ISSN: 0067-2904

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

Saba Nazeih Abdul-Ghaffar*[1], Rasmia Abed Abu-Risha[2]
[1]Department of Optics Techniques, Dijlah University College, Baghdad, Iraq.
[2]Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.

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), α-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

صبا نزيه عبد الغفار*[1]، رسمية عبد ابو ريشة[2]
[1] قسم تقنيات البصريات ، كلية دجلة الجامعة الاهلية ، بغداد ، العراق.
[2] قسم علوم الحياة ، كلية العلوم ، جامعة بغداد ، بغداد ، العراق.

الخلاصة

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

*Email: sabanazeih@yahoo.com

Hemolysin و Aerobactin siderophores ضمن العزلات المدروسة. وكانت نسبه انتشار الجينات *fimH* و *papC* و*ang43* و *hlyA* و *iutA* هي 88,37٪ و 72,09٪ و 18,60٪ و 51,16٪ و 79,06٪ على التوالي. وكان الجين *fimH* بنسبة وجوده 88,37٪ اكثر انتشارا 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 القثطرة وغير القثطرة البولية. واظهرت العزلات في هذه الدراسة 16 نمطا جينيا وكانت السلالات المعزولة من مرضى القثطرة البولية اكثر واكبر تباينا في الانماط الجينية بمقارنتها بعزلات غير القثطرة 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
fimH	For: TGCAGAACGGATAAGCCGTGG	508	[26]
	Rev: GCAGTCACCTGCCCTCCGGTA		[26]
papC	For: GTGGCAGTATGAGTAATGACCGTTA	200	[26]
	Rev: ATATCCTTTCTGCAGGGATGCAATA		[27]
agn43	For: ACGCACAACCATCAATAAAA	700	[28]
	Rev: CCGCCTCCGATACTGAATGC		[28]
hlyA	For: AACAAGGATAAGCACTGTTCTGGCT	1177	[29]
	Rev: ACCATATAAGCGGTCATTCCCGTCA		[29]
iutA	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, 21isolates 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), α-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 25µL included 12.5µL of PCR green master mix, 1µL of each primer, and 2µL of template DNA have been used, the rest volume was completed to 25µL 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.

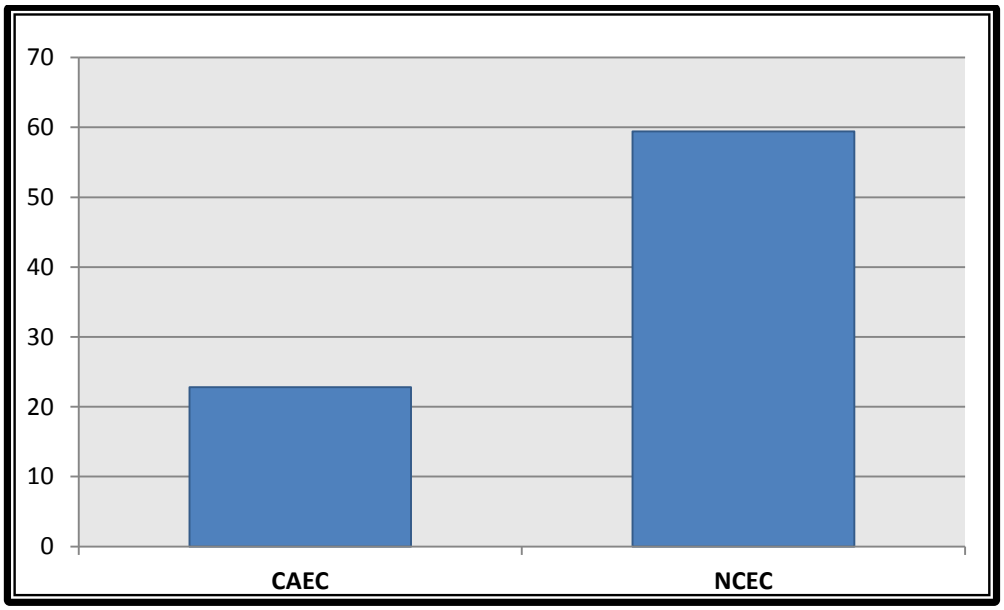

Figure 1- Percentage of catheterized associated *E. coli* (CAEC) and non-catheterized associated *E. coli* (NCEC).

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.

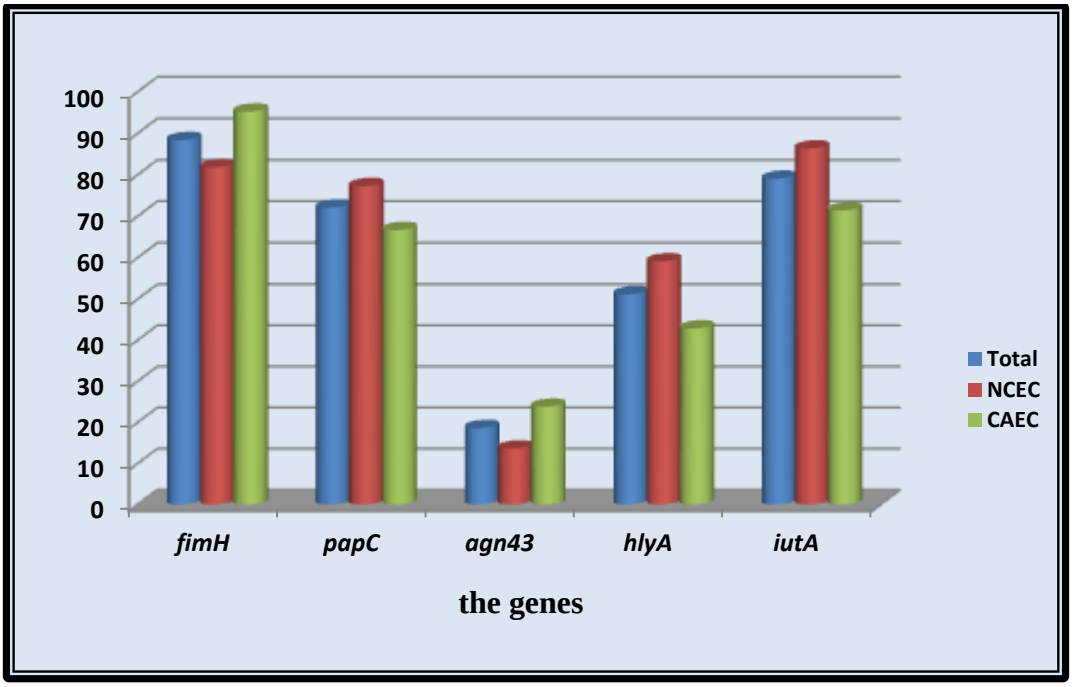

Figure 2- Prevalence of virulence factors genes in *Escherichia coli* isolates.

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

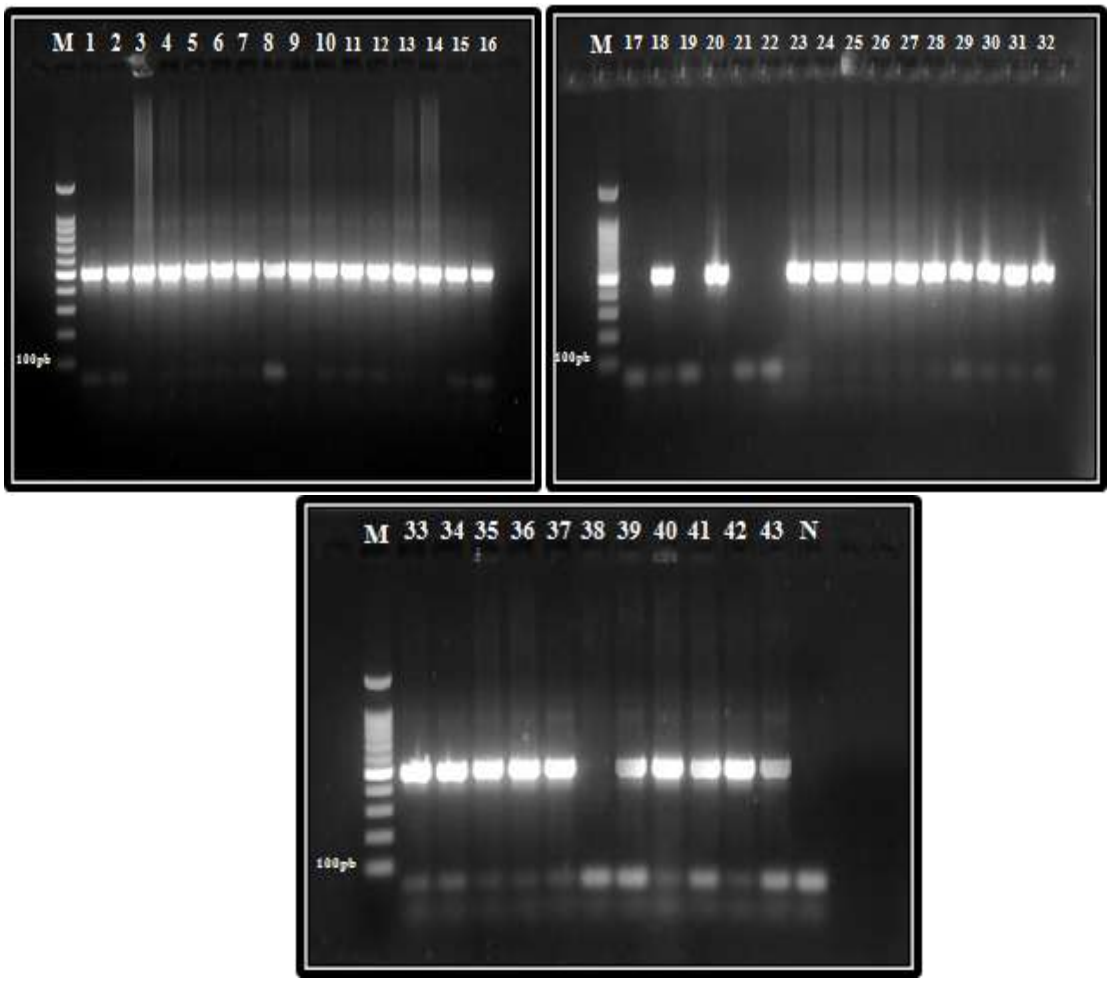

Figure 3- Electrophoresis of amplified *fimH* (508bp). Lanes 1-22 represent non-catheterized *E. coli* isolates and Lanes 23-43 represent catheterized *E. coli* isolates, respectively. M and N lanes represented 100pb ladder and negative sample, respectively. Running conditions: Agarose gel 2%, 75 volt 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. Avery 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 was 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.

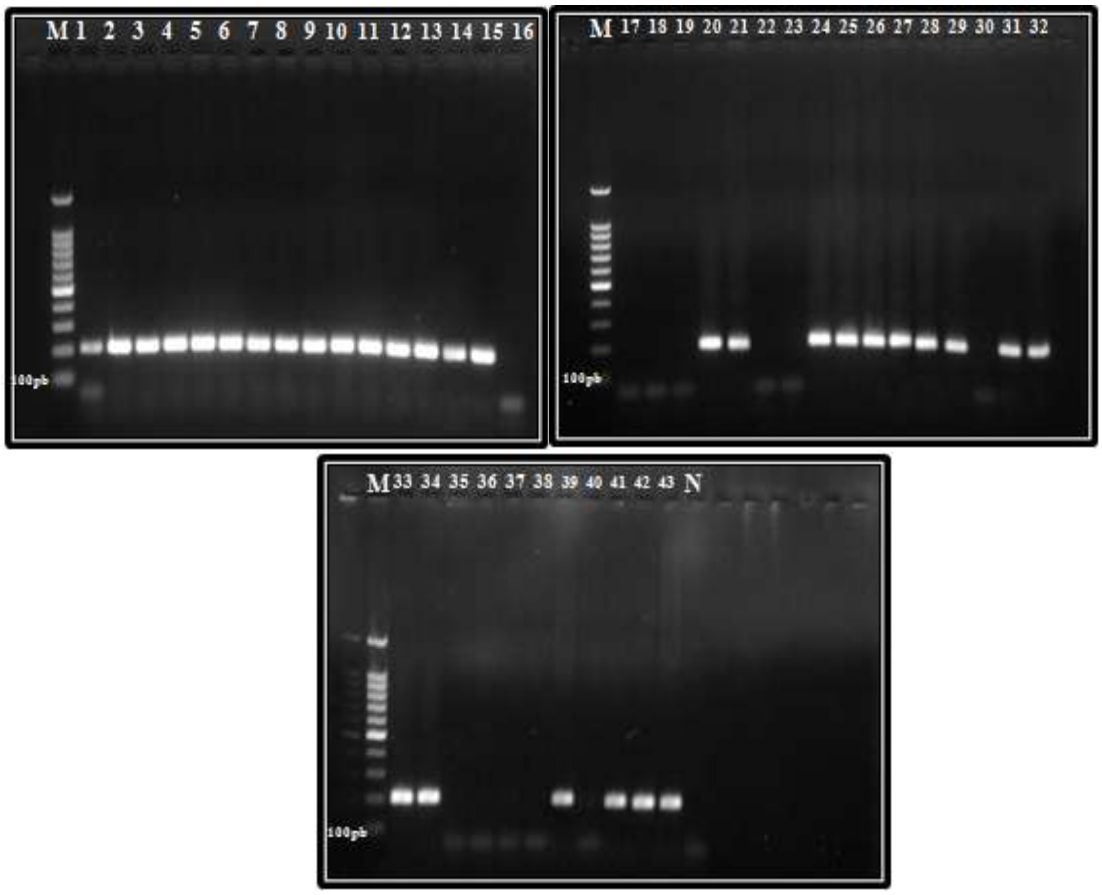

Figure 4- Electrophoresis of amplified *papC* (200bp). Lanes 1-22 represent non-catheterized *E. coli* isolates and Lanes 23-43 represent catheterized *E. coli* isolates, respectively. M and N lanes represented 100pb ladder and negative sample, respectively. Running conditions: Agarose gel 2%, 75 volt 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 *pap*C 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 Biros˘ova´ *et al*. [42] in total, 201 *α*-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.

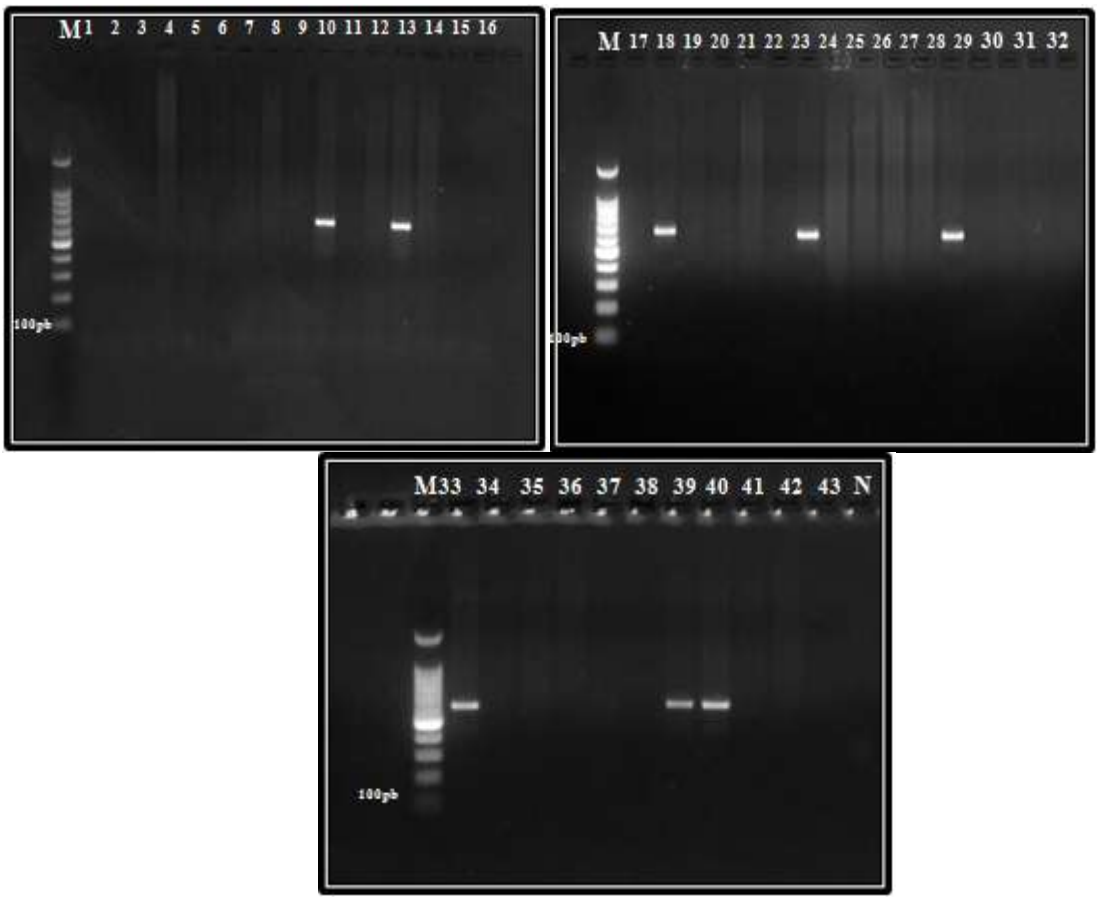

Figure 5- Electrophoresis of amplified *ang43* (700bp). Lanes 1-22 represent non-catheterized *E. coli* isolates and Lanes 23-43 represent catheterized *E. coli* isolates, respectively. M and N lanes represented 100pb ladder and negative sample, respectively. Running conditions: Agarose gel 2%, 75 volt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

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 Enteroaggregative *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.

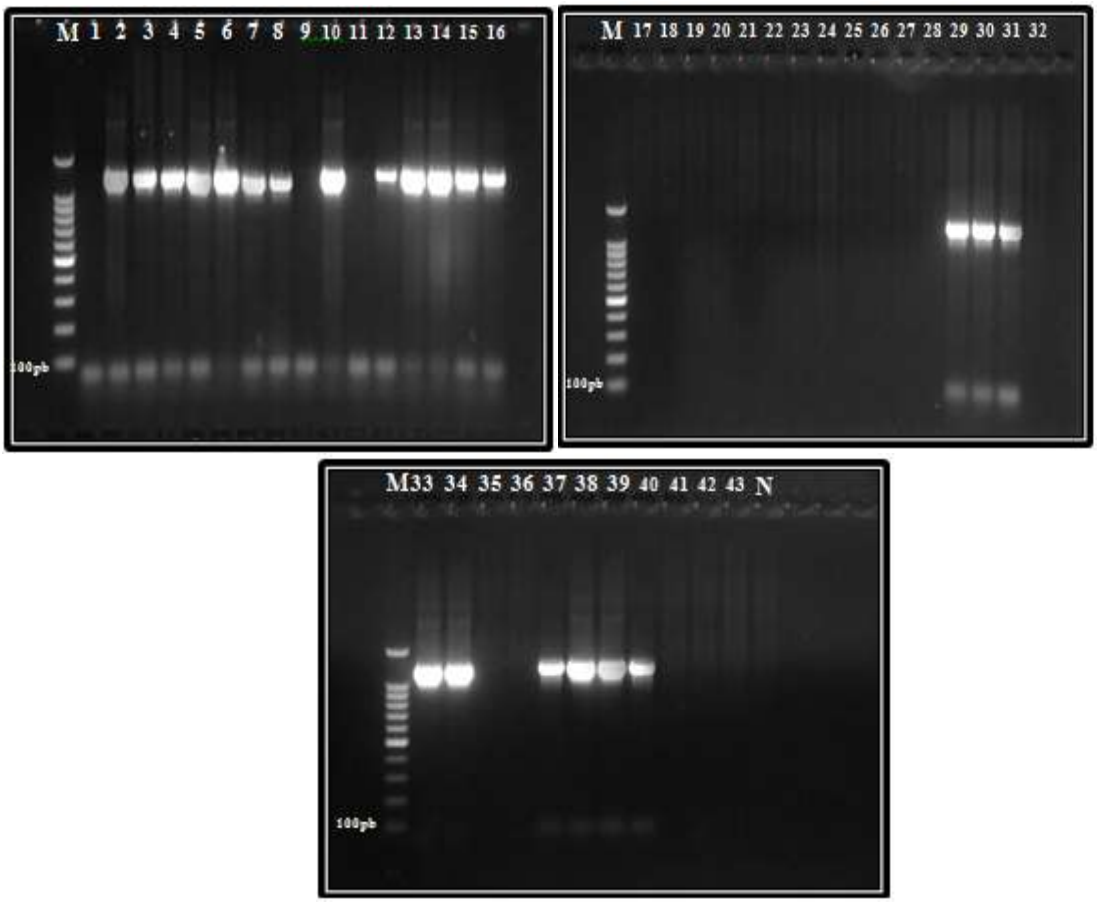

Figure 6- Electrophoresis of amplified *hlyA* (1177bp). Lanes 1-22 represent non-catheterized *E. coli* isolates and Lanes 23-43 represent catheterized *E. coli* isolates, respectively. M and N lanes represented 100pb ladder and negative sample, respectively. Running conditions: Agarose gel 2%, 75 volt 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 (*hly*A) 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.

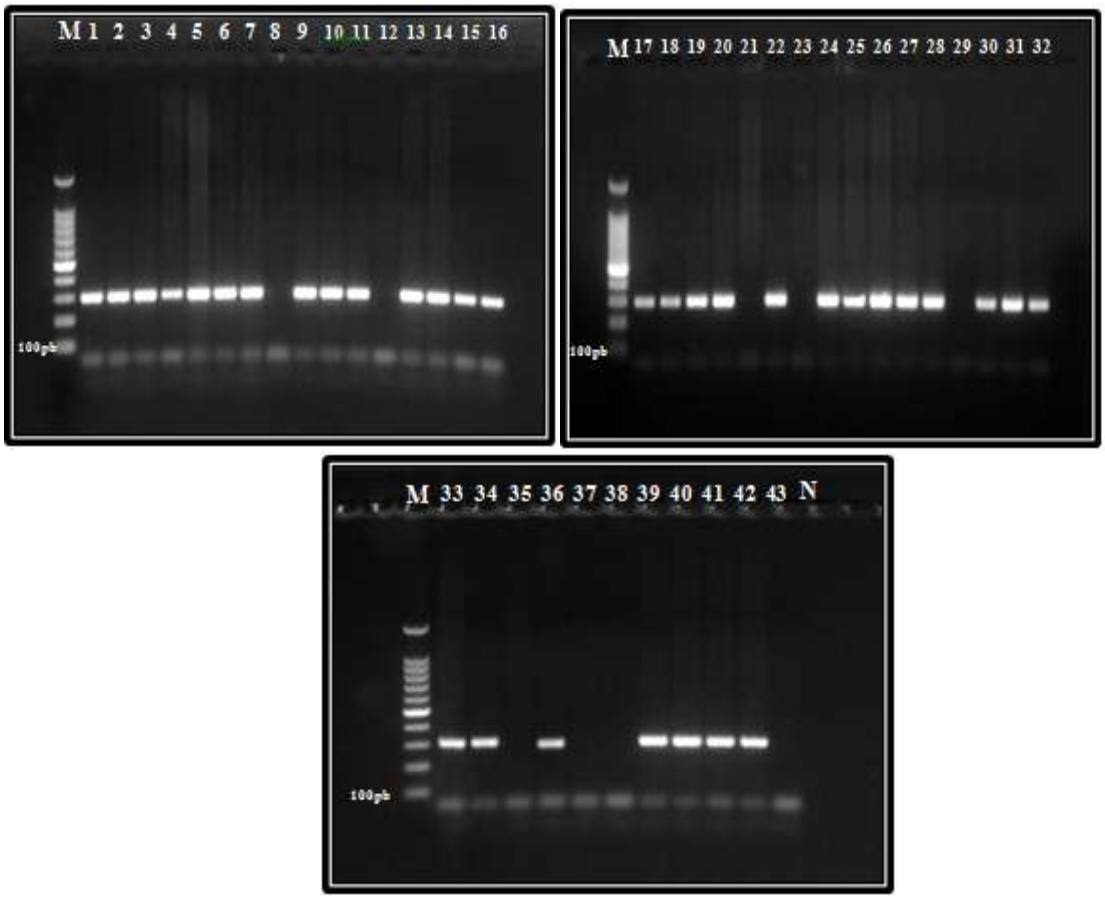

Figure 7- Electrophoresis of amplified *iutA* (300bp). Lanes 1-22 represent non-catheterized *E. coli* isolates and Lanes 23-43 represent catheterized *E. coli* isolates, respectively. M and N lanes represented 100pb ladder and negative sample, respectively. Running conditions: Agarose gel 2%, 75 volt 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 α-haemolytic *E. coli* isolates from various clinical materials were examined by PCR for the presence of the *α*-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[41] 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		
	fimH	***papC***	***agn43***	***hlyA***	***iutA***	**Tatal**	**NCEC**	**CAEC**
P_1	+	+	-	-	+	11	4	7
P_2	+	+	-	+	+	10	8	2
P_3	+	+	+	+	+	4	2	2
P_4	+	+	-	+	-	3	2	1
P_5	-	-	-	-	+	3	3	0
P_6	+	-	-	+	+	2	1	1
P_7	+	-	+	-	+	1	1	0
P_8	-	+	-	-	-	1	1	0
P_9	+	-	+	-	-	1	0	1
P_{10}	+	+	+	-	+	1	0	1
P_{11}	+	-	-	-	-	1	0	1
P_{12}	+	-	-	-	+	1	0	1
P_{13}	+	-	-	+	-	1	0	1
P_{14}	-	-	-	+	-	1	0	1
P_{15}	+	+	-	-	-	1	0	1
P_{16}	+	-	+	+	+	1	0	1

Four of the virulence gene patterns designated as P_5, P_8, P_{11} and P_{14} were characterized by the presence of only one gene, which was either *iutA*, *papC*, *fimH* or *hlyA* (6 strains). Four patterns (P_9, P_{12}, P_{13} and P_{15}) were represented by strains possessing a two gene association (4 strains). The patterns which included strains presenting three virulence genes (P_1, P_4, P_6 and P_7) were the best represented (17 strains). The association of four genes was recognized in (P_2, P_{10}, P_{16}) patterns (12 strains), followed by the P_3 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_2 pattern was the most prevalence with 8 isolates followed by P_1 (4 isolates). Among the strains isolated from patients with urinary catheter show multitude and greatest diversity of genes patterns; it seemed to be more aggresive than strains from non-catheterized patients. 13 of the 16 genetic virulence patterns were identified, in this collection P_1 pattern was the most frequently detected with 7 isolates followed by P_2 and P_3 (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.

	fimH	***papC***	***ang43***	***hlyA***	***iutA***
Total	38(88.37%)	31(72.09%)	8(18.60%)	22(51.16%)	34(79.06%)
NCEC	18(81.81%)	17(77.27%)	3(13.63%)	13(59.09%)	19(86.36%)
CAEC	20(95.23%)	14(66.66%)	5(23.80%)	9(42.85%)	15(71.42%)
P value	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 UTI's. 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.

References

1. Tambekar, D.H., Dhanorkar, D.V., Gulhane, S.R., Khandelwal, V.K. and Dudhane, M.N. **2006**. Antibacterial susceptibility of some urinary tract pathogens to commonly used antibiotics. *African Journal of Biotechnology*. **5**(17): 1562-1565.
2. Macleod, S. M. and Stickler, D. J. **2007**. Species interactions in mixedcommunity crystalline biofilms on urinary catheters. *Journal of Medical Microbiology*. **56**: 1549–1557.
3. Jacobsen, S.M., Stickler, D.J., Mobley, H.L.T. and Shirtliff, M.E. **2008**. Complicated Catheter-Associated Urinary Tract Infections Due to *Escherichia coli* and *Proteus mirabilis*. *Clinical Microbiology Reviews*. **21**(1): 26–59.
4. Lartigue, M., Poirel, L., Poyart, C., Réglier-Poupet, H. and Nordmann, P. **2007**. Ertapenem Resistance of *Escherichia coli*. *Emerging Infectious Diseases*. **13**(2): 315–317.
5. Ruiz, J., Simon, K., Horcajada, J.P., Velasco, M., Barranco, M., Roig, G., Moreno-Martínez, A., Martínez, J.A., de Anta, T.J., Mensa, J. and Vila, J. **2002**. Differences in Virulence Factors among Clinical Isolates of *Escherichia coli* Causing Cystitis and Pyelonephritis in Women and Prostatitis in Men. *Journal of Clinical Microbiology*. **40**(12): 4445–4449.
6. Connell, H., Agace, W., Klemm, P., Schembri, M., Marild, S. and Svanborg, C. **1996**. Type 1 fimbrial expression enhances *Escherichia coli* virulence for the urinary tract. *Proceedings of the National Academy of Sciences.* **93**: 9827-9832.
7. Al-Mayahie, S.M.G. **2013**. Vaginal Colonization by papG Allele II+ *Escherichia coli* Isolates from Pregnant and Nonpregnant Women as Predisposing Factor to Pyelonephritis. *Infectious Diseases in Obstetrics and Gynecology*. 6.
8. Islam, S. **2008**. Effect of nitric oxide on biofilm formation by *Echerichia coli*. M.Sc. Thesis. Department of Microbiology, Karolinska Institutet, Uppsala Universitit. Uppsala, Sweden.
9. Amalaradjou, M.A.R. and Venkitanarayanan, K. **2013**. Role of Bacterial Biofilms in Catheter-Associated Urinary Tract Infections (CAUTI) and Strategies for Their Control. *InTech.*
10. Reisner, A., Maierl, M., Reisner, M.R., Maierl, M., Jorger, M., Krause, R., Berger, D., Haid, A., Tesic, D. and Zechner, E.L. **2014**. Type 1 Fimbriae Contribute to Catheter-Associated Urinary Tract Infections Caused by *Escherichia coli*. *Journal of Bacteriology*. **196**(5): 931–939.
11. Bien, J., Sokolova, O. and Bozko, P. **2012**. Role of Uropathogenic *Escherichia coli* Virulence Factors in Development of Urinary Tract Infection and Kidney Damage. *International Journal of Nephrology*. **15**.
12. Snyder, J. A., Haugen, B. J., Lockatell, C.V., Maroncle, N., Hagan, E. C., Johnson, D.E., Welch, R.A. and Mobley, H.L.T. **2005**. Coordinate expression of fimbriae in uropathogenic *Escherichia coli*. *Infection and Immunity*. **73**: 7588–7596.
13. Aikman, P. **2009**.The effect of a sub-acute ruminal acidosis challenge induced by either grain or alfalfa-pellet based diets on the pathogenicity of rumen *Escherichia coli* populations in dairy cows. *Stapledon Memorial Trust Travelling Fellowship.*
14. Hasman, H.; Chakraborty, T. and Klemm, P. **1999**. Antigen-43-Mediated Autoaggregation of *Escherichia coli* Is Blocked by Fimbriation. *Journal of Bacteriology*. **181**(16): 4834–4841.
15. Roche, A.J., McFadden, J.P. and Owen, P. **2001**. Antigen 43, the major phase-variable protein of the *Escherichia coli* outer membrane, can exist as a family of proteins encoded by multiple alleles. *Microbiology*. **147**: 161–169.

16. Johnson, J. R. **1991**. Virulence factors in *Escherichia coli* urinary tract infection. *Clinical Microbiology Reviews.* **4**(1): 80-128.
17. Linggood, M. A. and Ingram, P.L. **1982**. The role of alpha haemolysin in the virulence of *Escherichia coli* for mice. *Journal of Medical Microbiology.* **15**: 23-30.
18. Slavchev, G.; Pisareva, E. and Markova, N. **2009**. Virulence of uropathogenic *Escherichia coli*. *Journal of culture collections.* **6**: 3-9.
19. Bagg, A. and Neilands, J.B. **1987**. Molecular mechanism of regulation of siderophore-mediated iron assimilation. *Microbiological Reviews.* **51**: 509-518.
20. Reisner, A., Krogfelt, K. A., Klein, B. M., Zechner, E. L. and Molin, S. **2006**. In Vitro Biofilm Formation of Commensal and Pathogenic *Escherichia coli* Strains: Impact of Environmental and Genetic Factors. *Journal of Bacteriology.* **88**(10): 3572-3581.
21. Lee, J.Y., Janes, B. K., Passalacqua, K. D., Pfleger, B. F., Bergman, N. H., Liu, H., Håkansson, K., Somu, R.V., Aldrich, C. C., Cendrowski, S., Hanna, P. C. and Sherman, D. H. **2007**. Biosynthetic Analysis of the Petrobactin Siderophore Pathway from *Bacillus anthracis*. *Journal of Bacteriology.* **189**(5): 698–1710.
22. Torres, A. G., Redford, P., Welch, R. A. and Shelley M. Payne, S. M. **2001**. TonB-Dependent Systems of Uropathogenic *Escherichia coli*: Aerobactin and Heme Transport and TonB Are Required for Virulence in the Mouse. *Infection and Immunity.* **69**(10): 6179–6185.
23. Shetty, S. K., Rao, S.P., Subbannayya, K. and Janakiram K. **2014**. Study of Prevalence of Virulence Factors in Extraintestinal Pathogenic *Escherichia coli* isolated from a tertiary care hospital. *International Journal of Current Microbiology and Applied Sciences.* 3(7): 1055-1061.
24. Kukanur, S., Meundi, M., Bajaj, A. and Kotigadde, S. **2015**. Co-Relation between Virulence Factors and Antibiotic Resistance of *E. coli*, With Special Reference to Uropathogenic *E. coli*. *IOSR Journal of Dental and Medical Sciences.* **14**(3): 15-21.
25. Hooton, T. M., Bradley, S. F., Cardenas, D. D., Colgan, R, Geerlings, S.E., Rice, J.C., Saint, S., Schaeffer, A. J., Tambayh, P. A., Tenke, P. and Nicolle. L. E. **2010**. Diagnosis, Prevention, and Treatment of Catheter-Associated Urinary Tract Infection in Adults: 2009 International Clinical Practice Guidelines from the Infectious Diseases Society of America. *Clinical Infectious Diseases.* **50**: 625–663.
26. Johnson, J. R. and Stell, A. L. **2000**. Extended Virulence Genotypes of *Escherichia coli* Strains from Patients with Urosepsis in Relation to Phylogeny and Host Compromise. *The Journal of Infectious Diseases*. **181**: 261.
27. Le Bouguenec, C., Archambaud, M. and Labigne, A. **1992**. Rapid and specific detection of the *pap, afa*, and *sfa* adhesin-encoding operons in uropathogenic *Escherichia coli* strains by polymerase chain reaction. *Journal of Clinical Microbiology.* **30**: 1189–93.
28. Mendez-Arancibia, E., Vargas, M., Soto, S., Ruiz, J., Kahigwa, E., Schellenberg, D., Urassa, H., Gascón, J. and Vila, J. **2008**. Prevalence of Different Virulence Factors and Biofilm Production in Enteroaggregative *Escherichia coli* Isolates Causing Diarrhea in Children in Ifakara (Tanzania). *The American Journal of Tropical Medicine and Hygiene*. **78**(6): 985–989.
29. Yamamoto, S., Terai, A., Yuri, K., Kurazono, H., Takeda, Y. and Yoshida, O. **1995**. Detection of urovirulence factors in *Escherichia coli* by multiplex polymerase chain reaction. *FEMS Immunology & Medical Microbiology.* **12**: 85–90.
30. Johnson, J. R. and Brown, J. J. **1998**. Colonization with and acquisition of uropathogenic *Escherichia coli* strains as revealed by polymerase chain reaction-based detection. *The Journal of Infectious Diseases.* **177**: 1120–4.
31. Wang, M-C, Tseng, C-C, Chen, C-Y, Wu, J-J and Huang, J-J. **2002**. The Role of Bacterial Virulence and Host Factors in Patients with *Escherichia coli* Bacteremia Who Have Acute Cholangitis or Upper Urinary Tract Infection. *Clinical Infectious Diseases.* **5**: 1161–6.
32. Usein, C-R, Damian, M., Tatu-Chitoiu, D., Capusa, C., Fagaras, R., Tudorache, D., Nica, M., Bouguénec, C.L. **2001**. Prevalence of virulence genes in *Escherichia coli* strains isolated from Romanian adult urinary tract infection cases. *Journal of Cellular and Molecular Medicine.* **5**(3): 303-310.
33. López-Banda, D. A., Carrillo-Casas, E. M., Leyva-Leyva, M., Orozco-Hoyuela, G., Manjarrez-Hernández, A. H., Arroyo-Escalante, S., Moncada-Barrón, D., Villanueva-Recillas, S., Xicohtencatl-Cortes, J. and Hernández-Castro, R. **2014**. Identification of Virulence Factors Genes

in *Escherichia coli* Isolates from Women with Urinary Tract Infection in Mexico. *BioMed Research International.* Volume 2014, Article ID 959206.

34. Karimian, A., Momtaz, H. and Madani, M. **2012**. Detection of uropathogenic *Escherichia coli* virulence factors in patients with urinary tract infections in Iran. *African Journal of Microbiology Research.* **6**(39): 6811-6816.

35. Tiba, M. R., Yano, T. and Leite, D. D. S. **2008**. Genotypic characterization of virulence factors in *Escherichia coli* strains from patients with cystitis. *The Revista do Instituto de Medicina Tropical de São Paulo.* **50**(5): 255-260.

36. Johnson, J. R., Kuskowski, M. A., Gajewski, A., Soto, S., Horcajada, J. P., de Anta, M. T. J. and Vila, J. **2005**. Extended Virulence Genotypes and Phylogenetic Background of *Escherichia coli* Isolates from Patients with Cystitis, Pyelonephritis, or Prostatitis. *The Journal of Infectious Diseases.* **191**: 46–50.

37. Johnson, J. R., Russo, T. A., Tarr, P. I., Carlino, U., Bilge, S. S., Vary, J. C. and Stell, A. L. **2000**. Molecular Epidemiological and Phylogenetic Associations of Two Novel Putative Virulence Genes, *iha* and *iroN*$_{E.}$ *coli,* among *Escherichia coli* Isolates from Patients with Urosepsis. *Infection and Immunity.* **68**(5), pp: 3040–3047.

38. Koga, V. L., Tomazetto, G., Cyoia, P. S., Neves, M. S., Vidotto, M.C.; Nakazato, G. and Kobayashi, R.K.T. **2014**. Molecular Screening of Virulence Genes in Extraintestinal Pathogenic *Escherichia coli* Isolated from Human Blood Culture in Brazil. *BioMed Research International.* **9**.

39. Bingen, E., Picard, B., Brahimi, N., Mathy, S., Desjardins, P. and Elion, J. **1998**. Phylogenetic Analysis of *Escherichia coli* Strains Causing Neonatal Meningitis Suggests Horizontal Gene Transfer from a Predominant Pool of Highly Virulent B2 Group Strains. *Journal of Infectious Diseases*. **177**: 642–50.

40. Archambaud, M., Courcoux, P. and Labigne, A. **1988**. Detection by molecular hybridization of PAP, AFA, and SFA adherence systems in *Escherichia coli* strains associated with urinary and enteral infections. *Annales de l'Institut Pasteur / Microbiologie*. (Paris). **139**: 575-588.

41. Firoozeh, F., Saffari, M., Neamati, F. and Zibaei, M. **2014**. Detection of virulence genes in *Escherichia coli* isolated from patients with cystitis and pyelonephritis. *International Journal of Infectious Diseases.* **29**: 219–222.

42. Biros˘ova´, E., Siegfried, L., Kmet'ova´, M., Makara, A., Ostro´, A., Gres˘ova´, A., Urdzı´k, P., Lipta´kova´, A., Moloka´c˘ova´, M., Ba´rtl, R. and Valansky, L. **2014**. Detection of virulence factors in α-haemolytic *Escherichia coli* strains isolated from various clinical materials. *Clinical Microbiology and Infection.* **10**: 569–573.

43. Tiba, M. R., Nogueira, G. P. and Leite, D. S. **2009**. Study on virulence factors associated with biofilm formation and phylogenetic groupings in *Escherichia coli* strains isolated from patients with cystitis. *Revista da Sociedade Brasileira de Medicina Tropical.* **42**(1): 58-62.

44. Kotlowski, R., Bernstein, C. N., Sepehri, S., Krause, D. O. **2007**. High prevalence of *Escherichia coli* belonging to the B2+D phylogenetic group in inflammatory bowel disease. *Gut*. **56**: 669–675.

45. Ejrnæs, K., Stegger, M., Reisner, A., Ferry, S., Monsen, T., Holm, S. E., Lundgren, B. and Frimodt-Møller, N. **2015**.Characteristics of *Escherichia coli* causing persistence or relapse of urinary tract infections: Phylogenetic groups, virulence factors and biofilm formation. *Taylor & Francis.* **2**(6): 528-537.

46. Qin, X., Hu, F., Wu, S., Ye, X., Zhu, D., Zhang, Y. and Minggui Wang, M. **2013**. Comparison of Adhesin Genes and Antimicrobial Susceptibilities between Uropathogenic and Intestinal Commensal *Escherichia coli* Strains. *Public Library of Science one.* **8**(4): e61169.

47. Ulett, G. C., Valle, J., Beloin, C., Sherlock, O., Ghigo, J. M., Schembri, M. A. **2007**. Functional analysis of antigen 43 in uropathogenic *Escherichia coli* reveals a role in long-term persistence in the urinary tract. *Infection and Immunity.* **75**(7): 3233–3244.

48. Santo, E., Macedo, C. and Marin, J. M. **2006**. Virulence factors of uropathogenic *Escherichia coli* from a university hospital In Ribeirão Preto, São Paulo, Brazil. *The Revista do Instituto de Medicina Tropical de São Paulo.* **48**(4): 185-188.

49. Maslow, J. N., Mulligan, M. E., Adams, K. S., Justis, J. C. and Arbeit, R. D. **1993**. Bacterial adhesins and host factors: role in the development and outcome of *Escherichia coli* bacteremia. *Clinical Infectious Diseases.* **17**: 89–97.

50. Adwan, G., Issa, B. and Adwan, K. **2015**. Virulence Profile, Fluoroquinolone and Quinolone Resistance of Uropathogenic *Escherichia coli* Isolates Recovered from Thabet Hospital-Tulkarm, Palestine. *British Microbiology Research Journal,* **5**(5): xxx-xxx.

51. Watts, R. E., Hancock, V., Ong, C.Y., Vejborg, R.M., Mabbett, A.N., Totsika, M., Looke, D. F., Nimmo, G. R., Klemm, P., Mark A. and Schembri, M. A. **2010**. *Escherichia coli* Isolates Causing Asymptomatic Bacteriuria in Catheterized and Noncatheterized Individuals Possess Similar Virulence Properties. *Journal of Clinical Microbiology,* **48**(7): 2449–2458.

52. Schlager, T. A., Whittam, T. S., Hendley, J. O., Wilson, R. A., Bhang, J., Grady, R. and Stapleton, A. **2000**. Expression of virulence factors among *Escherichia coli* isolated from the periurethra and urine of children with neurogenic bladder on intermittent catheterization. *Infectious Diseases Journal*, **19**(1): 37-41.

53. Mobley, H. L.T., Chippendale, G. R., Tenney, J. H., Hull, R.A. and Warren, J.W. **1987**. Expression of Type 1 Fimbriae May Be Required for Persistence of *Escherichia coli* in the Catheterized Urinary Tract. *Journal of Clinical Microbiology,* **25**(12): 2253-2257.

54. Venier, A.G., Talon, D., Patry, I., Mercier-Girard, D. and Bertrand, X. **2007**. Patient and bacterial determinants involved in symptomatic urinary tract infection caused by *Escherichia coli* with and without bacteraemia. *Clinical Microbiology and Infection,* **13**: 205–208.

55. Ong, C.Y., Ulett, G .C., Mabbett, A. N., Beatson, S. A., Webb, R. I., Monaghan, W., Nimmo, G. R. and Looke, D. F. **2008**. Identification of Type 3 Fimbriae in Uropathogenic *Escherichia coli* Reveals a Role in Biofilm Formation. *Journal of Bacteriology,* **190**(3): 1054–1063.