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DETECTION OF SOME VIRULENCE FACTORS AND ANTIBIOTICS SUSCEPTIBILITY OF *ESCHERICHIA COLI* ISOLATED FROM UTI PATIENTS

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ABSTRACT : This study have been included 300 samples of mid-stream urine, each 50 samples have been taken from different sources which are: Inpatients, Diabetic patients, Pregnant women, Primary school students, Patients with crystalluria and patients with failure to thrive. These samples have been collected from Baquba Teaching Hospital, Consultant Department of Al-Batool Hospital and primary coeducation schools of Al-Ghiraf and Al-Sabteia in Diyala Governorate, during the period from 26-10-2017 to 27-3-2018, these samples cultured on selective media for isolation and diagnosis of bacterial infection of urinary tract depending on cultural, microscopical and biochemical testes and molecular identification by PCR. The results of bacterial culture have been recorded that only 115 (38.33%) samples had positive results and*Escherichia coli*form 25 isolation (23.35%). Prevalence of *Escherichia coli* isolates more in female than in male (60% & 40%), patients in age group e"50 year were the commonest (48%), the prevalence in rural population (56%) was more than in urban population (44%) and the rate in the six groups: Inpatients, diabetic patients, pregnant women, primary school students, patients with crystalluria and patients with failure to thrive were (29%, 26%, 22.2%, 0%, 17.6%, 33.3%) respectively. The result of molecular identification of *Escherichia coli* isolation displayed that it was 16S rRNA carrier in 100%, and the result of detection for *kpsMII* and *fimH* genes showed that appearance rate 100% and 88%, respectively. Prevalence of virulent factors in isolates which include: Adhesion to epithelial cells, Capsule formation, Lipase production, Biofilme formation by tubes and Congo Red methods, Protease production and Hemolysin were as follow (100%, 88%, 84%, 64%, 84%, 12%, 0%) respectively. The isolates showed clear variations in resistance against antibiotics.

Key words : *Escherichia coli*, UTI, PCR, virulence gene, antibiotics.

INTRODUCTION

Urinary tract infection (UTI) refers to any infection of the Urinary tract by microorganism, which consists of urethra, urinary bladder, ureter and kidneys (Montini *et al*, 2011). Bacteria are the main causative agent of infection among other microorganisms such, Gram negative bacteria are the main cause of UTI, Gram positive bacteria also cause UTI but in less rate. *Escherichia coli* (*E. coli*) considered as the main cause of urinary tract infection (70-90%) of community acquired infection and (50%) of nosocomial infection (Kudinha *et al*, 2012). *E. coli* which is responsible for the UTI (Uropathogenic *E. coli* (UPEC)) has many traits that are lacking in commensal one. The main chromosome of this bacteria carries the gene clusters on the pathogenicity islands that encode a number of virulence factors). Whereas this bacteria possessesfimbiria or pili that enable them to adherent to the epithelial cells after entering the urinary tract, so this step is important for occurrence of infection, then bacteria extend to form small colonies in the mucous lining of the bladder to form the biofilm, which then begins with the gene expression of the genes that encode toxins, siderophoras, lipopolysaccharids (LPS) and capsule. These factors determine the severity of the disease and the virulence of the strains that cause UTI (Oliveira *et al*, 2011). In view of the importance of urinary tract infection, which is a common health problem in society, and because *E. coli* is the most common cause of infection, this study was carried out to isolate and diagnose these bacteria causing infection of different age groups and both gender from Inpatients, diabetic patients, pregnant women, primary school students, patients with crystalluria and patients with failure to thrive, detection antibiotic sensitivity and some virulence factors and the presence rate of genes responsible for adhesion and capsule formation of *E.coli* by PCR.

MATERIALS AND METHODS

Collection of samples

Total of 300 samples of mid-stream urine, these samples were 137 from male, 163 from female, from different age groups, 203 from rural population and 97 from urban population, and each 50 samples have been

taken from six different sources. These samples have been collected from Baquba Teaching Hospital, Consultant Department of Al-Batool Hospital and Primary Coeducation schools of Al-Ghiraf and Al-Sabteia in Diyala Governorate, during the period from 26-10-2017 to 27-3-2018 in sterile cans.

Culture of samples

Samples were cultured directly on Blood agar and Maconkey agar, Eosin Methylene Blue (EMB) medium the dishes were incubated for 24 hours at 37°C. The growth isolates on MacConkey agar were identified depending on the morphology, general characteristics of colony and biochemical tests (Collee *et al*, 1996).

Antibiotic sensitivity test

The Kirby-Bauer disc method was used for the purpose of sensitivity of *E. coli* for (18) antibiotics by disc diffusion technique by serial-cotton swabs on Mueller-Hinton agar. The inhibition zone diameter was measured and recorded and the results comprised with (CLSI, 2017).

Detection of Virulence factors of *Escherichia coli*

The detection of 0f *Escherichia coli* virulence factors according to Collee *et al* (1996).

Haemolycin production

The isolates of *E. coli* were cultured on bloodagar, the dishes were incubated at 37Cº for 24 hours. Hemolysis production was detected by the appearance of the hemolysis zones around the bacterial colonies.

Protease production

Skime-Milk-Agar medium inoculated with pure colonies of *E.coli*, dishes incubated at 37Cº for 24 hours, then the results were readon the base (Collee *et al*, 1996).

Lipase production

Egg-Yolk Agar medium inoculated with pure colonies of *E. coli* by making a line in the middle of the dish surface containing the medium, the dishes incubated for 24 hours at 37Cº. The appearance of the decomposition around the inoculated linewas the positive.

Adhesion of bacteria to epithelial cells

About 0.5 ml was taken from bacterial cell uspension which prepare from transfer of pure colonies to 4ml of normal saline and mix with 0.5mL of human epithelial cell suspension, which prepared. The mix incubation at 37ºC for 1 hour with shaking every 10 minutes, cells were washed after incubation period for four times by using the normal saline with centrifuge (1000 cycles/min) for five minutes for each time to eliminate of the non-adherent bacteria. Drop was taken from final suspension, put on a clean glass slide and left to dry at room temperature, fixed by flame, and stained with gram stain then the results of adhesion of the bacterial isolates to the epithelial cells were examined (Lomberg *et al*, 1986).

Possession of the capsule

The Shan method was used to detect the possession of *E. coli* isolates capsule. A small drop of Nigrosin stain was used, viewing a transparent halo around the bacterial cells indicated that the isolate possessecapsul (Tortora *et al*, 2007).

Molecular detection of virulence factors

The isolates of *E. coli* were diagnosed by detecting the 16S rRNA gene. *fimH* and *kpsMTII*genes were also detected in these isolates using PCR method. This method included the following steps:

Isolation of Genomic DNA

Genomic DNA is extracted from the isolates of *E.coli* using a commercially available kit (Wizard® Genomic DNA Purification Kit / Promega), according to the manufacturer's instructions installed in the working method. A Spectrophotometer NanoDrop was used to measure the concentration and purity of DNA (Sambrook and Russel, 2001).

Primers Preparation

The primers used in this study which are described in sequence and the size of their products, were presented in Table 1 were prepared according to the manufacturer's instructions installed in the working method ofequipped company (Alpha DNA). The PCR Mixture Preparation

Table 1 : Sequences and output size of productsof the primers used in the study.

Primer	The initiator sequence (3'- 5')	Output size bp	Reference
16S rRNA	F- GGAAGAAGCTTGCTTCTTTGCTGAC	544	Sabat *et al* (2000)
	R-AGCCCGGGGATTTCACATCTGACTTA		
fimH	F: TGCAGAACGGATAAGCCGTGG	508	Johnson and Stell (2000)
	R: GCAGTCACCTGCCCTCCGGTA		
kpsMTII	F: GCGCATTTGCTGATACTGTTG	272	Johnson and Stell (2000)
	R: CATCCAGACGATAAGCATGAGCA		

was prepared according to the manufacturer's instructions of Bionear. The machine was then programmed to provide optimal conditions for multiplication steps of used genes.

Gel Electrophoresis

The Agarose gel was prepared at 1.5% concentration, according to Sambrook and Russell (2001).

Statistical analysis

Chi-square test was done using SPPS version 7.5 computer software.

RESULTS AND DISCUSSION

The results of the diagnosis showed that the number of isolates of the Gram negative bacteria formed 60 (56.60%) of the total samples (300 samples) and that *E. coli* was the most 25 isolates (23.35%) characterized by its ability to ferment the lactose when growing on the MacConkey agar. All the isolates of *E. coli* were positive for the tests of Catalase, Methyl Red and Indole and negative for Oxidase, Vogues-Proskauer, Citrate Utilization, and Urease. The results of the molecular diagnosis by PCR to detect the presence of a 16s rRNA gene in 25 isolates which diagnosed by classic methods as *E. coli* showed that all isolates were carrier of the gene (100%) (Fig. 1).

Distribution of *Escherichia coli* isolates by gender, age and residence

The results of the study showed that *Escherichia coli* isolates for urinary tract infection were higher in females than males (9.25% and 7.24% respectively) and the study did not record significant difference. The present results were agreed with He *et al* (2018). The results of the diagnosis of isolated bacteria and by age group showed that the highest percentage was in the age group of 50 years or more (16.66%) with a significant statistical difference of (p = 0.001), the results did not agree with the findings of Hassooni *et al* (2018) in Al-Kut city where *E. coli* had more isolation from age group (10-40) years. The results showed that isolates were higher in rural than in urban (12.3% and 6.4%) this agreed with the study results of Sheikhs and Jassim (2017) (Table 2).

Table 2 : The distribution *of* 25 *Escherichia coli* isolate from UTI patient according to gender, age and residence.

Patient		No of sample	No of isolate	%	P.value
Gender	Male	137	10	7.24	0.317
	Female	163	15	9.25	
Age	1-10	85	5	5.88	0.001
	10-20	48	1	2.08	
	20-30	53	4	7.54	
	30-40	26	2	7.69	
	40-50	16	1	6.25	
	50≥	72	12	16.66	
Residence	Rural	203	13	6.4	0.841
	Urban	97	12	12.3	

Table 3 : Distribution of 25 *Escherichia coli* isolate according to source of sample.

Patient	No of sample	No of isolate	%	P.value
Inpatient	50	8	16	0.238
Diabetic Patients	50	6	12	
Pregnant Woman	50	4	8	
Primary School Students	50	0	0	
Patient with Crystalluria	50	3	6	
Patient with Failure to Thrive	50	4	8	

Table 4 : Virulence factors of (25) *Escherichia coli* isolates.

The virulence factor	Number	%
Hemolycin Production	0	0
Protease Production	3	12
Lipase Production	21	84
Adhesion to epithelial cells	25	100
Possession of the capsule	13	52

Distribution of *Escherichia coli* isolates by sample source

Table 3 shows the distribution of *E.coli* isolates according to the source of the sample. The percentages of Inpatients, Diabetic Patients, Pregnant women, Primary School Students, Patients with Crystalluria and Patients with Failure to Thrive were 29%, 26%, 22.2%, 0%, 17.6% and 33.3 respectively. The difference in Isolation rates may be due to the nature of the country, the environmental and living conditions of individuals.

Hemolysin production

Table 4 shows that all isolates were non-producing of hemolycin, the result is similar to the results of Al-Janaby and Al-Faham (2017). The study found that *E.coli* isolates from urine samples were produced by hemolysin.

Protease production

Table 4 showed that three isolates only were enzyme-producing (12%). This is an approach to Bhattacharyya *et al* (2016), who found that all isolates of *E.coli* were not enzyme-producing.

Lipase production

The results of the present study showed that 21 isolates (84%) produced lipase (Table 4). The results were similar to Jaafar *et al* (2015) in Tikrit where all isolates of *E. coli* isolated from cases of diarrhea in children were

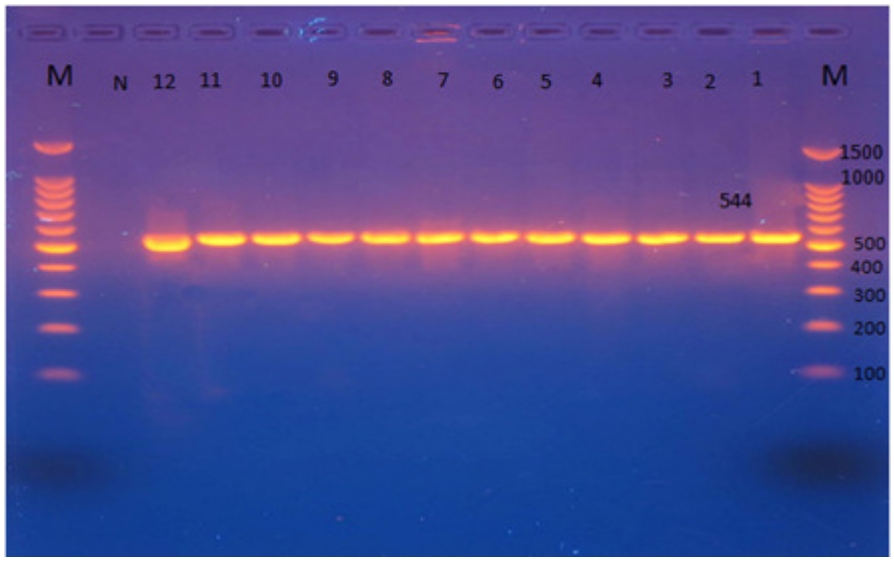

Fig. 1 : Electrophoresis of amplified 16S rRNA (544bp) for *Escherichia coli* by 1.5% agarose gel, using DNA Ladder, 1.5 V/cm for 1 hrs, stained with ethidium bromide dye and visualized under UV transilluminator documentation. All lanes are shown to be have the gene. N lane represents negative control (containing reaction mixture components except template DNA.

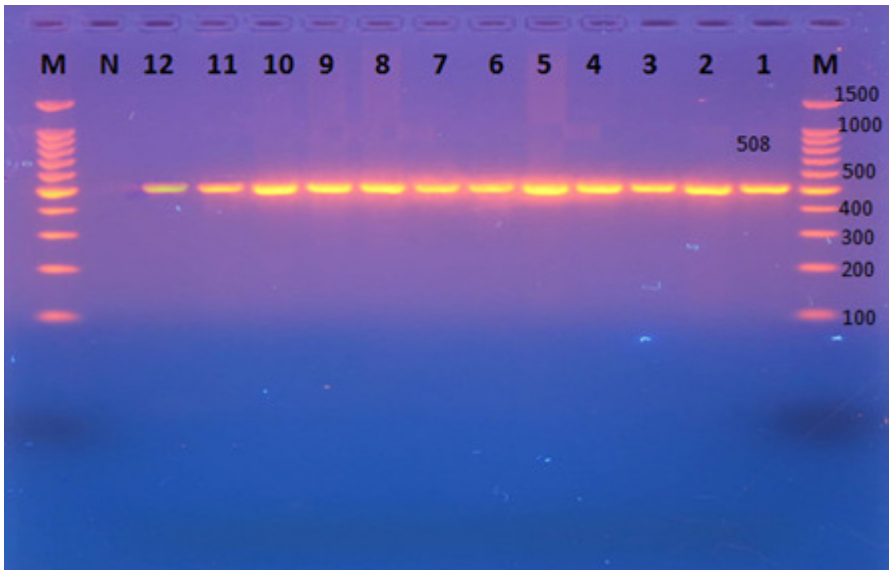

Fig. 2 : Electrophoresis of amplified *fimH* (508bp) for *Escherichia coli* by 1.5% agarose gel, using DNA Ladder, 65 V/cm for 1 hrs, stained with ethidium bromide dye and visualized under UV transilluminator documentation. All lanes are shown to be have the gene. N lane represents negative control (containing reaction mixture components except template DNA.

enzyme-producing.

Adhesion to epithelial cells

All isolates of *E. coli* had the ability to adhere to the epithelial cells (Table 4). The results of the present study corresponded to what reached by Al-Janabi and Al-Faham (2017) in Kufa.

Capsule possession

Table 4 showed that 13 isolates (52%) out of 25 isolates possesse capsule. The results were consistent with the findings of Al-Musawi and Al-Hassani (2012) in Diwaniyah which showed that (57.5%) of *E. coli* isolates from UTI had capsule.

Sensitivity of *Escherichia coli* to antibiotic

The results of the sensitivity test shown in Table 5 showed that isolates of *Escherichia coli* were 100% resistant to Rifampin, Ampicillin and Clarithromycin, the results were similar to those found in Assafi *et al* (2015) in Dohuk (Salih *et al*, 2016) in Tikrit and the resistance ratio to Imipenem (12%) was close to that of Hussein (2014) in Baghdad which found that resistance of *E. coli* to this antibiotic was 10.3%. The isolates were recorded less resistant to Amikacin (4%), the reults were similar to those of Al-Jebouri and Mdish (2013) in Tikrit where *E. coli* isolate from urinary tract infection was resistant

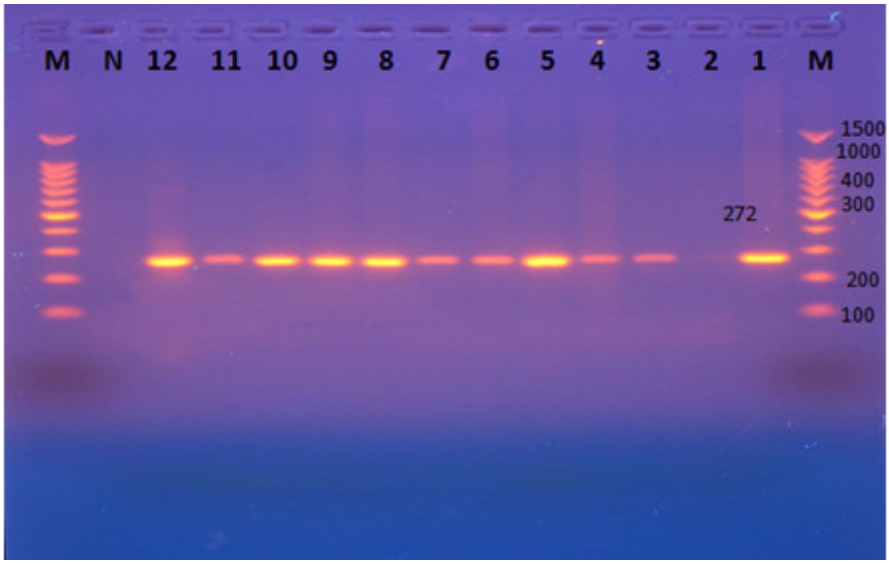

Fig. 3 : Electrophoresis of amplified *kpsMTII* (272bp) for *Escherichia coli* by 1.5% agarose gel, using DNA Ladder, 65 V/cm for 1 hrs, stained with ethidium bromide dye and visualized under UV transilluminator documentation All lanes are shown to be have the gene except lane 2 does not contain gene. N lane represents negative control (containing reaction mixture components except template DNA.

Table 5: Sensitivity of 25 *Escherichia coli* isolates to antibiotics.

Antibiotics	Sensitive		Resist	
	No	%	No	%
Cefuroxime (30ug)	1	4	24	96
Rifampin (5ug)	0	0	25	100
Ciprofloxacin(5ug)	19	76	6	24
Ampicillin (25ug)	0	025	100	
Aztreonam (30ug)	2	8	23	92
Nitrofurantion(30ug)	23	92	28	
Trimethoprim-Sulphamethoxazole (25ug)	11	44	14	56
Gentamicin(30ug)	20	80	5	20
Clarithromycin(15ug)	0	025	100	
Piperacillin (100ug)	3	12	22	88
Doxycyclin (30ug)	15	60	10	40
Amikacin (10ug)	24	96	1	4
Ceftriaxone (30ug)	3	12	22	88
Trimethoprim(5ug)	14	56	11	44
Imipenem(10ug)	22	88	3	12
Amoxicillin-Clavulaniacid(30ug)	2	8	23	92
Cefotaxime(30 ug)	1	4	24	96
Ceftazidime(30ug)	2	8	23	92

in (5%) to this antibiotic and was higher than that of Al-Hamdany (2018) in Babylon, where there was no *E.coli* isolate resistant to Amikacin.

Detection of adhesion gene(*fimH*)

The results of the detection of the *fimH* gene showed that all isolates (100%) were carrier (Fig. 2). The results were consistent with the findings of Al-Jebouri (2017) in Diyala, where the percentage of *fimH* in *E.coli* isolate which isolated from children with urinary tract infections was 100%. The *fimH* (type 1 fimbrial adhesion) gene regarded as virulence gene for adhesion factor proteins and this gene plays an important role in attachment to host cells and the formation of biofilm in *E. coli* pathogenic urinary tract.

Detection of capsule formation gene (*kpsMTII*)

The results of the *kpsMTII* gene revealed that 22 (88%) isolates carrier the gene (Fig. 3). The results were similar to that of Yun *et al* (2014) in Korea where (93.3%) *E. coli* isolates from UTI were carrier this gene, this result similar to Abed *et al* (2014) in Baghdad, where 72% of *E.coli* isolates in children with UTI were carrier this gene. *kpsMTII* regard as encoded gene to capsular proteins or polysaccharide lipid membrane.

CONCLUSION

The results indicate that the *Escherichia coli* isolates have many virulence factors that enhance their ability to UTI occurrence, including protease and lipase production, adhesion of human epithelial cells and capsule possession. *E.coli* showed variation in antibiotic sensitivity. The molecular detection of *fimH* and *kpsMII* distribution in bacteria revealed the presence of both genes by 100% and 88%, respectively.

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