Int J Basic Sci Med. 2023;8(4):167-172 Original Article

http://ijbsm.zbmu.ac.ir/ doi 10.34172/ijbsm.46649

Variability in Virulence Gene Prevalence Among Uropathogenic *Escherichia coli* Isolates: Implications for Understanding UTI Pathogenesis

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ARTICLE INFO
Article History:
Received: November 5, 2023
Accepted: December 17, 2023
ePublished: December 31, 2024

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Abstract

Introduction: Uropathogenic *Escherichia coli* (UPEC) is a common cause of urinary tract infections (UTIs), imposing a significant healthcare burden worldwide. Understanding the distribution of virulence genes among UPEC isolates is crucial for elucidating the pathogenesis of UTIs and developing therapeutic strategies.

Methods: In this study, a total of 100 UPEC isolates previously collected from UTI patients were analyzed. The prevalence of various virulence genes associated with iron acquisition, including siderophore receptors (*ireA*), hemolysin toxins (*hylB*, *hylC*, and *hylD*), and iron uptake systems (*feoB*, *fepC*, and *fyuA*), as well as putative iron transport genes (*modD*, *prrA*, and *yc73*), was assessed using the Multiplex PCR method.

Results: The results revealed notable variability in gene prevalence, with *fyuA* being the most frequently detected gene 63 (63%). However, *hlyD* was absent in all isolates. Other genes such as *feoB*, *fepC*, *hlyB*, *hlyC*, *ireA*, *modD*, *prrA*, and *yc73* exhibited frequencies ranging from 9 (9%) to 53 (53%). Notably, 76 of the isolates harboured multiple virulence genes associated with iron acquisition, suggesting their potential for enhanced pathogenicity and adaptation to the host environment.

Conclusion: The diverse prevalence of virulence genes underscores the dynamic nature of UPEC strains and their ability to adapt to host environments. Comprehending these patterns of gene prevalence offers invaluable insights into UTI pathogenesis, emphasizing the necessity for personalized therapeutic interventions. There is a pressing need for additional research elucidating the functional significance of these genes in UTI pathogenesis, aiming to formulate more efficient strategies to combat UPEC-induced UTIs.

Keywords: Uropathogenic *Escherichia coli*, Iron transporter, Virulence genes

Please cite this article as follows: Behshad E, Rashki A, Hadadi F, Shahkarami F, Shabani S. Variability in virulence gene prevalence among uropathogenic *Escherichia coli* isolates: implications for understanding uti pathogenesis. Int J Basic Sci Med. 2023;8(4):167-172. doi:10.34172/ijbsm.46649.

Introduction

Urinary tract infections (UTIs) are among the most common bacterial infections worldwide, impacting millions of people each year and placing a significant burden on healthcare systems.[1] Uropathogenic *Escherichia coli* (UPEC) is the principal causative agent, responsible for approximately 80% of community-acquired UTIs and 50% of hospital-acquired UTIs.[2-4] The ability of UPEC to colonize and persist within the urinary tract is facilitated by an array of virulence factors, which enable adherence to uroepithelial cells, invasion of host tissues, and evasion of host immune responses.[5]

Understanding the variability in the prevalence of virulence genes among UPEC isolates is crucial for unravelling the complex pathogenesis of UTIs. Several virulence-associated genes have been identified as key determinants in UPEC pathogenicity, including *feoB*, *fepC*, *hlyB*, *hlyC*, *ireA*, *fyuA*, *modD*, *prrA*, and *yc73*.[6,7] These genes encode various virulence factors, such as iron acquisition systems, adhesins, toxins, and regulatory proteins, which collectively contribute to the ability of UPEC to establish infection and cause tissue damage within the urinary tract.[7,8] The virulence genes under investigation play pivotal roles in the pathogenicity

of *E. coli* by mediating critical aspects of the infection process. For instance, *feoB* and *fepC* are involved in the acquisition of iron, a vital nutrient for bacterial growth and survival within the host.[9] The hemolysin genes *hlyB* and *hlyC* encode components of the hemolysin toxin, which disrupts host cell membranes and facilitates bacterial invasion and dissemination.[10] Additionally, *ireA* is associated with iron-responsive regulation, modulating the ability of *E. coli* to adapt to varying iron concentrations in the host environment.[11] The yersiniabactin receptor gene *fyuA* contributes to iron uptake and is implicated in bacterial persistence and virulence.[12] Furthermore, *modD*, *prrA*, and *yc73* encode regulatory proteins that modulate the expression of other virulence factors, coordinating the pathogenic arsenal of *E. coli*.[13,14] Understanding the prevalence and distribution of these virulence genes among UPEC isolates is essential for deciphering the intricate molecular mechanisms underlying UTI pathogenesis.[15] Despite the well-established role of these virulence genes in UTI pathogenesis, there exists considerable variability in their prevalence among UPEC isolates from different geographical regions and patient populations.[16] This variability may be attributed to genetic diversification, selective pressures within the host environment, and the dynamic interplay between UPEC and the host immune system.[17] However, the implications of such variability for UTI pathogenesis remain poorly understood. In this study, we aimed to investigate the variability in the prevalence of *feoB*, *fepC*, *hlyB*, *hlyC*, *ireA*, *fyuA*, *modD*, *prrA*, and *yc73* among UPEC isolates obtained from patients with UTIs. Characterizing the distribution and diversity of these virulence genes among UPEC isolates allows us to seek insight into the molecular mechanisms underlying UTI pathogenesis. Ultimately, our findings may have implications for the development of targeted therapeutic strategies and preventive measures to combat UTIs and reduce the associated morbidity and healthcare costs.

Materials and Methods

Sample Collection

Clean-catch midstream urine samples were collected from both outpatients and inpatients presenting symptoms suggestive of UTIs at Amir-al-Mommenin hospital in Zabol, Iran. Subsequently, a large loopful (10 µL) of the urine sample was cultured on both blood agar and Oxoid MacConkey Agar No. 3 plates. Following this, the plates were incubated for 24 hours at 37°C. In this study, a colony count of ≥ 100 000 (1×10^5) CFU/mL was considered indicative of a UTI.[18] A total of 100 UPEC isolates were identified by our group using standard biochemical and morphological assays and then preserved at -80 °C until further use.

For this research, bacterial isolates were cultivated on MacConkey (Oxoid, UK) Agar plates and incubated at 37 °C for 18-24 hours. Then, standard biochemical tests were conducted to detect the production of indole from tryptophan, followed by the Methyl Red (MR) and Voges-Proskauer (VP) (HiMedia) tests to assess mixed acid fermentation and acetoin production, respectively. Additionally, the citrate utilization test was performed, along with Triple Sugar Iron (TSI) (HiMedia) test to detect glucose, lactose, and sucrose fermentation. Urease production was assessed, and lysine decarboxylase activity was determined. Finally, motility was observed to confirm the characteristic motile behaviour of *E. coli*.[19]

DNA Extraction

All UPEC isolates were cultured in 5 mL of LB broth (Merck, Germany) for 16 hours at 37 °C in a shaking incubator running at 200 rpm. The boiling procedure was used to extract genomic DNA from the bacterial isolates.[20] In summary, 2 mL of fresh bacterial culture broth was pelleted by centrifugation at 3000 rpm for 5 minutes, resuspended in 200 µL of distilled water, and heated for 10 minutes at 95 °C. After 5 minutes of cooling the suspension on ice, the lysate was centrifuged for 5 minutes at 13 000 rpm. Finally, the genomic DNA-containing supernatant was collected and kept at -20 °C until needed.

Multiplex PCR

The prevalence of virulence genes (*fepC*, *modD*, *prrA*, *yc73*, *hlyB*, *hlyC*, and *hlyD*) as well as genes associated with iron acquisition (*feoB*, *fyuA*, and *ireA*) was determined in two distinct multiplex PCR assays (MPI/MPII) using five forward and reverse primers. MPprimer software (http://biocompute.bmi.ac.cn/MPprimer/) was used to design primers (Table 1).

The PCR was performed in a total volume of 25 µL containing 2 µL of template DNA, 1X PCR reaction buffer, 1.5 mM of $MgCl_2$, 200 mm of dNTPs (Invitrogen™), 0.2 µM of each primer (Pishgam, Iran), and 1 U of Ampliqon Taq DNA Polymerase.

The PCR assays were conducted under the following conditions: initial denaturation at 95 °C for 5 minutes, denaturation at 95 °C for 30 seconds, annealing at 58 for MPI/55 °C for MPII for 30 seconds, extension at 72 °C for 1 minute, and final extension at 72 °C for 10 minutes. PCR products underwent electrophoresis (85V) on a 1.5% agarose gel, ethidium bromide staining, and image analysis using a Gel Doc 1000 (Vilber Lourmat, France). A molecular weight marker (100 bp ladder, Fermentas) with increments of 100 bp was used to determine the size of the DNA fragments (Figure 1). To ensure the accuracy of the PCR results, some samples were sequenced.

Statistical Analysis

Descriptive statistics were used to assess the multiplex PCR assay data. As a percentage of the total number of

bacterial isolates, the frequency of each virulence factor gene was computed. Microsoft Excel was used to present the data as tables and graphs.

Results

In this investigation, we examined 100 UPEC isolates, 64 isolates from female patients and 36 isolates from male patients, to determine the frequency of virulence factor genes. Figure 2 reports the frequency of the virulence genes under study. We observed that the *fyuA* gene was the most frequently detected gene, present in 63 (63%) of the isolates. Conversely, *hlyD* had the lowest frequency in all isolates. The remaining virulence genes had the following frequencies: *ireA*: 10 (10%), *feoB*: 53 (53%), *fepC*: 53 (53%), *hlyB*: 37 (37%), *prrA*: 9 (9%), *modD*: 19 (19%) yc73: 27 (27%), and *hlyC*: 40 (40%) (Figure 2).

These findings show that the UPEC isolates under study varied greatly in terms of their virulence factor genes. Every *feoB*-positive isolate also carried the *fepC* gene. None of the eleven isolates had any virulence genes. All the isolates under study displayed 34 virulence gene patterns, or EC, based on the distribution of the different virulence determinants. Of these, the following gene associations were more common: *fyuA*, *hlyC*, *modD*, *feoB*, *fepC*, *hlyB*, and *ireA* (1 isolate), *feoB*, *fepC*, *fyuA*, *hlyB*, and *hlyC* (8 isolates), and *feoB*, *fepC*, *fyuA*, and *hlyB* (8 isolates). Only the *fyuA* gene (ferric yersiniabactin uptake receptor) was present in EC27, which was the most prominent pattern and was present in 7 isolates. Seventy-six isolates had different gene combinations (Table 2). These findings provide important insights into the distribution of virulence factor genes among UPEC isolates and may have implications for further research on the pathogenicity of these bacteria.

Table 1. List of Primers Used in the Multiplex PCR Assay

	Gene		Sequences	T (°C)	Size (bp)
MP1	*feoB*	F	TGGGTAACAGCAGCGAACTGCG	60	227
		R	CGCGCCAAAGTTGGTGACGTTG	60	
	fepC	F	ACACGCGCTCACGCAGATTGAA	60	430
		R	CTGCAGCAATTTGGCCTCGGGA	60	
	fyuA	F	GCAGCAGCATTATTCGCGCACC	60	522
		R	TTCTCGGCGACGAACGGTTTGG	60	
	hlyB	F	TTGCAAGGGCGCTGGTGAACAA	60	339
		R	AGCGCAACAGGAACTCGCTGAA	60	
	ireA	F	CGGGCATTGCCGTGATGTGTTC	59	108
		R	TGAGCTGCTGAGTGAACCCGGA	59	
MP2	hlyD	F	AGGCTGGAACAAACTCGGTA	56	755
		R	GCCTTTCCTTACACCCGATA	56	
	modD	F	TGCCTGTTGCGGACTAAAT	54	378
		R	GCAAACTCCACTCCAGCAC	54	
	Yc73	F	GCGAAGCCGTGCCTGATTAT	58	437
		R	TTGCTGAAGGTAAAGGTCTG	58	
	hlyC	F	CCAGTTCCCCATTACACAGA	54	311
		R	CACCCTGATGGCTCTGAATA	54	
	prrA	F	ATGGTGTTGATGGGCTGGC	58	479
		R	CCCTGAAAAGTCGGCTGTATC	58	

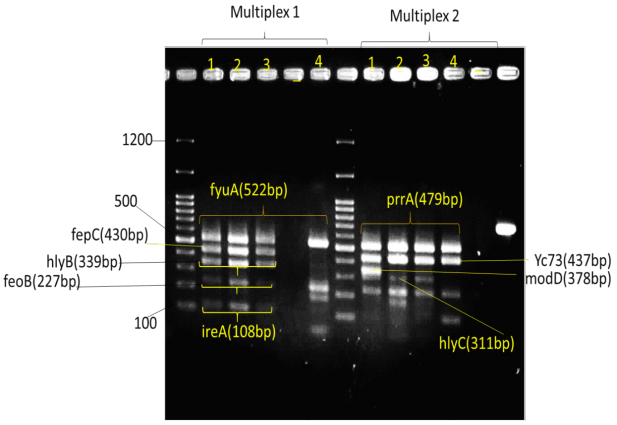

Figure 1. Multiplex-PCR Profiles Specific for Virulence and Iron Transport Factors in UPEC

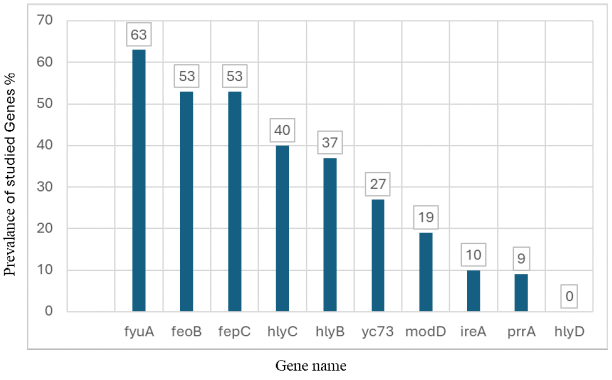

Figure 2. Prevalence of Virulence Factor Associated Genes among 100 UPEC Isolates

Discussion

The ferric yersiniabactin uptake system, which is encoded by the *fyuA* gene, is an essential part of the iron acquisition process in *E. coli*.[21] Iron is an essential nutrient for bacterial growth, and competition for iron is a major factor in the interaction between bacteria and their hosts.[22] The high frequency of the *fyuA* gene in our UPEC isolates suggests that iron acquisition may be an important factor in the pathogenicity of these bacteria.[22-24] None of the isolates had the *hlyD* gene, which codes for a part of the hemolysin secretion system. Hemolysins are pore-forming toxins that can damage host cells and are believed to be involved in the pathophysiology of UPEC infections.[25] The absence of the *hlyD* gene in our isolates may indicate that UPEC isolates are unable to produce hemolysins or that it uses alternative mechanisms for virulence. The other virulence factor genes analyzed in this study have previously been implicated in *E. coli* pathogenesis. The *hlyC*, *cy73*, and *prrA* genes encode

Table 2. Virulence Pattern among UPEC isolates

Pattern	*feoB*	*fepC*	*fyu*A	*hlyB*	*ireA*	*modD*	*yc73*	*hlyC*	prrA	*hlyD*	Number of Strains
EC1	+	+	+	+	+	+	-	+	-	-	1
EC2	+	+	+	+	+	-	+	+	-	-	1
EC3	+	+	+	+	+	-	-	+	-	-	2
EC4	+	+	+	+	-	-	+	+	-	-	3
EC5	+	+	+	+	-	+	+	-	-	-	1
EC6	+	+	+	+	-	-	+	-	-	-	4
EC7	+	+	+	+	-	-	-	+	-	-	8
EC8	+	+	+	+	-	-	-	-	-	-	8
EC9	+	+	+	-	+	+	+	+	+	-	1
EC10	+	+	+	-	-	+	-	-	-	-	2
EC11	+	+	+	-	-	+	-	+	-	-	2
EC12	+	+	+	-	+	+	-	-	-	-	1
EC13	+	+	+	-	-	-	+	-	-	-	2
EC14	+	+	+	-	-	-	-	-	-	-	6
EC15	+	+	+	+	-	-	-	-	-	-	1
EC16	+	+	+	-	-	-	-	+	-	-	1
EC17	+	+	+	-	-	-	-	-	-	-	1
EC18	+	+	-	+	+	-	-	+	-	-	2
EC19	+	+	-	+	+	-	-	-	-	-	1
EC20	+	+	-	+	-	+	-	+	-	-	2
EC21	+	+	-	-	-	-	-	-	-	-	2
EC22	+	+	-	-	+	-	+	+	-	-	1
EC23	-	-	+	-	-	-	-	-	-	-	1
EC24	-	-	+	-	-	+	+	+	-	-	1
EC25	-	-	+	-	-	+	+	-	+	-	1
EC26	-	-	+	-	-	-	-	+	-	-	2
EC27	-	-	+	-	-	-	-	-	-	-	7
EC28	-	-	+	+	-	-	+	+	-	-	2
EC29	-	-	+	+	-	+	+	-	-	-	1
EC30	-	-	+	-	-	-	-	+	-	-	2
EC31	-	-	+	-	-	-	+	-	+	-	3
EC32	-	-	-	-	-	+	-	-	-	-	3
EC33	-	-	-	-	-	+	-	+	+	-	3
EC34	-	-	-	-	-	-	+	+	-	-	4
EC35	-	-	-	-	-	-	+	-	-	-	2
EC36	-	-	-	-	-	-	-	+	-	-	2
EC37	-	-	-	-	-	-	-	+	+	-	1
EC38	-	-	-	-	-	-	-	-	-	-	12

components of the hemolysin secretion system, while the *modD* gene is involved in the modification of lipopolysaccharide, which is a key component of the bacterial outer membrane.[7,26] The *feoB* and *fepC* genes encode iron acquisition systems, while the *ireA* gene is involved in iron metabolism.[27] The *hlyB* gene encodes a component of the hemolysin transport system.[28]

The variability in the frequency of these virulence genes among the UPEC isolates analyzed may reflect differences in the pathogenic potential of these strains. Several factors can influence the distribution of virulence determinant genes among UPEC isolates. For example, the presence of certain virulence factors may be associated with specific ecological niches, such as the intestinal tract of certain animal species.[29] In addition, horizontal transfer of genetic elements, such as plasmids and bacteriophages,

can facilitate the spread of virulence factor genes between bacterial strains. [30]

Several research studies have investigated the prevalence of virulence factor genes in *E. coli* strains. Sarowska et analyzed *E. coli* isolates from different sources and observed that the most frequently detected virulence genes were *fimH* (96.8%), *traT* (83.2%), and *iutA* (78.9%).[31] In contrast, the genes *fyuA* and *hlyD* were found in only 5.3% and 1.1% of the isolates, respectively. This contrasts with our study, where the *fyuA* gene was observed in 63% of the isolates, while *hlyD* was identified in none of the isolates.

Chandra et al examined *E. coli* isolates in a tropical environment from various sources and observed that *fimH* (98.4%) was the most common virulence gene, followed by *iutA* (89.7%) and *traT* (88.9%). The genes *hlyA* and *hlyD* were detected in 18.3% and 5.6% of the isolates, respectively.[32] This is comparable to our investigation, in which 40% of the isolates had the *hlyC* gene, but none of the isolates had the *hlyD* gene. Moeinizadeh et al analyzed 100 *E. coli* isolates from individuals suffering from UTI and found that the *hlyD* gene had the highest frequency (95%) and the *hlyC* gene had the lowest frequency (23%). The genes *hlyA* and *hlyD* were found in only 50% and 43% of the isolates, respectively.[6] This contrasts with our study, where *hlyB* was observed in 37% of the isolates, while *hlyD* was observed in none of the isolates. Overall, these studies indicate that there is significant variability in the frequency of virulence genes among *E. coli* isolates from various sources. This variability may reflect differences in the pathogenic potential of these strains and highlights the importance of understanding the distribution of virulence determinants in *E. coli*.

Conclusion

In conclusion, our analysis of 100 UPEC isolates revealed a wide range of variability in the presence of virulence factor genes. The *fyuA* gene had the highest frequency, while the *hlyD* gene was not detected in any of the isolates. These findings provide important insights into the distribution of virulence factor genes among UPEC isolates and may have implications for further research on the pathogenicity of these bacteria. Future studies could explore the relationship between the presence of specific virulence factor genes and the clinical manifestations of UTIs and investigate the mechanisms underlying the variability in the distribution of virulence factor genes among different *E. coli* strains.

Acknowledgements

The authors extend their appreciation and acknowledgment to the faculty of Science and Veterinary Medicine at the University of Zabol, Iran.

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Competing Interests

The authors have no conflict of interests to declare.

Ethical Approval

This study was approved by the Iranian Ministry of Health and Medical Education and the local Research Ethics Committee (IR.UOZ.REC.1402.026). The patients' medical data and personal information were documented and kept confidential.

Funding

This research was supported by grant number IR-UOZ-GR-2331 University of Zabol.

References

1. Foxman B. Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. Infect Dis Clin North Am. 2014;28(1):1-13. doi:10.1016/j.idc.2013.09.003
2. Whelan S, Lucey B, Finn K. Uropathogenic *Escherichia coli* (UPEC)-associated urinary tract infections: the molecular basis for challenges to effective treatment. Microorganisms. 2023;11(9):2169. doi:10.3390/microorganisms11092169
3. Öztürk R, Murt A. Epidemiology of urological infections: a global burden. World J Urol. 2020;38(11):2669-2679. doi:10.1007/s00345-019-03071-4
4. Medina M, Castillo-Pino E. An introduction to the epidemiology and burden of urinary tract infections. Ther Adv Urol. 2019;11:1756287219832172. doi:10.1177/1756287219832172
5. Zhou Y, Zhou Z, Zheng L, et al. Urinary Tract Infections Caused by Uropathogenic Escherichia coli: Mechanisms of Infection and Treatment Options. Int J Mol Sci. 2023;24(13):10537. Published 2023 Jun 23. doi:10.3390/ijms241310537
6. Moeinizadeh H, Shaheli M. Frequency of hlyA, hlyB, hlyC and hlyD genes in uropathogenic *Escherichia coli* isolated from UTI patients in Shiraz. GMS Hyg Infect Control. 2021;16:Doc25. doi:10.3205/dgkh000396
7. Frick-Cheng AE, Sintsova A, Smith SN, Pirani A, Snitkin ES, Mobley HLT. Ferric citrate uptake is a virulence factor in uropathogenic *Escherichia coli*. mBio. 2022;13(3):e0103522. doi:10.1128/mbio.01035-22
8. Ons E, Bleyen N, Tuntufye HN, Vandemaele F, Goddeeris BM. High prevalence iron receptor genes of avian pathogenic *Escherichia coli*. Avian Pathol. 2007;36(5):411-414. doi:10.1080/03079450701589183
9. Sheldon JR, Laakso HA, Heinrichs DE. Iron acquisition strategies of bacterial pathogens. Microbiol Spectr. 2016;4(2). doi:10.1128/microbiolspec.VMBF-0010-2015
10. Wiles TJ, Mulvey MA. The RTX pore-forming toxin α-hemolysin of uropathogenic *Escherichia coli*: progress and perspectives. Future Microbiol. 2013;8(1):73-84. doi:10.2217/fmb.12.131
11. Li Y, Dai J, Zhuge X, et al. Iron-regulated gene ireA in avian pathogenic *Escherichia coli* participates in adhesion and

stress-resistance. BMC Vet Res. 2016;12(1):167. doi:10.1186/s12917-016-0800-y
12. Ikeda M, Kobayashi T, Fujimoto F, et al. The prevalence of the iutA and ibeA genes in *Escherichia coli* isolates from severe and non-severe patients with bacteremic acute biliary tract infection is significantly different. Gut Pathog. 2021;13(1):32. doi:10.1186/s13099-021-00429-1
13. Ranson-Olson B, Zeilstra-Ryalls JH. Regulation of the *Rhodobacter sphaeroides* 2.4.1 hemA gene by PrrA and FnrL. J Bacteriol. 2008;190(20):6769-6778. doi:10.1128/jb.00828-08
14. Tu J, Qi K, Song X, et al. Horizontal transfer and functional evaluation of high pathogenicity islands in avian *Escherichia coli*. Pol J Vet Sci. 2017;20(2):395-402. doi:10.1515/pjvs-2017-0048
15. Maniam L, Vellasamy KM, Ong TA, et al. Genotypic characteristics of uropathogenic *Escherichia coli* isolated from complicated urinary tract infection (cUTI) and asymptomatic bacteriuria-a relational analysis. PeerJ. 2023;11:e15305. doi:10.7717/peerj.15305
16. Vigil PD, Stapleton AE, Johnson JR, et al. Presence of putative repeat-in-toxin gene tosA in *Escherichia coli* predicts successful colonization of the urinary tract. mBio. 2011;2(3):e00066-00011. doi:10.1128/mBio.00066-11
17. Wiles TJ, Kulesus RR, Mulvey MA. Origins and virulence mechanisms of uropathogenic *Escherichia coli*. Exp Mol Pathol. 2008;85(1):11-19. doi:10.1016/j.yexmp.2008.03.007
18. Primack W, Bukowski T, Sutherland R, Gravens-Mueller L, Carpenter M. What urinary colony count indicates a urinary tract infection in children? J Pediatr. 2017;191:259-261.e1. doi:10.1016/j.jpeds.2017.08.012
19. Shah C, Baral R, Bartaula B, Shrestha LB. Virulence factors of uropathogenic Escherichia coli (UPEC) and correlation with antimicrobial resistance. BMC Microbiol. 2019;19(1):204. Published 2019 Sep 2. doi:10.1186/s12866-019-1587-3
20. Ribeiro Junior JC, Tamanini R, Fritegoto Soares B, et al. Efficiency of boiling and four other methods for genomic DNA extraction of deteriorating spore-forming bacteria from milk. Semin Ciênc Agrár. 2016;37(5):3069-3078. doi:10.5433/1679-0359.2016v37n5p3069
21. García-Suárez JF, Poza M, Bou G, Aranda J. Iron acquisition in gram-negative bacteria: mechanisms and regulation. Front Cell Infect Microbiol. 2011;1:8. doi:10.3389/fcimb.2011.00008
22. Nairz M, Weiss G. Iron in infection and immunity. Mol Aspects Med. 2020;75:100864. doi:10.1016/j.mam.2020.100864
23. Ghatpande N, Harrer A, Azoulay-Botzer B, et al. Iron regulatory proteins 1 and 2 have opposing roles in regulating inflammation in bacterial orchitis. JCI Insight. 2024;9(5):e175845. doi:10.1172/jci.insight.175845
24. Cornelis P, Andrews SC. Iron Uptake and Homeostasis in Microorganisms. In: Cornelis P, ed. Iron Uptake and Homeostasis in Microorganisms. Caister Academic Press; 2010:1-25.
25. Gu H, Cai X, Zhang X, et al. A previously uncharacterized two-component signaling system in uropathogenic *Escherichia coli* coordinates protection against host-derived oxidative stress with activation of hemolysin-mediated host cell pyroptosis. PLoS Pathog. 2021;17(10):e1010005. doi:10.1371/journal.ppat.1010005
26. Huang SH, Wan J H, Huang ZQ. The role of virulence factors in the pathogenicity of Escherichia coli infections. Microbiol Mol Biol Rev. 2017;81(4):e00015-17. doi:10.1128/mmbr.00015-17
27. Kim KS. Virulence factors in *Escherichia coli* strains causing urinary tract infection. Virulence. 2016;7(4):517-528. doi:10.1080/21505594.2016.1159578
28. Sun Z, Zhou N, Zhang W, Xu Y, Yao YF. Dual role of CsrA in regulating the hemolytic activity of *Escherichia coli* O157:H7. Virulence. 2022;13(1):859-874. doi:10.1080/21505594.2022.2073023
29. Wang L, Zhang TL, Xiang Q, et al. Selective enrichment of virulence factor genes in the plastisphere under antibiotic and heavy metal pressures. J Hazard Mater. 2024;465:133319. doi:10.1016/j.jhazmat.2023.133319
30. Mellata M. Human and avian extraintestinal pathogenic *Escherichia coli*: infections, zoonotic risks, and antibiotic resistance trends. Foodborne Pathog Dis. 2013;10(11):916-932. doi:10.1089/fpd.2013.1533
31. Sarowska J, Futoma-Koloch B, Jama-Kmiecik A, et al. Virulence factors, prevalence and potential transmission of extraintestinal pathogenic *Escherichia coli* isolated from different sources: recent reports. Gut Pathog. 2019;11(1):10. doi:10.1186/s13099-019-0290-0
32. Chandra M, Sharanappa V, Thomas P, Ravikumar KL, Shetty AS. Distribution of virulence genes and molecular fingerprinting of *Escherichia coli* isolates from different sources in a tropical environment. J Microbiol Immunol Infect. 2013;46(2):96-102. doi:10.1016/j.jmii.2012.03.002