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Adhesion Gene Profiles and Urothelial Adherence in Uropathogenic Escherichia coli: Evidence of Phenotypic Convergence

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ABSTRACT: Uropathogenic *Escherichia coli* (UPEC) utilise several adhesin systems to colonise the urinary tract, nevertheless, the connection between gene adhesin carriage and adherence to epithelial cells is not fully understood. Here we explored the prevalence of six adhesion related genes (*auf*, *csg, fimA, matA, papA* and *sfa*) in 25 clinical *E. coli* isolates and assessed their adhesion capacity to urothelial cells. Genotypic heterogeneity was found to be noteworthy with PCR screening showing six adhesin profiles. The presence of *csg*, *matA* and *papA* was observed in all of the isolates, whilst *fimA*, *auf*, and *sfa* showed variable expression. The most widespread profile was the one with all six genes (48%). Adherence to urothelia was determined by counting the cells of the epithelia on which the bacteria were bound. On comparison of the adherence among the genotypic profiles, there were no significant differences ($P > 0.05$). The isolates were stratified in the presence or absence of *auf*, *fimA*, or *sfa*, which also demonstrated overlapping interquartile ranges and lack of significant differences in median values of adherence. These results suggested that adherence capacity under the tested conditions cannot be predicted only by adhesin gene carriage. The preserved nature of *csg*, *matA*, and *papA* in all isolates could give a minimum adhesion functional attribute that fills in the variation in other adhesin genes, creating similar adherence levels among different genotypes. Altogether, the findings specify the multifacetedness of the UPEC adhesion process and indicate that regulatory and expression dynamics have a far greater impact on the phenotypic adherence than the mere presence of the genes.

INTRODUCTION

Urinary tract infections (UTIs) are one of the most prevalent bacterial infections exhibited on a global scale and remain a substantial clinical and economic problem (Yang et al. 2022). *Escherichia coli* has been the most common aetiological agent in both community-acquired and healthcare-associated UTIs (AL-Nasrawi et al. 2025). The factors that determine the ability of uropathogenic *E. coli* (UPEC) to develop an infection are highly reliant on its ability to adhere to the urothelial cells, resist the urine stream, and colonise. Adhesion is hence regarded as a key initial process in UTI pathogenesis and forms the basis of the following events including invasion, intracellular community development and persistence (Whelan et al. 2023). Uropathogenic *E. coli* (UPEC) expresses a wide range of fimbrial and afimbrial adhesins, which attach UPEC to different host receptors. FimA-encoded type 1 fimbriae promote mannose-sensitive adhesion of uroplakins and their colonisation of the bladder is indispensable (Torres-Puig et al. 2022). Deficiency of type 1 pili has also been recently reported to change immune recognition and lower the performance of host pathogen interaction (Schwan 2025). P fimbriae, the major subunit of which is encoded by *papA*, show topology towards Gal(α14) Gal-bearing receptors on renal epithelium and are closely linked to infection of the

upper tract (Wiles et al., 2008). S fimbries that are coded by *sfa* in recognising sialylated glycoconjugates and have been associated with recurrent or severe disease manifestations (Khalid and Abu-Resha 2024). Other adhesion correlated systems serve the role of epithelial interaction and environmental persistence. Curli fimbriae (coded by *csg*) increase colonisation on epithelial surfaces and promote colonisation in murine models of the urinary tract (Koodi et al. 2026). The mat operon (more recently renamed the *ecp* operon) is a conserved adhesin cluster that is involved in the epithelial attachment and colonisation of *E. coli* Common Pilus (ECP) (Rendon et al. 2007) and the *auf* operon has been associated with epithelial binding and colonisation fitness, but these systems are not well characterised (Flores-Oropeza et al. 2023). These adhesins are important virulence determinants, which are taken collectively but their distribution and functional contribution differ significantly among clinical isolates (Al-Rubaye and Al-Rubaye, 2025). Although the significance of these adhesion systems has been acknowledged, a limited number of studies have analysed the carriage of several adhesin genes along with the direct quantitative adherence of the adhesion systems to the urothelial cells. Genotypic profiling in itself might not be a consistent indicator of adhesion behaviour and phenotypic assays under molecular context cannot identify the underlying determinants that account for increased binding. It is thus important to understand the relationship between gene carriage and functional adherence in order to demystify the biology of UPEC colonisation. In this study, the adherence potential of clinical isolates of *E. coli* was investigated by combining molecular detection of six key adhesion-linked genes (*auf*, *csg*, *fimA*, *matA*, *papA*, and *sfa*) with the determination of adherence quantitatively onto urothelial cells. This integrated technique offers a better insight into the correlation between adhesin gene profiles and functional binding capacity and could be used in the identification of isolates with the greater propensity towards urothelial colonisation.

MATERIALS AND METHODS

Escherichia coli isolates

A total of 25 *E. coli* clinical isolates in the Microbiology Laboratory, Department of Biology, College of Science, University of Baghdad. All the isolates were kept under ordinary laboratory conditions and stored accordingly till use.

Adherence assay

Escherichia coli isolates were grown in Brain Heart Infusion (BHI) broth and incubated at 37°C over 24 h. Harvesting of the bacterial cells was done by centrifugation followed by subsequent washing with phosphate-buffered saline (PBS), then resettled to a final concentration of about 1 x 10^8 CFU/ml. Midstream urine of a 23-year-old healthy female volunteer was collected and used to get uroepithelial cells. The urine sample was centrifuged at 1000 xg for 5 min so as to harvest the epithelial cell. The pellet that resulted was washed thrice with PBS and resuspended to acquire clean epithelial cell suspension. The bacterial and epithelial cell suspensions were mixed in equal amounts and incubated at 37°C for 1 h to enable adherence to take place. Afterwards, centrifugation (at 1000 ×g for 5 min) and washing three times with PBS were used to remove any bacteria that were not attached after incubation. The last pellet was suspended once again in PBS, spread on clean glass slides, and dried at air. Methanol was then used to fix the slides and then stained with Gram staining technique. The count of bacteria attached to individual epithelial cell was determined using a compound light microscope. The complete adherence test was conducted thrice on each isolate and the analysis done on a mean basis (He et al. 2023).

DNA extraction and primer preparation

Genomic DNA was extracted from each isolate using the Presto™ Mini gDNA Bacteria Kit (Geneaid Biotech Ltd., Taiwan), following the manufacturer's instructions to ensure high-quality DNA suitable for PCR amplification. Primers for the target genes were synthesized by Biolab (USA) and received in lyophilized form. Each primer was reconstituted in 300 µl of sterile de-ionized distilled water DNase/RNase-free water (Bioneer corporation, USA) to a stock concentration of 100 pmol/µl. Working solutions were prepared by diluting 10 µl of stock primer into 90 µl of sterile de-ionized distilled water DNase/RNase-free water, giving a final concentration of 10 pmol/µl. These primer solutions were stored at –20 °C until use. Primers employed in the PCR-amplification of the adhesion-associated genes and the sizes of the corresponding amplicons are as follows, *csgA* gene amplification was done with the forward primer: GTACGGTGGCGGTAACTCTG and reverse primer: AAAGCCAACCTGAGTCACGT and the product was 280 bp long. *fimA* gene was amplified by using primers GTCCCTCAGTTCTACAGCGG (forward) and GGCAACAGCGGCTTTAGATG (reverse), which produced a 244 bp long amplicon (designed in this research work). GTCTCCCCATGACGCCTACT (forward) and TCTGACTTTCTGGCCGCAAT (reverse) were used to target the *matA* gene and gave a fragment of 213 bp. The amplification of *papA* was done under the conditions of TGTACTGGCCGTATCCTGGA (forward) and TCAATTCACGCTGACCACGA (reverse) that gave a 232 bp product. The *sfaA* gene amplification was done using

TGTACTGGCCGTATCCTGGA (forward) and GGCGTTGACTTACCTGTTGC (reverse) which gave a 432 bp amplicon. All these primers were designed in this study. Lastly the amplification of the *auf* gene was done with TGACTTATCTTCCTGGTAGC (forward) and GCTCCAGTTTACCTGCTG (reverse) resulting in an 838 bp product (Li et al. 2021).

PCR amplification

Each PCR reaction was prepared in a total volume of 25 µl, combining 1 µl of each primer, 12.5 µl of Taq 2x Master mix (CWbio/ Korea), and 2 µl of template DNA, with the remaining volume adjusted with sterile de-ionized distilled water DNase/RNase-free water. A negative control, in which DNA template was replaced with distilled water, was included in each run. PCR amplification was carried out using a Master cycler gradient PCR (Eppendorf, Germany). The cycling program was initiated with an initial denaturation step at 95 °C for 3 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 58**°**C (all genes except for *sfa*, which was optimized at 56°C) for 30 s, and extension at 72 °C for 1 min. A final extension step was performed at 72°C for 3 **min** to ensure complete synthesis of all PCR products. Subsequently, the PCR amplicons were separated in the electrophoresis process in 1.5 % agarose gel to confirm the size and integrity of the product.

Statistical analysis

Data normality was assessed using Shapiro-Wilk test. The non-parametric tests were carried out to investigate the statistical differences in the adherence data as they were not normally distributed. The Kruskal-Wallis test was used to determine differences in urothelial adherence across the adhesin genotypic profiles. Further comparisons regarding the existence of individual adhesin genes was measured by the Mann-Whitney U test. The data was displayed in the form of median and interquartile range (IQR). Any P-value less than 0.05 was taken to be statistically significant.

RESULTS

Distribution of Adhesin Genes Among UPEC Isolates

The screening of the six adhesion-related genes by PCR demonstrated genetic variation between the *E. coli* isolates with seven adhesion patterns formed. The 25 isolates possessed the *csg* and *matA* gene as well as the *papA* genes. Conversely, there was a mixed distribution of the rest of the adhesin genes with *fimA* being present in 16 (64%), *auf* in 21 (84%), and *sfa* in 20 (80%) of the isolates (Figure 1).

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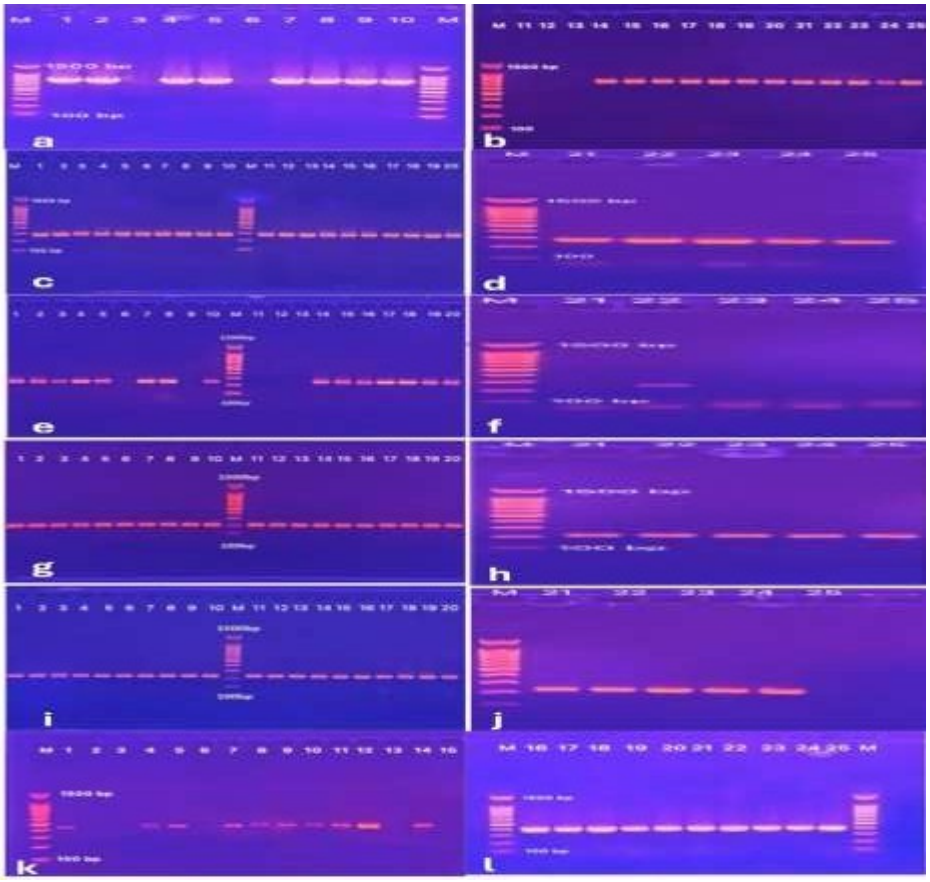

Fig. 1 PCR amplification patterns of the six adhesin genes of Escherichia coli isolates. All isolates (lanes 1-25) are represented with each pair of panels, on (A-B) *auf*; (C-D) *csg*; (E-F) *fimA*; (G-H) *matA*; (I-J) *papA*; (K-L) *sfa*. PCR products were separated using a 1.5% agarose gel with a current of 5 V/cm and observed and stained with ethidium bromide under UV light. M: molecular weight marker.

Adhesin Genotypic Profiles of the Isolates

The isolates were classified as eight genotypic profiles (G1-G8) based on the presence or absence of the genes (Table 1). The most common profile was the G8 which had all six genes intact ($auf^+/csg^+/fimA^+/matA^+/papA^+/sfa^+$) and this was observed in 11 (44.0%) isolates. Urothelial adherence was determined on the six genotypic profiles. The findings did not show statistically significant differences between the profiles ($H = 0.303$, $df = 6$, $P > 0.05$).

Table 1 Genotypic adhesion patterns of the *Escherichia coli* isolates (n=25)

Profile ID	Gene Pattern	Representative Isolates	Frequency (%)
G1	$auf^-/csg^+/fimA^-/matA^+/papA^+/sfa^-$	E38	1 (4)[a]
G2	$auf^-/csg^+/fimA^-/matA^+/papA^+/sfa^+$	E50	1 (4)[a]
G3	$auf^-/csg^+/fimA^+/matA^+/papA^+/sfa^-$	E16	1 (4)[a]
G4	$auf^-/csg^+/fimA^-/matA^+/papA^+/sfa^+$	E15	1 (4)[a]
G5	$auf^-/csg^+/fimA^+/matA^+/papA^+/sfa^+$	E22	1 (4)[a]
G6	$auf^+/csg^+/fimA^-/matA^+/papA^+/sfa^+$	E05, E08, E12, E32, E42, E46	6 (24)[b]
G7	$auf^+/csg^+/fimA^+/matA^+/papA^+/sfa^-$	E10, E33, E35	3 (12)[a]
G8	$auf^+/csg^+/fimA^+/matA^+/papA^+/sfa^+$	E02, E04, E09, E11, E17, E18, E19, E20, E29, E44, E47	11 (44)[c]

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Effect of Individual Adhesin Genes on Adherence

The presence or absence of *auf*, *fimA* and *sfa* was then compared in terms of adherence values (bacterial cells/ urothelial cell). When examining *auf*, the median adherence was 6.46 (IQR: 5.95 - 10.27) regarding gene-positive isolates and 10.64 (IQR: 6.12-14.19) for gene-negative isolates. Concerning *fimA*, the median of adherence was 6.79 (IQR: 5.95 - 10.12) in positive isolates and 7.48 (IQR: 6.13 - 13.51) in negative isolates. When considering *sfa*, the medians of the gene-positive and gene-negative isolates were 7.30 (IQR: 6.00 - 10.34) and 6.36 (IQR: 5.52 - 12.25), respectively. All these comparisons did not show statistically significant differences (Figure 2).

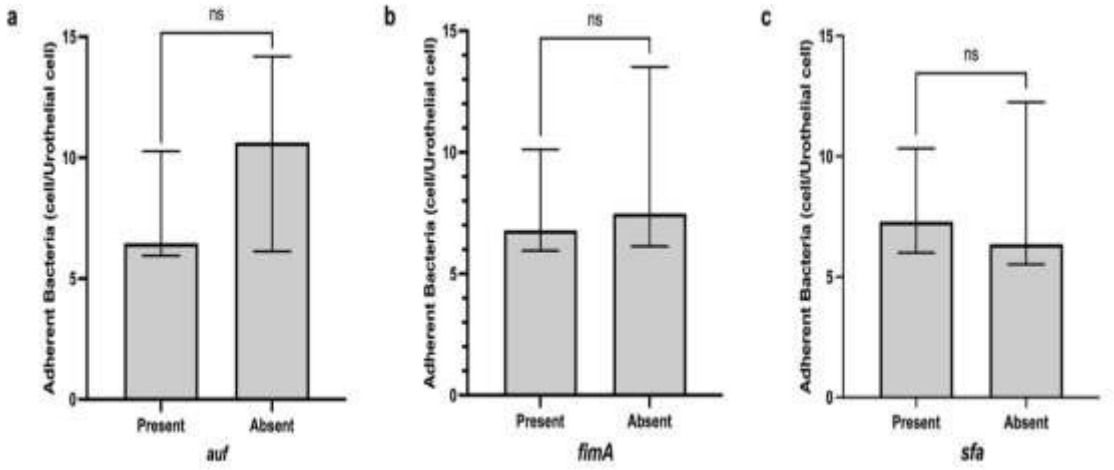

Fig. 2 Urothelial adhesion relative to the presence of adhesin genes. The isolates were grouped as (a) *auf*, (b) *fimA* and (c) *sfa* gene-positive and gene-negative. Error bars represent median with interquartile range.

DISCUSSION

The *E. coli* isolates showed a great genetic heterogeneity as the analysis of adhesion-related genes showed that six combinations of genes are possible. Despite the detection of *csg*, *matA* and *papA* in each of the isolates, indicating that these loci may be conserved adherence determinants in this population, the other genes (*fimA*, *auf* and *sfa*) were variably carried indicate that the adhesin repertoire is varied in this population. This heterogeneity agrees with the of adhesin genes may change significantly even within isolates of the same clinical background established genomic plasticity of UPEC and confirms previous results that the content (Whelan et al. 2023; Rashki et al. 2019).The most prevalent genotypic profile was the isolates with all six adhesin-related genes. The contemporary location of *fimA*, *papA* and *sfa* in a single strain indicates that the strain has multiple fimbrial systems whose functions are well described in the colonisation of the urethra (Torres-Puig et al. 2022). Type 1 fimbriae (encoded by *fimA*) are mediators of mannose-sensitive attachment to bladder uroplakins and are necessary in the initial colonisation of the bladder (Hamid and Khoshabeh 2024). Similarly, the S fimbriae (encoded by *sfa*) allow the detection of sialylated receptors and were associated with frequent or severe UTIs (Govindarajan et al. 2024). These three large systems of fimbriae, together with curli (encoded by *csg*) and MAT fimbriae (encoded by *matA*) indicate a wide adhesive repertoire of these isolates. Profiles that do not have one or more classical fimbrial genes but still have *csg* and *matA* suggest that the profiles depend on alternative adherence strategies. Curli fimbriae (encoded by *csg*) also play a role in surface adhesion, persistence in the environment and initial biofilm formation, and have been identified to increase colonisation of the urinary tract (Garcia-Garcia et al. 2025). The *mat* operon, which is also less well characterised, has also been incriminated in epithelial binding and colonisation of the bladder. These loci being found in all isolates supports the argument that curli and MAT fimbriae could be core adhesion systems that can counterbalance the difference in other adhesin genes. This is known to be a characteristic of UPEC pathogenesis (Lehti et al. 2010; Luna-Pineda et al. 2019). It is also notable how *auf* is widely present in a number of profiles. In spite of the fact that the *auf* operon is studied less well than fimbrial adhesins, there is the beginning of evidence that *auf* proteins could play a role in epithelial attachment or as secondary adhesins in certain conditions (Desvaux et al. 2020). It is detected often in this study the presence of which could suggest it is playing a greater role in colonisation than had been previously recognised. Although there was genotypic diversity, quantitative adherence to urothelial cells showed no significant differences in the seven profiles and there was no difference between isolates positive and negative of *auf*, *fimA* or *sfa*. It shows that the adherence capacity was not predictive by gene carriage only, with the in vitro conditions examined. This observation is consistent with the already known

fact that the expression of adhesins is strongly regulated and depends on environmental factors, phase variation and transcriptional regulation. It was demonstrated that adhesin genes, including *fimA*, *papA* and *sfa*, can be genomically silent and only become expressed under certain conditions of the host or the environment (Schwan 2025). Moreover, past experimental studies have demonstrated that a low level of type 1 fimbriae expression can be compensated by other adhesins including curli or autotransporter proteins (Luna-Pineda et al. 2019).

CONCLUSION

The patterns of adhesion genes detected in the current work indicate the plasticity of UPEC in the colonisation of the urinary tract. Though there was a difference in the genotypes, the difference did not translate into the observable phenotypic difference in adherence. This highlights the multifactoriality of UPEC adhesion and indicates that processes other than the presence of the genes especially the regulation of the genes, the dynamics of expression and environmental stimuli are central determinants in the determination of adherence behaviour. Studies that include gene expression (e.g. RT-qPCR), regulatory profiling or in vivo models will be necessary to understand the mechanisms behind the variability of adherence across these genotypic profiles.

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Data availability statement

10.6084/m9.figshare.31164316

Ethics approval and consent to participate

The bacterial strains used in this study were taken in a lab repository after their initial collection in research approved protocol. The Research Ethics Committee of the College of Science, University of Baghdad (Ref. No. CSEC/1124/0084) gave an ethical approval. All patients freely consented to provide their specimens for research purposes. All isolates were anonymized before analysis.

Patient consent for publication

Not applicable

Author declaration

The authors declare that they have no conflicts of interest.

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REFERENCES

[1] AL-Nasrawi M, Al-Draghi W, Aldujaili N (2025) Predominance of antibiotics resistance of Escherichia coli isolated from Urinary tract infection patients in diabetic and non-diabetic woman. Iraqi J Biotechnol 24(3):95-103.

[2] Al-Rubaye DS, Al-Rubaye TS (2025) Bioactivity of Streptomyces against colistin resistant K. pneumoniae isolated from different clinical samples. Iraqi J Agric Sci 56(2):767-775. doi:10.36103/46d38432.

[3] Desvaux M, Dalmasso G, Beyrouthy R, Barnich N, Delmas J, Bonnet R (2020) Pathogenicity Factors of Genomic Islands in Intestinal and Extraintestinal Escherichia coli. Front Microbiol 11:2065. doi:10.3389/fmicb.2020.02065.

[4] Flores-Oropeza MA, Ochoa SA, Cruz-Cordova A, Chavez-Tepecano R, Martinez-Penafiel E, Rembao-Bojorquez D, Zavala-Vega S, Hernandez-Castro R, Flores-Encarnacion M, Arellano-Galindo J, Velez D, Xicohtencatl-Cortes J (2023) Comparative genomic analysis of uropathogenic Escherichia coli strains from women with recurrent urinary tract infection. Front Microbiol 14:1340427. doi:10.3389/fmicb.2023.1340427.

[5] Garcia-Garcia JD, Contreras-Alvarado LM, Cruz-Cordova A, Hernandez-Castro R, Flores-Encarnacion M, Rivera-Gutierrez S, Arellano-Galindo J, S AO, Xicohtencatl-Cortes J (2025) Pathogenesis and Immunomodulation of Urinary Tract Infections

Caused by Uropathogenic Escherichia coli. Microorganisms 13(4). doi:10.3390/microorganisms13040745.

[6] Govindarajan DK, Eskeziyaw BM, Kandaswamy K, Mengistu DY (2024) Diagnosis of extraintestinal pathogenic Escherichia coli pathogenesis in urinary tract infection. Curr Res Microb Sci 7:100296. doi:10.1016/j.crmicr.2024.100296.

[7] Hamid SAA, Khoshabeh RM (2024) Antibiotic Resistance, Biofilm Formation, and Identification of FimH and FimA Adhesion Genes in Uropathogenic Escherichia Coli (UPEC) Isolated from Patients in Baghdad Province. Iraqi J Sci:5546-5554. doi:10.24996/ijs.2024.65.10.19.

[8] He R, Chen H, Chen W, Zhang M, Pei J, Chen W, Zhong Q (2023) Respiratory depression driven by membrane damage as a mechanism for linalool to inhibit Pseudomonas lundensis and its preservation potential for beef. J Appl Microbiol 134(3). doi:10.1093/jambio/lxad023.

[9] Khalid AN, Abu-Resha RA (2024) Expression of sfa and afa Genes in Uropathogenic Escherichia coli Under Probiotic Effect. Al-Rafidain J Med Sci 7(2):66-71. doi:10.54133/ajms.v7i2.1501.

[10] Koodi MK, Asra NA, Jalil aAA, Najeeb LM (2026) Expression of fimA and csgA genes in uropathogenic Escherichia coli treated with supernatant of Lactobacillus plantarum plus gentamicin. Int J Bioinf Res Appl 22(1):50-67. doi:10.1504/ijbra.2026.151285.

[11] Lehti TA, Bauchart P, Heikkinen J, Hacker J, Korhonen TK, Dobrindt U, Westerlund-Wikstrom B (2010) Mat fimbriae promote biofilm formation by meningitis-associated Escherichia coli. Microbiology 156(Pt 8):2408-2417. doi:10.1099/mic.0.039610-0.

[12] Li X, Zhou K, Wang J, Guo J, Cao Y, Ren J, Guan T, Sheng W, Zhang M, Yao Z, Wang Q (2021) Diagnostic Value of the Fimbriae Distribution Pattern in Localization of Urinary Tract Infection. Front Med (Lausanne) 8:602691. doi:10.3389/fmed.2021.602691.

[13] Luna-Pineda VM, Moreno-Fierros L, Cazares-Dominguez V, Ilhuicatzi-Alvarado D, Ochoa SA, Cruz-Cordova A, Valencia-Mayoral P, Rodriguez-Leviz A, Xicohtencatl-Cortes J (2019) Curli of Uropathogenic Escherichia coli Enhance Urinary Tract Colonization as a Fitness Factor. Front Microbiol 10:2063. doi:10.3389/fmicb.2019.02063.

[14] Rashki A, Rahdar M, Rashki Ghalehnoo Z (2019) Characterization of Uropathogenic Escherichia coli: Distribution of Adhesin-Encoding Genes and O-Serotypes Among Ciprofloxacin Susceptible and Resistant Isolates. Jundishapur J Microbiol 12(9). doi:10.5812/jjm.89179.

[15] Rendon MA, Saldana Z, Erdem AL, Monteiro-Neto V, Vazquez A, Kaper JB, Puente JL, Giron JA (2007) Commensal and pathogenic Escherichia coli use a common pilus adherence factor for epithelial cell colonization. Proc Natl Acad Sci USA 104(25):10637-10642. doi:10.1073/pnas.0704104104.

[16] Schwan WR (2025) Loss of Type 1 Pili and Flagella in Uropathogenic Escherichia coli Leads to Reduced Phagocytosis by Human and Murine Monocytes. Pathogens 14(10). doi:10.3390/pathogens14100968.

[17] Torres-Puig S, Garcia V, Staerk K, Andersen TE, Moller-Jensen J, Olsen JE, Herrero-Fresno A (2022) "Omics" Technologies - What Have They Told Us About Uropathogenic Escherichia coli Fitness and Virulence During Urinary Tract Infection? Front Cell Infect Microbiol 12:824039. doi:10.3389/fcimb.2022.824039.

[18] Whelan S, Lucey B, Finn K (2023) Uropathogenic Escherichia coli (UPEC)-Associated Urinary Tract Infections: The Molecular Basis for Challenges to Effective Treatment. Microorganisms 11(9). doi:10.3390/microorganisms11092169.

[19] Yang X, Chen H, Zheng Y, Qu S, Wang H, Yi F (2022) Disease burden and long-term trends of urinary tract infections: A worldwide report. Front Public Health 10:888205. doi:10.3389/fpubh.2022.888205.