Detection of virulence factors of E.coli in patients with acute UTI

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الخلاصة

تم في هذه الدراسة جمع 180 عينة إدرار من المرضى المصابين بالتهاب المجاري البولية من فئات عمرية مختلفة تتراوح بين اقل من 18 سنة الى اكثر من 80 سنة ولكلا الجنسين من المرضى الذين راجعوا مستشفى الديوانية التعليمي خلال الفترة من تشرين الثاني 2014 إلى آذار 2015 للتحري عن التهاب المجاري البولية المتسبب عن بكتريا *E.coli* , حيث بينت نتائج الاختبارات الكيموحيوية عائديه 137(76%) عينة *E.coli* من مجموع 180 عينة تم جمعها وعائدية 43 (24%)عزلة الى اجناس بكتيرية أخرى في عينات الإدرار, تم قياس بعض عوامل الضراوة باستخدام تقنية تفاعل إنزيم البلمره , نتائج PCR امتلاك جميع عزلات بكتريا *E.coli* التي اجري عليها الفحص لدى مرضى السكري المصابين بالتهاب المجاري البولية لجميع جينات الضراوة قيد الدراسة وبنسبة 100% , بينما تفاوتت الجينات في بقية عينات المرضى بين وجودها وعدم وجودها , وهذا الاختلاف بين وجود وعدم وجود الجينات أدى إلى الاختلاف بالاستجابة للمضادات الحياتية بين المرضى .

Abstract:

Aim: To define the most virulence factors of *E.coli* that causing acute urinary tract infection in some patients including diabetics, and showing the effect of this virulence on culture and sensitivity results in urine samples.

Patients and methods: 180 patients with age ranging from 18 up to 80 years of both genders with a features of acute UTI, *E Coli* was a causative agent found in 137 (76%) of them. Direct smear for microscopic features, culture urine samples on selective (Mackonky agar) and enrichment media (blood agar) to uptake standers colonies, then the bacterial was isolate and send for biochemical tests to identification the *E.coli* bacteria virulence factors then DNA samples using to detect virulence factors genes by PCR technique.

Result: 137 (76%) patients from 180 patients with acut UTI were causing by *E Coli*. The UTI shows that the youth age (20-30 years) and elderly age groups are more infected than others age group. Acute UTI are more commonly occur in female with a ratio about 72% of patients. Also UTI was more frequent in obese patients. 51 patients had poorly control diabetes mellitus. One or more of virulence factor genes were found in all patients while all virulence factor genes are seen in diabetic patients.

Conclusion: *E.coli* bacteria are more causative agent for UTI than other bacterial types and the virulancy of *E.coli* differ from patients to another as a result of chronic disease, pregnancy and other condition. Youth age group (20-30 years) are more exposure to infection with *E.coli* UTI , and the female had more chance for UTI than male.

Recommendation: Detection of virulence factors in infected individuals may be predict the response for treatment.

Introduction:

Urinary tracts infections (UTIs) are conditions where one or more structures in the urinary tracts become infected after microorganisms overcome its strong natural defenses. UTIs are the most common of all infections and can occur at any time in the life of the individual [1]. The commonest cause of UTIs represented by Gram negative bacteria belong to *Enterobacteriacea* family specially *Escherichia coli* accounting for as much as (80-85%) of community acquired UTIs. *Staphylococcus saprophyticus* , *kleibsella spp*, Pseudomonas and rarely viral or fungal infections such as candida albicans and *Enterococcus spp* can be a cause[2]. *E.coli* bacteria have several virulence factors such as hemolysin, cytotoxic necrotizing factor, aerobactin, biofilm and different types of adhesion factors which have been responsible for *E. coli* pathogenesity causing UTIs [2]. Hemolysin is a protein can induce osmotic lysis of erythrocyte because of its bore forming activity and cytotoxic to several types of human cell. *E. coli* can produce several types of hemolysin including extracellular protein (α-hemolysin), cell bound protein (β- hemolysin) and a hemolysin produced by nalidexic acid resistant mutant (γ-hemolysin) [3,4]. Biofilm is a micro colony of bacteria enclosed by complex materials of protein and polysaccharide that form on living tissue and medical device such as catheter [5]. Adhesion and biofilm formation protect the bacteria from tissue immunity, increasing antibiotic resistance and bacterial ability to multiply and invade host tissue, which lead to successfully initiation of infection. The genes responsible for adhesion called *Aap* and *Afa* genes. *Pap* gene coding for flagella formation aid bacteria to motile into tissue cells ,and afa gene coding for billi formation which aid bacteria to attach with epithelial cells of urinary tract [6]. Other important genes such as *Iha* that responsible for capsule formation to aid bacteria attachment and protect it from immune cells attack, and *Irp2* which responsible for iron up take for nutrition of bacteria(4). The virulence factors of *E. coli* are located on large plasmids and/or in particular region called pathogenesity island (PAIs) on the chromosome. The polymerase chain reaction (PCR) assay was developed which allow the simultaneous detection of virulence genes by using specific primers that amplify this virulence genes encoding for virulence pathogenic traits of uropathogenic bacteria(7).

The aims of this study are to define the most virulence factors of *E.coli* that causing UTI in some patients including diabetics, and showing the effect of this virulence in culture and sensitivity results in urine samples.

Patients and methods:

This is a prospective study done on 180 patients with age ranging from 18 up to 80 years of both genders with a features of acute UTI who were visited to the Al-Diwaniya Teaching Hospital from January 2014 to June 2015. Other 90 seem to be a health persons were taken as a control group .

The UTI patients were diagnosed according to the clinical and laboratory criteria. The clinical criteria based on the clinical symptoms and signs such as dysuria , frequency , urgency, fever , loin pain and other constitutional symptoms, while the laboratory criteria were including the general urine examination (GUE), define the turbidity of urine, and presence of leukocyte, albumin and some time bacteria seen, and presence of positive urine culture with at least 10^5 CFU of bacteria /ml.

The patients with any types of renal surgery, patient on steroid and other immunecomprmised drugs and pregnant women were excluded from this study.

All patient included in this study and the control group were undergo with detailed history and physical examination . Renal function tests, liver function tests, fasting blood sugar and HbA1c, and ultrasound of

abdomen were done to all patients. From all patient and control groups, Mid stream urine samples were taken in sterile cups and sends for:

1. Direct smear to study macroscopic and microscopic features by general urine examination.
2. Culture urine samples on selective (Mackonky agar) and enrichment media (blood agar) to uptake standers colonies.

After growth appear on culture, the bacterial was isolate and send for:

1. Biochemical tests to identification the *E.coli* bacteria virulence factors (oxidase, heamolysin, catalase, urase, gas production , motility test, citrate utilization, malonate utilization,methyl red test).
2. Culture and sensitivity done by cultured isolates on muller hinton agar to make dugs sensitivity test.
3. DNA extraction by taken some of isolates and saved in append of tube with 1 ml of normal saline to make DNA extraction by using DNA extraction kit .
4. DNA samples using to detect virulence factors genes by PCR technique and using specific thermal program and specific primers which shown in a picture and table (1 and 2).

Table (1): virulence factors genes and its function in this study

Viruleance factor genes	function
Pap	Pilli(fimbrial adhesion
Afa	flagella
Hly	Heamolysin
Iha	Capsule
irp2	Iron uptake

Table (2): show the figures of DNA primers.

Reference	Size	(5'-3')nitrogen base sequence	Primer type
(15)	336 bp	GCAACAGCAACGCTGGTTGCATCAT AGAGAGAGCCACTCTTATACGGACA	*Pap*
(16)	750 bp	GCTGGGCAGCAAACTGATAACTCTC CATCAAGCTGTTTGTTCGTCCGCCG	*Afa*
(17)	413 bp	AAGGATTCGCTGTTACCGGAC AACTCCTGATACAGGTGGC	*Irp 2*
(17)	824 bp	ACTATTCTCTGCAGGAAGTC CTTCCGATGTTCTGAACGT	*Tsh*
(15)	113 bp	AACAAGGATAAGCACTGTTCTGGCT ACCATATAAGCGGTCATTCCCGTCA	*Hly*
(18)	827 bp	CTGGCGGAGGCTCTGAGATCA TCCTTAAGCTCCCGCGGCTGA	*Iha*

Statistical analysis was performed by Social Science Statistics and the Statistical Package For Social Sciences version 17 for Windows Software and Microsoft Excel 2013. All these statistical tests considered that P- value less than the 0.05 level was statistically significant.

Results: From 180 patients with acute UTIs, one hundred thirty seven patients were the underlying microorganism (m.o.) was E.Coli, while other m.o. causing UTI to the other forty three. Microscopic examination show to the bacteria as bacilli shape ,aggregated in pairs and single, and gram negative , this results agreed with many studies done for detection of this bacteria [8,9]. The result of culture and identification E.coli bacteria showed the colonies was soft, small, in size about 1-3 mm, and surrounded by hemolytic zone (β - heamolysis) on blood agar , and cultured sample on Mackonky , the colonies appeared shiny, pink color , small and soft ,about 1-2 mm. Biochemical tests revealed positivity of E.coli to maltose, catalase, iosin methylene blue, indol test, methyl red, gas production test, manitol and lactose[10] .

Table (3) will revealed the patients and control candidates age distribution, which shows that the youth age (20-30 years) and elderly age groups are more infected than others age group. The mean age of patient group was 41.35±8.6 year with a male / female ratio was 28.5/71.5 while the age of control group was 40.13±7.23 year with a male/female ratio was 35/65
It is obviously that middle age group was the main group included in the study; which reflect the widely distributed of UTIs in this age group specially in female patient because increasing the incidence of UTIs and this agree with other study done in Vitnaam and america.

Table (3): case-control in the mean of age and gender.

	Patient (no.=137)	Control (no.=90)	P value
Age (year)			
Range	18-80	18-80	0.55 {Ns}
Mean ± SD	41.35± 8.6	40.13±7.23	
SE	1.06	1.94	
Gender	No **(%)**	No **(%)**	P value
Male	39(28.5)	31(35)	0.675 {Ns
Female	98(71.5)	59(65)	0.532 {Ns}

Table four would demonstrate the assessment of body mass index (BMI) in patient and control groups which is high in patients group. The BMI is high in patient group in comparing with control group which might be reflect the increase the incidence of UTIs in obese patient.

Table (4): case-control difference in the mean of BMI.

	Patient (N=137)	**Control (N=90)**	P value
BMI			
Range	21-39	22-36	
Mean ± SD	30±3.16	28±2.14	0.64 {Ns}
SE	0.28	0.32	

Table five will revealed the distribution of diabetes mellitus (DM) in patient group. In this table the UTIs patients will divided into two subgroups according to present or absent of DM. Also this table show the duration of DM and HbA1c range. Fifty one patient had diabetes: with duration ranging from less than one year to ten year, most of them are poorly control with mean HbA1c about 7.1 which still above our goal management of DM. more than 75% of diabetic patient are female which might reflect the high incidence of UTI in women might be related to the urethral anatomy in women.

Table (5): distribution of DM in patient group.

		No.	(%)	
Non DM		86	(62.7%)	
DM (female)		51 (39)	(37.3%)	
		Range(mo)	mean±SD	SE
	Duration	6-120	43±53	0.12
	HbA1c	4.5-14.6	7.1±61	0.24

Table six will show the presence of virulence factor genes in diabetic patient with acute UTIs comparing to non diabetic patients. The all virulence factor of *E. Coli* are present in patient with DM, this might reflect the acuteness and severity of UTI in diabetic patient.

Table (6): distribution of virulence factor genes in DM and non DM patient.

Virulence factors gene	DM	Non DM
Pap	yes	yes
Afa	yes	yes
Hiy	yes	No
Iha	yes	No
Irp2	yes	No

Table (7) distribution of virulence factors genes in patient group according to their age group

Genes	**<20**	**20-39**	**40-59**	**60-80**
Pap	Yes	Yes	Yes	Yes
Afa	No	No	Yes	Yes
Hiy	Yes	No	Yes	Yes
Iha	No	Yes	No	Yes
Irp 2	Yes	Yes	Yes	Yes

Table seven will appear the distribution of virulent factors according to the age group patient. All the virulent factors will present in the middle and elderly, this might be explained the severity of UTI in advance age, also this might be reflect the impairment of immunity in elderly patient.

Table (8): sensitivity and resistance to various type of antibiotic

Antibiotic	Sensitivity	Resistance
Amikacine	75%	25%
azithromycine	77%	23%
Gentamycne	32%	68%
Vancomycine	21%	79%
Doxycycline	13%	87%
ciprofloxacine	65%	35%
Amoxillin	31%	69%
Ceftriaxon	79%	21%
Cefotaxime	77%	23%
Cefixim	71%	29%

Table eight was clearly show the high resistance to most widely using antibiotic in our city to patients with UTI, this might be reflect the misusing the antibiotic. The most popular one, Amoxillin, will show very high resistance , also third generation cephalosprine antibiotic will show an increasing of resistance, and this a warning sign.

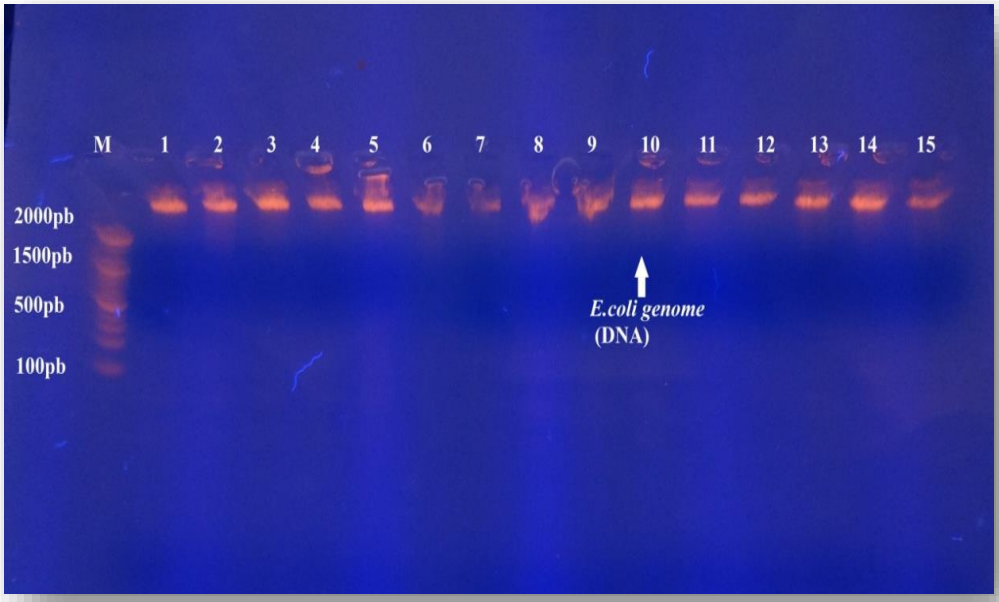

Image (1) Products of DNA Extraction in agarose gel (1.5 %) , volt (100) in about 1 hour for E.coli isolates by using Genomic DNA mini kit (M= DNA ladder 100- 1500 bp)

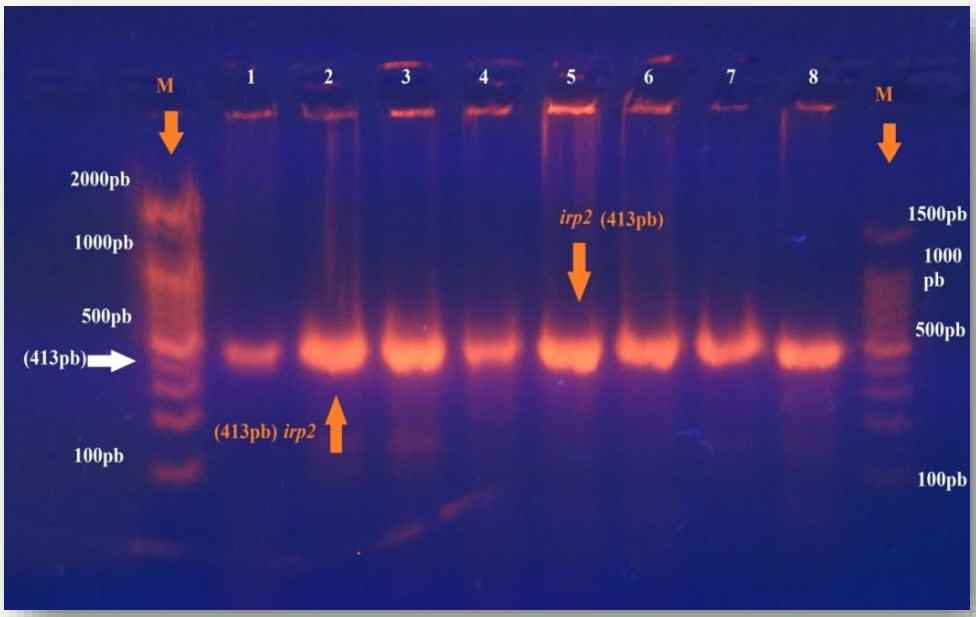

Image (2) Products of irp2 amplification in agarose gel (1.5 %) , volt (100) in about 1 hour for *E.coli* isolates by using Genomic DNA mini kit (M= DNA ladder 100-1500 bp)

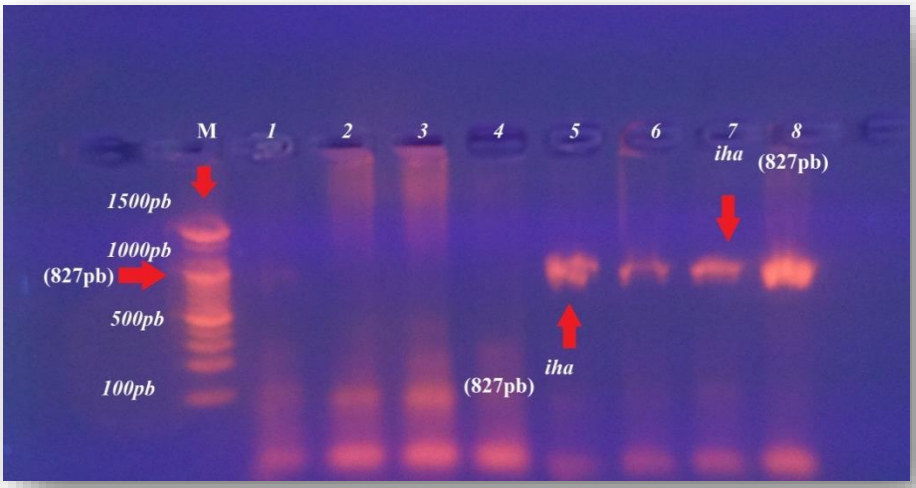

Image (3) Products of iha amplification in agarose gel (1.5 %) , volt (100) in about 1 hour for *E.coli* isolates by using Genomic DNA mini kit (M= DNA ladder 100-1500 bp)

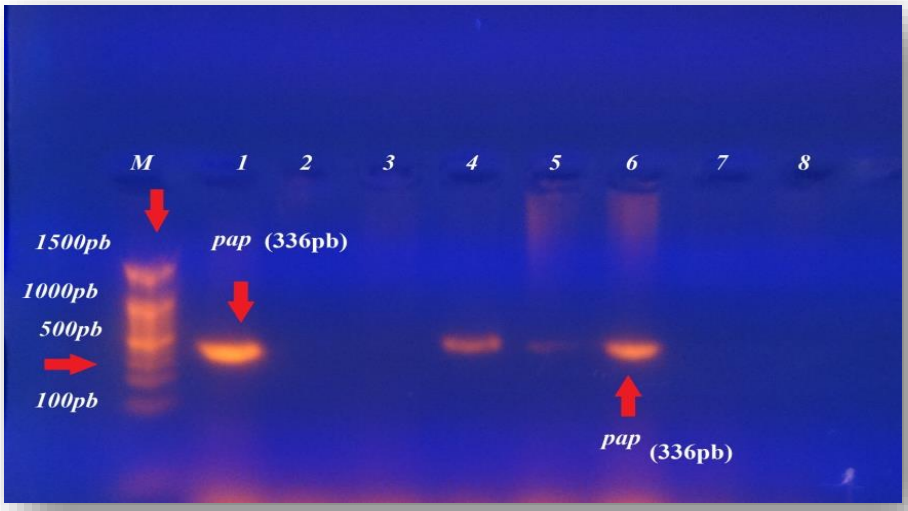

Image (4) Products of pap amplification in agarose gel (1.5 %) , volt (100) in about 1 hour for *E.coli* isolates by using Genomic DNA mini kit (M= DNA ladder 100-1500 bp)

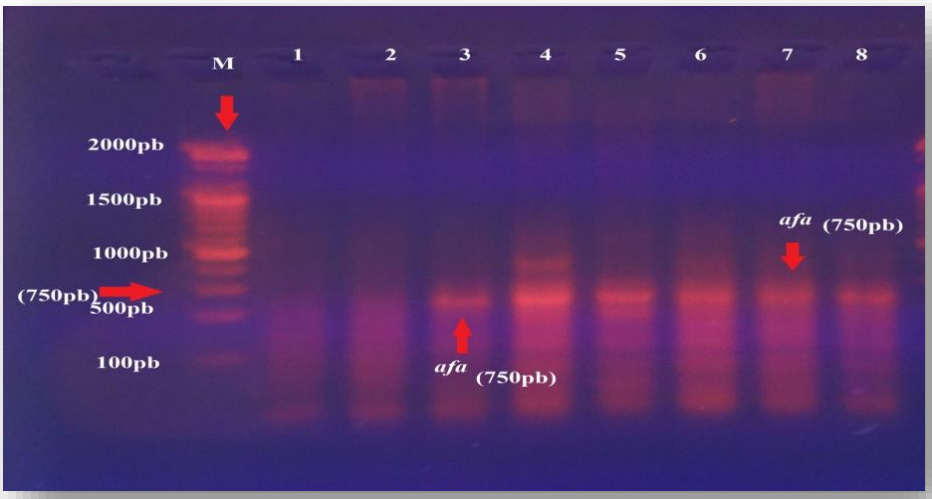

Image (5) Products of afa amplification in agarose gel (1.5 %) , volt (100) in about 1 hour for *E.coli* isolates by using Genomic DNA mini kit (M= DNA ladder 100-1500 bp)

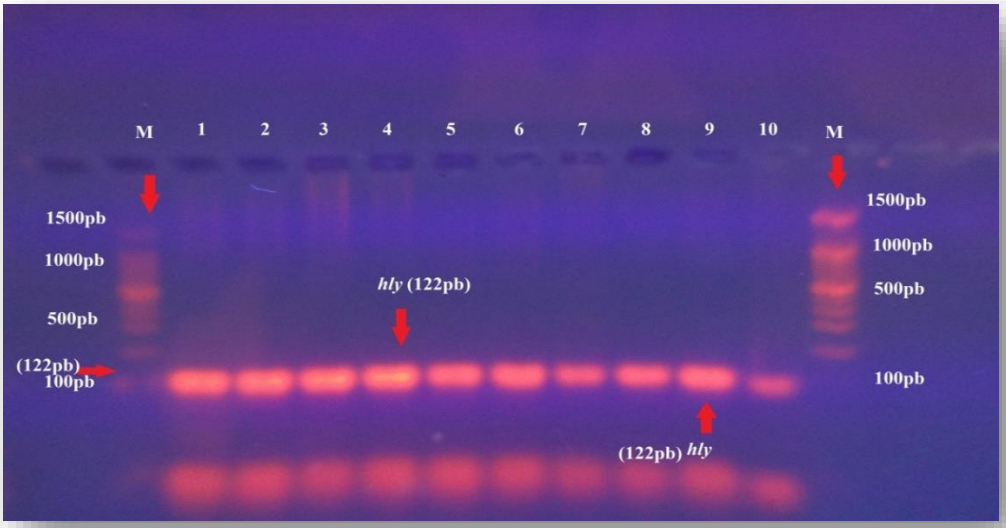

Image (6)): Products of *hly* amplification in agarose gel (1.5 %) , volt (100) in about 1 hour for *E.coli* isolates by using Genomic DNA mini kit (M= DNA ladder 100-1500 bp)

Discussion:

Urinary tract infection still the most common acute infection in the worldwide. Its incidence is high in women which might be related to their anatomical short urethra. In our study, 76% of patients with acute UTI causing by *E. Coli* bacteria while other 24% belong to other types of bacteria , this result agreed with many studies in America , Philippine, Germany and south Africa [11,12,13] . The diagnose of E Coli done by microscopic features, cultures finding and various biochemical study, the results agreed with study done by Johnson, J.R, et al [7,8,14].

The collected samples from patients with UTI in this study and used all investigations roles of safety. The high incidence of E Coli in acute UTI may be belong to ability of *E.coli* to adapt in urinary tract of human in highly degree because the ability to living in environmental conditions of this tract and having the virulence factors which are very important in infection such as attachment ability to epithelia cells receptors by using unique fimbrea , and this step considered the first step in infection and bacterial invasion, and having other virulence factors such as capsule synthesis , toxin productions , iron uptaking and other factors aid it to make infection, this result agreed with studies in south Japan and British [15,16], while other reasons make *E.coli* more than other bacteria in UTI by ability of bacteria to passing from anus it was found in it as normal flora to urethra and cause infection, this result agreed with study in America [17]. In field study of relationship between sex and UTI the result showed to infected of female with UTI is more than male , in this study infect 98 (78.5%) , in other hand the number of infected male reached to 39(21.5%), this agreed with Indonesia and Malaysia [18]. The reason of this result belong to nearing of anus to urethra and short urethra in female compares with male, in addition the presence of prostatic secretion which act as an antibacterial. In age field ,this study showed to youth group (20-30%) is more infected by UTI , 39 patients (28.49%) , followed by age group (40-50 years) , 36 patients (26.29%), this result agreed with studies in Sudia, south Africa , Egypt [10,19,20]. This finding might be related to high sexual activity in youth age group especially the females, other causes such as the changes in PH during menstrual cycle that lead to changes in normal flora and aid bacteria to grow in vagina and passing to urethra [21]. In

elderly the cause of infection belong to decrease in general immunity, presence chronic disease, decrease in systemic function such as staying urine residue in bladder, in male enlargement of prostate , all this reasons causes the high incidence of infection in old age group [20].in this study we used polymerase chain reaction technique (PCR) to detect some virulence factors genes, in *E.coli* bacteria, like *iha* (capsule formation gene), *pap* (fimbrea formation gene), *irp2* (iron uptake gene), *hly* (heamolysin production gene), afa (flagella formation gene). The results showed all these virulence factors genes had isolated in diabetic patients, the reasons belong to decrease in general immunity of patients with diabetes mellitus and that lead to impaired resistance from body tissue against bacterial virulence [22]. The results of antibiotics sensitivity tests showed the different between sensitivity and resistance among all patients, that mean the diabetic patients appear resistance for one antibiotic, while the non diabetic patients appear sensitivity for same antibiotic [23,24]. The high resistance rate was more common with Amoxillin and Doxycycline antibiotic which reflect the misuse of these antibiotic. Other antibiotic will show an increasing resistance.

Conclusion:

1. *E.coli* bacteria are more causative for UTI than other bacterial types.
2. The effect of antibiotics is vary among patients because of physiological and pathological changes.
3. Virulancy of *E.coli* differ from patients to another as a result of chronic disease, pregnancy and other condition.
4. Youth age group (20-30 years) are more exposure to infection with *E.coli* UTI , followed by (40-50 years) age group.
5. *E.coli* UTI is more common in female than male patients.

Recommendation:

Detection of virulence factors in infected individuals may be predict the response for treatment.

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