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Molecular Identification of Some Specific Virulence Genes in *Escherichia coli* Responsible for UTIs

Yasser Hamad Humada
Department of Biology University of Kirkuk, College of science, Iraq

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ABSTRACT

Objective: *This study aimed to determine the phylogroups and specific virulence genes of uropathogenic Escherichia coli (UPEC) in patients suspected of urinary tract infections (UTIs) at Al-Hawija General Hospital in Kirkuk.* ***Methods:*** *A total of 120 urine samples were collected from patients exhibiting clinical signs of UTIs. The presence of bacteria was confirmed using light microscopy and bacterial cell identification techniques. Positive samples underwent bacterial cultivation and DNA extraction using a specialized kit. Polymerase chain reaction (PCR) analysis was performed to identify virulence factor genes (chuA, fimH, uidA, and arpA) associated with pathogenicity.* ***Results:*** *Of the 120 analyzed samples, 90 (75%) showed bacterial growth, with 25 (27.78%) from males and 65 (72.22%) from females. PCR analysis confirmed the presence of virulence genes in E. coli isolates, linking these genes to the bacteria's role in causing UTIs.* ***Novelty:*** *This study provides a comprehensive analysis of UPEC phylogroups and virulence genes in UTI patients, highlighting gender-based prevalence. The use of PCR to identify pathogenic genes offers valuable insights into the molecular mechanisms of UPEC, which could inform future diagnostic and treatment strategies.*

INTRODUCTION

E. coli is a commensal bacterium raise in the intestines of humans and animals. In addition to being a typical intestinal flora, *E. coli* is recognized as the most prevalent bacterium responsible for urinary tract infections (UTIs). Pathogenic Escherichia coli strains are implicated in several illnesses, including toxin-mediated diarrhea, bloodstream infections, and wound infections. Moreover, infants and persons with compromised immune systems may be susceptible to infections caused by *E. coli* [1]. Uropathogenic *Escherichia coli* (UPEC), strains originate from many phylogenetic groupings and feature a range of virulence characteristics that enhance their capacity to circumvent diverse defensive systems and induce illness [2]. Fimbriae enhance bacterial adhesion and invasion, iron-acquisition systems support bacterial survival in iron-deficient urinary tracts, flagella enable motility, and toxins encourage bacterial dissemination, all of which are virulence factors encoded by virulence genes. The chromosome and/or transmissible genetic elements (plasmids) include virulence genes [3], which enable non-pathogenic strains to acquire more FVs from accessory DN [4]. A phylogenetic study has demonstrated that E. coli strains may be categorized mad about multiple phylogroups, each exhibiting similar ecological niches, features, and propensity to induce illness Clermont et al., 2013 and Gordon et al., 2008[5], [6]. Consequently,

determining the phylogroup of an unidentified strain helps enhance control and preventive initiatives as well as the treatment of illnesses [7].

Numerous studies indicate the disparity in virulence profiles and phylogenetic relationships of E. coli isolates obtained from UTIs, central nervous system infections, respiratory tract infections and bloodstream infections compared to commensal and diarrheagenic isolates [8]. UPEC, Neonatal Meningitis-Causing E. coli (NMEC), and sepsis-causing E. coli (SPEC) are generally referred to as ExPEC due to their shared genetic virulence characteristics, which enable them to circumvent host defenses and induce diverse illnesses in people and animals. [9], [10], [11].

Clinical detection techniques need hours (dipsticks) to days (culturing procedures), constraining prompt action. Implementing molecular approaches may enhance both speed and accuracy; nevertheless, their application is hindered by significant genetic heterogeneity across UPEC strains [12]. This study pursues to estimate the presence of chuA, fimH, uidA, and arpA, in E. coli isolated from UTIs samples collected from patients at Al-Hawija General Hospital in Kirkuk.

RESEARCH METHOD

A. Samples Collection

1. Bacterial Isolation and preservation

One hundred and twenty urine samples were collected from individuals afflicted with (UTI), aged between 10 and 60 years, from Al-Hawija General Hospital in Kirkuk from December 1, 2023, to the end of April 2024. Using the API 20E System, the Vitek 2 system, and cultural and biochemical characteristics, the samples were determined to be Escherichia coli. Before being sent to the laboratory, College of Science at the University of Kirkuk for culture, samples were placid using Copan's UriSponge® System for Urine Specimen Collection and Transport (USCT). Sorbitol Mac-Conkey agar and desoxycholate citrate agar cultivated the specimens (United Kingdom). Colonies exhibiting the macroscopic morphology of E. coli were cultivated on Müller Hinton agar plates (UK). After that, these colonies were re-incubated at 37 °C for a whole day. Uncontaminated colonies were identified using the VITEK-2 automated system bioMérieux, France and subjected to Gram staining. After being cultivated overnight in Luria Bertani (LB) broth "Hi-Media, India, the E. Coli isolates were stored for further analysis at -80 °C Thermo Fisher Scientific, Waltham, MA, United States of America" in a 50% (v/v) sterile glycerol solution. The isolates were analyzed by PCR to identify the genes chuA, fimH, uidA, and arpA.

B. Molecular Method

2. DNA extraction from E. coli isolates

Two milliliter's of LB broth were used to incubate the presumed isolates for the whole night. As modified from the article of Omar and Barnard, 2014 [13], overall genomic DNA, was extracted using the guanidium thiocyanate procedure " Sigma-Aldrich, USA ". The Nanodrop device (Genova, UK) was used to quantify the extracted

DNA's concentration and purity. 1.5% agarose gel electrophoresis was then used for detection.

3. Identification of urovirulence genes in isolates of *E. coli*

This work used Conventional PCR to discover two VFs of E. coli isolates from patients with UTIs at Al-Hawija General Hospital in Kirkuk, Iraq. Table 1 presents the primers utilized to identify UPEC virulence genes.

Table 1. Primers sequences used in this study.

Genes	Direction	Primer (5′-3′)	Amplicon size (bp)	Reference
chuA	F	GCTACCGCGATAACTGTCAT	221	[12]
chuA	R	TGGAGAACCGTTCCACTCTA		
arpA	F	AACGCTATTCGCCAGCTTGC-	400	[14]
arpA	R	TCTCCCCATACCGTACGCTA		
fimH	F	TGCAGAACGGATAAGCCGTGG	508	[15]
fimH	R	GCAGTCACCTGCCCTCCGGTA		
uidA	F	CGCCGATGCAGATATTCGTA	259	[12]
uidA	R	CTGCCAGTTCAGTTCRTTGT		

The total volume of each PCR reaction was 20µL. The PCR mixes for each gene were prepared according to the specifications described in Table 2. The mixes were homogenized using a vortex before use. The PCR protocol was conducted using the temperature parameters specified in Table 3.

Table 2. The components of conventional PCR.

S. No	PCR reaction mixture	Volume
1	EntiLink PCR master mix	10 µl
2	Primer forward (10µM)	1 µl
3	Primer reverse (10µM)	1 µl
4	DNA template	2 µl
	D.W up to	20 µl

Table 3. The thermal cycling conditions.

Stage	Temperature	Time	Number of cycle
Initial denaturation	95	3-5min	1
Denaturation	95	30sec	
Annealing	60	30sec	30 cycles
Extension	72	30	
Final elongation	72	5 min	1

RESULTS AND DISCUSSION

Result

A total of 120 samples were collected from Al-Hawija General Hospital province, specifically from male and female patients diagnosed with UTIs. The collection period spanned from December 1, 2023, to the end of April 2024. Among the 120 samples analyzed, a total of 90specimens (75%) exhibited noteworthy bacterial growth. Specifically, 65 specimens (72.22%) were derived from female subjects, while the remaining 25 specimens (27.78%) originated from male subjects, as indicated in Table 4.

Table 4. Distribution and percentages of infection according to gender.

Type of patient	No. of Sample	Percentage %	No. of Sample that bacterial growth appeared	Percentage %
Female	84	70%	65	72.30%
Male	36	30%	25	27.70%
Total	120	100%	90	100%

Discussion

This study reported a greater prevalence of UTIs in females 66% than in males 34%. These results were aligned with Qadir *et al*., 2018 [16], who raise that 86.2% females were infected by UPEC contrast to males 13.8% and, Hasan *et al*.,2022 [17], found that 71.4% females were infected with UPEC contrast to males 28.6% in addition to Gebissa Al-Hilali, 2015[18], infected youthful rate of 26.8% of those who pre-sented [18]. UTIs are more prevalent in girls than in males due to the anatomical structure of the female urethra, which is shorter and broader, rendering it less efficient in preventing bacterial colonization [19]. Escherichia coli is prevalent as it is part of the natural flora in the large intestine and can be readily transmitted by faecal pollution, particularly leading to ascending UTIs in females [20]. The peak occurrence of UTIs was noted in the age demographic of 26–45 years. This may be attributable to the sexual activity prevalent in this age range. Sexual intercourse may facilitate the admission of microorganisms into the bladder. Identifying VFs expressed by uropathogenic E. coli is crucial for understanding the pathophysiology and severity of UTIs and identifying targets for vaccine and therapeutic development [21].

Contemporary technology has significantly simplified the identification of possible virulence genes [22]. Infection models examining isogenic strains that vary by a specific virulence gene offer compelling evidence for pathogenic if infection is attributed to a singular property; however, uropathogenicity is characterized by many properties, predominantly exhibiting functional redundancy. Consequently, epidemiologic correlations with specific clinical symptoms of UTIs delineate the VFs that have facilitated uropathogenicity [23].

Some information about the relative prevalence of virulence genes is required to acquire an organism's pathogenic pathway. This study involved collecting 120 urine

samples from patients alleged of having a UTIs based on clinical symptoms. Sampling was predicated on UT conditions, such as congenital abnormalities or calculi within the urinary system. Pregnant females and diabetic patients were excepted due to their heightened susceptibility to diseases, as noted by Schneeberger [24].

In the present study, all isolates (100%) were positive for the *chuA* and *fimH* genes, and the *arpA* gene was current in all isolates as well. The *uidA* gene was identified.in individual 20% of the isolates. Merely 20% of the isolates have the uidA gene. An external membrane receptor protein that may be involved in the absorption of chemicals like heme is encoded by the chuA gene. This gene is common in pathogenic strains of E. coli and is linked to the genomic area that facilitates heme transport, which probably facilitates iron import [25], [26], [27]. The chuA gene is essential for UPECs to build intracellular bacterial communities exhibiting various biofilm-like characteristics. In order to endure a host immune response, these internal biofilms facilitate the formation of a reservoir of latent pathogenic cells inner bladder epithelial cells [28], [29]. Because of its anticipated function in the bladder during UTIs, chuA should be used in a detection system [12].

According to research from Baghdad 100% [17], Kirkuk City 100% [23], Romania 86% [30], Mongolia 89.9% [31], Iran 86.17% [32], 79.67% [33], and China 87.4% [34], the fimH adhesion gene was one of the maximum common and abundant genes in E. coli isolates causing UTIs. It is essential to target fimH as a vaccine candidate in order to prevent UTIs, and research is now being done on its potential as a vaccine candidate. Antibodies that target fimH prevent UPEC isolates from colonizing the urinary tract [35].

The beta-D-glucuronidase gene uidA, which is unique to E. coli, was expended as an inner amplification control. Because this gene creates an enzyme unique to E. coli, it is frequently used as a particular marker for E. coli in identification kits [12], [36]. It was found in 76.9% of the clinical specimens. An increase in the chuA, uidA, and fimH genes might be the cause of the 20% decrease in the arpA gene.

CONCLUSION

Fundamental Finding : This study highlights the prevalence and diversity of bacterial pathogens causing UTIs in the Al-Hawija district, with E. coli as the most dominant species. The identification of virulence genes provides crucial insights into the pathogenic mechanisms and persistence of these bacteria in the urinary tract. **Implication :** The findings underscore the importance of targeted diagnostic and treatment strategies for UTIs, focusing on the specific virulence factors of E. coli. This approach may improve patient outcomes and aid in developing tailored antimicrobial therapies. **Limitation :** The study was confined to a specific geographic area and primarily focused on E. coli, limiting the generalizability of the findings to broader populations or other bacterial species. **Future Research :** Further research should explore the molecular mechanisms of virulence genes in E. coli and other bacterial pathogens, alongside epidemiological

studies across diverse populations and regions to enhance understanding and management of UTIs.

REFERENCES

1. Kandi, V., Shahapur, P. R., Suvvari, T. K., Bharadwaj, V. G., Shahapur, R., Podaralla, E., & Godishala, V. (2024). Molecular characterization of Escherichia coli causing urinary tract infections through next-generation sequencing: a comprehensive analysis of serotypes, sequence types, and antimicrobial and virulence genes. Cureus, 16(3).
2. Dadi, B. R., Abebe, T., Zhang, L., Mihret, A., Abebe, W., & Amogne, W. (2020). Distribution of virulence genes and phylogenetics of uropathogenic Escherichia coli among urinary tract infection patients in Addis Ababa, Ethiopia. BMC infectious diseases, 20, 1-12.
3. Farshad, S., Ranjbar, R., Japoni, A., Hosseini, M., Anvarinejad, M., & Mohammadzadegan, R. (2012). Microbial susceptibility, virulence factors, and plasmid profiles of uropathogenic Escherichia coli strains isolated from children in Jahrom, Iran.
4. Johnson, J. R., Kuskowski, M. A., O'bryan, T. T., Colodner, R., & Raz, R. (2005). Virulence genotype and phylogenetic origin in relation to antibiotic resistance profile among Escherichia coli urine sample isolates from Israeli women with acute uncomplicated cystitis. Antimicrobial agents and chemotherapy, 49(1), 26-31.
5. Clermont, O., Christenson, J. K., Denamur, E., & Gordon, D. M. (2013). The C lermont E scherichia coli phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. Environmental microbiology reports, 5(1), 58-65.
6. Gordon, D. M., Clermont, O., Tolley, H., & Denamur, E. (2008). Assigning Escherichia coli strains to phylogenetic groups: multi-locus sequence typing versus the PCR triplex method. Environmental microbiology, 10(10), 2484-2496.
7. Müştak, H. K., Günaydin, E., Kaya, İ. B., Salar, M. Ö., Babacan, O., Önat, K., ... & Diker, K. S. (2015). Phylo-typing of clinical Escherichia coli isolates originating from bovine mastitis and canine pyometra and urinary tract infection by means of quadruplex PCR. Veterinary Quarterly, 35(4), 194-199.
8. Zeb, Z., Azam, S., Rehman, N., Khan, I., Afzal, S., Ullah, A., & Absar, M. (2021). Phenotypic and molecular characterization of virulence factors of extra-intestinal pathogenic Escherichia coli isolated from patients of Peshawar, Pakistan. Pakistan Journal of Pharmaceutical Sciences, 34(1).
9. Johnson, J. R., Kuskowski, M. A., Owens, K., Gajewski, A., & Winokur, P. L. (2003). Phylogenetic origin and virulence genotype in relation to resistance to fluoroquinolones and/or extended-spectrum cephalosporins and cephamycins among Escherichia coli isolates from animals and humans. The Journal of infectious diseases, 188(5), 759-768.
10. Johnson, J. R., & Russo, T. A. (2002). Extraintestinal pathogenic Escherichia coli:“the other bad E coli”. Journal of Laboratory and Clinical Medicine, 139(3), 155-162.
11. Smith, J. L., Fratamico, P. M., & Gunther, N. W. (2007). Extraintestinal pathogenic Escherichia coli. Foodborne pathogens and disease, 4(2), 134-163.
12. Brons, J. K., Vink, S. N., de Vos, M. G., Reuter, S., Dobrindt, U., & van Elsas, J. D. (2020). Fast identification of Escherichia coli in urinary tract infections using a virulence gene based PCR approach in a novel thermal cycler. Journal of microbiological methods, 169, 105799.
13. Omar, K. B., & Barnard, T. G. (2014). Detection of diarrhoeagenic Escherichia coli in clinical and environmental water sources in South Africa using single-step 11-gene m-PCR. World Journal of Microbiology and Biotechnology, 30, 2663-2671.
14. Alfinete, N. W., Bolukaoto, J. Y., Heine, L., Potgieter, N., & Barnard, T. G. (2022). Virulence and phylogenetic analysis of enteric pathogenic Escherichia coli isolated from

children with diarrhoea in South Africa. International Journal of Infectious Diseases, 114, 226-232.
15. Ahmed, N., Zeshan, B., Naveed, M., Afzal, M., & Mohamed, M. (2019). Antibiotic resistance profile in relation to virulence genes fimH, hlyA and usp of uropathogenic E. coli isolates in Lahore, Pakistan.
16. Qadir, H. A. H., Abdulla, A. B., & Abduljabbar, H. N. (2018). Molecular Study of Virulence Factors of Escherichia coli Isolated from Patient with urinary tract infection in Wasit Province. Al-Kut Univ. College Journal, 3(2), 39-50.
17. Hasan, R. N., Jasim, S. A., & Ali, Y. H. (2022). Detection of fimH, kpsMTII, hlyA, and traT genes in Escherichia coli isolated from Iraqi patients with cystitis. Gene Reports, 26, 101468.
18. Al-Hilali, S., & Hussein, M. (2015). Genetic affinities of multiple drug resistant uropathogenic Escherichia coli isolated from patients with urinary tract infection in Najaf. University of Kufa.
19. Themphachana, M., Kongpheng, S., Rattanachuay, P., Khianngam, S., Singkhamanan, K., & Sukhumungoon, P. (2015). Molecular characterization of virulence and antimicrobial susceptibility profiles of uropathogenic Escherichia coli from patients in a tertiary hospital, southern Thailand. Southeast Asian Journal of Tropical Medicine and Public Health, 46(6), 1021.
20. Dadi, B. R., Abebe, T., Zhang, L., Mihret, A., Abebe, W., & Amogne, W. (2020). Distribution of virulence genes and phylogenetics of uropathogenic Escherichia coli among urinary tract infection patients in Addis Ababa, Ethiopia. BMC infectious diseases, 20, 1-12.
21. Flores-Mireles, A. L., Walker, J. N., Caparon, M., & Hultgren, S. J. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nature reviews microbiology, 13(5), 269-284.
22. Humada, Y. H., Khalid, R. W., & Hussein, F. K. (2024). Molecular Study of K1, K2, MagA genes in High Virulent Kebsiella pneumonia in Kirkuk City, Iraq, *South Asian Res J Bio Appl Biosci*, 6, 242-245.
23. Aljebory, I. S., & Mohammad, K. A. (2019). Molecular Detection of Some Virulence Genes of Escherichia coli Isolated from UTI Patients in Kirkuk City, Iraq. Journal of Global Pharma Technology, 11(3), 349-355.
24. Schneeberger, C., Geerlings, S. E., Middleton, P., & Crowther, C. A. (2012). Interventions for preventing recurrent urinary tract infection during pregnancy. Cochrane Database of Systematic Reviews, (11).
25. Torres, A. G., & Payne, S. M. (1997). Haem iron-transport system in enterohaemorrhagic Escherichia coli O157: H7. Molecular microbiology, 23(4), 825-833.
26. Wyckoff, E. E., Duncan, D., Torres, A. G., Mills, M., Maase, K., & Payne, S. M. (1998). Structure of the Shigella dysenteriae haem transport locus and its phylogenetic distribution in enteric bacteria. Molecular microbiology, 28(6), 1139-1152.
27. Nagy, G., Dobrindt, U., Kupfer, M., Emödy, L., Karch, H., & Hacker, J. (2001). Expression of hemin receptor molecule ChuA is influenced by RfaH in uropathogenic Escherichia coli strain 536. Infection and immunity, 69(3), 1924-1928.
28. Anderson, G. G., Dodson, K. W., Hooton, T. M., & Hultgren, S. J. (2004). Intriguing questions for future study. Trends in Microbiology, 9(12), 424-430.
29. Reigstad, C. S., Hultgren, S. J., & Gordon, J. I. (2007). Functional genomic studies of uropathogenic Escherichia coli and host urothelial cells when intracellular bacterial communities are assembled. Journal of Biological Chemistry, 282(29), 21259-21267.
30. Usein, C. R., Damian, M., Tatu-Chitoiu, D., Capusa, C., Fagaras, R., Tudorache, D., ... & Le Bouguénec, C. (2001). Prevalence of virulence genes in Escherichia coli strains isolated

from Romanian adult urinary tract infection cases. Journal of cellular and molecular medicine, 5(3), 303-310.

31. Munkhdelger, Y., Gunregjav, N., Dorjpurev, A., Juniichiro, N., & Sarantuya, J. (2017). Detection of virulence genes, phylogenetic group and antibiotic resistance of uropathogenic Escherichia coli in Mongolia. The Journal of Infection in Developing Countries, 11(01), 51-57.
32. Momtaz, H., Karimian, A., Madani, M., Safarpoor Dehkordi, F., Ranjbar, R., Sarshar, M., & Souod, N. (2013). Uropathogenic Escherichia coli in Iran: serogroup distributions, virulence factors and antimicrobial resistance properties. Annals of clinical microbiology and antimicrobials, 12, 1-12.
33. Karimian, A., Momtaz, H., & Madani, M. (2012). Detection of uropathogenic Escherichia coli virulence factors in patients with urinary tract infections in Iran. African Journal of Microbiology Research, 6(39), 6811-6816.
34. Wang, Y., Zhao, S., Han, L., Guo, X., Chen, M., Ni, Y., ... & He, P. (2014). Drug resistance and virulence of uropathogenic Escherichia coli from Shanghai, China. The Journal of antibiotics, 67(12), 799-805.
35. Flores-Mireles, A. L., Walker, J. N., Caparon, M., & Hultgren, S. J. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nature reviews microbiology, 13(5), 269-284.
36. Cleuziat, P., & Robert-Baudouy, J. (1990). Specific detection of Escherichia coli and Shigella species using fragments of genes coding for β-glucuronidase. FEMS microbiology letters, 72(3), 315-322.

***Yasser Hamad Humada (Corresponding Author)**
Department of Biology University of Kirkuk, College of science, Iraq
Email: yasirhamad1974@uokirkuk.edu.iq