Iraqi Journal of Biotechnology, 2022, Vol. 21, No. 1, 79-88

The Prevalence of *FimH* and *TosA* Genes in *Escherichia coli* Isolated from Urinary Tract Infections

[1]Asmaa Ghafer , [1]Abdulameer M. Ghareeb , [1]Siham Hamel Mohaisen

Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Iraq.

Received: May 11, 2022 / Accepted: June 19, 2022

Abstract: Urinary tract infections (UTIs) are one of the main health problems caused by various microorganisms including uropathogenic *Escherichia coli* (UPEC). UPEC strains are the most frequent pathogens responsible for 85% and 50% of community and hospital acquired UTIs, respectively. UPEC strains have specific virulence factors that can result in worsening of UTIs, including adhesion trait. In this study, Fifty-four UPEC isolates were collected from patients with various clinical manifestations of UTIs attending different hospitals in Baghdad/Iraq from November 2020 to March 2021. These isolates were identified by culturing on differential media MacConkey agar and selective media Hardy chromo UTI agar in addition to the blood agar, then confirmed the identification using Vitek 2 automated system. The prevalence of fimbrial adhesion related gene (*fimH*) and non fimbrial adhesion related gene (*tosA*) gene were screened among 45 UPEC isolats using polymerase chain reaction assay with specific primers. The result reported the presence of studied genes was in high percentage (95.6%) for *fimH* coded gene while was in moderate percentage (47%) for *tosA* coded gene. thus, *fimH* and *tosA* could be used as a possible diagnostic marker in addition to indicate the level of UPEC pathogenicity.

Keywords: Urinary tract infections; Uropathogenic *Escherichia coli*; *fimH*; *tosA*

Corresponding author: (Email: ASmaaa@gmail.com).

Introduction

Urinary tract infections (UTIs) are widely globally distributed health problems and both men and women can be affected with UTIs which usually present with different symptoms and prognosis (1). These infections commonly cause morbidity and, if left untreated, may lead to high mortalities. Nowadays, UTIs are serious public health issues and are responsible for nearly 150 million disease cases every year worldwide (1).

UTIs are major causes of morbidity in boy infants, older men and females of all ages (2). After respiratory and gastro-intestinal infections, the UTI is considered as the third most common infection encountered by humans (3).

Extraintestinal pathogenic *E. coli* (ExPEC) are facultative pathogens that are part of normal human intestinal flora. However, their existence may be correlated with some infectious diseases including neonatal meningitis *E. coli* (NMEC), sepsis *E. coli* (SEPEC) In addition to the most prevalent infection which is UTI. Many UTIs are caused by a highly heterogeneous ExPEC group called uropathogenic *E. coli* (UPEC) (4).

UPEC strains encode a various number of virulence factors, which allow the microorganism colonize the urinary tract and persist in face of

highly effective host defence. UPEC isolates exhibit a high degree of genetic variety due to the possession of specialised virulence genes located on cellular genetic elements called pathogenicity islands (5).

Virulence factors of *E. coli* that have been potentially implicated as important to establish UTI can be divided into two groups: The first group of virulence factors associated with the surface of bacterial cell while the second group of virulence factors are secreted and exported to the site of action. The essential step for beginning and development of UTI is bacterial attachment to uroepithelial cells (6).

E. coli attachment is mediated by ligands of bacteria (generally small proteins placed at the tips of bacterial fimbriae) which bind to host cell wall carbohydrate residues, working as receptors (7). Therefore, the adherence of *E. coli* to host receptors is a function, usually mediated by adhesions of bacteria to host cell receptors (8).

The bacterial attachment permits bacteria to resist mechanical elimination by the flow of urine and bladder emptying and increasing persistence of *E. coli*. UPEC strains produce different types of adhesins, including type 1 fimbriae, which are essential for recognition and attachment to urinary tract receptors (9).

Among adhesions factors of UPEC, the adhesive subunit of type 1 fimbriae (*FimH*) is a major determinant, which has high tropism for urinary tract receptors; thus, *FimH* adhesion is important in colonizing different niches of *E. coli* (10).

Furthermore, Type 1 secretion A is described as a non-fimbrial adhesion that contributes to the etiology by binding to the host's surface receptors of epithelial cells derived from the upper urinary tract and is coded by *tosA* gene (11).

It was a potential marker of pathogenicity in these isolates and was associated with high colonization of the urothelium (12). Thus, current study aimed to determine the prevalence of both *fimH* and *tosA* genes among *Escherichia coli* isolated from Urinary tract infections.

Material and Methods

UPEC Bacterial Isolation and Identification

Three hundred midstream urine samples (MSU) were collected from patients of different ages of both genders, who have symptoms of UTI from three hospitals in Baghdad; AL-Yarmouk Teaching Hospital, AL-Karama Teaching Hospital and Al kidney Teaching Hospitals during the period from November 2020 to March 2021. The urine samples were plated on differential media MacConkey agar, blood agar and incubated at 37°C for 24 hrs. Bacterial isolates have been recognized and categorized consistent with microscopy (Gram stain smear), colony morphology, fermentation, pigmentation, and hemolysis on blood agar, and cultivation on selective media (Hardy chromo UTI agar). Afterward, Vitek-2 system used to affirm the identification.

Genomic DNA Isolation

The genomic DNA was extracted from the UPEC isolates upon overnight cultures at 37°C in Brain Heart Infusion broth using a commercial wizard genomic DNA purification kit (Wizard-GenomicDNA-Purification Kit,

Promega/ USA) according to the manufacturer's instructions.

After genomic DNA extraction, agarose gel electrophoresis has been adopted to confirm the presence and integrity of the extracted DNA (13). The concentrations and purity of extracted DNA were determined by NanoDrop device (Thermo Fisher Scientific/USA).

Molecular detection of *fimH* and *tosA* genes

Polymerase chain reaction (PCR) was used to confirm the presence or absence of the *fimH* and *tosA* genes in the 45 of UPEC isolates. The sequence of the primers that used for tested genes were showed in table (1) with expected product sizes (*fimH* 508bp and *tosA* 589bp) according to Reference. The general properties of these primers were checked by using the Oligocalc Oligonucleotide Porperties Calculator program.

The thermal cycler conditions were performed with an automated thermal cycler programmed as mentioned in table (2). The presence or absence of bands on agarose tested genes is distinguished by gel electrophoresis.

Table (1): The oligonucleotides sequences, amplicon sizes, and the references for the primers used in this study.

Name of primer	Sequence (5′→3′ direction)	Amplicon size (bp)	References
tosA	F-GCACAGCATAACGGGAAAAT	589	(Xicohtencatl, 2019) (12)
	R-CCAGCATGTTACCACGAATG		
fimH	F-TGCAGAACGGATAAGCCGTGG	508	
	R-GCAGTCACCTGCCCTCCGGTA		

Table (2): Thermal cycler amplification conditions For *fim*H and *tos*A genes.

Step	Temperature(^{o}C)	Time	No. of cycles
Initial denaturation	95	3 min	1
Denaturation	95	30 sec.	35
Annealing	57 for *fimH;* 55 for *tos*A	30 sec.	
Extension	72	30 sec.	
Final extension	72	5 min	1

Results and Discussion

UPEC Isolation and Identification

Phenotypic Characteristics

Colonies of *E. coli* cultivated on the MacConky agar were characterized by being small rod colonies of pink color with non- viscous in appearance, and lactose fermented. While on the blood agar *E. coli* appeared as a creamy form and on the Hardy chrom UTI agar appeared as pure blue as shown in figure (1).

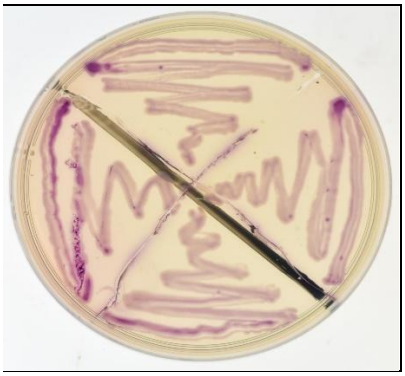

Figure (1): *Escherichia coli* colonies on Hardy chrom UTI agar.

Microscopic Examination

The results of the microscopic examination of *E. coli* after Gram staining of a smear taken from pure colonies showed that their cells are gram-negative, have a short stick shape arranged in single or pairs, and do not form spores. The identification of all isolates was confirmed using Vitec 2 system.

UPEC distribution According to Gender Factor

In this study, the number of *E. coli* isolates from females was 32 isolates (71.2%), while only 13 isolates (28.8%) were from males of a total of 45 *E. coli* isolates.

The ages of patients for both genders study were ranged between 10-80 years. According to this finding, the prevalence of *E. coli* isolates among females infected with UTI is higher than the rate among male patients (figure 2).

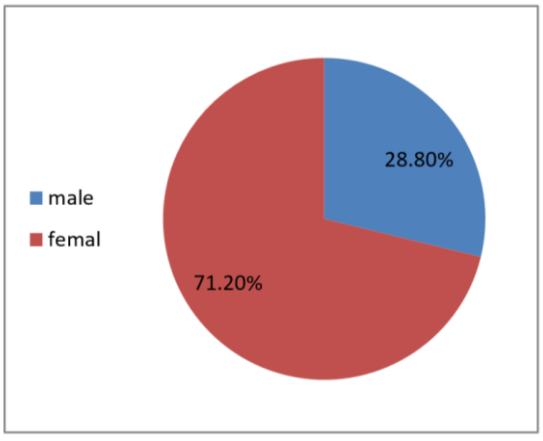

Figure (2): UPEC distribution according to gender factor.

This results is agreed with a study conducted in Iran by Neamati *et al*. (14) who reported that 78% of *E. coli* isolates were from females and 22% were from males of a total 150 urine samples collected from patients suffering from UTI.

Similar result was reported by Jameel and Artoshi (15) who showed that the infection occurrence in females was higher than males with percentage of 68% and 32% respectively of a total 316 samples collected from diabetic and non-diabetic patients in Zakho city in Iraq.

Another local study conducted in Baghdad by Ali and Khudhair (16) mentioned the number that 329.

The high incidence of UTIs in women increases with age due to several factors including women owning several receptors of the type 1 fimbria (*FimH*) that considered one of the virulence factors of UPECm (17).

Furthermore, urethra in the woman is short and near to the anus that facilities passing of UPEC to the bladder and cause infection, while the male urethra is longer which make it difficult for bacteria to infection the bladder (18،19).

Moreover, change in hormones such as decrease of estrogen hormone during menopause and pregnancy; all these factors lead to an increase of UTIs (20).

Molecular detection of *fim*H gene

All 45 DNA samples of UPEC isolates were subjected to a molecular detection by PCR amplification to determine the prevalence of the fimbrial coded gene (*fimH*) using a specific primer. The result showed 43 isolates of them (95.6%) gave positive results of sharp amplified bands with approximately a molecular size of 508bp as shown in figure (3).

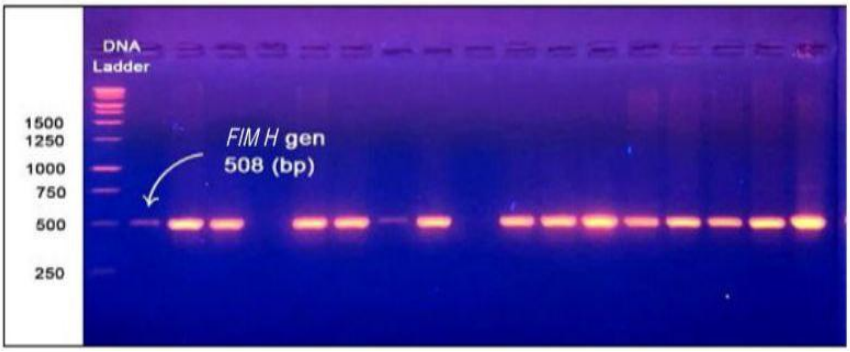

Figure (3): Gel electrophoresis of amplified *fimH* gene (508 bp) of uropathogenic *Escherichia coli*. Lane M: 1 kb DNA ladder; lanes (1-17): Amplicons of *FimH* gene on agarose 2% at 70 V for 80 min.

According to current finding, the prevalence percentage of *fimH* has been considered as a significantly high. This finding agreed with a numerous number of local studies (21, 22, 23, 24); who recorded 100%, 100%, 99%, 95%, 94.5% as prevalence rate respectively.

In addition, similar finding was recorded in international studies (25, 26, 27) who detected that 92.2%, and 91% of the isolates were positive for the *fimH* gene.

Furthermore, this result is slightly higher than prevalence rate which recorded in previous studies (28, 29, 30) who demonstrated that 84% 80% and 71% respectively, of the isolates were positive for *fimH* gene.

In other hands, the high prevalence of *fimH* gene in current study was in disagreement with finding

of few studies who showed lower percentage of prevalence as recorded in (31, 32) with 51.7% and 68% respectively. This difference may be due to the different in type and number of samples.

This results came in line with what mentioned by many researcher, who confirmed importance of the first type of fimbriae and its function in the induction of infection and promote the ferocity of *E. coli* bacteria in urinary tract infections.

This fact was supported by various studies such the work that conducted in Iraq by Al-Khafaji, (33) which showed the importance of the (type I fimbriae) and his relationship as a ferocity factor can participate in promote virulence of *E. coli* bacteria.

The importance of the *fimH* virulence gene in uropathogenic *E. coli* assimilate in its association with worsening of UTIs and it is a critical determinant of tropism for the urinary tract (34) and vaginal epithelium (35).

It was shown previously that naturally occurring amino acid replacements in *fimH* can modify its tropism towards uroepithelium and various components of basement membranes.

These replacements increase the monomannose (1M)-binding capability of *fimH* by affecting shear-dependent conformational properties of the protein (36).

Minor sequence variation within the *fimH* genes renders the *fimH* alleles possible for use in high-resolution typing method of *E. coli* (37). *fimH* gene is frequently associated with UPEC strains and it is more likely to be altered or modified due to selective pressure.

The phenotypic variants of *fimH* gene is earnestly associated with genetic variations thus, SNPs may contribute to the ability of organisms to cause illness conferring epidemic distribution or long term evolution of virulence (38).

SNP analysis in *fimH* gene has discriminating power for this locus and it may be accurate enough for investigating UTI caused by UPEC that occurs over limited time periods or in confined geographical settings (39).

Molecular detection of *tosA* gene

The same 45 isolates of UPEC which screened for *fimH* were subjected to all were a molecular detection by PCR amplification to determine the prevalence of the afimbrial coded gene (*tosA*) using a specific primer.

The result showed 21 isolates of them (47%) gave positive results according to successful amplified product with approximately a molecular size of 508bp as shown in figure (4).

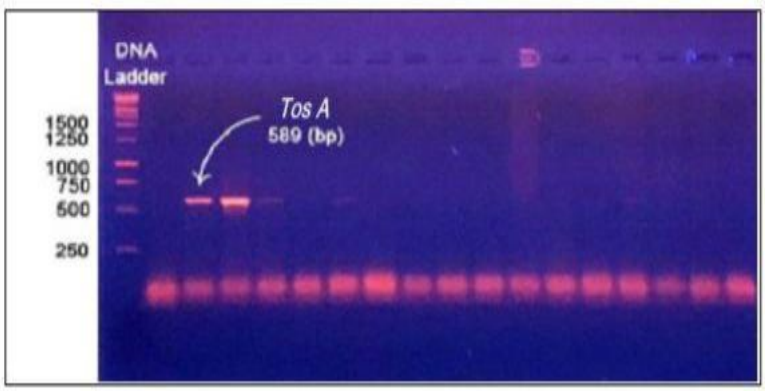

Figure (4): Gel electrophoresis of amplified *tosA* gene (589 bp) of uropathogenic *Escherichia coli*. Lane M: 1 kb DNA ladder; lanes (1-17): Amplicons of *tosA* gene on agarose 2% at 70 V for 80 min.

Current study has also shown the presence of *tosA* gene in moderate prevalence rate which about 47% while 24 isolates (53%) showed negative result who indicate the absence of the gene.

This finding is consistent with previous reports of gene prevalence among UPEC clinical isolatesws with UTIs which showed the presence of the *tosA* gene at a frequency of 36.9% in UPEC strains.

The *TosA* protein in UPEC strains has attracted interest due to its antigenicity, activity as afimbrial adhesion, and virulence marker properties (40, 41).

According to Vigil *et al*., (41) *tosA*-positive strains carry an unusually high number of the virulence or fitness genes.

In addition, the presence of *tosA* was predictive of successful colonization of a murine model of infection, even among fecal isolates, and can be used as a marker of pathogenic strains of UPEC.

Rapid identification of uropathogenic strains of *E. coli* may aid in the development of therapeutic and preventive therapies.

Ethical Clearance

The Research Ethical Committee at scientific research by ethical approval of both health and environmental and higher education and scientific research ministries in Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

Self-funding.

References

1. Motse, D. F. K.; Ngaba, G. P.; Foko, L. P. K.; Ebongue, C. O. and Adiogo, D. D. (2019). Etiologic profile and sensitivity pattern of germs responsible for urinary tract infection among under-five children in Douala, Cameroon: a Hospital-Based Study. Avicenna Journal of Clinical Microbiology and Infection, 6(2): 49-56.
2. Chaudhri, N.; Masud, M.; Shehzad, M.N.; Kumar, J.; Qureshi, M.A. and Sarwar, N. (2020). Antibiotic susceptibility and resistance patterns in urine cultures-a single center experience. Journal of Rawalpindi Medical College, 24(1): 74-79.
3. Najar, M.S.; Saldanha, C.L. and Banday, K.A. (2009). Approach to urinary tract infections. Indian Journal of Nephrology, 19(4): 129.

4. Sarowska, J.; Futoma-Koloch, B.; Jama-Kmiecik, A.; Frej-Madrzak, M.; Ksiazczyk, M.; Bugla-Ploskonska, G., *et al*. (2019). Virulence factors, prevalence and potential transmission of extraintestinal pathogenic *Escherichia coli* isolated from different sources: recent reports, Gut pathogens, 11(1): 10.
5. Wiles, T.J.; Kulesus, R.R. and Mulvey, MA. (2008). Origins and virulence mechanisms of uropathogenic *Escherichia coli*. Experimental and Molecular Pathology; 85(1): 11–19.
6. Emody, L.; Kerenyi, M. and Nagy, G. (2003). Virulence factors of uropathogenic *Escherichia coli*. International Journal of Antimicrobial Agents, 22: 29-33.
7. Eto, D. S.; Jones, T. A.; Sundsbak, J. L. and Mulvey, M. A. (2007). Integrin-mediated host cell invasion by type 1–piliated uropathogenic *Escherichia coli*. PLoS Pathogens, 3(7): e100.
8. Le Bouguénec, C. (2005). Adhesins and invasins of pathogenic *Escherichia coli*. International Journal of Medical Microbiology, 295(6-7): 471-478.
9. Oliveira, F.A.; Paludo, K.S.; Arend, L.N.; Farah, S.M.; Pedrosa, F.O.; Souza, E.M., *et al*. (2011). Virulence characteristics and antimicrobial susceptibility of uropathogenic *Escherichia coli* strains. *Gene*tics and Molecular Research, 10 (4): 4114–4125.
10. Sokurenko, E. V.; Feldgarden, M.; Trintchina, E.; Weissman, S. J.; Avagyan, S.; Chattopadhyay, S., *et al*. (2004). Selection footprint in the FimH adhesin shows pathoadaptive niche differentiation in *Escherichia coli*. Molecular Biology and Evolution, 21(7): 1373–1383.
11. Engstrom, M. D.; Alteri, C. J. and Mobley, H. L. (2014). A conserved PapB family member, TosR, regulates expression of the uropathogenic Escherichia coli RTX nonfimbrial adhesin TosA while conserved LuxR family members TosE and TosF suppress motility. Infection and Immunity, 82(9): 3644-3656.
12. Xicohtencatl-Cortes, J.; Cruz-Cordova, A.; Cazares-Dominguez, V.; Escalona-Venegas, G.; Zavala-Vega, S.; Arellano-Galindo, J., *et al.* (2019). Uropathogenic *Escherichia coli* strains harboring tosA gene were associated to high virulence genes and a multidrug-resistant profile, Microbial Pathogenesis, 134: 103593.
13. Sambrook, J. (1989). Molecular cloning: a laboratory manual. *Synthetic oligonucleotides*.
14. 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(2): e17514.
15. Jameel, A. Y. and Artoshi, D. M. E. (2019). Prevalence of Urinary Tract Infections and Their Antimicrobial Sensitivity Among Diabetic and Non Diabetic Patients in Zakho, Science Journal of University of Zakho, 7(4): 125–131.
16. Ali, M. R. and Khudhair, A. M. (2018). Detection of Colony Adhesion Factors and Genetic Background of Adhesion Genes Among Multidrug- Resistant Uropathogenic *Escherichia coli* Isolated in Iraq. Journal of Pure and Applied Microbiology, 12(4): 2017–2025.
17. Kolawole, A. S.; Kolawole, O. M.; Kandaki-Olukemi, Y. T.; Babatunde, S. K.; Durowade, K. A. and Kolawole, C. F. (2009). Prevalence of urinary tract infections (UTI) among patients attending Dalhatu Araf Specialist Hospital, Lafia, Nasarawa state, Nigeria. International Journal of Medicine and Medical Sciences, 1(5): 163–167.
18. McLaughlin, S. P. and Carson, C. C. (2004). Urinary tract infections in women. The Medical Clinics of North America, 88(2): 417–429.
19. Sheerin, N. S. (2011). Urinary tract infection. *Medicine*, 39(7): 384-389.
20. Hindi, N. K. K.; Hasson, S. O. and Hindi, S. K. K. (2013). Bacteriological study of urinary tract infections with antibiotics susceptibility to bacterial isolates among honeymoon women in Al Qassim Hospital, Babylon Province, Iraq. Biotechnology Journal International, 332–340.
21. Salih, E. G. M.; Nader, M. I. and Rasheed, M. N. (2015). Rapid Detection of Uropathogenic Escherichia coli virulence factors in Iraqi patients by multiplex polymerase chain reaction. Jundisher Journal Microbiology, 9(2). 12-19.
22. Al-Taai, H. R. R.; Al-Jebouri, Z. A. S.; Khalaf, B. H. and Mohammed, Y.Q. (2018). Antibiotic resistance patterns and adhesion ability of uropathogenic

Escherichia coli in children. Iraqi Journal of Biotechnology, 17(1): 18–26.
23. Kadhum, H.A. (2018). Bacteriological and molecular study for detection of uropathogenic *Escherichia coli* isolated from cases of urinary tract infection. M.Sc. Thesis. College of Biotechnology, University of Al Nahrain. Iraq.
24. Jabber, A.M. (2020). Detection of genes involved in biofilm formation by *Escherichia coli* isolated from patients Suffering from urinary tract infection. M.Sc. Thesis. Department of Biotechnology. College of Biotechnology. University of Al-Nahrain. Iraq.
25. Mihaylova, M.; Kostadinova, S. and Marhova, M. (2012). Distribution of virulence determinants and biofilm-forming among clinical urinary isolates, Journal of BioScience and Biotechnology, 2012, 45-51.
26. Merza, N. S. (2017). Prevalence and molecular Characterization of Fim H gene in *Escherichia Coli* isolates recovered From patients with UTIs. Medical Journal of Babylon, 14(3): 470-477.
27. Hojati, Z.; Zamanzad, B.; Hashemzadeh, M.; Molaie, R. and Gholipour, A. (2015). Detection of fimh gene in uropathogenic *Escherichia coli* strains isolated from patients with urinary tract infection. Jundishapur Journal of Microbiology, 8(2): 12–15.
28. Momtaz, H.; Karimian, A.; Madani, M.; Dehkordi, F. S.; Ranjbar, R.; Sarshar, M. *et al.* (2013). Uropathogenic *Escherichia coli* in Iran: serogroup distributions, virulence factors and antimicrobial resistance properties. Annals of Clinical Microbiology and Timicrobials, 12(1): 8.
29. Lopez-Banda, D. A.; Carrillo-Casas, E. M.; Leyva-Leyva, M.; Orozco-Hoyuela, G.; Manjarrez-Hernandez, A. H.; Arroyo-Escalante, S., *et al*. (2014). Identification of virulence factors genes in *Escherichia coli* isolates from women with urinary tract infection in Mexico. *BioMed Research International*, 2014, 959206.
30. AL- Abady, Y.M.R. (2021). Molecular Detection of some virulance Genes from Commensal *E.coli* Isolated from Children in AL-Muthannaa Governorate and its Association with UTI. M.Sc. Thesis. Institute of genetic engineering and biotechnology. University of Baghdad. Iraq.
31. Garofalo, C. K.; Hooton, T. M.; Martin, S. M.; Stamm, W. E.; Palermo, J. J.; Gordon, J. I., *et al*. (2007). *Escherichia coli* from urine of female patients with urinary tract infections is competent for intracellular bacterial community formation. Infection and Immunity, 75(1): 52–60.
32. Asadi, S.; Kargar, M.; Solhjoo, K.; Najafi, A. and Ghorbani-Dalini, S. (2014). The association of virulence determinants of uropathogenic *Escherichia coli* with antibiotic resistance. Jundishapur Journal of Microbiology, 7(5).
33. AL–Khafaji, Z.. (2013). Identification of some UTI causative agents using cultural and molecular methods and their correlation with interleukin-8 in children patients. M.Sc. Thesis. Department of Biology. College of Science for Women. University of Baghdad. Iraq.
34. Marrs, C. F.; Zhang, L. and Foxman, B. (2005). *Escherichia coli* mediated urinary tract infections: are there distinct uropathogenic *E. coli* (UPEC) pathotypes?. FEMS Microbiology Letters, 252(2): 183-190.
35. Russo, T. A. and Johnson, J. R. (2000). Proposal for a new inclusive designation for extraintestinal pathogenic isolates of *Escherichia coli*: ExPEC. The Journal of Infectious Diseases, 181(5): 1753-1754.
36. Sokurenko, E. V.; Chesnokova, V.; Dykhuizen, D. E.; Ofek, I.; Wu, X. R.; Krogfelt, K. A., *et al*. (1998). Pathogenic adaptation of *Escherichia coli* by natural variation of the FimH adhesin. Proceedings of the National Academy of Sciences, 95(15): 8922-8926.
37. Roer, L.; Tchesnokova, V.; Allesøe, R.; Muradova, M.; Chattopadhyay, S.; Ahrenfeldt, J., *et al*. (2017). Development of a web tool for *Escherichia coli* subtyping based on fimH alleles. Journal of Clinical Microbiology, 55(8): 2538-2543.
38. Lai, Y. M.; Norgainathai, R.; Zaw, M. T. and Lin, Z. (2015). A new primer set for detection of fimH gene in *Escherichia coli* isolates. International Journal of Collaborative Research on Internal Medicine and Public Health, 7(4): 65-71.
39. Abdallah, K. S.; Cao, Y. and Wei, D. J. (2011). Epidemiologic investigation of Extra-intestinal pathogenic *E. coli* (ExPEC) based on PCR phylogenetic group and fimH single nucleotide

polymorphisms (SNPs) in China. International Journal of Molecular Epidemiology and Genetics, *2*(4): 339.

40. Vigil, P. D.; Wiles, T. J.; Engstrom, M. D.; Prasov, L.; Mulvey, M. A. and Mobley, H. L. (2012). The repeat-in-toxin family member TosA mediates adherence of uropathogenic *Escherichia coli* and survival during bacteremia. Infection and Immunity, 80(2): 493-505.
41. Vigil, P. D.; Alteri, C. J. and Mobley, H. L. (2011). Identification of in vivo-induced antigens including an RTX family exoprotein required for uropathogenic *Escherichia coli* virulence. Infection and Immunity, 79(6): 2335-2344.