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Detection of Uropathogenic Virulence Genes in *Escherichia coli* isolated from Urine Samples of Children under Five Years

Ilham S. Banno[1], Ali F. Ahmed[2]

Biology Department, College of Education for Pure Science, Ibn, AL-Haythum, University of Baghdad

Abstract: ***Escherichia coli is the predominant pathogen causing urinary tract infection(UTI).A total of 72 uropathogenic E,coli strains were isolated from clinical samples were taken from children with (UTI), whome they were under the age of five years, male and female taken from Baghdad hospitals in the period from 1/11/2014 to 28/2/2015. The study results showed that the infection in urinary tract of female were39 isolates 54.2% of and that of males were 33 isolates 45.8%. All isolates were identified according to morphological and biochemical tests and results were confirmed by Api 20E system. Polymerase chain reaction (PCR) was used to find out two types of adhesion factors, Fim(H) and Pap G alleles genes, as they were the cause of the most common (UTI)s by using specialist primers for each gene. Results showed the existence of Fim(H) gene was 100%, while Pap G(I) gene19.4%, Pap G(lll) gene 38.8%, and the results of the test of Pap G(ll) gene were negative to all isolates.***

Keywords: *Escherichia coli*, urinary tract infection

1. Introduction

Urinary tract infection (UTI) is among the most common bacterial infectious diseases encountered at all ages. Escherichia coli is being the etiologic agent in 50-80%. Therefore, it is an important public health problem (1,&2).Many virulence factors contribute to the pathogenicity of these *E.coli* strains, which are termed uropathogenic *E.coli*(UPEC)(3). In ascending infections, fecal bacteria colonize the urethra and spread up the urinary tract to the bladder as well as to the kidneys causing pyelonephritis(4) or the prostate in males. Because woman have a shorter urethra than men, they are 14 times more likely to suffer from an ascending UTI(1). Uropathgenic *E. coli* use P fimbriae (pyelonephritis-associated pili) to bind urinary tract urothelial cells and colonize the bladder(5). Many adhesive factors are seen in *E.coli*, they play a major role in the colonization of *E.coli* in different tissues of human body(6). Urovirulence factors of *E.coli* analysed by molecular methods are useful markers for detection of uropathogenic *E.coli* isolates(7).The polymearas chain reaction(PCR) is a powerful technique that has rapidly become one of the most widely used techniques in molecular biology because it is quick, inexpensive and simple(8). The aim of this study to detect genes encoding type 1 fimbriae *Fim(H),*and pili associated with pyelonephritis *PapG* alleles.

2. Material and Methods

Isolation of bacteria

In this study, 110 urine samples were obtained from children with urinay tract infection, whome they were under the age of five years, male and female taken from three kids hospitals in baghdad city(Ibn Al-balady hospital for children, Al-Imamayn Al-Khademayn Teaching hospital and Al-A 'lwayya hospital for children), in the period from 1/11/2014 to 28/2/2015. The midstream urine sample collected from all patients. 0.01 ml of urine cultured on MacConky agar and Eosin Methylen Blue(EMB). The isolation and identification of *E.coli* were performed on the basis of culture characteristics (colonies phenotypic, Gram stain and biochemical tests according to (9) and confirmed by the Api 20E system(BioMerieux, France).

Bacterial culture and DNA extraction : *E.coli* strains were grown in Tryptic soy brith at 37º C overnight, DNA extraction of *E.coli* isolates was performed using (Geneaid kit).

PCR amplification

Specific primers which were provided in a lyophilized form by Bioneer(Korea), dissolved in sterile DDistilled water to give a final concentration of 100 pmol/µl as recommended by provider and stored in deep freezer until used in PCR amplification. Primers were used for detecting and amplify sequences of *E.coli* adhesion factors the *Fim(H*) gene and *Pap G* gene alleles (l, ll, lll). Details of primers sequences according to(10), shown in Table(1). PCR Master Mix contents was provided by the supplier(Bioneer) were listed in table(2).

Table 1: Oligonucleotide primers sequence used for PCR amplification of specific gene

5 ' – 3 ') (Fim (h)	**F:**	TGC AGA ACG GAT AAG CCG TGG	509 bp
	R:	GCA GTC ACC TGC CCT CCG GTA	
pap G allel I (5' – 3')	F:	TCG TGC TCA GGT CCG GAA TTT	461 bp
	R:	TGG CAT CCC CCA ACA TTA TCG	
pap G allel II (5 ' – 3 ')	F:	GGG ATG AGC GGG CCT TTG AT	190 bp
	R:	CGG GCC CCC AAG TAA CTC	
pap G allel III (5' – 3')	F:	GGC CTG CAA TGG ATT TAC CTG G	258 bp
	R:	CCA CCA AAT GAC CAT GCC AGA	

Table 2: PCR Master Mix provided from Bioneer

Volum µ l	Material	
1 U	Top DNA polymerase	1
10	Tris-HCl ,pH=9	2
250	dNTPs(dATP,dCTP,dGTP,dTTP)	3
30	KCl	4
1.5	$MgCl_2$	5
———	Stabilizer and tracking dye	6

PCR assay

All 72 E.coli isolates were analyzed by PCR. To each premix tube, the following reagents were added; 2µl of each of forwarded and Reverse primers,5µl of DNA samples, and the volum adjusted to 20µl by adding sterilized double distilled water. Table (3) showed program detection for *Fim(H)* gene fragment and Table(4) showed program detection for *Pap(G)* gene allels by PCR cycling conditions. The amplification was carried out in a ESCO Thermo cycler machine model 5000. A10µl aliquot of the PCR product underwent gel electrophoresis on 1% agarose, followed by staining with ethidium bromide solution. Amplified DNA fragments of specific sizes were detected by UV- induced fluorescence, and the size of the amplicons was estimated by comparing them with the 100bp DNA ladder(Promega) included on the same gel.

Table 3: PCR amplification program for *Fim(H)*gene

No. of cycle	*Time (min)*	*Temperature C°*	*stage*
1	5	94	DNA Intial Denaturation
30	1	94	DNA Denaturation
	1	60	Annealing
	1	72	Extention
1	7	72	Final Extention

Table 4: PCR amplification program for *Pap (G)* alleles

No. of cycle	Time (min)	Temperature C°	stage
1	4°	94	DNA Intial Denaturation
30	1	94	DNA Denaturation
	2	60	Annealing
	3	72	Extention
1	20	72	Final Extention

3. Results and Discussion

Out of 110 urine samples collected from children under five years old, from Baghdad hospitals. 72 Escherichia coli isolates were identify and characterize according to certain morphological, cultural and biochemical tests as described by (11), the identification results were confirmed using ApiE20.Table(1) showed that the infection in urinary tract of female were39 isolates 54.2% of and that of males were 33 isolates 45.8%. result show that females had tendency to get UTI more than males, this due to the anatomy and femal have a shorter urethra than male, they are 14 times more likely to suffer from an ascending UTI(12). This result was agreed with (13) that *E.coli* is the major causative agent in humen UTI. This study showed that children under two years infected more than older age due to many reasons,the more important, defect in immune system and infant artificial suckling (12).

Table 5: Number and percentage of E. coli isolates

Sex	*No. of E.coli isolates*	%
Male	33	45.8%
Female	39	54.2%
Total	72	100%

Genotypic detection of isolates

PCR technique is a primer mediated enzymatic amplification of specifically cloned or genomic DNA sequences. PCR has become fast reliable assay for molecular biology based diagnosis of the variety of many diseases, because of its speed and versatility. In present study PCR technique was used to detect genes responsible for fimbrial adhesive system. The DNA of 72 *E.coli* isolates was extracted and detected by electrophoresis on 1% agarose and exposed to UV light in which the DNA appered as compact bands figure(1).

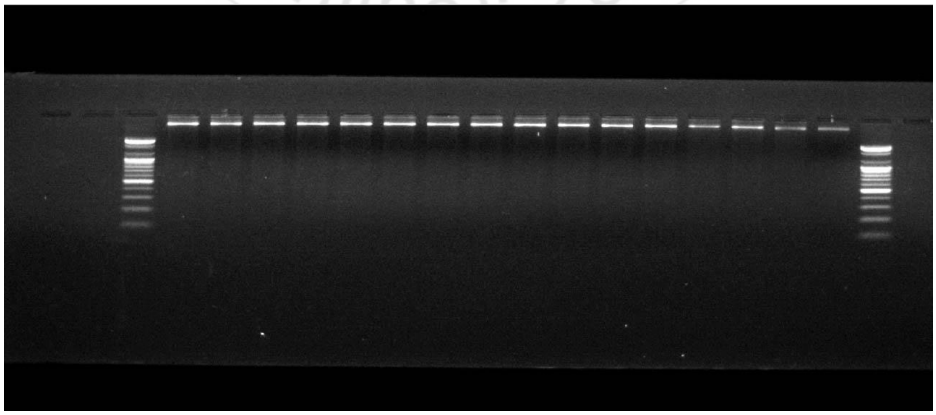

Figure 1: Total genomic DNA extracted from E.coli isolates using 1% agarose gel electrophoresis 70 volt, 1.5 hour

Detection of *Fim(H)* gene:

All 72 E.coli isolates were genotypically characterized by the use of PCR assay, for virulence factor encoding *FimH*. Polymerase chain reaction showed 100% positive result ,gave clear bands of the same amplified *FimH* gene, on 1% agarose gel as a result of PCR reaction Figure (2), the size of the amplified gene *FimH* gene was 508 bp. As compared with 100 bp DNA ladder which represents the whole size of *FimH* gene and other virulence gene since it was picked up from the upstream and downstream region of the

gene(14).This study agreed with(10,15&16) found 100% of *E.coli* isolates were positive for *FimH* adhesive gene which was the most prevalent virulence factor detected, also agreed with(17)and(18) found that 95%,92% of *E.coli* isolates were positive for *Fim H* gene respectively.(19) and(13) found 71%,68% of *E.coli* isolates only have *FimH* gene respectively, whlile our study is agreement with(20) found 98% of *E.coli* isolated from children with UTI. *FimH* mediated adherence by enhancing the inflammatory response and contribute to colonization of the bladder and initiate infection by binding to the superficial bladder epithelial cells that line the luminal surface of the bladder(11).

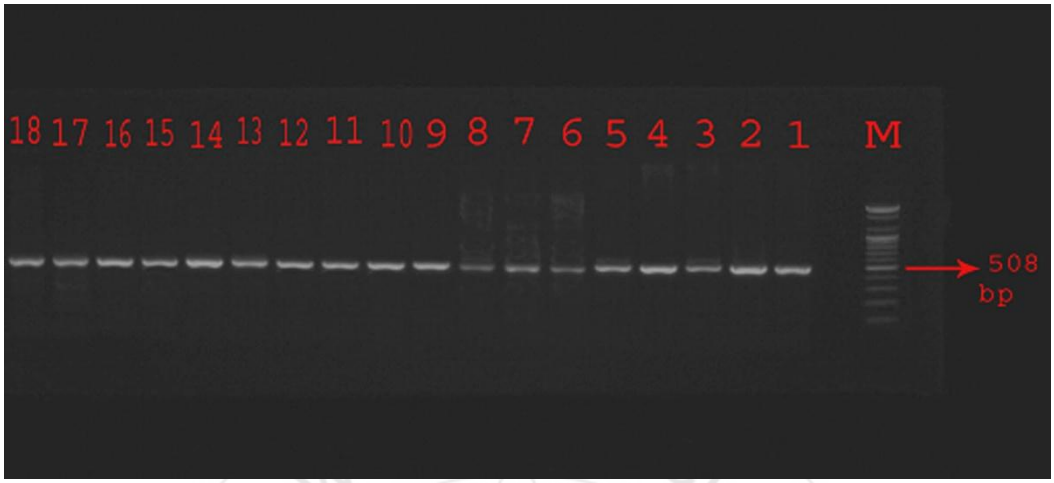

Figure 2: Agarose gel electrophoresis (1% agarose, 70 volt, for 1.5 hour) and Ethidium bromide staining to detect Fim(H) gene size product(band508bp)

Detection of *Pap (G)* alleles I II III

Atotal of 72 *E.coli* isolated from UTI children patients were detected for virulence factor encoding *Pap(G)* alleles by PCR technique.Results from polymerase chain reaction showed that the prevalence of virulent genes *PapG* I occurred in 14 isolates19.4%, Figure(3), results for *PapG* III occurred in28 *E.coli* isolates38.8%, Figure(4), while result detection for *PapG* II showed negative for this gene for all *E.coli* isolates.(21) found the prevalence of *PapG* I 6% and G II 30% in *E.coli* isolates, but in the study of(22) showed that *PapG* III detected in 27% and *PapG* II 7% of *E.coli* isolated from urinary tract infection, while (10) found that the prevalence of *PapG* II in 32.7% *E.coli* isolates from urine samples.PapG fimbrial adhesion recognize in adhering to uroepithelial cells in urinary tract and endothelial cells on receptor Gal ($\propto 1-4$) ,mediating bacterial adherence and initiate infection.

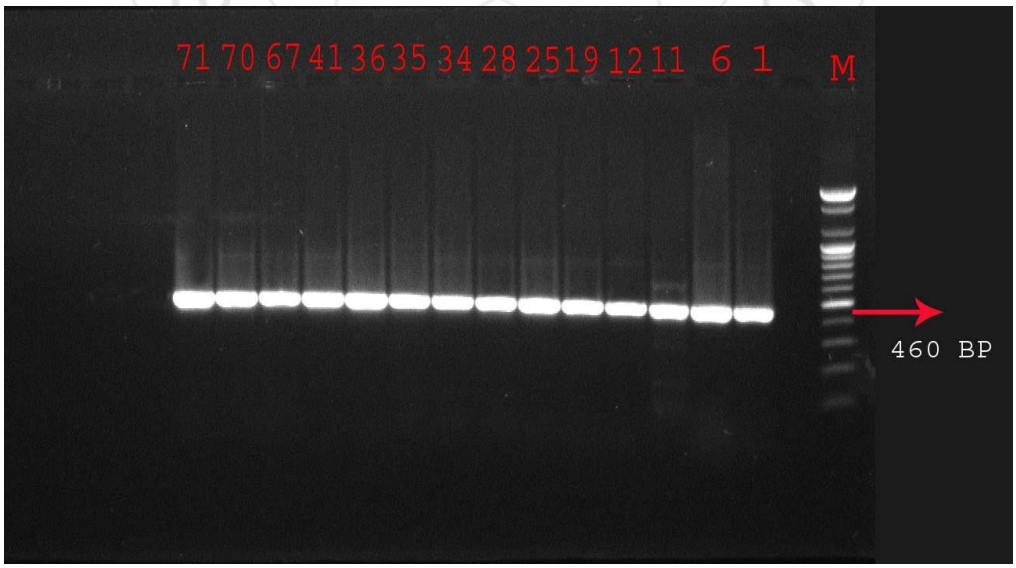

Figure 3: Agarose gel electrophoresis (1% agarose, 70 volt, for 1.5 hour) and Ethidium bromide staining to detect *PapG* I gene size product(band460bp) .

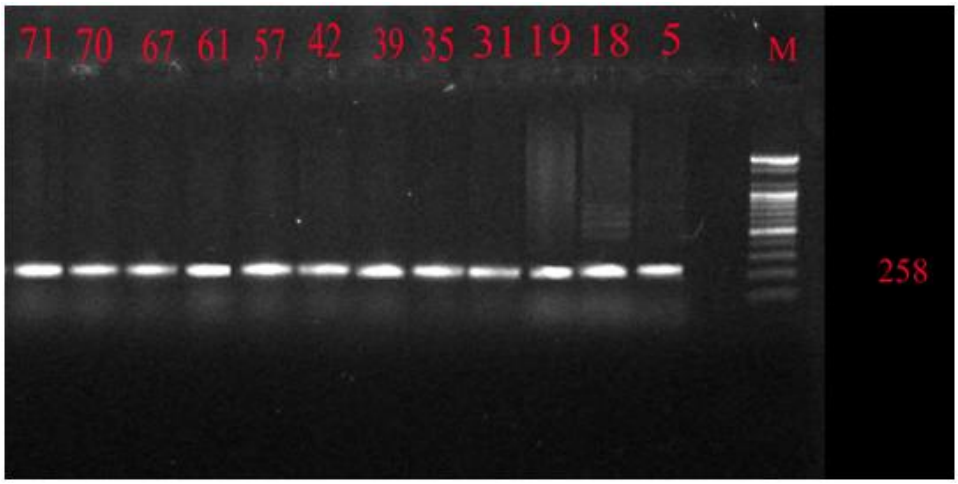

Figure 4: Agarose gel electrophoresis(1% agarose, 70 volt, for 1.5 hour) and Ethidium bromide staining to detect *PapG* III gene size product(band258bp)

P Fimbriae, the principal mannose-resistant adherence organelles of extraintestinal pathogenic *Escherichia coli*, are known to contribute to pathogensis by promoting bacterial colonization of host tissues and by stimulating an injurious host inflammatory response(23) *E.coli* causes the vast majority of UTI in both ambulatory and hospitalized patients(13).The degree of severity depends on the virulence of the responsible strains and on the susceptibity of the host, particularly if there is a concomitant urological illness (24). Genes coding for fimbrial adhesive systems represent the most common factors for the virulence of *E.coli* in UTI.The distribution of these genes in our strains is in agreement with other published data (25). Our results show a higher frequency of fimH compared with the rest of the genes, which may indicate a crucial role of the virulence genes in *E.coli* causing UTI. Regarding P fimbriae, our results were in agreement with those of many studies, indicating that among patients with acute Pyelonephritis and cystitis, posses P fimbriae(13). Moreover an important role of pap adhesion genes in the pathophysiology of pyelonephritis caused by E.coli has been reported in several studies (26).

In conclusion, our study showed, a high variability was found among UPEC isolates regarding the distribution of virulence genes and futher studies are needed to identify E.coli virulence factors responsible for UTI in children under five years and to determine the physiopathology of these infections of these infections to consider possible prevention measures and means.

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