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RESEARCH ARTICLE OPEN ACCESS

Immunological and Molecular Study of Interleukin-17A and Uropathogenic *E. coli* among Patients in Holy Karbala, Iraq

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Abstract

The current study amid to investigate association of Interleukin-17A with uropathogenic *Escherichia coli* among patients with urinary tract infection in karbala province, Iraq. Bacterial infections are widespread in urinary tract infections with a global extention. Uropathogenic *E.coli* (UPEC) is the most common causes of these infections. Out of 110 patients were examined by urologists for urinary tract infection, 25 patients showed positive result for UPEC and other 25 showed positive result for other bacterial pathogens. UPEC were diagnosed depended on the cultural, microscopical, biochemical examinations and confirm the identification by using Vitek2 system. Polymerase chain reaction was used to detection of four genes (*pap C, cnfA, fim H, and fyu A)*. Interleukin-17A concentration in urine was measured by using ELISA kit. out of 110 urine samples, 56 (44.90%) with significant bacteriuria, 44(40%) with non-significant bacteriuria and 10 (9.09 %) with negative culture. The presence of UPEC among significant bacteriuria was 25/56 (44.64 %). The distribution of *pap C, cnfA, fim H, and fyu A* genes among UPEC were 17(68%), 17(68%), 16(64%) and 15(60%) respectively. Through UTI patients, 50 gave positive (121.70) pg/ml results compared to 30 of control (13. 94) pg/ml. Among uropathogenic *Escherichia coli* patients, 25 gave positive (92.80) pg/ml results, while 25 of other bacterial pathogens gave positive (15.40) pg/ml results.

Keywords: Uropathogenic *E.coli*, Virulence genes, Molecular diagnosis, IL-17A.

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INTRODUCTION

Urinary tract infections (UTIs) are the most common type of infection in the human[1]. *Escherichia coli (E. coli)* are responsible for about 80% of these infections that have not passed the urinary bladder[2]. Uropathogenic *E. coli* has been seen to employ multiple virulence factors that facilitate the colonization and infected the urinary tract. These virulence factors involve adherence molecules (FimH and PapC adhesion molecules)[3], iron chelating molecule (like FyuA molecule) that bind ferric iron, and finally secreted toxin like alpha-hemolysin and cytonecrotizing factor 1 (CNF 1)[4]. One of the most immune defenses against uropathogenic *E.coli* is the IL-17A. It is upregulated from six hours to one week after inoculation, and remained over the baseline through the two-week of experimental duration[5]. Interleukin-17A plays role in the innate immune response of infection by enhancing neutrophil migration to infected tissue[6]. The aim of the study was to evaluate the role of IL-17A as the immunological marker and fimberial adhesin of UPEC in patients with urinary tract infections.

MATERIALS AND METHODS

Patients

One hundred and ten patients were clinically diagnosed that have UTIs in the outpatient urology clinic in Imam AL-Hussain Teaching Hospital in Karbala City/Iraq. The study was performed between September 2017 to the February 2018.

Diagnosis of bacteria

All urine samples were cultured on MacConkey agar and incubated overnight at 37°C. *E. coli* identification was done depending on morphological features biochemical tests (methyl red, indole, Voges-Proskauer, Simmons citrate and urease production)[7] and confirmation by using Vitek-2 system (bioMerieux) .

Molecular diagnosis of uropathogenic *E.coli* genes

Extraction of the DNA

Bacterial DNA was extracted by using commercial kit from Geneaid manifecture's (Korea). The primers for (*pap C, cnfA, fim H, and fyu A*) genes were according to[8,9,10]. The sequence of the primer and amplification condition of PCR as in Table 1.

Table 1. Primers sequence and PCR amplification conditions

Genes	Primer sequence (5'-3')	PCR condition	Size (bp)	Reference
papC	F-GACGGCTGTACTGCAGGGTGTGGCG	94°C/5min 1x	328	Le bouguenec *et al.,* 1992
	R-ATATCCTTTCTGCAGGGATGCAATA	94°C/2min 65°C/1min 25x 72°C/2min 72°C/7min 1x		
fimH	F-TGCAGAACGGATAAGCCGTGG R-GCAGTCACCTGCCCTCCGGTA	94°C/5min 1x 94°C/1min 63°C/30s 30x 72°C/3min 72°C/3min 1x	508	Obata-Yasuoka *etal.,* 2002
cnfA	F- GCAGTCACCTGCCCTCCGGTA R-CATTCAGAGTCCTGCCCTCATTATT	95°C/3min 1x 95°C/1min 60°C/1.5min 35x72°C/3min 72°C/8min 1x	498	Johnson and Stell, 2000
fyuA	F-TGATTAACCCCGCGACGGGAA R-CGCAGTAGGCACGATGTTGTA	95°C/3min 1x 95°C/1min 60°C/1.5min 35x72°C/3min 72°C/8min 1x	880	Johnson and Stell, 2000

Detection of PCR products

PCR products were separated on a 1.5% gel electrophoresis agarose and visualized by ultraviolet light after addition of ethidium bromide.

IL-17A ELISA Test

Urine level of interleukin 17A was determined by using Human Interleukin 17 ELISA Kit (IL-17A, IL17) from CUSABIO/ USA.

Statistical analysis

Data were analyzed using SPSS statistical package version 21. Chi square (x^2), were used to determine the incidence of UPEC among significant bacteriuria. t- test was used to estimate the significant level of the IL-17A[28].

RESULTS AND DISCUSSION

Patients and Clinical Isolates

Out of 110 urine samples, 56 (50.90%) found to be with significant bacteriuria and 44(40%) with non-significant bacteriuria and 10 (9.09%) with negative culture. The presence of UPEC among significant bacteriuria in this study was (25/56) 44.64% and 31 (55.36%) other bacterial pathogens as shown in (Fig. 1).

UPEC is the main causative agent of community-acquired UTIs which colonize the urinary bladder using a different virulence agents that play important roles in the pathogenesis of UTI[4] these involve surface antigenic structures, such as lipopolysaccharide (LPS), polysaccharide capsule, flagella, outer-membrane vesicles, pili, curli, non-pilus adhesins, as well as secreted toxins, secretion systems, and TonB-dependent iron-uptake receptors[11].

The results show significant difference ($P < 0.05$) between the incidence of UPEC and other pathogens among significant bacteriuria. The significant bacteriuria among results was (50.90%) of specimens. These results were higher than results of[12] and[13], whose found that the significant bacteriuria were (22.7%) and (20.8%) respectively, while, lower than result of[14], who reported that the significant bacteruria was (54.80%). As expected *E. coli* was the most frequently encountered species in the present study (44.64%) which was in the same line with the study conducted in Najaf city by[13], who found that *E.coli* (41.3%) was the predominant causative agent of UTI. On the other hand, these results were lower than the results of[15,16,17], whose found that the presence of UPEC were (58.57%), (57.7%) and (67.6%) respectively, while higher than the result of[18], who revealed that the presence of *E.coli* was (33.8%). The differences of these results may be due to different environmental condition and sample size. The highest isolation rate of *Escherichia coli* infection in the urinary tract may be attributed to the contaminated with fecal flora such as virulence *E. coli* that facilitate the ascent of bacteria from fecal flora, up the urethra in to the bladder. The urine negative culture may be attributed to other infections such as viral or fungal infections or may be due to recent use of antibiotic[19].

Molecular characterization of UPEC virulence factors

There was significant difference between the incidence of UPEC and other pathogens at

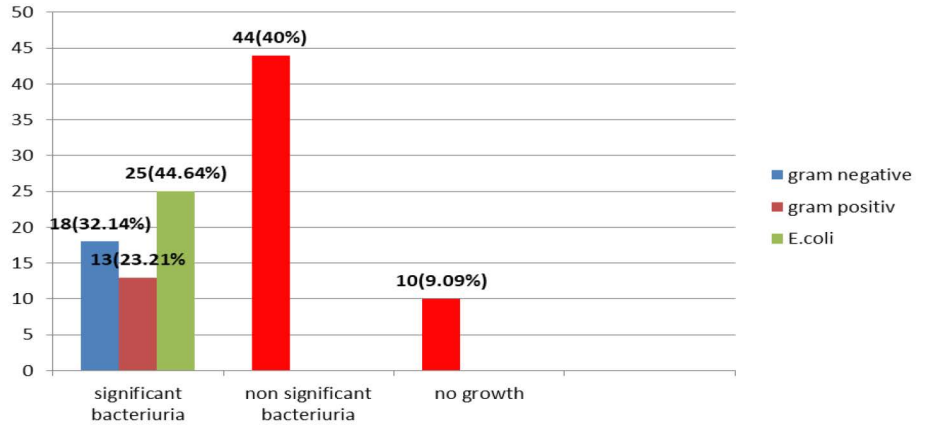

Fig. 1. The percentage of significant bacteriuria, non-significant bacteriuria and negative culture.

*P< 0.05***.** Polymerase chain reaction was used in the current study to identify four potential virulence genes (*papc, fimH, fyuA ,*and *cnfA*) in uropathogenic *E.coli* (Fig. 2-5). Out of 25 uropathogenic *E.coli*, only 5(20%) have all four genes, while the percentage of *papc, fimH, cnfA , and fyuA* were 17(68%), 16(64%), 17(68%) and 15(60%) respectively (Table 2). These genes were present at (328, 508, 498 and 880) bp respectively in PCR amplification.

Table 2. The distribution of uropathogenic *E. coli* virulence genes

Genes	Positive	Negative
papC	17(68%)	8(32%)
fimH	16(64%)	9(36%)
cnfA	17(68%)	8(32%)
fyuA	15(60%)	5(40%)
All genes	5(20%)	20(80%)

A genotypic assay was used to detection of different virulence factors of uropathogenic *Escherichia coli* such as adhesion-encoding genes and other virulence factors that can also associated to virulence in the urinary tract infection. In the present study, these adhesive agents, P fimbriae represents the most common adhesin in UPEC followed by type I fimbriae (*fimH*)[20]. The *FimH* adhesin mediates the bacterial adherence and

Table 3. IL-17A urine level in Patients with UTI and controls

Subject	Number	IL-17A mean (pg /ml ± SE*)
UTI patients	50	(121.70 ± 22.14)**
Control	30	(13.94 ±1.77)

* SE (Standard Error), **Significance *(P < 0.001)*

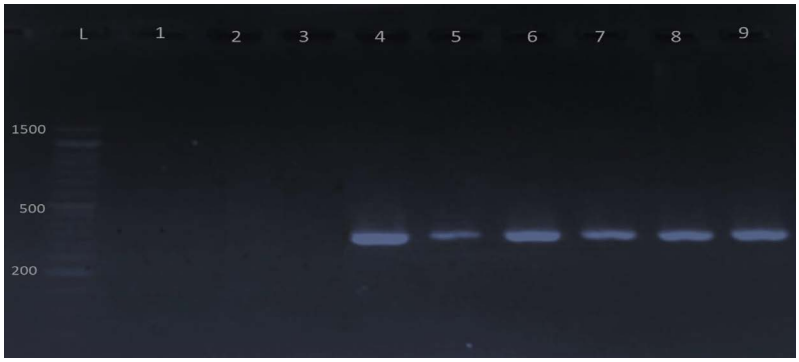

Fig. 2. Gel electrophoresis of PCR products of *papC* gene visualized under U. V light after mixed with ethidium bromide. L: 1500 bp DNA marker; lane 4-9 positive for *papC* gene and lane 1-3 negative results. The product size is 328 bp.

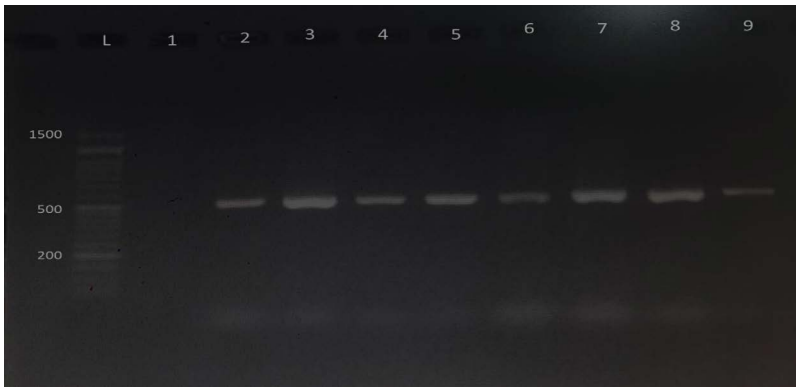

Fig. 3. Gel electrophoresis of PCR products of *fim H* gene visualized under U. V light after mixed with ethidium bromide. L: 1500 bp DNA marker; lane 2-9 positive for *fim H* gene and lane 1 negative result. The product size is 508 bp.

Table 4. IL-17A urine level of *E.coli* and other pathogens patients

Subject	Number	IL-17A mean (pg /ml ± SE*)
E.coli patients	25	(92.80±10.05)**
Other bacterial pathogens	25	(15.40±4.70)

* SE (Standard Error), **Significance *(P < 0.001)*

invasion of host cells and associated with the development of intracellular biofilms formation by UPEC[21]. The current study were differed from the study of[22], who found that *fimH* (97%) and *papC* (43%) in UPEC isolates respectively. The second highest prevalence belongs to cytotoxic necrotizing factor A (68%), which encoded by *cnf1* gene. The current study result in the same line with study conducted by[23], which revealed that the prevalence of *cnf* gene with the rate (67%). But the present results were lower than the result of[24], who found that the prevalence rate for *cnf* was (79.67%).

In this study, the percent of ferric Yersiniabactin uptake *(fyuA)* gene was (60%). The results of this study were lower than a study conducted by[22], who reported that the percentage of *fyuA* gene was (83%) in UPEC. Also[25], revealed that *fyuA* gene was (94%) which is also higher than result of current study. The differences in UPEC

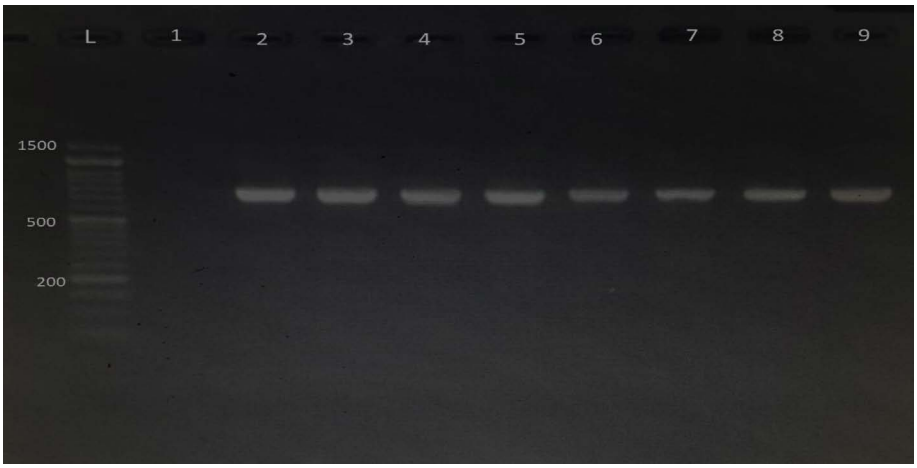

Fig. 4. Gel electrophoresis of PCR products of *fyuA* gene visualized under U. V light after mixed with ethidium bromide. L: 1500 bp DNA marker; lane 2-9 positive for *fyuA* gene and lane 1 negative result. The product size is 880 bp.

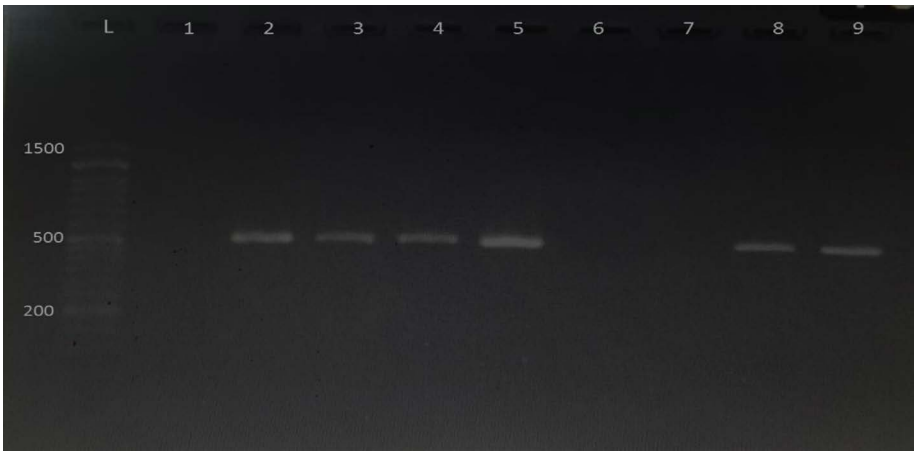

Fig. 5. Gel electrophoresis of PCR products of *cnfA* gene visualized under U. V light after mixed with ethidium bromide. L: 1500 bp DNA marker; lane 2-5,8,9 positive for *cnfA* gene and lane 1, 6,7 negative result. The product size is 498 bp.

virulence genes prevalence may be attributed to the climate of different geographic region[23,24].

Serum Level of Interleukin -17A

In present study among 50 patients of urinary tract infection compared to 30 of control, The IL-17A mean urine level of patients with UTI were (121.70) pg/ml, but it was (13.94) pg/ml for control and *E.coli* patients were (92.80) pg/ml, but it was (15.40) pg/ml for other bacterial pathogens patients. There was highly significant difference *(p <0.001),* (Table 3 and Table 4).

The role of cytokine IL-17A was characterized during urinary tract infection caused by UPEC. The result showed that the men of IL-17A were higher in UTI caused by uropathogenic *E.coli* than UTI caused by other pathogens and in control groups. This results was agreed with result of studies on mice, and because many of the genes influenced by IL-17A have the same function during UTI in mice and humans[26], we expect that all IL-17A results in mice also have the same causes during UTI in humans. IL-17A which is an immune-modulatory cytokines, considers as an innate immune response important factor in the UTI associated with UPEC[27]. IL-17A contributed to innate clearance of UPEC through a mechanism involving secretion of cytokines and chemokine and cells influx such as neutrophils and macrophages to the bladder[5]. The γδ-positive cells is the key source of IL-17A production[27].

In the study of murine model, the mice that lacking of IL-17A display deficient cytokine transcript up regulation and cellular responses during acute urinary tract infection, resulting in suboptimal clearance of *uropathogenic E. coli,* This clearance defect is likely a result of deficient cytokine and chemokine transcription and impaired of macrophage and neutrophil influx during infection, this result detect IL-17A seems to be unnecessary for the generation of protective immunity, and also IL-17A seems to play a role in regulating the response of innate immunity to UTI[27.] IL-17A transcript is up regulated in response to acute bladder infection by uropathogenic *E. coli* time points during a 28-day period, and remains elevated throughout two weeks. A study on infected mice with uropathogenic *E. coli*- reported increased in the IL-17A, with median values peaking at 48 hours post infection, while other cytokines will be retained to near base line level one week's post infections, refers to a role for IL-17A in the response of innate immunity to UTI[5,27].

CONCLUSION

The current study is the first study that revealed the association between human urine level of Interleukin -17A and *Escherichia coli* in Iraq and the world. The results of current study confirmed that the urine level of Interleukin 17A for *E.coli* patients were more than that in patients with other bacterial pathogens and control groups.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHORS' CONTRIBUTION

All authors have made substantial, direct and intellectual contribution to the work and approved it for publication.

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None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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