GENOTYPIC DETECTION OF THE VIRULENCE FACTORS OF UROPATHOGENIC *ESCHERICHIA COLI* (UPEC) STRAINS ISOLATED FROM PREGNANT FEMALES AND THEIR CORRELATION WITH ANTIBIOTIC RESISTANCE PATTERN

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ABSTRACT

Urinary Tract Infections (UTIs) during pregnancy are among the most common infections worldwide and can lead to poor perinatal and maternal outcomes, especially in developing countries and requires medical treatment as soon as it is detected. Virulent and resistant strains of *Escherichia coli* are the most important causative agents for UTIs. The present investigation aimed to detect virulence factors of Uropathogenic *Escherichia coli* (UPEC), including *pap, fim, sfa, aer* and *hly* genes in isolates collected from symptomatic and asymptomatic pregnant females by multiplex polymerase chain reaction (PCR) assay and determine their correlation with antibiotic resistance patterns. Two hundred urine samples collected from pregnant females with or without symptoms of UTI were admitted to antenatal clinic at Obstetrics and Gynecology Department of Al- Zahraa University Hospital in Cairo. Out of 200 cultured urine samples, the cultures that showed $\geq 10^5$ CFU/ml were identified to have significant growth. Found significant bacteriuria was detected in 61 samples and these samples selected for further analysis in this study, the overall prevalence of UTI among pregnant females was (30.5%). Symptomatic pregnant females with UTI were (48.5%) more than asymptomatic (11.3%). The most frequently isolated species was *S. saprophyticus* (35.0%), followed by *E. coli* (26.2%), *S .aureus* (19.4%), *C .albicans* (5.8%), *S. epidermidis* (2.9%) and each of *K. pneumonia, K .oxytoca, P .vulgaris, E.fecalis, Bacillus spp* were (1.9%), while *P. auroginosa* had the least percentage of isolation (1.0%).

Antimicrobial susceptibility pattern was done for 27 UPEC isolates showed the highest level of resistance (100%) against β-lactams as (Ampicillin, Penicillin and Ceftriaxone), nitrofurantoin, and ampicillin/sulbactam, while the lowest level of resistance (66.7%) against Cotrimoxazole. Multiple drug resistance (resistance to two or more drugs) was observed in (100%) of the UPEC isolates. The higher level of resistance to antibiotics was observed in symptomatic bacteriuria more than asymptomatic. Among two predominant uropathogens, *E. coli* showed complete resistance up to 5 different antibiotics of total 11 antibiotics (45.5%) while *S. saprophyticus* showed complete resistance (18.2%), so *E. coli* showed higher virulence and resistance than *S. saprophyticus* to cause UTI, so subjected to Multiplex PCR to detect its virulence factors, which included *pap, fim, sfa, aer* and *hly* genes The adhesive *fimH* gene was the most frequent in UPEC isolates (92.6%) . These virulence genes detected from total UPEC isolates of symptomatic cases were higher than asymptomatic. On the other hand, UPEC strains carrying the virulence genes were more resistant to the antibiotics used, so these observations confirm the important role of virulence genes of UPEC in in existence of symptoms of UTI and the drug resistance.

Keywords: Uropathogenic *Escherichia coli*, Virulence factor, *fimH*, pregnant, multiplex PCR, UTI.

Introduction

Urinary tract infection (UTI), which is caused by the presence and growth of microorganisms in the urinary tract is considered as one of the commonest health problems affecting women due to shorter urethra, pathogens entry facilitated by sexual intercourse and close proximity of the anus with vagina **(Oladeinde *et al.*, 2015 and Akpan *et al.*, 2017).**Pregnant women are more susceptible to UTIs due to a combination of hormonal and physiologic changes that predispose them to bacteriuria. Firstly, the weight of the gravid uterus on the renal system often leads to the accumulation of fluid in the ureter, known as hydroureter. Also, there is decrease in the bladder tone which may lead to the accumulation of urine up to twice the normal urinary volume without discomfort **(Aseel et al.,2011).** Gestational glycosuria, proteinuria and elevated levels of progesterone can decrease the muscle tone of the ureter and bladder. It can result in vesico-ureteric reflux. These provide enriched culture media for bacteria pathogens that may invade the urinary system **(Chaliha and Stanton, 2002 and Mazor *et al.*,2009).**

Pregnancy UTI is classified into two categories of symptomatic and asymptomatic. Symptomatic bacteriuria is divided in to lower tract (acute cystitis) or upper tract (acute pyelonephritis) infections. Cystitis is defined as significant bacteriuria with associated bladder mucosal invasion, whereas pyelonephritis is defined as significant bacteriuria with associated inflammation of the renal parenchyma, calices and pelvis. **(Emamghorashi *et al.*, 2012).**

Asymptomatic bacteriuria (ASB) is the most common cause of UTI during pregnancy, it refers to the presence of significant quantity of uropathogenic bacteria in a properly collected urine sample from an individual without signs or symptoms of UTIs **(Alemu *et al.*,2012).** Untreated ASB could lead to adverse obstetric and maternal outcomes like prematurity, low-birth weight, abnormalities in babies, and higher fetal mortality rates in several documented researches **(Ade-Ojo *et al.*, 2013).** The prevalence of ASB or UTI is influenced by several factors like socio-economic status of patients, increased maternal age, high parity, poor perineal hygiene, sexual activity, anatomical abnormalities of the urinary tract, previous history of catheterization, use of contraceptive, history of recurrent UTIs and diabetes mellitus **(Ezechi *et al.*,2013 and Akpan *et al.*, 2017)**.

UTI is mostly caused by a wide range of Gram-negative aerobic pathogens found in gastrointestinal tract of mammals like *Escherichia coli, Klebsilla pneumoniae*, *Pseudomonas aeruginosa, Proteus mirabilis and Enterobacter*. Other pathogens that cause UTI include *Enterococcus species, Serratia species, staphylococcus epidermidis* and *Staphylococcus saprophyticus* **(Beyene and Tsegaye ,2011).** Uropathogenic *E. coli* (UPEC) is the most common cause of UTIs both in community and hospital settings with significant morbidity and mortality worldwide**.** Previous investigations have shown that UPEC strains encode widespread virulence factors closely related to colonization, persistence and pathogenesis of bacteria in the urinary tract. The most important of these factors include adhesins or fimbriae, biofilm formations and toxins such as hemolysin **(Bien *et al.*, 2012).**

UTI has become more complicated and difficult to treat because of appearance of mutant uropathogens that are resistant to the commonly used antimicrobial drugs. As in many hospitals in developing countries, routine urine culture is not carried out even for antenatal mothers. Currently, most patients are treated empirically without culture and antimicrobial susceptibility testing (AST) and treatment is therefore based on empiric guidelines that are rarely updated **(Beyene and Tsegaye,2011).**

The successful management of patients suffering from UTI in pregnancy depends upon proper screening for bacteriuria regardless of its symptoms. Symptomatic bacteriuria can easily be diagnosed and treated because of its overt symptoms, but asymptomatic bacteriuria can be easily missed. As it is very common in pregnant women, early detection and identification of the organisms and the selection of an effective antibiotic against the organism keeping in mind the stage of pregnancy; will decrease the risk of complications in pregnancy **(Matuszkiewicz *et al*., 2015).** Therefore, this current survey was performed in order to determine the virulence factors of UPEC strains isolated from pregnant women with or without clinical signs and symptoms of (UTIs) and their correlation with antimicrobial resistance pattern and to find out the important associations between socio-demographic characteristics, obstetric history, medical complications and practices of personal hygiene with UTI among pregnant women.

Materials and Methods:

Samples and Escherichia coli identification

Twenty-seven *E. coli* isolates with significant count ($\geq 10^5$CFU/ml) were obtained from 200 urine samples collected from pregnant women with or without symptoms of UTI admitted to antenatal clinic at Obstetrics and Gynecology Department of Al- Zahraa University Hospital in Cairo during the period from March 2016 to May 2019. The pregnant women who have taken any antibiotics for UTI and other infections for the last two weeks were excluded from this study. Urine samples were cultured on Cysteine Lactose Electrolyte Deficient agar, Blood agar, MacConkey agar and Eosin Methylene Blue agar using a standard calibrated 4mm wire loop (0.01ml). Streaked culture plates were incubated at 37^0 C overnight. Identification of all isolates was done on the basis of Gram staining and routine biochemical tests including Indole as a key test, then Urease , Methyl red, Voges-Proskauer, Hydrogen Sulfide, Citrate Utilization ,Triple Sugar Iron agar (TSI), Gelatin hydrolysis, Motility , Oxidase test and hemolysis on blood agar (according to CLSI, 2017 guidelines) were used for identification of bacteria **(Bahalo *et al*., 2013).**

Pregnant women profile

Included, socio-demographic data, medical complications and obstetric history, in addition to, personal hygiene.

Antimicrobial Susceptibility patterns

The antibiotic susceptibility patterns were determined using Kirby-Bauer disc diffusion method **(Oxoid, Ltd, England)** recommended by the National Committee for Clinical Laboratory Standards (CLSI 2017) as documented. The following 12 different antimicrobials were tested: Ampicillin (10µg), Amoxicillin- clavulanic acid (30µg), Penicillin (10µg), Ceftriaxone (30µg), Cefuroxime (30µg), Nitrofurantoin (300µg), Streptomycin (10µg), Gentamicin (10µg), Oxacillin (1µg), Co-trimoxazole

(Trimethoprim/Sulfamethoxazole) (1.25/23.75µg) and Azithromycin (15µg) Ampicillin / Sulbactam (10/10 µg). Results were interpreted as susceptible or intermediate or resistant according to criteria recommended by the CLSI.

DNA-extraction

UPEC isolates were grown on nutrient agar for an overnight then used for DNA extraction by Boiling-centrifugation method as described by **(Soumet et al., 1994 and Yamamoto *et al.*, 1995),** then 2 or 3 pure colonies were inoculated on Mueller- Hinton broths and incubated for 24 hrs at 37^0C,then one ml aliquots of enrichment were centrifuged at 13,000 x*g* for 3 minutes. The pellets were resuspended in 100 µl of sterile distilled water, heated to 95ºC in a dry block for 10 min, cooled in ice and centrifuged at 13,000 xg for 3 min. These suspensions were used for PCR assay.

Polymerase chain reaction

Specific primers were used in this study to amplify the specific genes of *E. coli* were synthesized by Invitrogen under ThermoFisher Scientific corporation headquartered in California which were fimbrial adhesin type1*(fimH),* pyelonephritis associated pili *(pap),* S fimbriae *(sfa),* hemolysin *(hly)* and aerobactin (*aer)*genes as shown in Table (1). The multiplex PCR assay was carried out in a total volume of 25 µl of mixture containing 2 µl Maxime PCR Premix (GeneDireX®) containing (PCR buffer, Taq DNA polymerase, dNTP gel loading dyes and fluorescence dye), 0.5 µl of each of the virulence gene-specific primers, forward and reverse primers for each gene (a total of 5 µl for the 5 target genes , 2 µl of template DNA and 16 µl of deionized water) **(Hassan *et al.*, 2018).** The amplification conditions were done using thermocycler included three steps: heating at 94°C for 3 min; 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 30 s, and extension at 72°C for 30 s; and the final extension at 72°C for 7 min **(Jalali *et al.*, 2015).** Amplification was done using thermocycler (TECHNE, Model FTC51H2D, UK).

Table (1): Primers used for detection of virulence genes of UPEC strains.

Identified gene	***Primer***	***Primer sequence (5'-3')***	***Product size(bp)***
papE/F	*pap3*	F: GCAACAGCAACGCTGGTTGCATCAT	336
	pap4	R: AGAGAGAGCCACTCTTATACGGACA	
fimH	*fim1*	F: GAGAAGAGGTTTGATTTAACTTATTG	508
	fim2	R: AGAGCCGCTGTAGAACTGAGG	
sfaD/E	*sfa1*	F: CTCCGGAGAACTGGGTGCATCTTAC	410
	sfa2	R: CGGAGGAGTAATTACAAACCTGGCA	
Aer	*aer1*	F: TACCGGATTGTCATATGCAGACCGT	602
	aer2	R: AATATCTTCCTCCAGTCCGGAGAAG	
hlyA	*hly1*	F: AACAAGGATAAGCACTGTTCTGGCT	1177
	hly2	R: ACCATATAAGCGGTCATTCCCGTCA	

F: Forward **R**: Reverse

Visualization of the PCR products

All PCR products were analyzed by electrophoresis (120 V/208 mA) and visualized on 1.5% agarose gel in Tris- Acetate- EDTA Buffer (TAE Buffer) ,100-3000 bp DNA ladder H3 Ready-to-Use (GeneDireX®) was used as size standard to determine product size **(Jalali *et al*., 2015 and Hassan *et al.*, 2018).**

Quality Control

All Culture media were tested for sterility and performance as directed by the instructions provided by **(Chessbrough, 2017)**.

Statistical analysis

Statistical analysis was done using IBM SPSS® Statistics version 22 (IBM® Corp., Armonk, NY, USA). Numerical data were expressed as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. Pearson's Chi-square test or Fisher's exact test was used to examine the relation between qualitative variables. Comparison between two groups for normally distributed quantitative data was done using Student's t-test while for not normally distributed quantitative data, comparison was done using Mann-Whitney test (non-parametric t-test). All tests were two-tailed. A p-value < 0.05 was considered significant.

Results

1. Prevalence of UTI in relationship with profile of pregnant females

A total of 200 urine samples were collected from pregnant females attending antenatal clinic at Obstetrics and Gynecology Department of Al- Zahraa University Hospital in Cairo. Out of 200 cultured urine specimens, the cultures that showed $\geq 10^4$ CFU/ml were identified to have significant growth, significant bacteriuria was detected in 61 samples, and these samples were selected for further analysis in this study. The overall prevalence of UTI among pregnant females was (30.5%). Of the 103 significant etiologic agents isolated from urine cultures of pregnant females, *S.saprophyticus* had the highest percentage of isolation (35.0%), followed by *E. coli* (26.2%), *S.aureus* (19.4%), *C.albicans* (5.8%), *S. epidermidis* (2.9%) and each of *K. pneumonia, K.oxytoca, P.vulgaris, E.fecalis, Bacillus spp* were (1.9%), while *P.auroginosa* had the least percentage of isolation (1.0%).

Among pregnant female profile, as observed in Table (2), the demographic characteristics showed that the highest prevalence of UTI was observed in the age group of 14 to 20 years (37.5%), while the age group of 21 to 30 years showed the highest prevalence of *E. coli* infection (57.6%). The prevalence of bacteriuria was increased in illiterate (42.1%) and pregnant with lower economic level (32.9%), also the higher prevalence of *E. coli* infection was found among illiterate pregnant women by ratio (50.0%) and those with lower economic level by ratio (45.7%). The obstetric history and medical complication data showed that the most of the patients with bacteriuria were in the third trimester (33.6.6%) followed by first (31.3%) and second trimester (17.1%) of pregnancy, the higher percentage of pregnant women infected with *E. coli* (48.0%) was in the third trimester. Higher prevalence of UTI was recorded in pregnant females who previously used IUD contraceptive (30.9%) than those who were not using

this method (30.2%), also the higher prevalence of *E. coli* infection was among pregnant women who previously used IUD contraceptive (55.1%).

Among the 200 pregnant females 103 were symptomatic (51.5%) and 97 were asymptomatic (48.5%). Out of the 103 symptomatic pregnant females 50 of them were with significant bacteriuria (48.5%), whereas out of the 97 asymptomatic 11 were with significant bacteriuria (11.3%). The higher prevalence of *E. coli* infection was observed in symptomatic (48.0%) more than asymptomatic pregnant women (27.3%). There is a statistically significant correlation between higher prevalence of UTI and symptomatic bacteriuria pregnant females compared with asymptomatic bacteriuria p-value <0.05. Also, *E. coli* infection showed a higher prevalence in pregnant women with past history of UTI by ratio (35.1%) and (50.0%) respectively. The higher prevalence of UTI was observed in diabetic and anemic pregnant women (50.8%) and (44.9%) respectively, also a higher prevalence of *E. coli* among pregnant women was observed in diabetic and anemic (62.5%) and (51.6%) respectively. The personal hygiene data showed that a higher prevalence of UTI was observed in pregnant women who was washing without drying the perineum after urination (52.9%), in those (31.0%), were not changing underwear daily (36.9%), in those who were washing their genitals from back to front (36.0%) and in those who used materials other than cotton for underwear clothes (56.6%). The majority of pregnant women with *E. coli* infection were observed in those who washed without drying, those who not washed before and after intercourse, those that not changed underwear daily, washed their genitals from back to front and those used materials other than cotton for underwear clothes (54.1%), (51.9%),(45.8%),(48.1%) and (53.3%) respectively.

Table (2): Prevalence of UTI in relation to significant pregnant Socio-demographic and Clinical data.

patients' profiles		Frequency No. examined)	Positive culture No.	Percentage % of UTI	Percentage % of infection with *E. coli*	P-Value
1-Socio-demographic characteristics	**Maternal age (years)**					
	14-20	16	6	37.5%	33.3%	
	21-30	116	33	28.4%	57.6%	**0.701**
	31-42	68	22	32.4%	27.3%	
	Socioeconomic level					
	Low	140	46	32.9%	45.7%	
	Intermediate	44	12	27.3%	41.7%	**0.444**
	High	16	3	18.8%	33.3%	
	Educational level					
	Illiterate	57	24	42.1%	50.0%	
	Primary	75	21	28.0%	38.1%	**0.141**
	Secondary	45	11	24.4%	45.5%	
	Higher education	23	5	21.7%	40.0%	
2- Obstetric history and medical complications	**Gestational age**					
	First trimester	16	5	31.3%	40.0%	
	Second trimester	35	6	17.1%	16.7%	**0.165**
	Third trimester	149	50	33.6%	48.0%	
	Previous use of (IUD)Contraceptive					**0.919**
	Yes	94	29	30.9%	55.17%	
	No	106	32	30.2%	34.4%	
	Symptoms of UTI					
	Yes	103	50	48.5%	48.0%	**< 0.001**
	No	97	11	11.3%	27.3%	
	History of DM					
	Yes	63	32	50.8%	62.5%	**0.001**
	No	137	29	21.2%	24.1%	
	Hemoglobin concentration					
	<11mg/dl (Anemia)	69	31	44.9%	51.6%	**0.001**
	≥11mg/dl (Not Anemia)	131	30	22.9%	36.7%	
	Past history of UTI					
	Yes	97	34	35.1%	50.0%	**0.175**
	No	103	27	26.2%	37.0%	
3- Personal hygiene	**Washing and drying after urination**					**<0.001**
	Wash with dry	130	24	18.5%	29.2%	
	Wash without dry	70	37	52.9%	54.1%	
	Washing before and after intercourse					**0.885**
	Yes	113	34	30.1%	38.2%	
	No	87	27	31.0%	51.9%	
	Frequency of changing underwear daily					
	Non	130	48	36.9%	45.8%	**0.025**
	Once	50	10	20.0%	40.0%	
	Twice	20	3	15.0%	33.3%	
	Direction of wash genitals					
	Front to back	50	7	14.0%	14.3%	
	Back to front	150	54	36.0%	48.1%	**0.003**
	Types of underwear cloths					
	Cotton					
	Other materials	147	31	21.1%	35.5%	**0.001**
		53	30	56.6%	53.3%	

2.Antibiotic susceptibility pattern of UPEC*:*

Uropathogenic *E. coli* showed the highest level of resistance (100%) against β-lactams (ampicillin, penicillin and ceftriaxone), also (100%) against nitrofurantoin, and ampicillin/sulbactam, while the lowest level of resistance (66.7%) against cotrimoxazole as observed in the table (3) and figure (1). Multiple drug resistance (resistance to two or more drugs) was observed in all of 27 isolates of *E. coli* which were detected complete resistance up to 5 different antibiotics from a total 11 antibiotics used (45.5%).

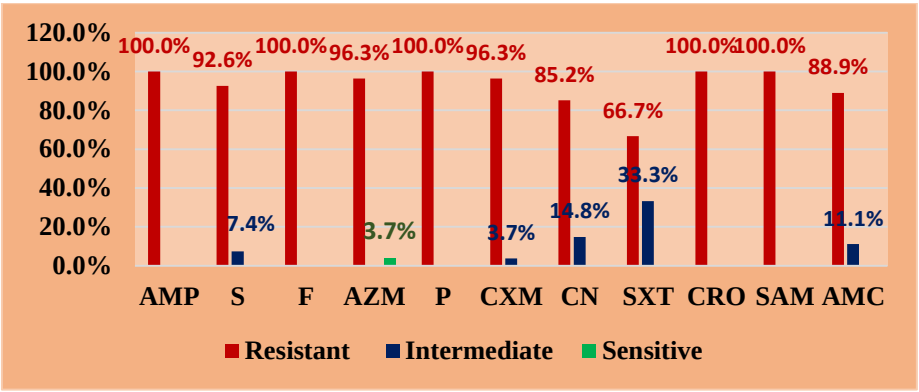

Figure (1): Antibiotic susceptibility profile of UPEC.

Table (3): Antibiotic susceptibility profile of UPEC.

Antibiotic	Sensitive No. & %	Intermediate No. & %	Resistant No. & %
AMP	0 (0.0%)	0 (0.0%)	27 (100%)
S	0 (0.0%)	2 (7.4%)	25 (92.6%)
F	0 (0.0%)	0 (0.0%)	27 (100%)
AZM	1 (3.7%)	0 (0.0%)	26 (96.3%)
P	0 (0.0%)	0 (0.0%)	27 (100%)
CXM	0 (0.0%)	1 (3.7%)	26 (96.3%)
CN	0 (0.0%)	4 (14.8%)	23 (85.2%)
SXT	0 (0.0%)	9 (33.3%)	18 (66.7%)
CRO	0 (0.0%)	0 (0.0%)	27 (100%)
SAM	0 (0.0%)	0 (0.0%)	27 (100%)
AMC	0 (0.0%)	3 (11.1%)	24 (88.9%)

R*=resistant, **I*=**intermediate, **S*=**sensitive, **AMP** = Ampicillin, **S**= Streptomycin, **F**=Nitrofurantoin, **AZM**=Azithromycin, **P**=Penicillin, **CXM**= Cefuroxime, **CN**= Gentamicin, **SXT**= Co-trimoxazole, **CRO**= Ceftriaxone, **SAM**= Ampicillin / Sulbactam, **AMC**=Amoxiclav.

3. Antimicrobial resistance profile among pregnant women with symptomatic or asymptomatic bacteriuria:

There was correlation between resistance to all antibiotics tested in symptomatic bacteriuria compared with asymptomatic bacteriuria. The higher level of resistance to antibiotics was observed in symptomatic bacteriuria more than asymptomatic, as shown in the table (4)

Table (4): Comparative resistance to antibiotics between symptomatic and asymptomatic bacteriuria:

Antibiotic	Frequency of *E. coli* resistant to the antibiotic	Symptomatic bacteriuria resistance (No.)	Symptomatic bacteriuria resistance (%)	Asymptomatic bacteriuria resistance (No.)	Asymptomatic bacteriuria resistance (%)
AMP	27	18	66.7%	9	33.3%
S	25	17	68%	8	32%
F	27	18	66.7%	9	33.3%
AZM	26	17	65.4%	9	34.6%
P	27	18	66.7%	9	33.3%
CXM	26	17	65.4%	9	34.6%
CN	23	16	69.6%	7	30.4%
SXT	18	11	61.1%	7	38.9%
CRO	27	18	66.7%	9	33.3%
SAM	27	18	66.7%	9	33.3%
AMC	24	17	70.8%	7	29.2%
OX	27	18	66.7%	9	33.3%

4. Distribution of UPEC virulence genes in urine samples:

fimH gene found in 25 isolates (92.6%), *sfaD/E* found in 12 isolates (44.4%), *hlyA* found in 11 isolates (40.7%), *papE/F* virulence gene found in 2 isolates (7.4%), while *Aer* gene found in only one isolate (3.7%). It was found that *fimH* gene is the most frequent gene among UPEC isolates, while *Aer* is the lowest one as observed in the table (5) and figure (2).

Table (5): Frequency of UPEC virulence genes in urine samples:

Virulence gene	Frequency No. and %	
	+ve	-ve
fimH	25(92.6%)	2(7.4%)
sfaD/E	12(44.4%)	15(55.6 %)
hlyA	11(40.7%)	16(59.3%)
papE/F	2(7.4%)	25(92.6%)
Aer	1(3.7%)	26(96.3%)
Total no of *E. coli* isolates	**27**	

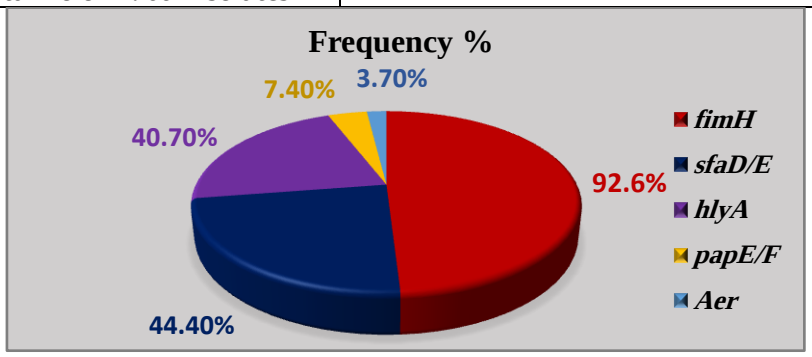

Figure (2): Frequency of UPEC virulence genes in urine samples.

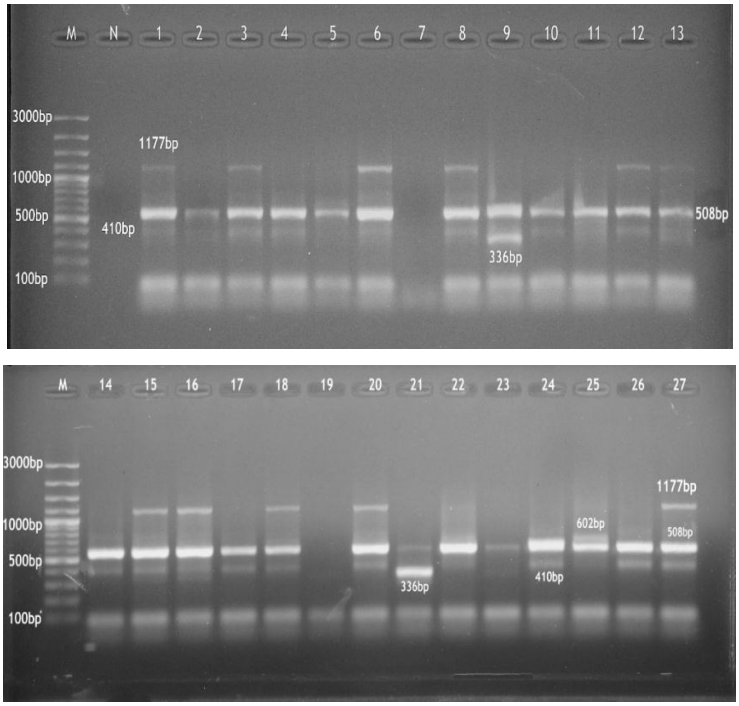

Figure (3): Results of the gel electrophoresis for confirmation of *E. coli* in the genomic DNA extracted from the bacterial colonies

5. Prevalence of UPEC virulence genes in relationship to symptomatic and asymptomatic bacteriuria:

fimH gene was found in (72.0%) of women with symptomatic bacteriuria while, it was found in (28.0%) of asymptomatic ones, *sfaD/E* gene found in (83.3%) of women with symptomatic bacteriuria while, it was found in (16.7%) of asymptomatic ones. *hlyA* gene found in (72.7%) of women with symptomatic bacteriuria while it was found in (27.3%) of asymptomatic ones. *papE/F* gene was found in symptomatic and asymptomatic case of bacteriuria with (50%) whereas, *Aer* gene found only in symptomatic bacteriuria case (100%). It has been observed that symptomatic bacteriuria was more associated with virulence factors as compared to asymptomatic bacteriuria. As shown in table (6) and figure (4).

Table (6): Prevalence between virulence factors of UPEC and symptomatic bacteriuria compared with asymptomatic bacteriuria.

Virulence gene	Frequency No. of genes	Symptomatic bacteriuria with gene No. (%)	Asymptomatic bacteriuria with gene No. (%)
fimH	25	18 (72.0%)	7(28.0%)
sfaD/E	12	10 (83.3%)	2(16.7%)
hlyA	11	8 (72.7%)	3(27.3%)
papE/F	2	1 (50%)	1(50%)
Aer	1	1(100%)	0(0.0%)

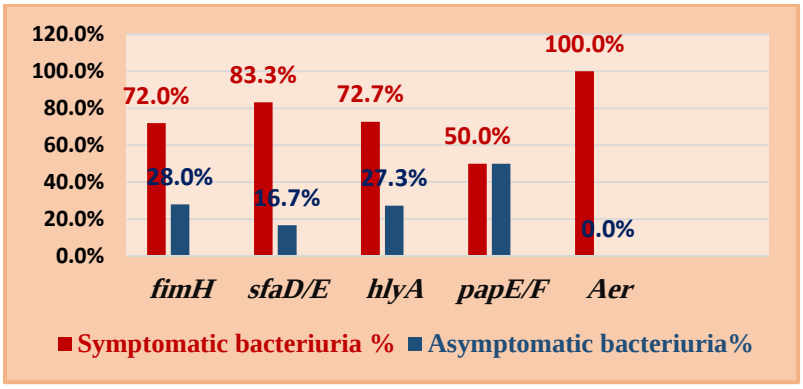

Figure (4): Prevalence of UPEC virulence genes in relationship with symptomatic and asymptomatic bacteriuria

6. Relationship between antimicrobial resistance and virulence factors of UPEC:

From a total 27 UPEC isolates, two isolates with *pap* virulence gene showed resistance to all antibiotics except cotrimoxazole, only one isolate with *Aer* gene was also resistant to all antibiotics. All of 25 isolates (100%) containing *Fim* gene were resistant to penicillin, ampicillin and ceftriaxone, nitrofurantoin and ampicillin/sulbactam while, 24 (96%) of them were resistant to streptomycin, azithromycin and cefuroxime, 22 (88%) of them were resistant to gentamicin and amoxiclav and 16 (64%) of them were resistant to cotrimoxazole. All of the12 isolates (100%) containing *Sfa* gene were resistant to β-lactams (penicillin, ampicillin, ceftriaxone), nitrofurantoin, amoxiclav and ampicillin/sulbactam, while 11 (91.7%) of them were resistant to streptomycin, azithromycin and cefuroxime, 9 (75%) of them were resistant to gentamicin, 6 (50%) of them were resistant to cotrimoxazole. All of the 11 isolates (100%) containing *Hly* gene were resistant to β-lactams (penicillins and cephalosporins), streptomycin, nitrofurantoin and ampicillin/sulbactam, but 10 (90%) of them were resistant to azithromycin and amoxiclav, 8 (72.7%) of them were resistant to gentamicin while 6 (54.5%) of them were resistant to cotrimoxazole. It has been observed that strains carrying the virulence genes were more resistant to the antibiotics. As shown in the table (7).

Table (7): The relationship between the presence of UPEC virulence genes and antimicrobial susceptibility pattern.

Antibiotic	Virulence genes											
	Pattern	*Pap* (2)		*Fim* (25)		*Sfa* (12)		*Aer* (1)		*Hly* (11)		Total
		+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	
AMP	S	0	0	0	0	0	0	0	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	0	0
	R	2(100%)	25	25(100%)	2	12 (100%)	15	1 (100%)	26	11 (100%)	16	27
S	S	0	0	0	0	0	0	0	0	0	0	0
	I	0	2	1(4%)	1	1 (8.3%)	1	0	2	0	2	2
	R	2 (100%)	23	24 (96%)	1	11 (91.7%)	14	1 (100%)	24	11 (100%)	16	25
F	S	0	0	0	0	0	0	0	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	0	0
	R	2 (100%)	25	25 (100%)	2	12 (100%)	15	1 (100%)	26	11 (100%)	16	27
AZM	S	0	1	1(4%)	0	1 (8.3%)	0	0	1	1(10%)	0	1
	I	0	0	0	0	0	0	0	0	0	0	0
	R	2(100%)	24	24(96%)	2	11 (91.7%)	15	1(100%)	25	10(90%)	16	26
P	S	0	0	0	0	0	0	0	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	0	0
	R	2(100%)	25	25(100%)	2	12(100%)	15	1(100%)	26	11(100%)	16	27
CXM	S	0	1	1(4%)	0	1(8.3%)	0	0	1	0	1	1
	I	0	0	0	0	0	0	0	0	0	0	0
	R	2(100%)	24	24(96%)	2	11(91.7%)	15	1(100%)	25	11(100%)	15	26
CN	S	0	0	0	0	0	0	0	0	0	0	0
	I	0	4	3(12%)	1	3(25%)	1	0	4	3(27.3%)	1	4
	R	2(100%)	21	22(88%)	1	9(75%)	14	1(100%)	22	8(72.7%)	15	23
SXT	S	2(100%)	7	9(36%)	0	6(50%)	3	0	9	5(45.5%)	4	9
	I	0	0	0	0	0	0	0	0	0	0	0
	R	0	18	16(64%)	2	6(50%)	12	1(100%)	17	6(54.5%)	12	18
CRO	S	0	0	0	0	0	0	0	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	0	0
	R	2(100%)	25	25(100%)	2	12(100%)	15	1(100%)	26	11(100%)	16	27
SAM	S	0	0	0	0	0	0	0	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	0	0
	R	2(100%)	25	25(100%)	2	12(100%)	15	1(100%)	26	11(100%)	16	27
AMC	S	0	0	0	0	0	0	0	0	0	0	0
	I	0	3	3(12%)	0	0	3	0	3	1(10%)	2	3
	R	2(100%)	22	22(88%)	2	12(100%)	12	1(100%)	23	10(90%)	14	24

Discussion

The results of the present investigation revealed that the overall prevalence of urinary tract infection among pregnant women was (30.5 %). This is similar to the previous studies conducted in Libya(30%) by **(Tamalli *et al.*, 2013)** and , in Egypt

(32%) by **(Shaheen *et al.*, 2017).**while lower than other studies done in Southeastern Nigeria (61.5%) by **(Ezeigbo *et al.*, 2016)** and in India (76.29%) by **(Latika *et al.*, 2015),** on the other hand, it is more than that of Khartoum (14%) by **(Hamdan *et al.*, 2011).**

The incidence of UTIs is due to increasing in the pregnant urinary concentration of amino acids and lactose which provide an excellent culture medium for bacterial growth. Also, higher levels of progesterone and estrogens induced by gestation may lead to a decreased ability of the lower urinary tract to resist invading bacteria **(Obiogbolu, 2004).** Moreover, the variation in prevalence of UTI in different studies may be explained by the fact that differences exist in the environment, cultures, social habits of the community and religious behaviors related to sexual contact, the standard of personal hygiene and health education practices and may be attributed to difference in UTI perception, methodologies used and mode of screening **(Tsegay, 2014).** The decreased incidence of UTI in the current study is because of the better toilet hygiene in the pregnant females due to religious reasons **(Ramzan *et al.*, 2004).**The reported prevalence of symptomatic UTI among pregnant females in this present study was (48.5%), this is in agreement with previous studies reported from Nigeria by **(Akobi *et al.*, 2014).** Symptomatic patients whose urine culture didn't show appreciable significant growth might be due to anatomical changes associated with pregnancy induced changes on urinary system as increasing uterus weight which compress on the urinary bladder causing block of the drainage of urine from the bladder leading to bacterial proliferation in urine and some symptoms like UTI symptoms as dysuria, urgency and frequency. Also due to other less frequent UTI causing microorganisms, such as parasites, fungi and viruses **(Bonadio *et al.*, 2001).** The reported prevalence of asymptomatic UTI among pregnant females in this present study was (11.3%). This is in agreement with a study reported from Nigeria (13.8%) by **(Alfred *et al.*, 2014)**. This current study reported that significant association between maternal anemia, previous history of diabetes and poor personal hygiene through washing genitals without drying, not changing underwear daily, washing and drying genitals from back to front direction and using materials other than cotton for underwear and development of UTI among pregnant females (p-value < 0.05).Whereas, there is no statistically significant association between each of maternal age, gestational age, education status, socio-economic level, washing before and after intercourse, previous use of IUD, past history of UTI, and development of the UTI among pregnant females (p-value > 0.05) .The higher prevalence of UTIs among pregnant women was within the younger age groups (14 to 20) (37.5%). This observation is in harmony with the findings in Tanzania by (**Masinde *et al.*, 2009)** and in India by **(Priscilla *et al.*, 2017)** who found that the higher incidence of UTIs (57.35%) was in the age group (18 to 25). While the higher incidence of E. coli infection was within the age groups (21 to 30) (57.6%) this is in agreement with **(Akpan *et al.*, 2019).**

Considering socio-demographic characteristics, the increased prevalence within this age groups may be due to the fact that the women in this age group were more productive and sexually active so early and intensive sexual intercourse caused minor urethral trauma and transfer bacteria from the perineum into the urethra and the bladder therefore were more prone to UTIs, this is in harmony with **(Derese *et al.*, 2016) .** The highest proportion of UTI and *E. coli* infection were among lower economic level

(32.9%), (45.7%) respectively, this similar to the study conducted in Saudi Aribia by **(Ashshi *et al.*, 2013)** .This is explained by the relation of socio-economic status with nutrition and immunity especially in pregnant women **(Emiru *et al.*, 2013).** The higher prevalence of UTI and *E. coli* infection were in illiterate (42.1%), (50.0%) respectively which was in agreement with another study done in Egypt by **(Dimetry *et al.*, 2007)**. Illiterate pregnant females were infected mostly due to absence of clinical data on signs and symptoms of UTI and lack of awareness with the required personal health care and hygiene during pregnancy.

Considering obstetric history and medical complications, the higher rate of UTI and *E. coli* infection were during the third trimester (33.6%), (48.0%) respectively, which is concordant with a previous study done in Baghdad by **(Ali *et al.*, 2016).** This can be justified scientifically by several anatomical and hormonal changes in pregnancy lead to urethral dilatation and pressure of gravid uterus on the ureters causing urinary stasis which is increased with advancing pregnancy leading to taking longer for urine to pass through urinary tract giving more time for bacteria to multiply and allowing some strains of bacteria to grow which contributed to increased risk of developing UTI **(Abdullah and Al-Moslih, 2005).** In addition to, great abdominal distention during last stage of pregnancy with the subsequent ease of contamination with fecal flora next, the poor personal hygienic practices and bad clean-up of anus properly after defecating and difficult to clean their genitals after urination which facilitate UTI **(Moyo *et al.*, 2010 and Akban *et al.*, 2017).** The higher rate of UTI and *E.coli* infection were recorded among pregnant women who previously used IUD as a mean of contraceptive as a mean of contraceptive (30.9%),(55.17%) respectively as compared to those using no contraceptives, this is in harmony with a study done in Nigeria by **(Onwuezobe and Orok, 2015)**.This could be attributed to the mechanical interference of IUD with the subsequent frequent inflammation caused by its physical presence for long periods, also increasing chance of introducing of pathogens to genitals which may be transferred later to urethra causing UTI **(Harrington and Hooton, 2000).**The higher prevalence of UTI and *E.coli* infection were among diabetic pregnant females (50.8%),(62.5%) compared to non-diabetic ,this is in agreement with a study done in Egypt by **(Shaheen et al., 2017)**.The proposition mechanism for increased susceptibility of diabetic patient to get UTI involved diminished antibacterial activity of urine as a result of defect in cellular immunity in diabetic patient, moreover, hyperglycemia induces glucosuria giving good culture medium boosts and encourages bacterial growth in the urine **(Patterson and Andriole, 1997 and Johnson *et al.*, 2013).** The higher prevalence of UTI and *E. coli* infection were among pregnant females with lower hemoglobin concentration <11mg/dl (anemia) (44.9%), (51.6%) respectively, than without anemia, this in agreement with a study conducted in Ethiopia by **(Emiru *et al.*, 2013)**.The high probability of developing UTI among anemic pregnant may be related immunity, as hemoglobin deficiency may suppress the immune system giving a chance for attacking, invasion and colonization by pathogenic microbes. The higher percentages of UTI and *E.coli* infection were recorded in women with past history of UTI (35.1%), (50.0%) than those not previously infected ,this is in harmony with a study conducted in Nashik by **(Agrawal and Batavia, 2017)** .This is also justified scientifically by ineffective treatment or presence of drug resistance strains from uropathogens from those who had previous history of UTI **(Tsegay, 2014).**

Considering personal hygiene, the current study found that, the higher incidence of UTI and *E. coli* infection observed in pregnant females who did not dry the wet areas after washing (52.9%),(54.1%) respectively compared to those that dried after washing ,this is in agreement with a study conducted in Egypt by **(Badran *et al.*, 2015)**.This is because of drying the wet areas of genitals and urethra reduces their moisture which is considered as a good media for bacterial growth and hence increasing opportunity of UTI **(Ashshi *et al.*, 2013).**The higher prevalence of UTI and *E. coli* infection in pregnant females that not washing before and after the intercourse (31.0%),(51.9%) respectively than those who washed, this is in harmony with a study done in Egypt by **(Badran *et al.*, 2015)** .Infact, not washing before and after intercourse can create a conducive environment for microbial growth as washing results in removing the pathogens and reduce their opportunity for invasion and colonization of urethra and bladder **(Shaheen *et al.*, 2017)**.

The higher prevalence of UTI and *E. coli* infection were found among pregnant females who did not change their underwear clothes daily (36.9%), (45.8%) respectively. According to a study done in Egypt by **(Shaheen *et al.*, 2017).** This is because of not changing underwear daily may keep vaginal secretions which create an environment which is favorable and conducive to microbial growth. The higher prevalence of UTI and *E. coli* infection was observed in those that washed the genitals from back to front direction (36.0%), (48.1%) respectively compared to those who washed from front to back direction. This is in agreement with a study conducted in Egypt by **(Elzayat *et al.*, 2017).** According to **(Obiora *et al.*, 2014)** washing of genitals from back to front is more likely to lead to the spread of anal or vaginal flora into the urethra. This study observed that the higher prevalence of UTI and *E. coli* infection observed in pregnant females that used underwear clothes made of materials other than cotton (56.6%), (53.3%) compared to that used cotton underwear clothes ,this is in harmony with a study conducted in Baghdad by **(Ali et al., 2016)** . The underwear materials other than cotton, on the contrary of cotton ones, can keep the moisture and secretions that favor the conditions for bacterial growth and hence the increasing opportunity of UTI **(Dimetry *et al.*, 2007).**

This current study found that *E. coli* showed a highest level of resistance (100%) against β-lactams (ampicillin, penicillin), this is similar to a previous study performed in Ethiopia by **(Tsegay, 2014)**.The higher resistance rate of this pathogenic micro-organism can be explained by the production of lactamase enzymes and other alternative penicillin-binding proteins which can help the organism to become resistant to β-lactam antibiotics in addition to, indiscriminate, prolonged use and incomplete course of these antibiotics causing ecological disturbances in the normal intestinal microflora and promote antimicrobial-resistant strains **(Beerepoot *et al*, 2011 and Gebremariam *et al.*, 2019)**. Among the most predominant bacterial uropathogens causing UTI, all 27 isolates of E. coli showed complete resistance up to 5 different antibiotics (with different classes and generation) from 11 used, while all 36 isolates of S. saprophyticus showed complete resistance to two different antibiotics, so *E. coli* was the most virulent and resistant(45.5%) than S. saprophyticus(18.2%) to cause UTI. This is in agreement with a study performed in Ethiopia by **(Tsegay, 2014)**. This is due to the number of *E. coli* virulence factors specific for attachment to the uroepithelial cells and preventing bacteria from urinary lavage, allowing for multiplication, proliferation and tissue invasion, resulting in invasive infection and pyelonephritis in pregnancy **(Amiri**

***et al.*, 2009 and Lavigne *et al.*, 2011)**.There was a correlation between resistance to all antibiotics tested and symptomatic bacteriuria compared with asymptomatic bacteriuria, as it was observed that the higher level of resistance to antibiotics observed in symptomatic bacteriuria pregnant females more than asymptomatic bacteriuria pregnant females. A similar observation was reported in Iran by **(Tabasi *et al.*, 2015)**. In the developing world where apart from high level of poverty and ignorance, there is also a widespread of antibiotics misuse, in addition to, their low-cost easy availability in the community without prescription which make patients with symptomatic UTI subject to abuse then result in increasing the drug resistance **(Abubakar, 2009).**The current work observed that *fimH* (the adhesive subunit of type 1 fimbriae of UPEC) was the most prevalent virulence factor detected in UPEC strains and presented with the highest frequency (92.6%) in urine isolates as compared to the rest of the genes detected , this is similar to studies performed in Iran by **(Asadi *et al.*, 2014 and Ghazvin *et al.*, 2019)** and in Sudan by **(Hassan *et al.*, 2018)**. This reconfirms the crucial and essential role of *fimH* gene in *E. coli* to cause UTI among pregnant females, that is because of the higher binding ability of *fimH* which could result in the increased pathogenicity of UPEC strains as most strains of (UPEC) encode filamentous adhesive organelles called type 1 pili which is important for the invasion, adherence and persistence of the UPEC in the urinary bladder after its colonization which enhanced by adhesin *FimH* **(Hannan *et al.*, 2012).** The second most prevalent virulence factor detected in this study was *sfa* gene (the adhesive subunit of S fimbriae of UPEC) which was b/detected in 44.4% of UPEC isolates, this is similar to a study done in Egypt by **(Khairy *et al.*, 2019)**. The dissemination of bacterium within the host tissue was for S-fimbriae adhesins which encoded by sfa gene **(Lee *et al.*, 2016).** According to **(Derakhshandeh *et al.*, 2015)** genes encoding adhesins are the most frequently occurring virulence factors in UPEC and the prevention of *E. coli* infections may be achieved by blocking bacterial attachment.

The prevalence of *hlyA* gene (secreted virulence factor of lipoprotein toxin called α-hemolysin and encoded by hlyA) in present study was 40.7%, and approximately similar to the results obtained by **(Jalali *et al.*, 2015)**. There is a clear association between the presence of hemolysin and tissue damage as *hlyA* is an extracellular cytolytic protein, lyses leukocytes, erythrocytes and renal tubular cells by the formation of pores in the cell membrane **(Eto *et al.*, 2007).** Also, this toxin causes bladder cell exfoliation and increases bacterial access to the underlying tissue **(Bien *et al.*, 2016)**.The prevalence of pap gene (the adhesive subunit of P fimbriae of UPEC which was associated with pyelonephritis) was 7.4%, this is approximately similar to a study performed in Egypt by **(Khairy *et al.*, 2019)** who found 16.5% pap positive gene in UPEC isolates. The pap gene strains have the ability to colonize the kidneys and generate pyelonephritis **(Tarchouna *et al.*, 2013)**. The prevalence of these virulence genes varies on the basis of clinical representation and geographical distribution **(Basu and Mukherjee, 2018).** Among the 27 isolates of *E. coli*, 2 isolates were negative for *hlyA, fimH, papE/F, sfaD/E* and *Aer* genes, with the possible explanation that these isolates could be a part of the normal flora of the gastrointestinal tract. A positive PCR usually confirms the presence of the virulence genes **(Jalali *et al.*, 2015).** This study found that symptomatic bacteriuria cases were more associated with virulence factors compared to asymptomatic bacteriuria as virulence genes detected from UPEC strains isolated from symptomatic UTI has higher frequency than in asymptomatic UTI , this is in agreement with a previous studies done in Iran by **(Tabasi *et al.*, 2015)** and in Spain

by **(Blanco *et al,* 1996)**. Also, in this study, 2 isolates of UPEC isolated from asymptomatic pregnant women were negative for virulence factors, this is similar to a study done in Iran by **(Jalali *et al.,* 2015).**This observation also reflected the important role of virulence factors of UPEC in existence and severity of symptoms of UTI as virulence factors of UPEC specific for adherence and invasion of the urinary epithelium increased induction of the mucosal inflammation and pain associated with UTIs **(Sheffield and Cunningham, 2005).**There was relationship between antimicrobial resistance and virulence genes as, 92.6% of UPEC isolates carried the virulence genes and showed resistance to all antibiotics used and showed 100% multidrug resistant as demonstrated resistance to two or more of the antibiotics were tested, this is similar to some studies conducted in India by **(Mukherjee *et al.,* 2013)** and Iran by **(Neamati et al., 2015)**. In addition, in this study the strains that carried the virulence genes were more resistant to the antibiotics, this observation reflects the important role of virulence genes of UPEC in the drug resistance, this agrees with the previous studies done by **(Oliveira *et al.,* 2011 and Schwartz *et al.,* 2013) .**On contrast, several studies indicate that resistance to some antibiotics is associated with decreased virulence traits among clinical *E. coli* isolates **(Johnson et al., 2003 and Moreno *et al.,* 2006)**. Resistance to antimicrobial agents is often associated with the spread of transmissible plasmids, which may also carry virulence determinants. The acquisition of resistance and virulence traits may provide a benefit for the survival of microorganism. This situation may lead to ecological changes and domination of virulent antibiotic-resistant bacteria in the environment **(Da Silva and Mendonça, 2012)**.

Conclusion

The present study revealed that lower hemoglobin concentration in pregnant females, previous history of diabetes and poor personal hygiene through washing genitals without drying, not changing underwear daily, washing and drying genitals from back to front direction and using materials other than cotton for underwear were important risk factors and significantly associated with development of UTI. Whereas, each of maternal age, gestational age, education status, socio-economic level, washing before and after intercourse, previous use of IUD and past history of UTI, had been non-significantly associated with developing the UTI. Among predominant pathogens causing UTI, *E. coli* showed the higher virulence and resistance. Also, this study reported that UPEC strains isolated from pregnant females with or without clinical symptoms of UTI have a different virulence gene when compared with other studies. These virulence genes detected from total UPEC isolates of symptomatic cases were higher than asymptomatic. On the other hand, this study observed that UPEC strains carrying the virulence genes were more resistant to the antibiotics used, thus this confirms the correlation between the presence of UPEC virulence genes and antimicrobial susceptibility patterns, also these observations confirm the important role of virulence genes of UPEC in in existence of symptoms of UTI and the drug resistance.

Conflict of Interests

The authors declare no conflict of interest.

REFERENCES

Abdullah AA and Al-Moslih MI (2005): Prevalence asymptomatic bacteriuria in pregnant women in Sharjah, United Arab Emirates. *East Mediterr Health Journal* 11: 5-6.

Abubakar EM (2009): Antimicrobial susceptibility pattern of pathogenic bacteria causing urinary tract infections at the Specialist Hospital, Yola, Adamawa State, Nigeria. *J. Clin. Med. Res.,* 1(1): 001-008.

Ade-Ojo IP, Oluyege AO, Adegun PT, Akintayo AA, Aduloju OP and Olofinbiyi BA (2013): Prevalence and antimicrobial susceptibility of asymptomatic significant bacteriuria among new antenatal enrollees in South West Nigeria. *International Research Journal of Microbiology* 4(8): 197-203.

Agrawal V and Batavia A (2017): Prevalence of the urinary tract infections among pregnant women Sch. *J. App. Med. Sci*.; 5(2A):376-378.

Akobi OA, Inyinbor HE, Akobi EC, Emumwen EG, Ogedengbe SO,Uzoigwe, EO, Amlscn , Abayomi RO, Emumwen EF, Amlscn, Okorie IE and Bmls (2014): Incidence of urinary tract infection among pregnant women attending antenatal Clinic at Federal Medical Centre, Bida, Niger-State, North.

Akpan NG, Onwuezobe IA and Antia UE (2017): Asymptomatic bacteriuria among pregnant women at University Hospital in Uyo, Nigeria: Prevalence risk factors and characteristics. *Asian Journal of Medicine and Health* 3(3):1-9.

Akpan NG, Umoyen AJ, Luka TT, Onwuezobe IA, Antia UE and Okon AS (2019): Asymptomatic uropathogenic bacteriuria among pregnant and non-pregnant women at St Luke's Hospital Anua, Offot Ukwa District Uyo: *A Reassessment Case-Control Approach,* 4(1): 1-10.

Alemu A, Moges F, Shiferaw Y, Tafess K, Kassu A, Anagaw B and Agegn A (2012): Bacterial profile and drug susceptibility pattern of urinary tract infection in pregnant women at University of Gondar Teaching Hospital, Northwest Ethiopia. *BMC Research Notes,* 5:197.

Alemu AT, Moges SW, Shiferaw ST, Tafess ST, Kassu, TS, Anagaw SV and Agegn GS (2012): Recent development in the diagnosis and treatment of urinary tract infections. Niger. Br. *J. Obstet. Gynaecol,* 11(12): 23 30.

Alfred AO, Matie O, Gbenga SA, Chiedozie and Martin DU (2014): Diagnostic characteristics of urine microscopy in detecting asymptomatic bacteriuria of pregnancy using urine culture as gold standard. *e-ISSN: 2279-0853, p-ISSN: 2279-086*1. Volume 13, Issue 1 Ver. VI. PP 87-90.

Ali AM, Sajem KM and Tawfeq AS (2016): Urinary tract infection as a health problem among pregnant women in Baghdad \ AL Sadder City *International Journal of Scientific and Research Publications,* Volume 6, Issue 5,503 ISSN 2250-3153.

Amiri FN, Rooshan MH, Ahmady MH and Soliamani MJ (2009): Hygiene practices and sexual activity associated with urinary tract infection in pregnant women. *East Mediterr Health* 15: 105-108.

Asadi S, Kargar M, Solhjoo K, Najafi A and Dalini SG (2014): The association of virulence determinants of uropathogenic *Escherichia coli* with antibiotic resistance. *Department of Jundishapur J Microbiol*; 7(5): e9936.

Aseel M, Meer FA, Kuwari MA, and Ismail M (2011): Prevalence and predictors of asymptomatic bacteriuria among pregnant women attending primary Health Care in Qatar. *Qatar National Research Strategy*, 1715.

Aseel TM, Mohamed Al-Meer, Ghaith Al-Kuwari M and Ismail MF (2009): Prevalence and predictors of asymptomatic bacteriuria among pregnant women attending primary health *care in Qatar. Middle East J Fam Med*,7:10–13.

Ashshi AM, Faidah HS, Saati AA, Abou El-Ella GA, Al-Ghamdi AK and Mohamed AM (2013): Urinary tract infections in pregnant women, assessment of associated risk factors in Makkah, KSA. *Biosciences Biotechnology Research Asia*. Vol. 10 (1), proof.

Badran YA, El‑Kashef TA, Abdelaziz1 AS and Mohamad M (2015): Impact of genital hygiene and sexual activity on urinary tract infection during pregnancy. Vol 7. Issue 4. IP: 84.107.141.8.

Bahalo S, Tajbakhsh E, Tajbakhsh S, Momeni M, Tajbakhsh F (2013): Detection of some virulence factors of *Escherichia coli* isolated from urinary tract infection isolated of children in Shahrekord Iran by Multiplex PCR. *Middle-East J. Sci. Res*. 14(1):29-32.

Basu, S and Mukherjee M (2018): Incidence and risk of co-transmission of plasmid mediated quinolone resistance and extended spectrum β-lactamase genes in fluoroquinolone resistant uropathogenic *E. coli*: a first study from Kolkata, India. *Journal of Global Antimicrobial Resistance* 14: 217-233.

Beerepoot MA, TerRiet G, Nys S, (2011): Cranberries antibiotics to prevent urinary tract infections: a randomized double-blind noninferiority trial in premenopausal women. *Arch Intern Med*; 171:1270-8.

Beyene G and Tsegaye W (2011): Bacterial uropathogens in UTI and antibiotic susceptibility pattern in Jimma University specialized hospital. *Ethiopia Journal of Health Sci*. 21(2):141-146.

Bien J, Sokolova O and Bozko P (2016): Role of Uropathogenic *E. coli* virulence factors in development of urinary tract infection and kidney damage. *Int J Nephrol.*Vol.681473.

Blanco M, Blanco JE, Alonso MP and Blanco J (1996): Virulence factors and 0 groups of *Escherichia coli* isolates from patients with acute pyelonephritis, cystitis and asymptomatic bacteriuria. *European Journal of Epidemiology* 12: 191-198.

Bonadio M, Meini M, Spitaleri P and Gigli C (2001): Current microbiological and clinical aspects of urinary tract infections. *Euro J of Urology*, 40, 439-445.

Chaliha C and Stanton SL (2002): Urological problems in pregnancy. *British Journal of Urology International* 89: 469-476.

Cheesbrough M (2017): Medical laboratory manual for tropical countries II. 255- 275.

Da Silva GJ and Mendonça N (2012): Association between antimicrobial resistance and virulence in *Escherichia coli*; 3:18-28.

Derakhshandeh A, Firouzi R, Motamedifar M, Arabshahi S, Novinrooz A, Boroojeni AM, Bahadori M and Heidari S (2015): Virulence characteristics and antibiotic resistance patterns among various phylogenetic groups of uropathogenic *Escherichia coli* isolates. *Japanese Journal of Infectious Diseases* 68: 428-431.

Derese B, Kedir H, Teklemariam Z, Weldegebreal F and Balakrishnan S (2016): Bacterial profile of urinary tract infection and antimicrobial susceptibility pattern among pregnant women attending at antenatal Clinic in Dil Chora Referral Hospital, Dire Dawa, Eastern Ethiopia Ther. *Clin. Risk Manag*. 12 251.

Dimetry SR, El-Tokhy HM, Abdo NM, Ebrahim MA and Eissa M (2007): Urinary tract infection and adverse outcome of pregnancy. *Egypt Public Health Assoc J*; 82:203–l8.

Elzayat MAA, Barnett-Vanes A and Dabour MFE (2017): Prevalence of undiagnosed asymptomatic bacteriuria and associated risk factors during pregnancy: *a cross-sectional study at two tertiary centers in Cairo, Egypt. BMJ*;7: e013198. doi:10.1136/ bmjopen--013198.

Emamghorashi F, Mahmoodi N, Tagarod Z (2012): Meternal urinary tract infection as a risk factor for neonatal urinary tract infection. *Iran J Kidney Dis;*6(3):178-80.

Emiru T, Beyene G, Tsegaye W and Melaku S (2013): Associated risk factors of urinary tract infection among pregnant women at Felge Hiwot Referral Hospital,Bahir, *North west Ethiopia. BMC Res notes* 6:292.

Eto DS, Jones TA, Sundsbak JL and Mulvey MA (2007): Integrin-mediated host cell invasion by type 1–piliated uropathogenic *Escherichia coli*. PLoS Pathogens 3: e100.

Ezechi OC, Gab-Okafor CV, Oladele DA, Kalejaiye OO, Oke BO, Ekama SO, Audu RA, Okoye RN and Ujab I. A. (2013): Prevalence and risk factors of asymptomatic bacteriuria among pregnant Nigerians infected with HIV. *Journal of Matern Fetal Neonatal Med*. 26(4):402-4.

Ezeigbo OR, Nnadozie RI, Asuoha-Chuks N, Ojiako VU, Awurum IN and Ugochukwu MG (2016): Incidence of urinary tract infection (UTI) among pregnant women attending antenatal clinics at some selected hospitals in Aba, Southeastern Nigeria. *Int. J. Curr. Microbiol. App. Sci*. 5(1):193-9.

Gebremariam G, Legese H, Woldu Y, Araya T and Hagos K and Wasihun AG (2019): Bacteriological profile, risk factors and antimicrobial susceptibility

patterns of symptomatic urinary tract infection among students of Mekelle University, *northern Ethiopia. BMC Infectious Diseases,*19:950.

Ghazvin H, Taher K, Edalati E, Sedighi M and Mirkalantari S (2019): Virulence factors and antimicrobial resistance in uropathogenic *Escherichia coli* strains isolated from cystitis and pyelonephritis. *Turk J Med Sci* 49: 361-367:10.3906/sag-1805-100.

Hamdan HZ, Ziad AM, Ali SK and Adam I (2011): Epidemiology of urinary tract infections and antibiotics sensitivity among pregnant women at Khartoum North Hospital. Ann Clin Microbiol Antimicrobe; 10:2.

Hannan TJ, Totsika M, Mansfield KJ, Moore KH, Schembri MA and Hultgren SJ (2012): Host–pathogen checkpoints and population bottlenecks in persistent and intracellular uropathogenic *Escherichia coli* bladder infection. *FEMS Microbiol. Rev*. 36:616-648.

Harrington R and Hooton T (2000): Urinary tract infection risk factors and gender. *J Gend Specif Med;* 3(8): 27-34.

Hassan HM, Altayb HN, Mogahid M, Hassan MM, Elmekki MA (2018): Genotypic detection of the virulence factors of uropathogenic *Escherichia coli* isolated from diarrheic and urinary tract infected patients in Khartoum State, Sudan, *African Journal of Microbiology Research,* Vol. 12(9), pp. 230-236.

Jalali HR, Pourbakhsh A, Fallah F and Eslami G (2015): Genotyping of virulence factors of uropathogenic *Escherichia coli* by PCR. *Noveltyin Biomed*. 3(4):177-181.

Johnson JR, Kuskowski MA, Owens K, Gajewski A and Winokur

Johnson JR, Murray AC, Gajewsk A, Sullivan M, Snippes P, Kuskowisk MA and Smith KE (2003): Isolation and molecular characterization of nalidixic acid resistant extraintestinal pathogenic *Escherichia coli* from retail chicken products. Antimicrobe. *Agents Chemother*. 47:2161-2168.

Johnson KM, Ptaszynska A, Schmitz B, Sugg J, Parikh SJ and List JF (2013): "Urinary tract infection in patients with diabetes treated with *dapagliflozin Journal of Diabetes and Its Complications,* vol.27, no.5, pp. 479–484.

Khairy RM, Abdel ghaniy HM and Abdelrahim SS (2019): Phylogenic classification and virulence genes profiles of uropathogenic *E. coli* and diarrhegenic *E. coli* strains isolated from community acquired infections.Vol.0222441.

Latika J, Vaghela GM and Mahida H (2015): Urinary tract infection: bacteriological profile and its antibiotic susceptibility in western india. *National journal of medical research*. Volume 5. Issue 1. ISSN: 2249 4995.

Lavigne JP, Boutet-Dubois A, Lqouini D, Combescure C, Bouziges N, Mares P and Sotto A (2011): Virulence potential of *E. coli* strains causing asymptomatic bacteriuria during pregnancy. *Journal Clin Microbiol*. 49(1): 3950-3953.

Lee JH, Subhadra B, Son YJ, Kim DH, Park HS, Kim JM (2016): Phylogenetic group distributions, virulence factors and antimicrobial resistance properties of uropathogenic *Escherichia coli* strains isolated from patients with urinary tract infections in South Korea. *Lett Appl Microbiol*. 62 (1):84–90.

Masinde A, Gumodoka B, Kilonzo A and Mshana SE (2009): Prevalence of urinary tract infection among pregnant women at Bugando Medical Centre, Mwanza, Tanzania. Tanzania *Journal of Health Research* Vol. 11, No. 3.11, 154-159.

Matuszkiewicz JR, Małyszko J and Wieliczko M (2015): Urinary tract infections in pregnancy: *old and new unresolved diagnostic and therapeutic problems*

Mazor Dray ME, Levy A, Schlaeffer F and Sheiner E (2009): Maternal urinary tract infection: is it independently associated with adverse pregnancy outcome. J Maternal Fetal Neonatal Med; 22 /2: 124–128. 8.

Moreno E, Prats G, Sabaté M, Pérez T, Johnson JR, Andreu A (2006): Quinolone, fluoroquinolone and trimethoprim/sulfamethoxazole resistance in relation to virulence determinants and phylogenetic background among uropathogenic *Escherichia coli. J Antimicrobe Chemother;* 57:204‑11.

Moyo SJ, Aboud S, Kasubi M and Maselle SY (2010): Bacterial isolates and drug susceptibility pattern of urinary tract infection among pregnant women at Mihimbili National Hospital in Tanzania. *Tanzania Journal of Health Research* 12(4): 233-236.

Mukherjee M, BaSu S, Mukherjee SK and MajuMder M (2013): Multi drug resistance and extended spectrum beta lactamase production in uropathogenic *E. coli* which were isolated from hospitalized patients in Kolkata, India. *Journal of Clinical and Diagnostic Research: JCDR* 7:449.

Neamati F, Firoozeh F, Saffari M and Zibaei M (2015): Virulence genes and antimicrobial resistance pattern in uropathogenic *Escherichia coli* isolated from hospitalized patients in Kashan, Iran. *Jundishapur Journal of Microbiology* 8: e17514.

Obiogbolu CH (2004): Incidence of Urinary Tract Infection amongst Pregnant women within Akwa Metropolis. A B.Sc. Project in the Department of Applied Microbiology and Brewing, Nnamdi Azikwe University, Awka, Anambra State, Nigeria p. 55.

Obiora CC, Dim CC, Ezegwui HU (2014): Asymptomatic bacteriuria among pregnant women with sickle cell trait in Enugu, South Eastern Nigeria. *Niger J Clin Pract* 17:95–9.

Oladeinde BH, Omoregie R and Oladeinde OB (2015): Asymptomatic urinary tract infection among pregnant women receiving ante-natal care in a traditional birth home in Benin City, Nigeria. *Ethiop Journal of Health Science* 25(1): 3-8.

Oliveira F, Paludo K, Arend L, Farah S, Pedrosa F, Souza E, Surek M, Picheth G and Fadel-Picheth C (2011): Virulence characteristics and antimicrobial susceptibility of uropathogenic *Escherichia coli* strains. *Genet Mol Res* 10: 4114-25.

Onwuezobe A and Orok EE (2015): Associated risk factors of asymptomatic urinary tract infection among pregnant women attending antenatal care in a secondary health care facility in a South Nigerian City. *Int.J.Curr.Microbiol.App*.4(8): 223-230. 2319-7706 Volume 4 Number 8 pp. 223-230.

Patterson JE and Andriole VT (1997): "Bacterial urinary tract infections in diabetes." *Infect. Dis. Clin. N. Am;*11: 735-750.

Priscilla R, Latha G, Rajan D and Sultana M (2017): Prevalence and antimicrobial resistance pattern of bacterial strains isolated from patients with urinary tract infection Vol. 5 Issue 1 224-605.

Ramzan M, Bakhsh S, Salam A, Majid G and Mustafa G (2004): Risk factors in urinary tract infections. *Gomal Journal of Medical Sciences* July–Dec.Vol. 2, No. 2.

Schwartz DJ, Kalas V, Pinkner JS, Chen SL, Spaulding CN, Dodson, KW and Hultgren SJ (2013): Positively selected *FimH* residues enhance virulence during urinary tract infection by altering *FimH* conformation. Proceedings of the National Academy of Sciences 110: 15530-15537.

Shaheen HM, Farahat TM and Hammad NAE (2017): Prevalence of urinary tract infection among pregnant women and possible risk factors. Menoufia Med J 29:1055–1059 © Faculty of Medicine, Menoufia University 1110‑2098.

Sheffield JS and Cunningham FG (2005): Urinary tract infection in women. Obstetrics & Gynecology 106, 1085–1092.

Solberg OO, Ajiboye R and Riley LW (2006): Origin of class 1a integron and gene cassettes in a population-based sample of uropathogenic *Escherichia coli*. *Journal of Clinical Microbiology* 44: 1347-1357.

Soumet C, Ermel G, Fach P and Colin P (1994): Evaluation of different DNA extraction procedures for the detection of Salmonella from chicken products by polymerase chain reaction. *Lett. Appl. Microbiol*., 19, 294-298.

Tabasi M, Karam MRA, Habibi M, Yekaninejad MS and Bozari S (2015): Phenotypic assays to determine virulence factors of uropathogenic *Escherichia coli* (UPEC) isolates and their correlation with antibiotic resistance pattern. *Osong public health Res Perspect* .6(4),261-268.

Tamalli M, Sangar B and Alghazal M (2013): Urinary tract infection during pregnancy at Al-khoms, Libya. *International Journal of Medicine and Medical Sciences* ISSN: 2167-0404 Vol. 3 (5), pp. 455-459.

Tarchouna M, Ferjani A, Selma WB and Boukadida J (2013): Distribution of uropathogenic virulence genes in *Escherichia coli* isolated from patients with urinary tract infection. Int. *J. Infect. Dis.*17:450-3.

Tsegay E (2014): Bacterial profile and drug suscptibility pattern of urinary tract infection in pregnant women attending antenatal care at Mekelle Hospital, Northen Ethiopia. Addis Ababa, Ethiopia. 5(160): 1-9.

Yamamoto S, Terai A, Yuri K, Kurazono H, Takeda Y, Yoshida O (1995): Detection of urovirulence factors in *Escherichia coli* by multiplex PCR. FEMS Immuno. Med. *Microbiol.* 12:85-90.

الكشف عن النمط الوراثي لعوامل الضراوة لسلالات الإشريكية القولونية البولية المعزولة من الإناث الحوامل وعلاقتها بنمط المقاومة للمضادات الحيوية

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الملخص:

تعد التهابات المسالك البولية أثناء الحمل من بين أكثر أنواع العدوى شيوعًا في جميع أنحاء العالم ويمكن أن تؤدى الى خطورة كبيرة على كل من الأم والجنين في فترة الحمل ، خاصة في البلدان النامية وتتطلب علاجًا طبيًا بمجرد اكتشافها. وتعد السلالات ذات الضراوة والمقاومة من الايشيريشيا القولونية هي أهم العوامل المسببة لأعراض عدوى المسالك البولية. هدفت الدراسة الحالية إلى الكشف عن عوامل الضراوة لعصيات الإشريكية القولونية البولية في العزلات التي تم جمعها من الإناث الحوامل اللاتى يعانين من اعراض عدوى المسالك البولية والاتى لم يعانين منها عن طريق فحص سلسلة تفاعل البلمرة المتعددة وتحديد ارتباطها بأنماط مقاومة المضادات الحيوية. وقد تم جمع مائتي عينة بول من السيدات الحوامل اللاتى يعانين من اعراض عدوى المسالك البولية والاتى لم يعانين منها في قسم أمراض النساء والولادة بمستشفى الزهراء الجامعي بالقاهرة. وقد تم فحص وزراعة مائتى عينة بول و تم اجراء الكشف الكمى للكائنات الحية الدقيقة بهذة العينات حيث وجد النمو البكتيري فى ٦١ عينة بول من مختلف السيدات الحوامل وكانت نسبة العدوى ٣٠.٥% وقد تم اختيار هذة العينات الاخيرة لاجراء المزيد من التحاليل عليها وكانت نسبة الإناث الحوامل المصابات بأعراض عدوى المسالك البولية (٤٨.٥%) اكثر من النساء التى لم تظهر عليهن اى من الاعراض(١١.٣%). وكانت اكثر انواع البكتيريا التى تم عزلها هى المكورات العنقودية المترممة وكانت نسبتها (٣٥.٠%) يليها الايشيريشيا القولونية بنسبة (٢٦.٢%) ثم المكور العنقودى الذهبى بنسبة (١٩.٤%) وفطر الكانديدا البيكانز بنسبة (٥.٨%) المكورات العنقودة الجلدية بنسبة (٢.٩%) ثم كلا من الكلبسيلا الرئوية والكلبسيلا اوكسيتوكا والبروتيس فولجاريس والمكورات الرئوية والباسيليس بينما تتواجد الزائفة الزنجارية بنسبة (١%) . تم اجراء اختبار نمط الحساسية لمضادات الميكروبات لعدد ٢٧ عزلة من الايشيريشيا القولونية وقد اظهرت أعلى مستوى من المقاومة (١٠٠٪) ضد الأمبيسيلين والبنسلين والسيفترياكسون والنيتروفورانتوين والأمبيسيلين / سولباكتام ، بينما أدنى مستوى مقاومة (٦٦.٧٪) ضد الكوتريموكسازول. وقد لاحظت هذة الدراسة ان جميع العزلات الايشيريشيا القولونية مقاومة لاثنان او اكثر من المضادات الحيوية ولاحظت الدراسة ايضا ان البكتيريا التى تم عزلها من النساء الحوامل اللاتى يشعرن باعراض عدوى المسالك البولية اكثر مقاومة للمضادات الحيوية من البكتيريا التى تم عزلها من النساء الحوامل اللاتى لايشعرن باى من اعراض هذة العدوى ومن بين الاكثر نوعين من البكتيريا المسببة للعدوى اظهرت جميع عزلات الايشريشيا القولونية مقاومة كاملة لخمس من المضادات الحيوية المختلفة من بين احدى عشر من المضادات الحيوية المستخدمة فى الدراسة حيث كانت بنسبة ٤٥.٥% ولكن جميع عزلات المكورات العنقودية المترممة اظهرت مقاومة كاملة للمضادات الحيوية بنسبة١٨.٢ ٪ وبالتالى كانت الايشيريشيا القولونية اكثر ضراوة وشراسة ومقاومة من المكورات العنقودية المترممة. وكانت عوامل الخطورة بعزلات الايشيريشيا القولونية هى جينات *hly، pap, fim, sfa, aer* وتم الكشف عنها عن طريق تفاعل متعدد البلمرة المتسلسل وكان جين اللاصق (*fimH*) هو الاكثر تواجدا فى عزلات الايشيريشيا القولونية حيث تواجد بنسبة ٩٢.٦% وتواجدت هذه الجينات فى الايشيريشيا القولونية المعزولة من الحوامل الذين يشعرون باعراض عدوى الجهاز البولى بنسبة اكبر من تواجدها فى الايشيريشيا القولونية المعزولة من الحوامل الاتى ليس لديهم اى اعراض للعدوى من ناحية أخرى ، وعلى الجانب الاخر لاحظت هذه الدراسة أن سلالات الايشيريشيا القولونية التي تحمل جينات الضراوة كانت أكثر مقاومة للمضادات الحيوية المستخدمة وبالتالي تؤكد هذة الملاحظات ان هذة الجينات تلعب دورا هاما فى ظهور اعراض عدوى والتهاب المسالك البولية عند المرضى بالاضافة الى دورها فى جعل الايشيريشيا القولونية اكثر مقاومة للمضادات الحيوية.

الكلمات المفتاحية : الإشريكية القولونية البولية ، عامل الضراوة ، *fimH* ، حامل ، تفاعل سلسلة البلمرة المتعددة، التهاب المسالك البولية.