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Prevalence of virulence factors genes of Escherichia coli isolated from pregnant and non-pregnant women with urinary tract infection in Diyala Iraq

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ABSTRACT

Uropathogenic Escherichia coli (UPEC) is an essential cause factor of complicated and uncomplicated urinary tract infections. It is in charge of approximately 80% of community gained and 50% of nosocomial infection. The current study included collection of one hundred and eighty samples among different cases (pregnant and non-pregnant women) by urinary tract infection in Al – Batoul Teaching Hospital in Baquba, during the period from November 2017to February 2018. All isolates were identification as E. coli by using Gram – staining. EMB agar and conformation in VITEK2 compact system. It was found 96 E. coli isolates; it was carried out to 38 E. coli isolates. 18 isolates from pregnant and 20 isolates from non – pregnant women. In this study , E.coli isolates were resistance against different type antibiotics , it was pipracillin 84.38% ; tetracycline 70.83% ; Rifampicin 63.54% ; Levofloxacine 59.37% ; ciprofloxacin 54.17% ; tobramycin 47.9% ; Augmentin 39.58% ; gentamicin 35.49% ; chloramphenicol 30.21% ; Imipenem 26.04% .The molecular detection of virulence factors genes shows the prevalence of genes fimH , papC , hlyA , sfaS , iutA , ibeA were 100% , 94.44% , 55.5% , 72.2% , 66.66% , 22.22% respectively in pregnant case according to the mentioned tract infection. The prevalence of gene fimH , papC , hlyA , sfaS , iutA , ibeA were 85% , 60% , 25% , 45% , 45% , 5% respectively in non-pregnant women with urinary tract infection . All the studied strains showed 16 of virulence gene patterns, among isolates from pregnant 12 patterns, on the other hand the isolates from non-pregnant showed diversity of genes patterns.

Keywords: Escherichia coli, urinary tract infection, virulence factors genes.

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INTRODUCTION

Urinary tract infection (UTIS) is inflammatory disturbance which occurred due to abnormal proliferation of pathogens in the urinary system, while causing changes in the normal functioning of the Kidneys and urinary tract and it's acts as one of the extreme popular diseases faced in medical practice lately (Tambekar *etal* , 2006) . UPEC is an essential case factor of complicated and uncomplicated types of UTIS. It responsible for an approximately (80%) of community acquired and (50%) of nosocomial acquired UTIS, it is isolated from the urine of about 30% of patients experiencing urinary tract infection (Macleod and sticker, 2007). Several hours after birth, UPEC settles on the human intestine which consider as one reason of normal microbiota. However, it can cause various diseases such as diarrheal, UTI, and meningitis (Kaper *etal*, 2004). Antibiotic resistance of pathogens in the management of complicated and uncomplicated community acquired UTIS is a serious medical problem. However widespread use of antibiotics utilization has drove to the emergence of resistant bacteria (lartigue *etal*, 2007). Thus, Uropathogenic strains are confirmed to show a virulence variance especially for E. coli determinants have been related to the development of UTI. Among these factors: siderophores, toxins, capsules, fimbriae and other have been described (Ruiz *etal*, 2002). E. coli settlement during various anatomical sites is partially due to genome plasticity and remodelling by genetic material earning or losing process according to gained resistance or virulence factors. Thus, adaption and evolution have a horizontal transfer which consider as an essential element of E. coli to various niches (Mellata *etal*, 2010). Bacteria and epithelial cells is a multifactorial and complicated phenomenon which enfolds several adhesins production due to the infection level, in the other hand, adherence to epithelial cells is considered to be prim reason for colonization and establishment succession; gene can be performed by encoding each of toxins siderophores, LPS, and invasions specifies the severe level of disease as well as the strains virulence (Hilbert *etal*, 2008). Iron is 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 (Reisner *etal*, 2006). Acute infections and recurrent infection can be

caused by UPEC strains which are not react o popular antimicrobial treatment. UTI treatment generally includes β-lactam antibiotics, quinolones, trimethoprim/ sulfamethoxazole (Molina *etal*, 2011). Bacterial genome changing due to mutation or even acquisition led to resistance increment (Moura etal,2009 ; Hong *etal* , 2009) .Lately studies which attempt to characterize *E.coli* profile from pregnant and non-pregnant women, with described and applied tract infection by virulence gene (*fim*H , *pap*C , *hly*A , *sfa*S , *iut*A , *ibe*A) and their resistance to antibiotics to treatment of UTI.

MATERIAL AND METHOD

Collection of samples

During the period from November 2017 to February 2018 , a total of (180) clinical urine specimens were collected for suffering patients from urinary tract infection with (15-45) year and both pregnant and non-pregnant women admitted to Al- Batoul Teaching Hospital in Baquba; 97 samples from pregnant women and 83 samples from non-pregnant women .

Identification of *E.coli* isolates

The specimens received were inoculated on MacConkey, Eosin Methglene Blue and blood agar plates. Then all plates were incubated a 37ºC for 24 hrs. The isolates were show characteristic growth colour and identified as *E.coli* by using Gram- staining , conformation in VITEK2 compact system (Bio merieux / USA) .

Antimicrobial susceptibility Test

Ninety six of *E.coli* isolates and according to its resistance were tested against the following (10) antibiotic , Augmentin piperacillin , Imipenem , Tobramicin , Gentamicin, Levoflocacin, ciprofloxacin, Tetracyline, Rifampicin, chloramphenicol using VITEK2 compact system (Vitek2 Kit sensitive , Biomerieux / USA) .

DNA Extraction

The DNA of thirty eight *E. coli* isolates that high resistant was extracted according to the instruction of the promega kit Nanodrop spectrophotometer was used for measured the DNA concentration and purity. The extracted DNA was electrophoresed by gel electrophoresis system.

Preparation the primers

Primers were prepared according to the instruction of manufactured company. The primers selected in this study shown in Table 1.

Table 1: sequence of primers of virulence genes

Gene	Primers sequence 5 → 3	Ann. Temp.	Product Size (bp)	Reference
fimH	F- TGC AGAA CGG ATA AGCCG TGG R-GCAGTCACCTGCCCTCC GGTA	63°C	508	Abdul-Ghaffar, and Abu-Rish ,2017
papC	F-GTGGCAGTAGAGTAATGA CCGTTA R-ATATCCTTTCTGCAGGGA TGCAATA		200	
hlyA	F-AACAAGGATAAGCACTGT TCTGGCT R-ACCATATAAGCGG TCATTCCCGTCA		1177	
sfaS	F- GTGGATACGACGATTACTGTG R- CCG CCA GCATTCCCTGTATTC	55°C	646	Lopez Banda *etal* , 2014
iutA	F-GGCTGGACAT CATGGGAACTGG R-CGTCGGGAACGGGTA GAATCG		300	
ibeA	F- AGCCAGGTGTGCGCCGCGTAC R-TGGCATACCAACC AATGCGAG		900	

PCR amplification

PCR assay was performed in a monoplex. It were carried out 38 *E.coli* isolates, 18 isolates were from pregnant and 20 isolates were from non-pregnant women amplify different virulence factors genes. *fimH, papC , hlyA , sfaS , iutA , ibeA.* PCR mixture was set up in total volume of 25 µl included

125µl of PCR green master mix , 1 µl of each primer , and 2 µl of template DNA have been used , the rest volume was completed to 25 µl with sterile nuclease-free water . The PCR thermo cycler program is described in Table2.

Table 2: PCR thermo cycler program for DNA amplification of *E.coli* genes.

Stage	Temperature	Time	Number of cycles
Initial denaturation	95°c	5min	1
Denaturation	95°c	30s	
Annealing	X°c	30s	30
Extension	72°c	1min	
Final extension	72°c	7min	1

X: annealing temperature for each primer of virulence gene as follow : *fimH , papC , hlyA , sfaS* , 63°C , while *iutA* , *ibeA* were 55°c respectively .

RESULTS AND DISCUSSION

Isolation and identification of *E.coli* isolates

After performance the identification tests , it was found that in total of 96 *E.coli* isolates , 54 isolates from pregnant and 42 isolates from non-pregnant women , 38 (39.58%) *E.coli* isolates, 18 (33.33%) isolates were from pregnant patients women and 20 (47.6%) isolates were from non-pregnant patients women of urine specimens in Figure 1 .

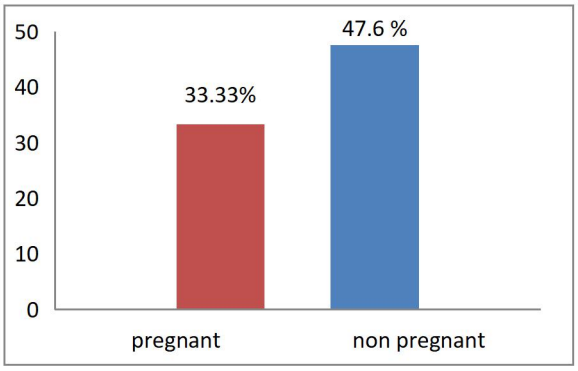


Figure 1: percentage of pregnant and non-pregnant associated *E.coli.*

Antibiotic susceptibility tests

From the results of the present study , various levels susceptibilities of different antibiotics among (96) isolates were observed, Table 2 .These results in recent study showed that *E. coli* isolates were resistant to pipracillin 84.38% , tetracycline 70.83% , Rifampicin 63.54% , Levofloxacin 59.37% , ciprofloxacin 54.17% , tobramycin 47.9% , Augmentin 39.58% , gentamicin 35.42% , chloramphenicol 30.21% , Imipenem 26.04% .

Table 2: Resistant of *E. coli* isolates to different antibiotic

Antibiotic name	*Resistant*	*Intermediate*	*Sensitive*
piperacillin	*84.38%*	*3.12%*	*12.5%*
Tetracyilne	*70.83%*	*3.13%*	*26.04%*
Rifampicin	*63.54%*	*0%*	*36.46%*
Levofloxacin	*59.37%*	*0%*	*40.63%*
ciprofloxacin	*54.17%*	*0%*	*45.83%*
Tobramycin	*47.9%*	*0%*	*52.1%*
Augmentin	*39.58%*	*0%*	*60.42%*
Gentamicin	*35.42%*	*2.08%*	*62.5%*
Chloramphenicol	*30.21%*	*0%*	*69.795*
Imipenem	*26.04%*	*2.08%*	*71.88%*

These results were closely to the results of (Al – Ezee *etal* , 2019) , they found the percentage of *E.coli* isolates resistance to Augmentin 40% tetracycline 73.3% ; Rifampicin 66.7% , Chloramphenicol 33.3% imipenem 66.7% , gentamicin 40% and ciprofloxacin 60% . However, the study results are agreed with agreed with the results of (Lopez – Banda *etal*, 2014) who found the percentage of *E.coli* resistance of Augmentin 31.3%, Imipenem 1.9%, pipracillin 51.6%, gentamicin 27.8%, tabromycin 43.5%, Levofloxacin 60%, and ciprofloxacin 62.5%. Study results showed resistance that might has a relation to bacteria genemoe changes due to mutation or even acquisition (Moura *etal* , 2009 ; Hong *etal* , 2009) .

Genetic study of *E. coli* isolates

DNA extraction

The DNA was extracted from *E.coli* isolates it were carried to 38 *E.coli* isolates, 18 isolates from pregnant and 20 isolates from non-pregnant women , *E.coli* DNA was with good quantitative and qualitative states that showed one band of DNA when analysis by the electrophoresis methods.

Detection of virulence factors genes

The infection caused by virulence factors of microorganism provides a clinician permit to anticipate the evolution of host infection. All of the genes were detected in different percentages; Results are presented in Figure 2.

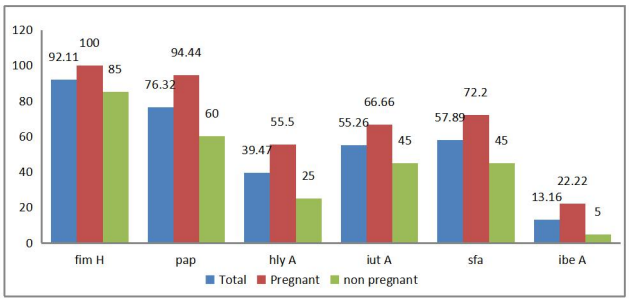


Figure 2: prevalence of virulence factors genes in E. coli isolates

Detection of *fimH* gene in *E. coli* isolates

From thirty eight *E. coli* isolates ; the monoplex PCR assay for *fimH* gene revealed high presence of *fimH* gene 35 (92.11%) in a total, 18 (100%) in pregnant patients women and 17 (85) in non-pregnant patients women,Figure3.

This results are agreed with the results of Al – Mayahie , 2013 who found 100% of *fimH* virulence gene was recorded in pregnant and non-pregnant women . wang *etal* , 2002 , studied the pathogenic role of host and E. coli virulence factors in the development of E. coli bacteremia in patients with upper urinary tract infection . There was a high prevalence (92%) of the genetic determinant of *fim*A gene . A similar results 86.1% of *fimH* gene was recorded by (Lopez – Banda *etal* , 2014) , 162 uropathogenic *E.coli* (UPEC) strains from patients with cystitis were genotypically characterized by PCR assay results identified 158 *fimH* (97.5%)(Tiba *etal* , 2008) .

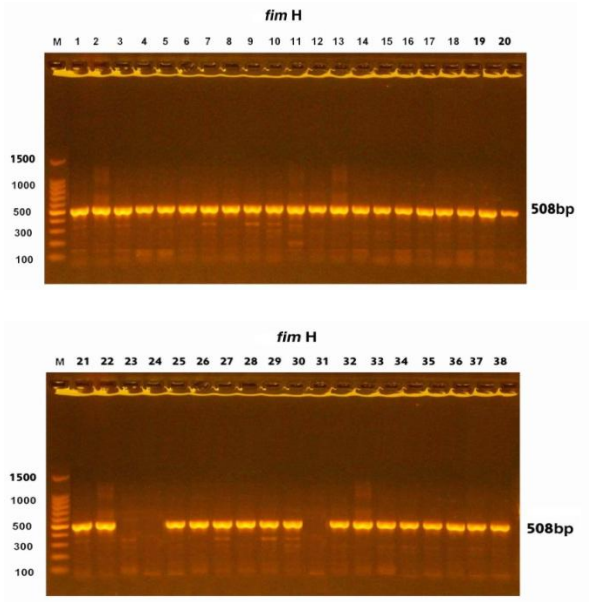


Figure 3 : Electrophoresis of amplified PCR product for the detection of *fim*H gene (508bp) lanes 1-18 represent pregnant E. coli isolates and Lanes 19-38 represent non pregnant E. coli isolates respectively. Run on 1% agarose (90 min at 100 volt) stained with ethidium bromide.M:marker DNA ladder (100pb)

Detection of *papC* gene in *E. coli* isolates

The adhesin *pap*C fimbriae gene were showed a percentage 29 (76.32%) in a total; 17 (94.44%) in pregnant patients women and 12 (60%) in non-pregnant patients women, Figure 4.

This results closely with (Lopez – Banda *etal* , 2014) that found the prevalence of *pap*C gene in his study was 62% in E. coli isolates from Mexican women. Al-Mayahie , 2013, who found 100% of *pap*C virulence gene was recorded in pregnant and non-pregnant women. Johnson *etal* ,2000 , studied bacterial adhesins in patients with *E.coli* urosepsis and *pap*C adhesin was (82%). In a study done by Birosova *etal* ,2014 , in total 201 E. coli isolates from various clinical materials (urine , vaginal and rectal swabs) were examined by PCR for the presence of *pap* gene was 89% .

Detection of *hlyA* gene in *E. coli* isolates

The PCR assay results identified haemolysin producing gene *hly*A , this study showed 15 (39.47%) in a total , 10 (55.5%) in pregnant patients women and 5 (25%) in non-pregnant patients , women , Figure 5 .

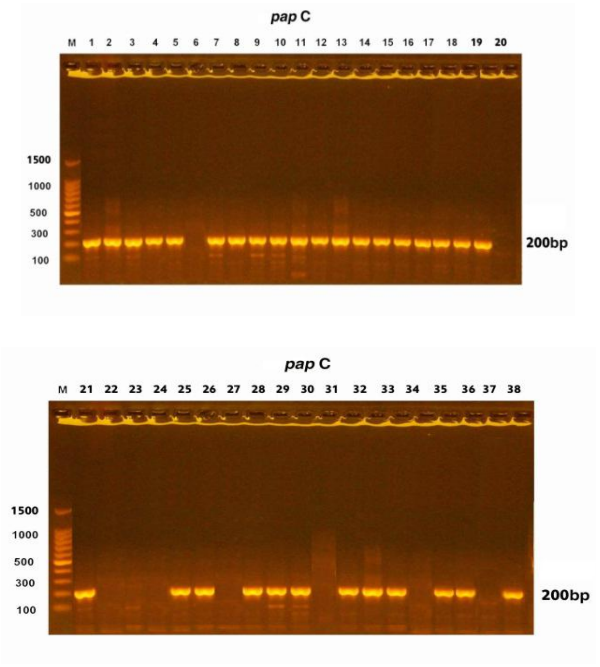
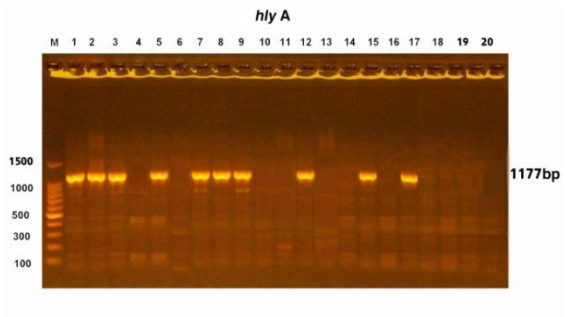


Figure 4: Electrophoresis of amplified PCR product for the detection of *pap*C gene (200 bp) . Lans 1 – 18 represent pregnant E. coli isolates and 19-38 represent non pregnant E. coli isolates respectively. Run on 1% agarose (90min at 100 volt) stained with ethidium bromide. M: marker DNA ladder (100bp) .



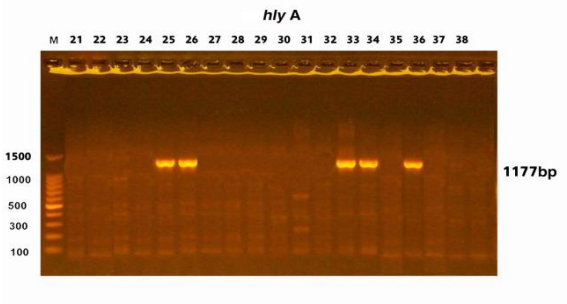
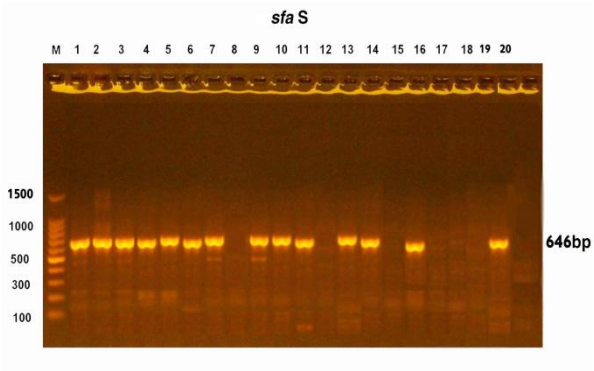


Figure 5: Electrophoresis of amplified PCR product for the detection of *hly*A gene (1177bp) lanes 1-18 represent pregnant *E.coli* isolates and represent non pregnant *E.coli* isolates respectively . run on 1% agarose (90 min at 100 volt) stained with ethidium bromide M : marker DNA ladder (100bp) .

In study done by Karimian *etal* ,2012 in Iran was found in a total of 123 strains of *E.coli* isolated from patients with urinary tract infection were tested in PCR for detection of E. coli virulence factors ; *hly*A gene presence was 50.4% in isolates, while similarly isolates recoverd from 75 patients , with E. coli bacteraemia caused upper UTI was 45% (wang *etal* , 2002). In contrast a high haemolysin percentage was recorded (25%) UPEC from patients (Tiba *etal* , 2008) Escherichia coli haemolysin (*hly*A) is define as a pore - forming exotoxin which might participate to bacterial virulence during bloodstream infection and sepsis (Koga *etal* , 2014).

Detection of *sfaS* gene in *E. coli* isolates

The frequency of *sfa*S gene in E. coli isolates, it was 22 (57.89%) in a total, 13 (72.2%) in pregnant patients women and 9 (45%) in non-pregnant women, Figure 6.



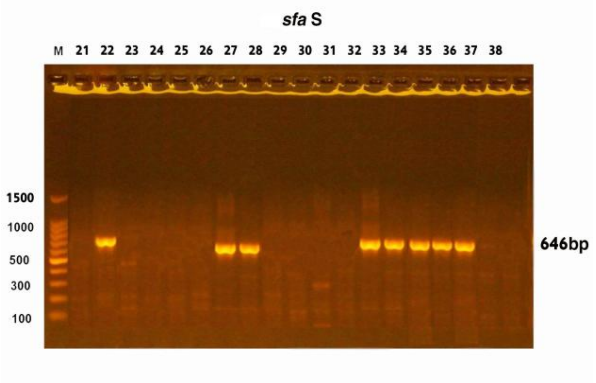


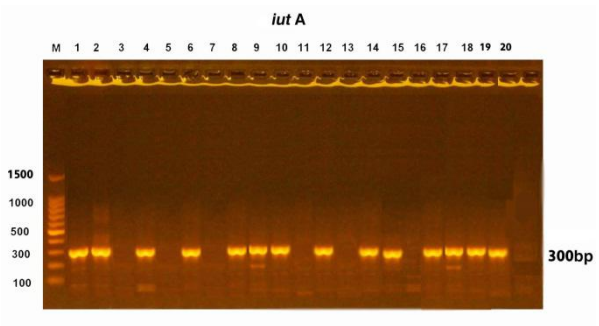
Figure 6: Electrophoresis of amplified PCR product for the detection of *sfa*S gene (646 bp) Lanes 1-18 represent pregnant E. coli isolates and 19 – 38 represent non pregnant *E.coli* isolates respectively , run on 1% agarose (90 min at 100 volt) stained with ethidium bromide . M : marker DNA ladder (100bp) .

This results a greedment with (Al-Mayahic ,2013) who found the prevalence of *sfa*S gene was 60% in pregnant women's isolates and 50 % in non-pregnant women's isolates . In study done by Lopez – Banda *etal* (2014) in Mexico women clinically diagnosed with urinary tract infection .

Detection of *iutA* in *E.coli* isolates

In this study the detection of *iut*A gene coding for aerobatic siderophores revealed 21 (55.26%) in a total 12 (66.66%) in pregnant patients women and 9 (45%) in non-pregnant patients women , Figure 7 .

Adwan *etal*, 2015, confirms the prevalence 74% of *iut*A gene in fifty clinical E. coli isolates were previously recoverd from urine specimens obtained from patients suffered from urinary tract infection in Tulkarm – Palestine in contrast the study In total, 201 E. coli isolates from various clinical materials were examined by PCR for the presence of virulence factors; The aerobatic in genes were found less frequently (59%) in a total and 53% of urine samples. (Firoozah *etal*, 2014), and previous study showed that E. coli strains isolated from children with UTI 39.75% was positive for presence of *iut*A(Karimian *etal* 2012).



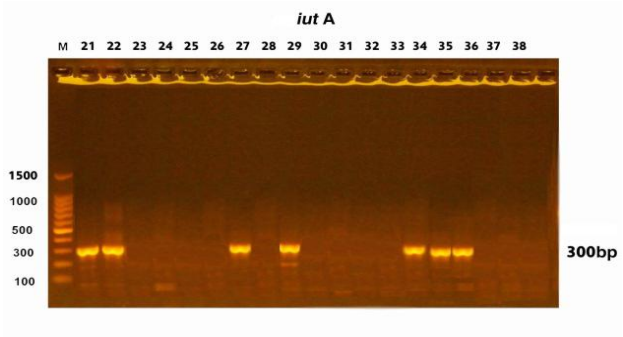


Figure 6 : Electrophoresis of amplified PCR product for the detection of *iut*A gene (300bp) Lanes 1-18 represent pregnant E. coli isolates and 19 – 38 represent non pregnant E. coli isolates respectively , run on 1% agarose (90 min at 100 volt) stained with ethidium bromide . M : marker DNA aldder (100bp) .

Detection of *ibeA* gene in *E. coli* isolates

In this study the detection of *ibe*A gene in E. coli isolates 5 (13.16%) in a total, 4 (22.22%) in pregnant patients women and 1 (5%) in non-pregnant patients women , Figure8.

This results agreed with (Lopez – Banda *etal* ,2014) , who found 2.8% of *ibe*A related to studied Mexican women cases and are clinically diagnosed according to urinary tract infection .

Virulence gene patterns

Based on the distribution of the various targeted sequence all the studied strains exhibited 16 virulence gene patterns and showed in Table 4.

Three of virulence gene patterns specified as P2, P3, and P10 were recognized by five different gene presence (5 strains). The patterns which included strains presenting three virulence genes (P7, P9, P11, P12, and P15) were the best represented (14 strains). The association of four genes was recognized in (P4 , P5 , P6 , P8) patterns (10 strains) , the P13 , P14 patterns were represented by strains possessing a two genes association (4 strains) , followed by the P1 patterns , which encompassed the six genes positive strains (2 strains) and the P16 patterns , which encompassed the six genes negative strains (3 strains), the association of presents of the virulence factor patterns in relationship with the different source of the isolates , the strains isolates from pregnant patients women exhibited 12 patterns , P4 , P6 patterns were the most prevalence with 3 isolates for both patterns , followed by P2 , P5 , patterns (2 isolates) for both patterns . Among the strains isolated from non-pregnant patients women show diversity of genes patterns. In comparison to this study , (Usein *etal* , 2001) recorded in a total of 78 E. coli strains isolated from different types of urinary tract infections all the studied strains exhibited 21 virulence gene patterns . Similar results were documented by Firoozeh *etal*, 2014, nineteen different virulence patterns were found among the UPEC strains regarding the frequency of virulence determinants.

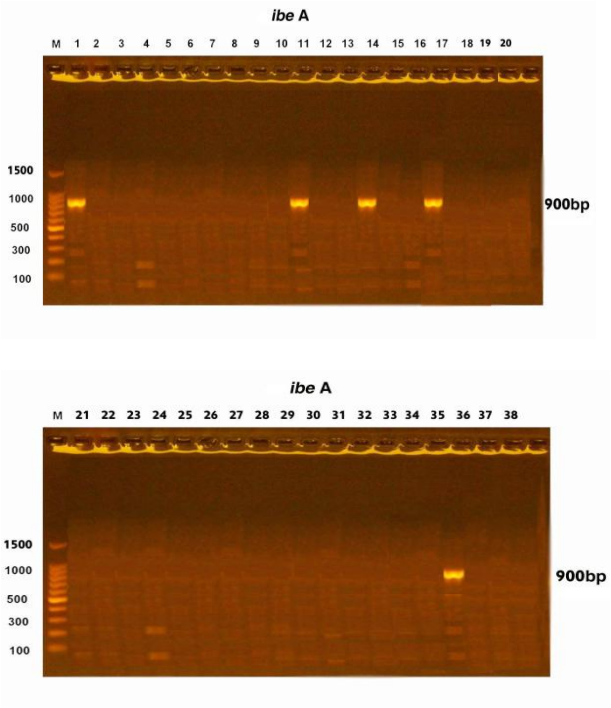


Figure 8: Electrophoresis of amplified PCR produced for the detection *ibe*A gene (900 bp) lanes 1-18 represent pregnant E. coli isolates and 19-38 represent non pregnant E. coli isolates respectively , run on 1% agarose (90min at 100 volt) stained with ethidium bromide . M: marker DNA ladder (100bp).

Table 4: Virulence pattern identified a mong *E. coli* isolates

patterns	Virulence genes	No. isolates

	*fim*H	*pap*C	*hly*A	*sfa*S	*iut*A	*ibe*A	Total	pregnant	Non pregnant
P1	+	+	+	+	+	+	2	1	1
P2	+	+	+	+	+	-	3	2	1
P3	+	+	+	-	+	+	1	1	0
P4	+	+	+	+	-	-	4	3	1
P5	+	+	-	+	+	-	2	2	0
P6	+	+	+	-	+	-	3	3	0
P7	+	-	-	+	+	-	5	1	4
P8	+	+	-	+	-	+	1	1	0
P9	+	+	-	-	+	-	2	1	1
P10	+	+	-	+	+	+	1	1	0
P11	+	+	-	+	-	-	1	1	0
P12	+	+	-	-	+	-	4	1	3
P13	+	+	-	-	-	-	3	0	3
P14	+	-	-	+	-	-	1	0	1
P15	+	+	+	-	-	-	2	0	2
P16	-	-	-	-	-	-	3	0	3

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