Original Article

Microbial Biosystems 10(2) (2025) 2025.352629
10.21608/mb.2025.352629.1228

Contents lists available at Egyptian Knowledge Bank
Microbial Biosystems
Journal homepage: http://mb.journals.ekb.eg/

Distribution and association of an *usp* genotoxin gene with biofilm formation in *E. coli*

Husam Sabah Auhim*, Hassan Majeed Rasheed

Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.

ARTICLE INFO

Article history
Received 13 January 2025
Received revised 2 Febrauary 2025
Accepted 15 May 2025
Available online 1 June 2025

Corresponding Editors
Al-Abedi, H.
Meskini, M.
Odaa, N. H.

Keywords
Gene detection,
UPEC,
Urinary tract infections,
usp gene,
USP protein.

ABSTRACT

Uropathogenic specific protein is a genotoxic protein targeting the DNA, leading to mutations and modifications in the normal cell's DNA and subsequently, cancer development. This study aims to determine the prevalence of the *usp* gene in Uropathogenic *Escherichia coli* isolated from females with urinary tract infections and study its correlation with biofilm formation. One hundred and five urine specimens were collected from female patients (20 to 55 years old) with urinary tract infections attending hospitals. Traditional laboratory methods using selective and differential culture media were used for initial bacterial isolation and identification, and molecular techniques that targeted a segment of the *16SrRNA* gene with a specific primer pair were used to confirm the bacterial identification and *usp* gene detection using a conventional polymerase chain reaction. A microtiter plate method was used to assess the ability of isolates to produce biofilm. The bacterial isolation and identification results revealed (54.28%, 57/105) of isolates were *Escherichia coli*. The results of molecular detection of the *usp* gene revealed a considerable prevalence (98.2%, 56\57) in Uropathogenic *Escherichia coli* and a 100% ability to form a biofilm. The isolates exhibited different biofilm formation abilities, with a higher ability to form strong biofilm (42%, 24/57) followed by moderate and weak biofilm formation (35%,20/57) and (23%, 13/57), respectively. However, no statistical correlation between the *usp* gene and different abilities for biofilm formation has been found. The study's limitation is that there is a small number of specimens due to the difficulty in specimen collection. In conclusion, the high prevalence of the *usp* gene in Uropathogenic *Escherichia coli*, although it does not correlate with biofilm, suggests its essential role in bacterial pathogenicity and the possibility of cancer disease in females with UTIs.

Published by Arab Society for Fungal Conservation

Introduction

Escherichia coli (E. coli) is one of the well-known normal flora of the human and animal large intestine. However, some *E. coli* strains acquired a specific genomic locus, known as a pathogenic island, via different genetic material transfer methods or gained a plasmid. The pathogenic islands carry genes for several virulence factors, which turn the normal strains into pathogenic ones (Braz et al.2020, Desvaux et al. 2020). Pathogenic *E. coli* is responsible for many diseases, such as septicemia, newborn meningitis, chronic diarrhoea, colorectal cancer, urinary tract infections (UTIs), and others (Whelan et al. 2023). Different pathotypes of *E. coli* were categorised based on

* *Corresponding author Email address*: husam.auhim@sc.uobaghdad.edu.iq **(Husam Sabah Auhim)**

the infection location, mechanisms of pathogenicity, and the presence of virulence factors, including, (i) Enterotoxigenic *E. coli* (ETEC), (ii) Uropathogenic *E. coli* (UPEC), (iii) Diffusely adherent *E. coli* (DAEC), (iv) Enteropathogenic *E. coli* (EPEC), (v) Enteroaggregative *E. coli* (EAEC), (vi) Neonatal meningitis *E. coli* (NMEC), (vi) Enterohemorrhagic *E. coli* (EHEC), (vii) Enteroinvasive *E. coli* (EIEC), (viii) Septicemia-associated *E. coli* (SEPEC), and (ix) Adherent-Invasive *E. coli* (AIEC) (Pokharel et al. 2023). The most common one is the UPEC; this pathotype is responsible for about 80–90% of community-acquired UTIs; recently, UPEC gained more interest owing to its ability to produce several genotoxins and is linked to some UPEC strains with colorectal cancer disease. The survival-infected cells with genotoxins will be genomically unstable and acquire malignant features (Wang & Flu 2023).

Genotoxins are a group of proteins or chemicals that target the DNA, leading to DNA damage and mutations, resulting in the malignant transformation of normal cells and, eventually, cancer development. Some UPEC strains produce more than one genotoxin protein, such as cytolethal distending toxins (Chen et al. 2023), colibactin (Chat et al.2023), and Uropathogenic-specific protein (USP) (Rihtar et al.2020). These toxins are different in their molecular structure and mode of action; however, they are targeted and damage the DNA. The molecular structure of the USP protein has not been solved yet; therefore, the actual function of the protein domains is unknown. However, bioinformatic analysis showed that the USP protein has two domains: pyocins and DNase-like colicins (Nipi et al. 2013). Most of the virulence factors have been found to be linked together, and this linkage could affect the pathogenicity of the pathogen positively or negatively. It has been found that the presence of hemolysin and colibactin exhibits advantages in colonisation and the progression of colorectal cancer (Yoshikawa et al. 2020). A study by Morgan and his colleagues on rats as a model for ascending urinary tract infection indicated that *E. coli* possessing colibactin and *cnf-1* genes induced severe illness within 48 to 72 hours. Furthermore, colibactin-producing isolates statistically correlate with biofilm formation (Morgan et al. 2019). However, a study of Rijavec et al.(2008) reported that there was no statistical correlation between the existence of some toxin genes in *E coli*, such as *hlyA*, *cnf1*, and *usp*, and biofilm formation. In the same previous study, the *usp* gene was statistically associated with non-immunocompromised patients as a highly prevalent gene. In addition, the *usp* gene is statistically associated with non-multi-drug-resistant bacteria.

In Iraq, some of the genotoxic proteins in UPEC have been studied. However, the association of the *usp* gene with biofilm formation has not been well established. Therefore, this study aims to investigate the prevalence of the genotoxic *usp* gene in UPEC and determine the potential association with different biofilm formation abilities. Knowing the possible association between the *usp* gene and biofilm formation could help to develop new strategies to eliminate its detrimental effect.

Material and methods

Specimens' collection, bacterial isolation, and identification

Mid-stream urine samples (*n*= 105) were collected from females (20-55 years old) with UTIs who attended Iraqi Hospitals in Baghdad from September to November 2024. Ethical approval (CSEC\1223\0132) for this investigation has been given by the ethics committees of the Department of Biology, College of Science, University of Baghdad. A sterile screw-cap container was used for urine sample collection and transported to the microbiology laboratory at the University of Baghdad. First, the samples were centrifuged at 5000 rpm for 10 min, and then, the precipitate was suspended in 1 ml of nutrient broth, then cultured on MacConkey agar, and incubated for 24 hours at 37°C. The isolated bacteria that showed a positive result for *E. coli* on MacConkey agar were subjected to primary identification by culturing on selective and differential media for *E. coli*, which are Eosin methylene blue (EMB) agar and 4-Methylumbelliferyl beta-D-Glucuronide (MUG) agar. The identification of suspected bacterial isolates was confirmed by amplifying a segment of the *16S rRNA* gene using conventional polymerase chain reaction (PCR) with specific primers. After bacterial identification, *E. coli* isolates were maintained at 4ºC for subsequent experiments.

Amplification of target genes by polymerase chain reaction (PCR)

The polymerase chain reaction (PCR) technique was employed to amplify a segment of two target genes: 16*S rRNA* for bacterial identification and *usp* for genotoxin detection. Primers were manufactured and provided in a lyophilised form by Macrogen Company. For *16SrRNA*, the forward primer 5`-TAGCTGGTCTGAGAGGATGA CCA-3` and reverse primer 5`-CCAACATTTCACAACA CGAGCTGAC -3`, with a 802 bp amplicon size, were used (Odaa & Rasheed 2025). For *usp*, the forward primer 5`-ACATTCACGGCAAGCCTCAG-3`, and reverse primer 5`-AGCGAGTTCCTGGTGAAAGC-3`, with a 435 bp amplicon size, were used (Khalaf & Flayyih 2024). The rapid bacterial DNA extraction and gene detection were performed as described by Auhim & Odaa (2025), briefly, 100 µl of PCR reaction mixture contains 50 µl Go Taq G2 Green Master Mix (2X, Promega, USA), 5 µl of each forward and reverse primers (10µM/ µl), and 40 µl of

nuclease-free water. 9 µl of the reaction mixture was aliquoted into the PCR tubes, and 1 µl of bacterial supernatant-containing DNA was added. The gene amplification was performed using an Applied Biosystems thermocycler with the following amplification conditions: 95°C for 5 min (initial denaturation) and 35 thermocycles (denaturation for 30 s at 95°C, annealing for 30 s at 58°C for*16SrRNA*, and at 55°C for *usp*, and extension for 1 min at 72 °C) terminated by a final extension for 7 min at 72 °C. The PCR product (5 µl) was electrophorised against 3 µl of DNA marker (100 pb, Promega, USA) for 60 min at 100 volts utilizing 2% agarose gel containing ethidium bromide (0.5µg/mL), then the bands of DNA were depicted using a UV illuminator system (Fisher Scientific, UK).

Assessment of biofilm formation by Uropathogenic E. coli isolates

A microtiter plate technique was used to assess the ability of UPEC isolates for biofilm formation as described by Djordjevic et al. (2002) Microplate ELISA readers (Diagnostic Automation, Inc., USA) was employed to measure the optical density (OD) at 580 nm for microtiter plate. The UPEC isolates have been classified into four groups according to the cut-off value (ODC): non-biofilm producer (OD≤ODC), weak biofilm producer (ODC<OD≤2× ODC), moderate biofilm producer (2×ODC<OD≤4×ODC), and strong biofilm producer (4×ODC<OD). The ODC, which is the cut-off optical density known as three standard deviations over the mean optical density of the negative control, was determined as described by Djordjevic et al.2002 ODC= mean optical density of the negative control + (three × standard deviations of the negative control). Nutrient broth was utilized as a negative control for ODC calculation, and no positive control was used.

Statistical analysis

All features were expressed as frequency and percentage, using a Pearson-Chi-square (P≤0.05) as a non-parametric test to describe the significant difference in percentages. Statistical software SPSS v. 22.0 and Excel 2013 were employed to analyse the results.

Results

Identification of isolated bacteria

From 105 urine samples, 57 UPEC isolates (54.2%, 57/105) were isolated and identified based on the cultural characteristics of three different selective and differential media for *E. coli*. UPEC isolates appeared on MacConkey agar as pink colonies, on EMB agar as green metallic sheen, and on MUG agar as fluorescence colonies under the UV illuminator system, as shown in Fig.1. Molecular identification by amplifying a segment of the *16S rRNA* gene using specific primers for *E. coli* revealed a single clear amplified DNA band with a 802 bp fragment size, as depicted in Fig. 2 (results for 19 isolates were shown). These results validated the bacterial identification of isolates as *E. coli.*

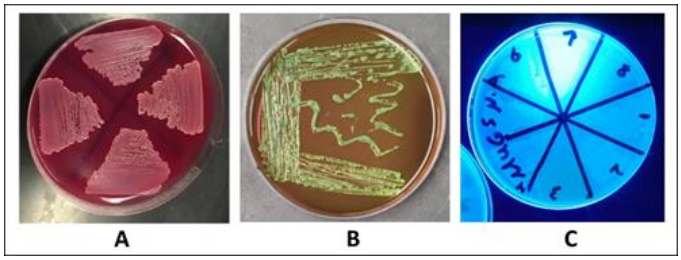

Fig 1. UPEC colonies on selective and differential media after culturing for 24 hours at 37ºC. A. MacConkey agar, B. EMB agar, C. MUG nutrient agar under UV illuminator system, number 7 represents *E. coli* isolate with fluorescence colonies, while other numbers represent other bacterial species that were cultured for comparison reasons.

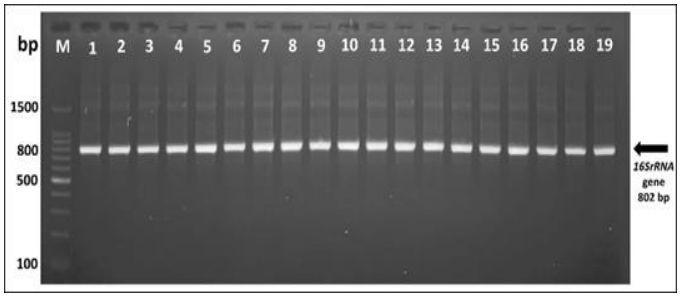

Fig 2. Image of an agarose gel electrophoresis (2%) shows the amplicon of the *16S rRNA* gene (802 bp) for 19 isolates. Numbers 1 to 19 refer to the UPEC isolates. M refers to the DNA marker (100 bp).

Biofilm formation by Uropathogenic E. coli isolates

The results exhibited that all UPEC isolates were biofilm-producing isolates with 100% biofilm formation ability under laboratory conditions. However, UPEC isolates showed different biofilm formation abilities. Strong formers (42 %), moderate formers (35 %), and weak formers (23%) with no significant differences (P <0.05), as illustrated in table 1**.**

Molecular detection and the frequency of usp gene

The results of molecular detection revealed that 98.2% (56\57) of UPEC isolates habour *usp* gene, compared to 1.8 % (1\57) that lack *usp* gene, with significant differences (p>0.01), as demonstrated in Fig. 3 and (Table 2).

Table 1 Different biofilm formation abilities of UPEC isolates.

Type of biofilm former	Number of isolates	Percentage
Strong former	24	42 %
Moderate former	20	35 %
Weak former	13	23 %
Total	57	100%
P value	P<0.05	

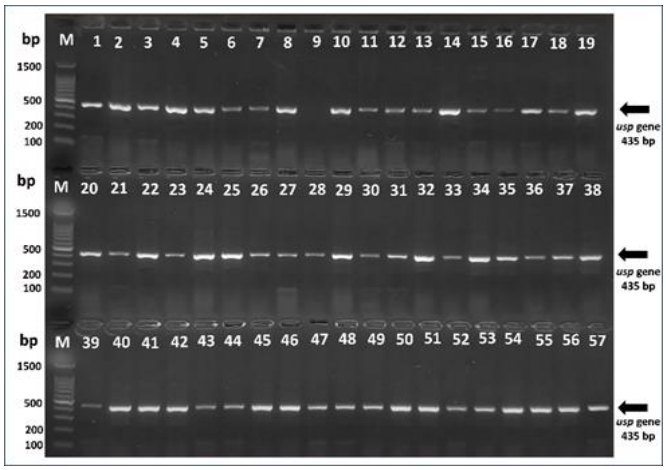

Fig 3. Image of an agarose gel electrophoresis (2%) shows the *usp* gene amplicon (435 bp). Numbers 1 to 57 refer to the UPEC isolates. M refers to the DNA marker (100 bp).

Table 2 The frequency of *usp* gene in UPEC isolates

UPEC isolates	Frequency	Percentage (%)	P-value
usp- positive	56	98.2	
usp- negative	1	1.8	P>0.000
Total	57	100%	

Association of usp gene with biofilm formation in Uropathogenic E.coli isolates

Results in (Table 3) revealed that *usp* gene was distributed among all biofilm-forming UPEC isolates with no significant differences (P <0.05). However, most *usp*-positive isolates exhibited strong biofilm formation ability, followed by moderate and weak formation abilities.

Table 3 Distribution of *usp* gene among biofilm-forming UPEC isolates.

Type of Biofilm	Number of *usp*-positive isolates	Percentage
Strong	24	100 % (24/24)
Moderate	19	95 % (19/20)
Weak	13	100 % (13/13)
Total	56	100%
P value	P<0.05	

Discussion

Urinary tract infections are known as a common health issue in communities. These infections result from bacterial infections (the main causative agent) or other causes, such as fungi and viruses. *E. coli* is known as the main agent for UTIs in Iraq and worldwide compared with other bacterial species such as *Proteus mirabilis*, *Staphylococcus* spp, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa,* and *Enterococcus faecalis* (Mancuso et al.2023). To isolate the bacteria under investigation from the UTIs, it is important to employ the selective and differential media for isolation, and this will aid in the inhibition and differentiation of other UTI causative agents rather than bacteria under investigation, leading to saving resources and time (Auhim & Odaa 2025). *E. coli* is a lactose-fermenting bacterium; therefore, it appeared as pink colonies on MacConkey agar. Also, *E. coli* exhibited a distinct colour on EMB agar, which is a green metallic sheen owing to their ability to form strong acid byproducts during fermentation with the metachromatic features of the dyes and the movement of bacteria with flagella (Basavaraju et al. 2023).

In addition to the above two selective and differential media, a third differential medium for *E. coli* can be applied to initiate bacterial identification, which is MUG nutrient agar. This medium contains fluorogenic substrate 4-Methylumbelliferyl beta-D-Glucuronide (MUG), bacteria that have the ability to produce b-glucuronidase enzyme, such as *E. coli* releases 4-Methylumbelliferone, which is a fluorogenic substance, making the bacterial colonies appear fluorescent under UV light (Shadix et al.1993).

The identification of bacteria based on amplifying a conserved housekeeping gene in bacterial species is considered reliable and widely utilised in pathogenic bacterial identification (Zhang et al. 2022). In the current study, all primarily identified bacteria gave positive results by targeting a fragment of the *16S rRNA* gene that confirmed the bacterial identification as *E. coli*. Similarly, Muhaimeed et al. (2023) reported that 100% of the primary identified *E. coli* were positive for *16S rRNA* gene detection using specific primers.

The isolation percentage of *E. coli* (54%) from females with UTIs in the current study is considered normal compared with other local studies that reported approximately similar isolation percentages. Salumi and Abood (2022) found that the isolation percentage of *E. coli* from urine was 58.09%, while another study conducted by Ebraheem & Alwendawi (2015) showed a slightly higher percentage, which was 65.8%. However, this high *E. coli* isolation percentage compared with other bacterial isolations from females with UTIs may be linked to the capacity of bacteria to settle into the urinary tract's and their

capacity to endure these environmental conditions. Furthermore, it exhibits significant virulence factors and has the capacity to produce biofilms. Moreover, as a result of the differences in urinary system anatomy between females and males, females exhibit more vulnerability to UTIs than males (Khan et al. 2024).

The main virulence factor in pathogenic strains is biofilm formation, which facilitates bacterial colonisation, genetic material transfer, increased antibiotic resistance, and disease development. UPEC isolates under investigation exhibited 100% ability to form a biofilm under laboratory conditions. This finding aligns with another study demonstrating that all UPEC isolates were biofilm producers (Rafaque et al.2020). However, UPEC isolates exhibited different abilities for biofilm formation, with the highest being strong, followed by moderate, and weak. Several factors are involved in biofilm formation and affect the initial attachment of *E.coli* on biotic and abiotic surfaces, including the presence of biofilm-related genes and adhesion elements (Silva et al.2014); the varying distribution of these genes and elements influences the initial number of cells that successfully adhere and form biofilms, beside the environmental factors, may elucidate the different abilities for biofilm formation.

The genotoxic protein produced by bacteria gained more interest from researchers due to the link between the producing bacteria of these toxins and some cancer diseases (Dalmasso et al.2024). USP toxin consists of 346 amino acids encoded by *usp* gene (1038 bp) located on a small pathogenicity island (PAIusp) and has been reported as a genotoxic toxin produced by *E. coli* (Rihtar et al.2020). The distribution of this genotoxic in *E. coli* is concerning, particularly since this bacterium has a high frequency in UTIs, as mentioned above. The result of this investigation revealed a considerable prevalence of USP in UPEC isolates under study, which was 98.2% (56/57). Isolate number 9, which lacks the *usp* gene, could be contaminated by another infection source. This result is in line with another study that demonstrated roughly similar distribution results; it was found that the *usp* gene in UPEC isolates obtained from pyelonephritis was 93.4% (Yamamoto et al. 2001). While the distribution of the *usp* gene in *E. coli* isolated from stool was significantly lower, a local study reported that 6.6 % of isolates from stool harbour the *usp* gene (Khalaf & Flayyih 2024). These results indicated the important role of USP toxin in UTIs. Furthermore, USP is considered a crucial element in UPEC infection due to its ability to pass infectious potential upon non-pathogenic *E. coli* (Yamamoto et al. 2001).

Several genes and factors regulate biofilm formation in bacteria; therefore, some virulence factors are associated with biofilm formation (Zhao et al.2023). However, the current investigation found no statistical correlation between the *usp* gene and biofilm formation, as the *usp* gene was distributed amongst all UPEC isolates that exhibited different abilities for biofilm formation. This result is in line with another investigation conducted by Rijavec et al.(2008), they revealed that, *usp* gene has no correlation with biofilm formation and multi-drug-resistant bacteria. The high prevalence of *usp* gene with no correlation with either biofilm formation or antibiotics resistance sheds light on its essential role in bacterial pathogenicity.

The study's limitations, such as sample size, hygienic habits, specimen collection, bacterial isolation procedure, and environmental factors, could lead to differences between the current results and other studies. For instance, incorrect specimen collection and bacterial isolation from a urine sample lead to a reduced isolation percentage; consequently, different results regarding study parameters.

Conclusion

The current finding exhibited a considerable prevalence of a genotoxic in UPEC isolates from females with UTIs, with no statistical association with biofilm formation abilities. This high prevalence of *usp*-positive isolates in females is a concern in the distribution of genotoxic isolates that could lead to cancer development in the urinary tract system or genital system. A possible correlation of *usp* gene with other virulence factors and toxins needs to be investigated. Further studies are required to solve the molecular structure of USP toxin to understand the structure-function relationship and design a possible inhibitor to eliminate the detrimental genotoxic effect.

Acknowledgements

The authors thank Dr Zainab Zamel Khalaf for kindly providing the *usp* gene primers.

Conflict of interest

The authors state no conflicts of interest.

References

Auhim HS, Odaa NH. (2025). Molecular detection and the frequency of a pore-forming toxin in *Enterococcus faecalis* isolated from urinary tract infections. Microbes and Infectious Diseases 6,2, 948-956. doi: 10.21608/mid.2024.324353.2252.

Basavaraju M, Gunashree BS.(2023). *Escherichia coli*: An Overview of Main Characteristics. *Escherichia coli* - Old and New Insights. IntechOpen, 1-21. http://dx.doi.org/10.5772/intechopen.105508.

Braz VS, Melchior K, Moreira CG. (2020). *Escherichia coli* as a Multifaceted Pathogenic and Versatile Bacterium. Frontiers in cellular and infection

microbiology 10, 1-9. https://doi.org/10.3389/fcimb.2020.548492.

Chat H, Dalmasso G, Godfraind C, Bonnin V, Beyrouthy R, Bonnet M, et al. (2023). Cytotoxic necrotising factor 1 hinders colon tumorigenesis induced by colibactin-producing *Escherichia coli* in *ApcMin/+* mice. Gut microbes 15, 2229569. https://doi.org/10.1080/19490976.2023.2229569.

Chen H, Ang CJ, Crowder MK, Brieher WM, Blanke SR. (2023). Revisiting bacterial cytolethal distending toxin structure and function. Frontiers in cellular and infection microbiology 13, 1289359. https://doi.org/10.3389/fcimb.2023.1289359.

Dalmasso G, Cougnoux A, Faïs T, Bonnin V, Mottet-Auselo B, Nguyen HTT, et al. (2024). Colibactin-producing *Escherichia coli* enhance resistance to chemotherapeutic drugs by promoting epithelial to mesenchymal transition and cancer stem cell emergence. Gut Microbes 16, 1–15: https://doi.org/10.1080/19490976.2024.2310215.

Desvaux M, Dalmasso G, Beyrouthy R, Barnich N, Delmas J, Bonnet R. (2020). Pathogenicity Factors of Genomic Islands in Intestinal and Extraintestinal *Escherichia coli*. Frontiers in microbiology 11, 2065. https://doi.org/10.3389/fmicb.2020.02065.

Djordjevic D, Wiedmann M, McLandsborough LA.(2002). Microtiter plate assay for assessment of *Listeria monocytogenes* biofilm formation. Applied and Environmental Microbiology 68, 2950–2958. https://doi.org/10.1128/AEM.68.6.2950-2958.2002.

Ebraheem A A, Alwendawi S A. (2015). Screening for in Vitro Biofilm Formation Ability of Locally Isolated Uropathogenic *Escherichia coli* (UPEC). Iraqi Journal of Science, 56, 1310-1314.

Khan A, Saraf VS, Siddiqui F, Batool T, Noreen Z, Javed S, et al. (2024). Multidrug resistance among uropathogenic clonal group A *E. Coli* isolates from Pakistani women with uncomplicated urinary tract infections. BMC Microbiology 24, 74. https://doi.org/10.1186/s12866-024-03221-8.

Mancuso G, Midiri A, Gerace E, Marra M, Zummo S, Biondo C. (2023). Urinary Tract Infections: The Current Scenario and Future Prospects. Pathogens 12, 623. https://doi.org/10.3390/pathogens12040623.

Morgan R N, Saleh S E, Farrag HA, Aboulwafa M M. (2019). Prevalence and pathologic effects of colibactin and cytotoxic necrotising factor-1 (Cnf 1) in *Escherichia coli*: Experimental and bioinformatics analyses. Gut Pathogens 11,1–18. https://doi.org/10.1186/s13099-019-0304-y.

Muhaimeed AA, Ghareeb AM. (2023). Prevalence of Multi-Antibiotic Resistance marA and Quorum Sensing luxS Genes and Evaluation of Biofilm Formation in Uropathogenic *Escherichia coli*. Iraqi Journal of Biotechnology 22, 252–61.

Nipic D, Podlesek Z, Budic M, Crnigoj M, Zgur-Bertok D. (2013). *Escherichia coli* uropathogenic-specific protein, Usp, is a bacteriocin-like genotoxin. The Journal of Infectious Diseases 208, 1545–1552.

Odaa, N. H., Rasheed, H. M. (2025). Distribution of cytotoxic necrotizing factor type 1 in clinical isolates of *Escherichia coli* isolated from urinary tract infections in Iraq. Microbial Biosystems, 10 (1), 33-38.

Pokharel P, Dhakal S, Dozois CM. (2023). The Diversity of *Escherichia coli* Pathotypes and Vaccination Strategies against This Versatile Bacterial Pathogen. Microorganisms 11, 344. https://doi.org/10.3390/microorganisms11020344.

Rafaque Z, Abid N, Liaqat N, Afridi P, Siddique S, Masood S, et al. (2020). In-vitro Investigation of Antibiotics Efficacy Against Uropathogenic *Escherichia coli* Biofilms and Antibiotic Induced Biofilm Formation at Sub-Minimum Inhibitory Concentration of Ciprofloxacin. Infection and Drug Resistance 13, 2801–2810. https://doi.org/10.2147/IDR.S258355.

Rihtar E, Žgur Bertok D, Podlesek Z. (2020). The Uropathogenic Specific Protein Gene usp from *Escherichia coli* and *Salmonella bongori* is a Novel Member of the TyrR and H-NS Regulons. Microorganisms 8, 330. https://doi.org/10.3390/microorganisms8030330.

Rijavec, M., Müller-Premru, M., Zakotnik, B., & Žgur-Bertok, D. (2008). Virulence factors and biofilm production among *Escherichia coli* strains causing bacteraemia of urinary tract origin. *Journal of medical microbiology*, *57*(Pt 11), 1329–1334. https://doi.org/10.1099/jmm.0.2008/002543-0

Salumi ZN , Abood ZH. (2022). Phenotypic diagnosis of efflux pump of *Escherichia coli* isolated from urinary tract infections. Iraqi Journal of Biotechnology 21, 21-31.

Shadix LC, Dunnigan ME, Rice EW. (1993). Detection of *Escherichia coli* by the nutrient agar plus 4-methylumbelliferyl beta-D-glucuronide (MUG) membrane filter method. Canadian journal of microbiology 39(11), 1066–1070. https://doi.org/10.1139/m93-161.

Silva VO, Soares LO, Silva Júnior A, Mantovani HC, Chang YF, Moreira MA. (2014). Biofilm formation on biotic and abiotic surfaces in the presence of antimicrobials by *Escherichia coli* Isolates from cases of bovine mastitis. Applied and environmental

microbiology 80, 6136–6145. https://doi.org/10.1128/AEM.01953-14.

Wang Y, Fu K. (2023). Genotoxins: The Mechanistic Links between *Escherichia coli* and Colorectal Cancer. Cancers 15, 1152. https://doi.org/10.3390/cancers15041152.

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, 2169.

Yamamoto S, Nakano M, Terai A, Yuri K, Nakata K, Nair GB, Kurazono H, Ogawa O. (2001). The presence of the virulence island containing the *usp* gene in uropathogenic *Escherichia coli* is associated with urinary tract infection in an experimental mouse model. The Journal of urology 165, 1347–1351.

Yoshikawa Y, Tsunematsu Y, Matsuzaki N, Hirayama Y, Higashiguchi F, Sato M, et al. (2020). Characterization of Colibactin-Producing *Escherichia coli* Isolated from Japanese Patients with Colorectal Cancer. Japanese journal of infectious diseases 73, 437–442. https://doi.org/10.7883/yoken.JJID.2020.066

Zhang M, Wu J, Shi Z, Cao A, Fang W, Yan D, et al.(2022). Molecular Methods for Identification and Quantification of Foodborne Pathogens. Molecules. 27: 1–11. https://doi.org/10.3390/ molecules27238262.

Zhao A, Sun J, Liu Y. (2023). Understanding bacterial biofilms: From definition to treatment strategies. Frontiers in cellular and infection microbiology 13, 1137947.https://doi.org/10.3389/fcimb.2023.1137947