allows the entry of pathogens into the bladder. The presence of a foreign body also leads to the formation of a biofilm, which helps pathogens to proliferate and cause infection. Therefore the *E. coli* isolates responsible for CAUTI harbor fewer virulence factors than isolates causing classical UTI.

There is speculation that in the presence of a catheter these *E. coli* isolates possess the virulence factors associated with infection and do not simply represent random colonization by avirulent organisms [53].

However Johnson *et al.* [37] mentioned that most VGs repertoire were similarly prevalent between isolates from compromised versus noncompromised hosts and between nosocomial versus community-acquired isolates.

Patients with underlying urinary catheter are more susceptible to colonization of the urinary tract by conventional *E. coli*. The mechanisms by which CAUTI *E. coli* strains adhere to the surfaces of

urinary catheters have not been well described. It can be speculated that some of the known adhesins that UPEC uses during UTIs could be expressed during CAUTIs [55].

### Conclusion

Uropathogenic *Escherichia coli* (UPEC) is the primary causative agent of complicated and uncomplicated UTIs. In this study five virulence genes in *E. coli* isolates from urinary catheterized and non-catheterized patients has been detected and all of the genes were detected in different percentages. That *fimH* gene had a highest prevalence of virulence genes in patients with UTIs. Statistically in the present study, there was no significant difference prevalence of genes profiles of *E. coli* isolates causing UTI in non-catheterized and catheterized patients but the strains isolated from patients with urinary catheter show multitude and greatest diversity of genes patterns than strains from non-catheterized patients. This study is the first to demonstrate associations between *E. coli* virulence genes and UTI compared to CAUTI.

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