

Isolation of Multi Antibiotics Resistance *Escherichia coli* from urinary tract infection and the Detection of *PapC* and *fimH* virulence genes by Polymerase chain reaction Technique

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Abstract

One hundred fifty urine samples were collected from urinary tract infections. patient ages is ranging with two days to 73 years, 92 females , 47 males and 11 cases of children for both sex. A total of 65 isolates of *E. coli*. Drug sensitivity of all *E. coli* isolates was evaluated using a disc diffusion method. Most *E. coli* strains showed variable resistance to different antibiotic ,showed high degree of sensitivity towored Nitrofurantoin Ciprofloxacin, Pifloxacin, and Norfloxacin showed the isolates Approximately 100% resistance to Amoxicillin/clavulanic acid and Cloxacillin in all tested isolates. And local isolate showed multidrug resistance range from (28% - 100%). The expression of adhesion factor type P fimbriae (CAFI, CAFII, CAFIII) was determined as followed (89%, 54% , 55%) respectively .As well as adherence of *E. coli* isolates to uroepithelial cells is showed 100% in the local isolates . A total of 28 UPEC strains were selected for genotypic characterization of virulence factor gene (*fimH* and *papC*) by using PCR to detect the relation between phenotype determine with present gene in all of these isolate . The results indicated that the occurrence of *fimH* was detected in (71%) of isolates ,while *pap C* was detected in (79%) of isolates .These results demonstrated the variable phenotypic expression when compared to genotypic detection .

Key words: *E. coli*; UPEC; Virulence genes; ; *pap C*; *fim H*;PCR; Multi -antibiotic resistance; UTI.

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عزل بكتريا *Escherichia coli* ذات المقاومة المتعددة للمضادات الحيوية من اخماج المسالك البولية وتحديد جينات الضراوة *PapC* و *fimH* باستخدام تقنية التفاعل التضاعفي لسلسلة الدنا

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المخلص

جمعت 150 عينة ادرار من المرضى المصابين بالتهابات المسالك البولية. وتم الحصول على 65 عزلة لبكتريا *Escherichia coli* , تراوحت اعمار المرضى من اطفال بعمر يومين الى 73 سنة , وكانت نسبة الاناث 92 , الذكور 47 والاطفال 11 لكلا الجنسين . واختبرت حساسية العزلات للمضادات باستخدام طريقة انتشار القرص. اظهرت معظم سلالات *E. coli* مقاومة متغايرة للمضادات الحيوية , حيث اظهرت العزلات درجة عالية من الحساسية لمضادات Nitrofurantoin و Ciprofloxacin , Pifloxacin , Norfloxacin بينما اظهرت العزلات نسبة مقاومة 100% لكل من Cloxacillin و Amoxicillin / clavulanic acid . وكشفت عزلاتنا المحلية عن مقاومة متعددة تراوحت نسبتها بين (28% - 100%) . كان التعبير عن عامل الالتصاق خمل النوع P (CAFI, CAFII, CAFIII) كالاتي (55% , 54% , 89%) على التوالي . بالاضافة الى انه نتائج الالتصاق لعزلات *E. coli* على الخلايا الطلائية كانت 100% للعزلات المحلية . حددت جينات عوامل الضراوة *fimH* و *papC* لـ 28 عزلة من عزلات *E. coli* الممرضة للمسالك البولية UPEC حيث استخدم طريقة PCR للكشف , وتحديد العلاقة بين الصفات المظهرية والكشف عن وجود الجينات في كل هذه العزلات . لاحظنا وجود جينات *fimH* بنسبة (71%) , بينما *pap C* بنسبة (79%) . كشفت هذه النتائج اختلاف الكشف المظهري عند مقارنته بالكشف الجيني .

الكلمات المفتاحية : - بكتريا القولون , عنصر الضراوة , مقاوم المضادات المتعددة .

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INTRODUCTION

Urinary tract infections (UTI) are the most common nosocomial infections which accounts for 40% of hospital acquired infections(1).Urinary Tract Infections (UTIs), including cystitis and pyelonephritis, are among the most frequent human extraintestinal infections. *Escherichia coli* is the major causative agent, and it also is the predominant facultative member of the normal human intestinal flora(2). This primary agent of UTIs accounts for greater than 80% of these infections. The bladder is the primary site of infection in about 95% of all UTIs. In most cases, uropathogenic clones are selected from the fecal flora and colonize the vaginal and periurethral tissue, and infected the urinary tract by the ascending manner (3).

Extraintestinal pathogenic *Escherichia coli* (ExPEC) are a diverse group of strains infecting extraintestinal locations, including the urinary tract (infected by uropathogenic *E.coli* (UPEC), the blood stream (infected by sepsis-associated pathogenic *E.coli*), and the meninges of the neonates (infected by newborn meningitis-associated *E. coli* (NMEC) (4).Uropathogenic *Escherichia coli* (UPEC) harbor numerous virulence factors including alpha-hemolysin, cytotoxic necrotizing factor, adhesins and iron acquisition systems.These factors support their ability to adhere to uroepithelial cells, help resist the bactericidal effect of serum and augment cell surface hydrophobicity thereby leading ultimately to tissue damage(5). Adherence to the urinary tract mucosa might protect bacteria from urinary lavage and in turn augment their ability to survive and invade renal tissues (6).

The pathogenic potential of *E. coli* strains is thought to be dependent on the presence of virulence factors (VFs)(7). Uropathogenic *Escherichia coli* (UPEC) isolates are a genetically heterogeneous group that exhibit several virulence factors associated with colonization and persistence of the bacteria in the urinary tract. The virulent strains of UPEC that cause cystitis typically produce, at least, one adhesion system. Adhesins can also contribute to virulence, promoting colonization, invasion and replication within uroepithelial cells (3).

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Virulence factors (VFs) associated with UPEC include adhesins (P fimbriae, type 1 fimbriae, S and F1C fimbriae, afimbrial adhesin), toxins (hemolysin, and cytotoxic necrotizing factor), siderophores (the aerobactin system) and polysaccharide coatings (group II capsules) (8,9). Specific adhesion is mediated by certain adhesins which can be differentiated based on their receptor binding specificity. P fimbriae that are encoded by the *E.coli* pap (pyelonephritis associated pilus) operon are the most important mannose-resistant adhesins, although they are expressed by only a limited number of *E. coli* serotypes (6).

Urovirulence factors of *E. coli* analyzed by molecular methods are useful markers for detection of uropathogenic *E.coli* strains using conventional PCR(7).

Aim of this study:The aim of this study was to detect the virulence genes *fimH* and *pap C* of *E.coli* strains isolated from patients with clinical signs of UTI infection in multi antibiotic resistance *E. coli*,PCR method was used for this purpose.

MATERIAL AND METHODS

Collection of Urine Samples:One hundred fifty urine samples were collected during June to November 2011 from 51 subjects (2 days to 73 years) for isolation of *E. coli*. A total of 65 isolates of *E. coli* were studied and taken from UTI patients who were studied clinically suspected to suffer from urinary tract infection according to (10).

Isolation and identification of *E. coli*: For isolation of bacteria urine samples were first inoculated into MacConkey agar and incubated at 37°C for 24 h, after which a loopful was spread onto plates of Eosin Methylene Blue and further incubated at 37°C for 24 h. Isolates were further purified by picking discrete colonies (green metallic sheen) and subculturing onto fresh plates of EMB and further incubating for 18 to 24 h at 37°C. After incubation, 1 to 2 discrete colonies were inoculated into the presumptive diagnostic medium sulfide-indole-motility medium (SIM) and incubated at 35°C for 24 h. Further characterization of isolates was carried out using the IMVIC test. Isolates that were indole positive, hydrogen sulfide negative, non-motile, as well as methyl red, Voges- Proskauer and citrate utilization tests

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were identified as *E. coli* ., fermentation of lactose,ability to produce indole, reaction on triple sugar iron(TSI) medium, hemolysis on blood agar, citrate utilization and motility of organism (10).The identifications were confirmed by the API 20E test system (Bio- Merieux).

Detection of Susceptibility to Antibacterial Agents: Susceptibility of all the isolates to different antibiotics was determined by the disc diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI) (11).The antibiotic discs used in this study were Amoxicillin/clavulanic acid (30mg; 20:10), Cloxacillin(1mg),Nalidixic acid (30 µg), CoTrimoxazole (Trimethoprim-Sulfamethaxazole)(1.25/23.75mg), , Nitrofurantoin(300 µg), ciprofloxacin (5 µg), Ofloxacin(5mg) , Norfloxacin(10mg) , Pifloxacin(10mg) , Cefotaxim(30mg) .Each antibiotic concentration was applied on the surface of Muller -Hinton agar plates inoculated with *E. coli* isolates and incubated at 37°C for 24 h. (12).

Minimum inhibitory concentration (MIC):was determined by agar dilution method in Muller Hinton agar (Oxoid). Serial two-fold dilution was incorporated in media plates from 0.25 to 512 mg/L as recommended by Clinical and Laboratory Standards Institute (11)Antibiotic concentration were prepared from each antibiotics used (ciprofloxacin, norfloxacin, nalidixic acid, pifloxacin) (13).

Hemagglutination and expression of type 1 and P fimbriae: The expression of type 1 fimbriae, or D-mannose-resistant fimbria types by the bacterial strains was determined by agglutinating human and chicken red blood cells in the presence or in the absence of D-mannose, as described by (14).

Adherence to uroepithelial cells: The adherence capacity of the different bacterial isolates to uroepithelial cells was assayed as described by(15).

Preparation of bacterial DNA: The DNA to be amplified was extracted from whole organisms by boiling method. The bacteria were harvested from 1.5 ml of an overnight Luria-Bertani broth culture, suspended in sterile distilled water, and incubated at 95 C° for 10

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min. Following centrifugation of the lysate, the supernatant was stored at -20 C° as a template DNA stock.(7) .

PCR amplification procedure: Detection of *papC*, *fim H* genes was performed by amplifying the genes by PCR. The primers sequences were previously reported by (8) and obtained from Alpha DNA company (USA). Descriptions and sequences of the PCR primers used in this study are displayed in Table 1. Amplification was performed in a thermal cycler (Eppendorf,Germany) according to the methods described by (16). Two program ,For *fim H* the reactions mixtures included an initial denaturation at 94°C for 5 min consisted of 30 cycles of 94 °C for one min, specific annealing temperature 63 C° for one min and 68 °C for three min, and a final extension at 72 °C for seven min in a Thermal Cycler (3). where as for *pap C* the reactions mixtures included an initial denaturation at 94°C for 5 min ,specific annealing temperature 63 C° for one min and 68 °C for three min, and a final cycle of primer extension at 72°C for 7 min. The thermocycler reaction conditions for each primer as fallow pair were calculated on the basis of the annealing temperature and the length of the product size. PCR amplified DNA was analyzed on 0.8 to 1% agarose gels by electrophoresis. The phylogenetic group to which the *E. coli* strains belonged was determined by a PCR-based method, as described by Clermont et al. (17).

Table 1:Primer sequences and predicted sizes of the amplified products of PCR

Primer		Oligonucleotide sequences (5'-3')	Size of amplicons
<i>papC</i>	F	GACGGCTGTACTGCAGGGTGTGGCG	328 bp
	R	ATATCCTTTCTGCAGGGATGCAATA	
<i>fimH</i>	F	TGC AGA ACG GAT AAG CCG TGG	508bp
	R	GCA GTC ACC TGC CCT CCG GTA	

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RESULTS

Isolation of *Escherichia coli* :

Among the 150 clinical samples *Escherichia coli* isolated from urine of UTI patients, their ages ranging from two days to 73 years, 92 females and 47 males. The majority of *Escherichia coli* in UTI cases, 67 cases, were married adult women. 25 cases of *E.coli* in girls (3-18)years which not married,and 11 cases of *E.coli* from children for both sex . All the patients were hospitalized, and suffering from one or more of UTI symptoms, UTI diagnoses were established by the hospital medical staff based on clinical symptoms and laboratory investigation. Urinary frequency, internal dysuria and suprapubic or pelvic pain are the characteristic symptoms of cystitis.

In general, acute pyelonephritis presents fever, flank pain, nausea and vomiting. The laboratory criterion for acute *E. coli* UTI was the presence of a positive culture response with at least 10^5 Colony forming unites of *E. coli* per mLof clean-voided urine. Among the subjects there were 15 cases of pyelonephritis. Table2 shows demographic data of *Escherichia coli* UTI patients.

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Table- 2: Demographic data of *Escherichia coli* UTI patients

Variable	Data
Age of patients	2 days-73 years
Sex of patients	Female : male
Female : male	92:47
Clinical case in adult women UTI	67 cases
Clinical case in girls UTI	25 cases
Clinical case in men UTI	47 cases
Clinical case in children UTI	11 cases

Identification of isolates: Identification and confirmation were done on the basis of morphological, biochemical and phenotypic characteristics. All isolates were found to be Gram negative rods. Biochemical tests oxidase-negative, glucose, lactose and sucrose fermenting , IMVIC test showed indole positive , positive for methyl red , negative for Voges- Proskauer and citrate utilization tests .The have ability of reaction on triple sugar iron(TSI) medium,. The identifications were confirmed by the API 20E test system (Bio-Merieux).

The disc diffusion method :The arrows on each zone of inhibition of the bacteria were measured. Different diameters were measured with a ruler and were recorded on a data sheet as shown in Table 3. The clear zones around the antibiotic disc show the susceptibility of the bacteria against the antimicrobial .There were also isolates that were resistant to many antibiotics and no clear zone was observed. The resistance of the different antibiotics varies as

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the diameters of the zone of inhibition differ. Table 3 shows the resistance and susceptibility of the isolated *E. coli* to different antibiotics as represented by the diameter in mm. The high light indicates cases considered to be resistant to their respective antibiotics. All the isolated *E. coli* show resistance to one or more antibiotics. Out of the 65 isolates, 100% were resistant to Amoxicillin/clavulanic acid, 22% were resistant to Nitrofurantoin, and 78% were susceptible to this particular antibiotic. 99% of the isolates were resistant to Cloxacillin, with a percentage of susceptibility of 1%. Co-Trimoxazole (Trimethoprim-Sulfamethaxazole) and Cefotaxim showed a resistance 72% of all the isolates. 37% isolates were resistant to Ciprofloxacin. The percentage of resistance to Nalidixic acid is 62%. Ofloxacin had a percentage of resistance 37% of the isolates were resistant to Norfloxacin was 35%. Pifloxacin showed a resistance was 34%.

Table-3 Percentage of isolates resistant to different antibiotics.

Antibiotics	Resistant %
Amoxicillin/clavulanic acid	100
Nitrofurantoin	22
Cloxacillin	99
Co Trimoxazole (Trimethoprim-Sulfamethaxazole)	72
Cefotaxim	72
Ciprofloxacin	37
Nalidixic acid	62
Ofloxacin	37
Norfloxacin	35
Pifloxacin	34

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Determination of Minimum inhibitory concentration MIC: MICs of 4 antimicrobial agents (Nalidixic acid,Ciprofloxacin,Norfloxacin, Pifloxacin)for each isolate were determined by agar dilution method. Table 4 indicates the resistance pattern of clinical isolates against 4 antimicrobial agents. The over all resistances offered by the isolates against different antibiotics are as follows: 20% of isolate resistance offered to Nalidixic acid and Ciprofloxacin and susceptible for both Norfloxacin and Pifloxacin .54% of isolate resistance to all antimicrobial agents (Nalidixic acid ,Ciprofloxacin,Norfloxacin, Pifloxacin).8% of isolate susceptible to all isolate .5% of isolate showed that only resistance to Ciprofloxacin and susceptible to antimicrobial agents(Nalidixic acid ,Norfloxacin, Pifloxacin).3%of isolate showed that only susceptible to Nalidixic acid and resistance to antimicrobial agents(Ciprofloxacin,Norfloxacin, Pifloxacin).6% of isolate showed that only resistance to Nalidixic acid and susceptible to antimicrobial agents(Ciprofloxacin,Norfloxacin, Pifloxacin).5%of isolate showed that only susceptible to Pifloxacin and resistance to antimicrobial agents(Ciprofloxacin,Norfloxacin, Nalidixic acid).

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Table- 4 Percentage of resistant isolates against different antibiotics by MIC .

NO of resistance isolate	Equivalent MIC (P) Breakpoint s (Mg/ml) ≥8)/ S(≤2)(R	Equivalent MIC (NOR) Breakpoint s (Mg/ml) ≥16)/ S(≤4)(R	Equivalent MIC (CIP) Breakpoint s (Mg/ml) ≥4)/ S(≤1)(R	Equivalent MIC (NA) Breakpoint s (Mg/ml) ≥32)/ S(≤8)(R	percentage % N65
13	S	S	R	R	20
35	R	R	R	R	54
5	S	S	S	S	8
3	S	S	R	S	5
2	R	R	R	S	3
4	S	S	S	R	6
3	S	R	R	R	5

Hemagglutination and expression of type 1 and P fimbriae: Bacterial adherence and colonization of the urinary tract by uropathogenic *E. coli* strains are mediated by the expression of several types of fimbrial and nonfimbrial adhesions (18,19). Under our experimental conditions, when using anti-P fimbria serum and red blood cell agglutination, 55 strains (84.6%) were able to express type 1 fimbriae. 43 (66%) also expressed type P fimbriae and another strains expressed type P but not type 1 fimbriae, detection of 58 (89%) colony adhesion factor (CAFI) The expression of type 1 fimbriae, or D-mannose-resistant fimbria types by the bacterial strains was determined by agglutinating human blood. also given 35 (54%) colony adhesion factor (CAFII) The expression of type 1 fimbriae, or D-mannose-resistant fimbria types by the bacterial strains was determined by agglutinating

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Shaymaa K.Abass .; Mun'im R. Ali .;Sawsan H.Authman.

chicken red blood cells in the presence or in the absence of D-mannose, and 36 (55%) colony adhesion factor (CAFIII). The expression of P fimbriae by the bacterial strains was determined by agglutinating human blood cells in the presence of Tannic acid.

Adherence to uroepithelial cells: Adherence of *E. coli* isolates to uroepithelial cells is used. It was shown that 100% of the isolates presented adherence that mean number of bacteria adhered to uroepithelial cells ranged from 8 to 63. All isolates showed a mean number of bacteria/cells very close to that needed for a strain to be considered uropathogenic. They produced P fimbriae but some isolates had no capacity for expression of any other biological characteristic. On the other hand, isolates (DA with D-mannose). These same isolates showed biological characteristics normally considered to be pathogenic traits and produced type 1 fimbriae, but not type P fimbriae. Table 5 indicates the adhesion factor and Percentage of colony adhesion factor pattern of clinical isolates.

Table 5 The adhesion factor and Percentage of colony adhesion factors pattern of *E. coli* clinical isolates :

Adhesion factor	NO of adhesion isolate	Percentage % (N 65)
Adherence to uroepithelial cells	65	100
CAFI	58	89
CAFII	35	54
CAFIII	36	55

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Shaymaa K.Abass .; Mun'im R. Ali .;Sawsan H.Authman.

PCR Amplification : A total of 28 UPEC strains from patients with UTI were genotypically characterized by the use of PCR assay, for virulence factor encoding *fimH* and *pap C*. we used these isolate depending upon high degree of multi antibiotic resistance . Polymerase chain reaction showed that the prevalence of virulent genes occurred in 20 *fimH*(71%), and it was detected in 22*Pap C*(79%) (Table 6).

Table-6: Distribution (number and percentage) of virulence factor genes in uropathogenic *E. coli* isolates from patients of UTI.

Virulence factors (genes)	Number of positive strains	Percentage of positive strains % N28
<i>Pap C</i>	22	79
<i>FimH</i>	20	71

The *papC* gene in the operon encoding P-pili was found in four isolate belonging to positive after amplification is shown in figure (1) The *FimH* gene in the operon encoding type 1 fimbria was found in three isolate belonging to positive after amplification is shown in figure (2).

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Shaymaa K.Abass .; Mun'im R. Ali .;Sawsan H.Authman.

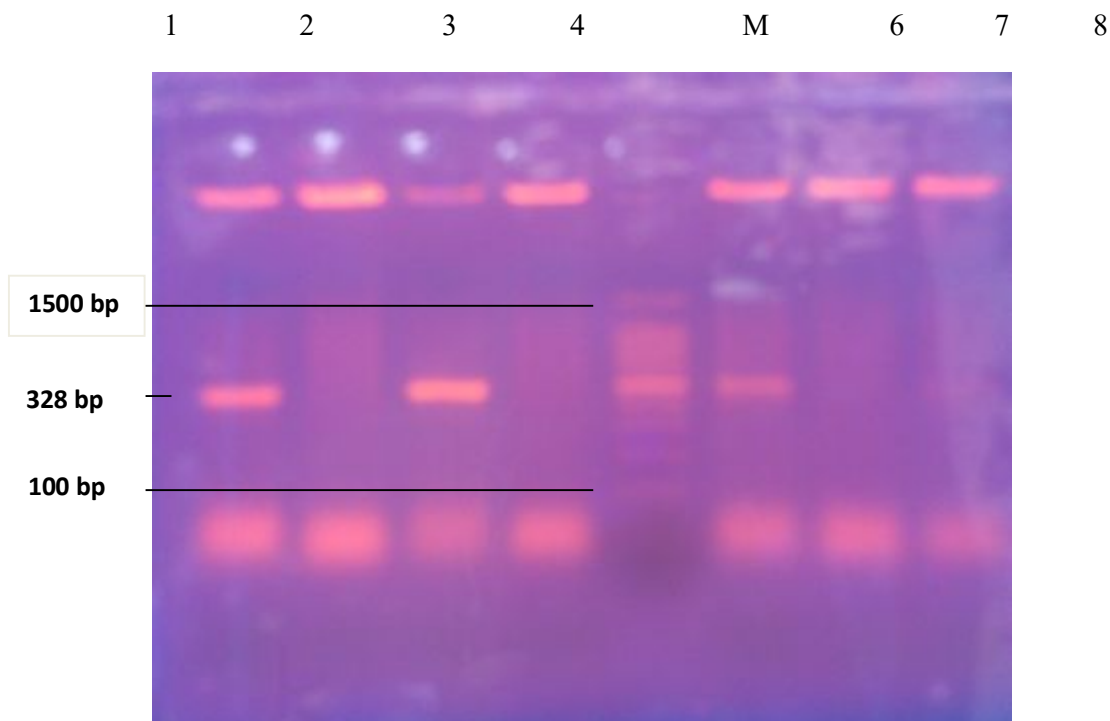


Figure 1: 1%Agarose gel electrophoresis and Ethidium bromide staining. to detect *papC* gene size product (band 328bp) Lane M, molecular size DNA ladder (123 bp DNA Ladder); lanes 1-4,5-8, DNAs isolated from *E. coli* samples and only lanes 1,3,6 showed Positive PCR bands; lanes 2,4,7,8 negative;; The PCR was carried out in duplicate. The photocomposition of the figure was obtained with Gel documentary Camera.

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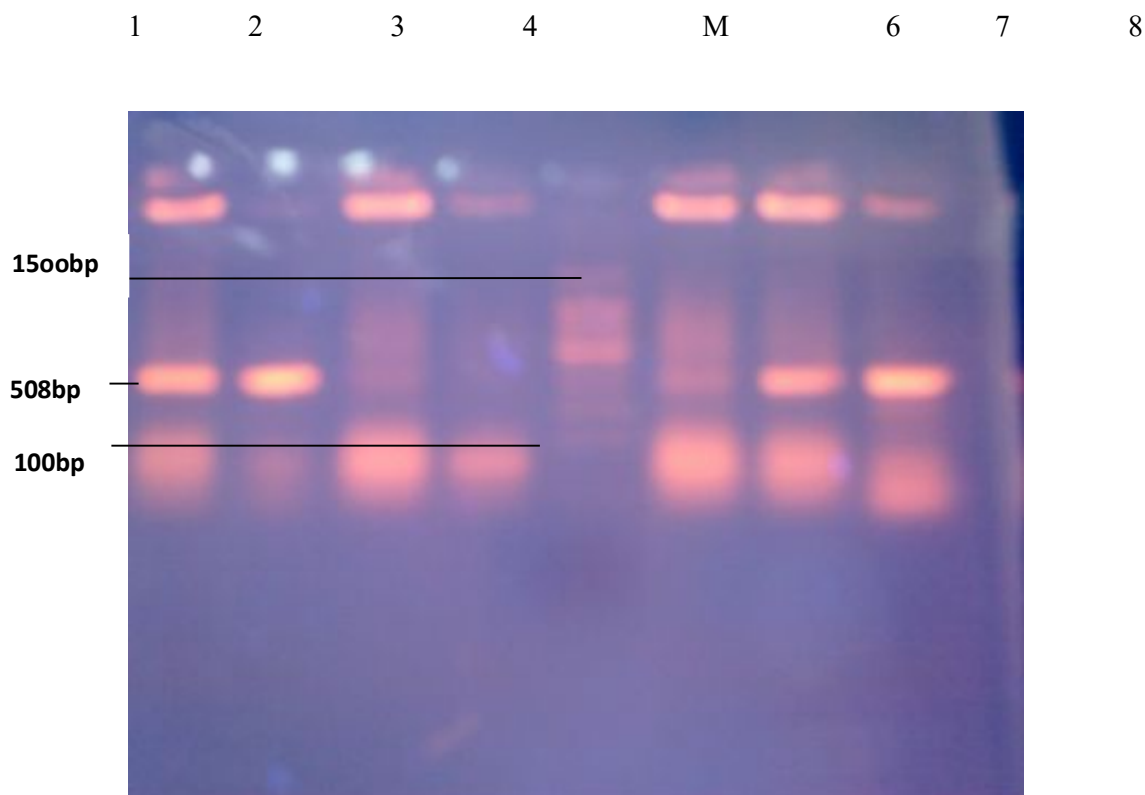


Figure 2: 1%Agarose gel electrophoresis and Ethidium bromide staining. to detect *FimH* gene size product (band 518bp) Lane M, molecular size DNA ladder (123 bp DNA Ladder); lanes 1-8, DNAs isolated from *E. coli* samples and only lanes 1,2,7,8 showed Positive PCR bands; lanes 3,4,6 negative;; The PCR was carried out in duplicate. The photocomposition of the figure was obtained with Gel documentary Camera.

Discussion

E. coli is the major causative agent in human UTIs, one of the most common bacterial infections. In most cases, uropathogenic clones are selected from the fecal flora, and the pathogenic potential of *E. coli* strains is thought to be dependent on the presence of VFs (20).In this study showed a higher proportion of UTI in females 92 (61.3%) than in males 47 (31.3%). This is understandable due to the anatomy and is a consistent trend worldwide .The

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Shaymaa K.Abass .; Mun'im R. Ali .;Sawsan H.Authman.

study is agreement with (21). Peak in the incidence of UTI was observed in the age groups 11–21 and 60–71 years. Among these, elderly patients are likely predisposed to conditions such as urinary tract obstruction, poor bladder emptying, and diabetes mellitus, etc. These factors favor colonization of bacteria and play an important role in UTI(22). Other studies have also reported similar findings(23).Iraq has a large infection burden and the genito-urinary infections are very prominent, this may be due to a less affordable personal/community hygiene for some of the economically back word populations.

We targeted these groups and analyzed strains obtained from such communities who reported for the UTI. As such there is not much information available from Iraq on the genetic and phenotypic diversity of UPEC. This information should therefore be construed as the first systematic analysis performed on a diverse type of patients samples. In the present study, 89% of the UTI isolates showed the presence of type 1 fimbriae (Mannose sensitive hemagglutination -MSHA). While only 54% of *E.coli* from UTI isolates exhibited P fimbriae (Mannose resistant hemagglutination -MRHA). Expression of type 1 fimbriae was more discerned in isolates from the cases of simple UTI and cystitis. In our observation, maximum P fimbriae positive isolates were highly associated with simple UTI, while only 10% of such isolates caused pyelonephritis. In the same manner Johnson JR(24) showed there is no relation among the occurrence or severity of symptoms and the site of infection and fimbrial expression (of P or type1) in case of bacterial isolates from urine. Antibiotic sensitivity test showed nitrofurantoin as the most effective followed by Ciprofloxacin , Pifloxacin and Norfloxacin in our isolates. All the strains showed the most resistance to Amoxicillin/clavulanic acid and Cloxacillin (Table 3). The result of this study was in agreement with the finding of (25) that showed It is well known that resistance to quinolones and fluoroquinolones such as nalidixic acid Ciprofloxacin , Pifloxacin, Ofloxacin, and Norfloxacin is an increasing issue in many parts of the world (26). According to recently published data, the rate of quinolone resistant *E. coli* in the urine has reached 25% which corresponds to our results 36 (55.3%) (27). It has also been shown that quinolone and fluoroquinolones resistant uropathogenic *E.coli* strains (UPEC) express fewer virulence factors

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than quinolone susceptible strains. Correspondingly, the present data showed that nalidixic acid-resistant uropathogenic strains were significantly less likely than their susceptible counterparts to harbor *papC* and *fimH* operons. This finding suggests that quinolone resistance could be directly associated with decreased prevalence of virulent genes, as suggested in a previous study(28). Detection and identifying the presence of potential uropathogenic *Escherichia coli* virulence factors genes for *papC* and *fimH* operons done by using PCR method which is highly specific, informative and a powerful genotypic assay, used for detection of adhesin-encoding operons and other virulence factors that can also contribute to virulence in UTI. In this study, we confirmed *fimH* among UPEC strains in 20 (71%) strains. 20 strains presented only adhesin-encoding operon *fimH*, and in 8 of these strains no *papC* gene was detected. This result demonstrated that type 1 fimbriae is an important and relevant VF, and that it can also contribute to virulence in *E.coli* strains. This study is agreement with (7). Type 1-mediated adherence has been proposed to play a role in the induction of inflammation, enhancing *E. coli* virulence for the urinary tract. Furthermore, we demonstrated that 71% *fimH* strains, occurred with at least one adhesin, such as *pap*, *sfa*, or *afa*. This result corroborates the occurrences presented in the literature, where *fimH* was found in associations with P fimbriae and S fimbriae in UPEC(29). P fimbriae, the principal mannose-resistant adherence organelles of extraintestinal pathogenic *Escherichia coli*, are known to contribute to pathogenesis by promoting bacterial colonization of host tissues and by stimulating an injurious host inflammatory response(30). *E. coli* strains were examined for *pap* genotype and specific primers were utilized to detect genes associated with outer membrane protein (*papC*). The study is agreement with Ananias and Yano (31) who have found that nearly 71% of strains were positive for *papC*, suggesting that other adhesin genes were present or *papC* may be deleted. While Watt *et al.*, (32) have found *papC* associated with group B2 of *E. coli* isolated from clinical samples. Another study by Nowrouzian *et al.*, (33) has demonstrated that half of commensal group B2 *E. coli* have carried *papC* genes which is persistent in gastrointestinal. In the other hand Wu *et al.*, (34) have observed that *papC* assay is the specific and sensitive method in the detection of UPEC strains and the 328 bp sequence

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may be proved to be valuable for the identification of UPEC strains as the specific molecular marker. As well as Marklund *et al.*, (35) have proposed that *E. coli* have acquired the *papC* locus after the speciation of *E. coli* and suggested that the *papC* genes could have been acquired by horizontal gene transfer. Moreover, they proposed that the recent genetic exchanges involving the entire pilin gene clusters have occurred in response to selection pressures exerted by the host. The structure and function of the *PapC* gene is required for P pilus biogenesis in uropathogenic *E. coli*. (36). Our observation was in agreement with another study (37) where in the presence of type 1 fimbriae was seen in 71% of isolates of *E. coli* from UTI and in 65 of isolates of *E. coli* from the control fecal flora. Taken together, UTI associated isolates did not show any significant expression of MSHA compared to controls, however, Najar *et al.* (37) showed an overall higher expression of type 1 fimbriae in both the UTI isolates and controls. Many studies have demonstrated the presence of P fimbriae as maximum in UTI isolates than in fecal isolates of healthy persons (37,38).

Conclusion

A relationship between UPEC reduced virulence and multidrug resistance. This study evaluated the antibiograms of *E. coli* strains having virulence genes and those not having virulence genes *papC* and *fimH* genes, we found relation between virulence genes and the resistance strains.

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