RESEARCH ARTICLE

MCBS
Mol Cell Biomed Sci. 2025; 9(2): 69-75
DOI: 10.21705/mcbs.v9i2.589

# Increased expression of *pap* and *sfa* Genes in Biofilm-Forming Uropathogenic *Escherichia coli* Associated with Urinary Tract Infections

Rini Purbowati, Sri Lestari Utami

*Biomedical Department and Biomolecular Research, Faculty of Medicine, Universitas Wijaya Kusuma Surabaya, Surabaya, Indonesia*

**Background:** Urinary tract infections cover a broad spectrum of infectious syndromes and affect the urinary tract from the urethra to the kidneys. Generally caused by uropathogenic *Escherichia coli* (UPEC), and their pathogenesis is greatly influenced by biofilm formation, which results in persistent and recurrent infections. UPEC uses filamentous adhesive structures such as pili or fimbriae, pyelonephritis-associated pili, and S fimbriae, which are regulated by the *pap* and *sfa* operons, respectively. The purpose of the study was to detect the effects of two virulence genes, (*pap 7* and *sfa 9*) on biofilm-forming UPEC associated with urinary tract infections.

**Materials and methods:** A total of 123 UPEC isolates were collected from clinical microbiology laboratory section of a general hospital in Surabaya, Indonesia. Urine samples yielded UPEC with significant counts (≥$10^5$ CFU/ml), and the biofilm development was analyzed using the Congo red agar method. The presence of *pap 7* and *sfa 9* genes in the isolates was determined using PCR assay.

**Results:** Among the 123 UPEC isolates, 66 isolates were able to form biofilms, as determined using the Congo red agar (CRA) method. Biofilm-forming UPEC isolates exhibited a high positivity frequency for the *pap 7* gene (65.85%), while the positivity frequency for the *sfa 9* gene was significantly lower (14.63%).

**Conclusion:** An increase in th expression of *pap 7* and *sfa 9* are is associated with the ability to form biofilms, which could serve as a diagnostic marker for biofilm formation potential vaccine target.

**Keywords:** *biofilm, pap, sfa, uropathogenic, Escherichia coli, UTI*

## Introduction

Urinary tract infection (UTI) is a general term that includes a broad spectrum of infectious syndromes that affect the urinary tract from the urethra to the kidneys. It is one of the most common infections, and it is reported that 50%-60% of women suffer from at least one UTI in their life.[1] UTI can also be exacerbated by several risk factors leading to treatment failure, recurrent infections, or significant morbidity and mortality with poor outcomes.[2] Uropathogenic Escherichia coli (UPEC) is the main etiologic agent of UTIs, including

Submission: November 13, 2024
Last Revision: January 28, 2025
Accepted for Publication: February 3, 2025

**Corresponding Author**:
Rini Purbowati
Biomedical Department and Biomolecular Research, Faculty of Medicine
Universitas Wijaya Kusuma Surabaya
Jl. Dukuh Kupang XXV No.54, Surabya 60225, Indonesia
e-mail: rini.purbowati@uwks.ac.id

Cell and Biopharmaceutical Institute

Print ISSN: 2527-4384, Online ISSN: 2527-3442
DOI: 10.21705/mcbs.v9i2.589

cystitis, pyelonephritis, and serious infectious complications that can lead to acute renal failure.[3] A research showed that among 54 research subjects classified as catheter-associated urinary tract infection (CAUTI), urine culture results showed the growth of *E. coli* bacteria (44.4%).[4] The nosocomial pathogen UPEC is linked to biofilm formation and urinary tract infections, both of which increase antibiotic resistance.[5] The pathogenesis of UPEC is strongly influenced by biofilms (intracellular bacterial communities), resulting in persistent and recurrent infections. Amyloid curli fibers (a protein) and cellulose phosphoethanolamine (pEtN) are the two major extracellular polymeric substances (EPS) components of UPEC biofilms. EPS has an impact in the pathophysiology and persistence of UPEC, including its resistance to treatment with antibiotics and adhesion to urothelial cells. EPS can prevent antibiotics from penetrating the biofilm, which prevents bacteria from being killed and makes medicines less efficient.[6]

Biofilm formation begin only when bacteria are in close contact with a surface and attach to the surface of a living or non-living object. Attachment to a surface is the initial stage of biofilm formation.[7] The major filamentous adhesive structures used by UPEC to achieve adhesion are pili or fimbriae, type 1 fimbriae, pyelonephritis-associated pili (*pap*), and S fimbriae. These structures are regulated by the operons for *fim, pap,* and *sfa*, respectively. A complementary extracellular protein named P fimbriae aids in UPEC's adhesion to uroepithelial cells. The glycosphingolipid globoseries is attached by P fimbriae. Because P fimbriae mediate attachment to epithelial cells in the kidney and renal tubules, they are more often associated with upper urinary tract infections and pyelonephritis (P fimbriae for pyelonephritis fimbriae). The *pap* operon is the chromosomal encoder of P fimbriae.[8]

S fimbriae is a mannose-resistant adhesin, binds to glycoproteins of urothelial tissue in the bladder and kidney and is commonly detected in strains that form robust biofilms *in vitro*.[9] The S fimbria are encoded by the *sfa* gene. S fimbriae mediate interactions with sialic acid-containing receptors, bladder epithelium, and urethral tract, and facilitate pathogenicity on host tissue surfaces.[10] All components of these virulence factors can be attractive candidates for next generation antimicrobials (NGA), which are compounds target bacterial virulence factors to disrupt potential pathogens. NGA makes pathogens more susceptible to clearance by the immune system and potentially makes more susceptible to conventional antibiotics.[11]

Research on the detection and prevalence of biofilm-coding genes has been carried out on various types of test pathogens; for example, previous research has reported a pap operon prevalence of 14-28% in UPEC which causes cystitis, 5-7% in *E. coli* and 71-91% of UPEC isolated from patients with pyelonephritis.[8] From 40 urine samples in UTI patients inpatients at a hospital in Semarang, 10 isolates of ESBl-producing *E. coli* were obtained and 8 samples were positive for pap C (80%) and 10 (100%) for the fim A gene.[12] It is known that the majority of UPEC bacteria (97.36%) are positive for both the *csgD* and *luxS* genes, while the remaining bacteria (92.10%) are positive for the *luxS* gene.[13] This study set out to determine the frequency of the genes pap, which codes for P fimbriae, and *sfa*, which codes for S fimbriae in Biofilm-forming UPEC isolated from UTI patients at one of the largest general hospitals in Jawa Timur. The urgency of this research is designing effective strategies and measures for the prevention and management of UTIs, particularly severe, recurrent, and complicated UTIs, suggests an understanding of the link between biofilm formation and the presence of virulence genes in UPEC strains. Biofilm formation is important as a form of support in the focus area of health-drug research, especially molecular, to realize independence and sovereignty in the field of medical devices. The purpose of the study was to detect the effects of two virulence genes, (*pap 7* and *sfa 9*) on biofilm-forming UPEC associated with urinary tract infections. The hypothesis of this study is that the presence of the *pap 7* and sfa 9 genes is related to UPEC's ability to form biofilms.

## Material and methods

### *Strains and Growth Conditions*

The UPEC isolates used in this study were obtained from urine samples of inpatients admitted to the clinical microbiology laboratory section of a general hospital in Surabaya, Indonesia. The urine samples yielded UPEC with significant counts ($\geq 10^5$ CFU/ml). To ensure the purity of the test bacteria, the isolates were grown in eosin methylene blue (EMB) media (Merck Company, Germany) for 24 hours at a temperature of 37°C so that the colonies that grew had metallic green and fluorescent characteristics. UPEC isolates were cultured in Luria-Bertani (LB) media and then incubated for 24 hours at 37°C so they were ready to be used for further tests.

***Biofilm Detection***

Biofilm detection was carried out through a slime formation test using the Congo red agar (CRA) Method.[14] Strains producing dark black, black, and reddish black colonies are considered to make slime, while red and reddish colonies are classified as not producing slime. *E. coli* S4 was used as a negative control which was known not to form biofilms.

***DNA Extraction***

One hundred twenty-three UPEC isolates were subjected to polymerase chain reaction (PCR) testing to detect the presence of genes associated with biofilm. The UPEC bacterial DNA genome was extracted using the NEXprep™ Cell/Tissue genomic DNA preparation kit. The extracted suspension was stored at -20°C and used as DNA templates.

***PCR Analysis and Virulence Factor Detection***

Amplification of the virulence genes *pap 7* and *sfa 9* was carried out by PCR using published primer pairs (Macrogen Singapore) (Table 1). Primer pairs were created with Primer3 using gene sequences from the National Center for Biotechnology Information (NCBI) genome databases. PCR was consistently performed using a mixture of 20 µL, consisting of 8.4 µL DNA templates, 0.8 µL each primer, and 10 µL PCR master mix. Amplification was carried out using a thermal sickle with the thermal cycle stages as follows: initial denaturation at 95° C, (denaturation at 95° C, annealing at 54° C, and extension at 72° C) for 40 cycles, and final extension at 72° C. The amplified products were stained with a DNA-safe marker and then viewed using UV transillumination imaging equipment. The amplified products were separated on a Merck 1% agarose gel using 0.5X tris borate EDTA buffer and a suitable molecular size marker (100 bp Plus DNA ladder) for 30 minutes at a voltage of 80 volts. *Staphylococcus aureus* ATCC 25923 was used as a positive control strain or the model strain and sterile distilled water was used as a negative control.

***Statistical Analysis***

Statistical analysis was performed using SPSS software 16.0 version (IBM Corp., New York, United States). The Chi-square test was used to evaluate the correlations between variables. A *p*-value of less than 0.05 was considered statistically significant.

## Results

***Slime Production in UPEC Colonies on CRA Indicated Biofilm Formation***

The morphological examination of UPEC isolates on CRA revealed distinct differences in colony appearance related to slime production. Red colonies (Figure 1A) indicated the absence of slime production, while black (Figure 1B) and deep black colonies (Figure 1C) demonstrated the production of slime, a key characteristic of biofilm formation.

***Presence of pap 7 and sfa 9 Genes in UPEC Isolates***

PCR amplification detected the presence of the *pap 7* and *sfa 9* genes in UPEC isolates (Figure 2). Isolates positive for the *pap 7* gene (110 bp) displayed clear bands on agarose gel, indicating gene presence, while some isolates were negative. Similarly, isolates harboring the *sfa 9* gene (459 bp) showed bands correlating with gene presence.

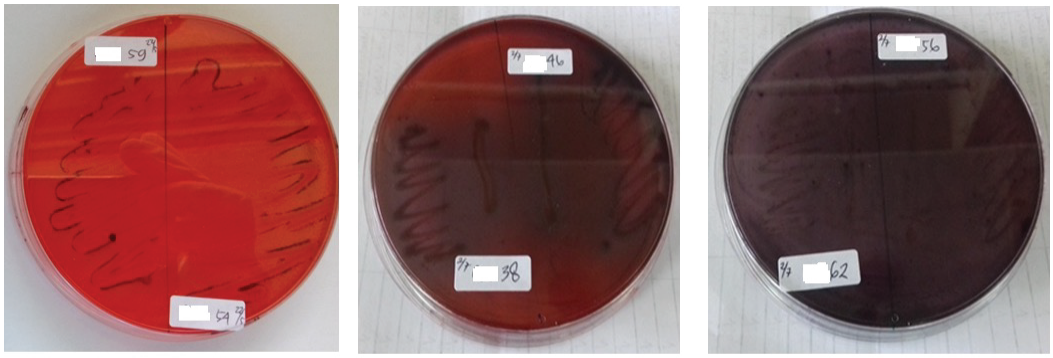
***Positive Association Between pap 7 and sfa 9 Genes and Biofilm Formation in UPEC Isolates***

Isolates positive for the *pap 7* gene showed a significantly higher tendency to form biofilms compared to those negative

**Table 1. Sequences of oligonucleotide primers used for PCR amplification of *pap7* and *sfa9*-associated genes in UPEC.**

| Gene | Sequences | Annealing Temperature (ºC) | Amplicon Size (bp) |
|---|---|---|---|
| ***pap 7*** | F: TACCAGGCACGCTATTACCT<br>R: GCCTGCATCTCTCCTTTCAG | 54 | 110 |
| ***sfa 9*** | F: TCTGCTGTGTTTGCTGGTTC<br>R: GACCGTCGCTTTTACATCCC | 54 | 489 |

Print ISSN: 2527-4384, Online ISSN: 2527-3442
DOI: 10.21705/mcbs.v9i2.589



A B C

**Figure 1. Morphology of UPEC isolates on CRA medium.** A: Red colonies (no slime produced). B: Black colonies (slime produced). C: Deep black colonies (slime produced).

for the gene. Similarly, isolates positive for the *sfa 9* gene exhibited a higher rate of biofilm formation compared to their sfa 9-negative counterparts, with both associations yielding statistically significant results.

## Discussion

This research showed that most UPEC isolates can form biofilms using the CRA method. The type of colony color when biofilm-positive in each bacterial species was also clearly visible, and criteria for biofilm-positive can be determined. CRA method is simple and has a high detection rate as a test for detecting biofilm formation capacity in clinical practice.[15] Biofilm detection by the CRA method may vary in this qualitative test possibly due to variations in the media used as well as subjective errors made during interpretation. In this study, the persistence of black pigmentation in bacterial colonies on CRA, which is an indication of biofilm formation, decreased over time (2-4 days), whereas on modified CRA, the staining increased and remained constant over time (after 3 days).[16]

This research showed that the presence of the *pap 7* gene is significantly correlated with biofilm formation in UPEC. P fimbriae are accessory extracellular proteins that play a role in the attachment of UPEC to uroepithelial cells.[7] P fimbriae are chromosomally encoded by the *pap* operon. The structure of the main fimbriae is encoded by *papA, papEF* encodes the adapter subunit and *papG* encodes the terminal adhesive protein. Therefore, the *papG* gene is usually used as a molecular marker to determine the prevalence of P fimbriae among UPEC. The establishment of differential methylation patterns as a result of competition between DNA adenine methylase (Dam) and leucine responsive regulatory protein (Lrp) for binding to two overlapping sets of binding sites within the pap regulatory region is the basis for phase variation-based regulation of the *pap* operon. Two genes comprise the regulatory region. The first, *pap*I, engages with the Lrp-*pap* DNA complex to activate phase-on when

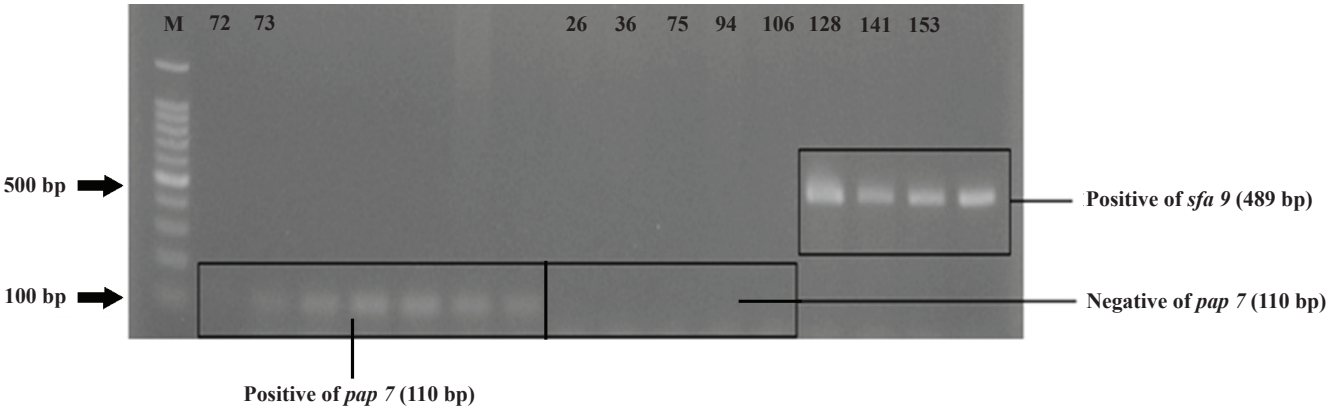



**Figure 2. Genes involved in the development of biofilms can be identified using PCR amplification of the *pap* 7 and *sfa* 9 genes in UPEC bacteria isolated for UTI patients.** Lane 1: ladder (50-1000 bp). Lane 2-7: positive for pap 7 gene (110 bp). Lane 8-12, negative for pap 7 gene (110 bp). Lane 13-16: positive for sfa 9 gene (459 bp).

**Table 2. Positive association was found between *pap* 7 and *sfa* 9 genes and biofilm formation in UPEC isolates.**

| Virulence Factors | Number of Isolates n (%) | Biofilm Formation n (%) | p-value |
|---|---|---|---|
| ***pap* 7** | | | |
| Positive | 81 (65.85) | 66 (81.48) | 0.001* |
| Negative | 42 (34.14) | 15 (35.71) | |
| ***sfa* 9** | | | |
| Positive | 18 (14.63) | 66 (27.27) | 0.004* |
| Negative | 105 (85.37) | 15 (14.29) | |

*Significant (Chi-square test, $p<0.05$).

Dam methylates particular GATC sequences. The second is *pap*B, which both represses transcription when protein concentrations are high and stimulates *pap*I transcription.[8] Regulation of *pap* pili expression is described as follows: The core pap locus contains 11 genes. Transcription of the pap locus is controlled by the *pap*BA promoter and regulated by a phase variation mechanism (ON and OFF) through the regulatory proteins *pap*B and *pap*I. In *pap* pili, *pap*A is the main gyre, while *pap*J, *pap*K, *pap*E, and *pap*F are the tip fibrillum. *pap*G is a galactose-binding adhesin and *pap*H is a pilus anchor. Two other gene products, *pap*D (periplasmic chaperone) and *pap*C (OM, usher), assist in the assembly of CUP-based *pap*pili.[17]

P fiber is implicated in the pathophysiology of all forms of UTIs as it helps in bacterial attachment to urothelial cells through its *pap*G adhesin. The *pap*G component has so far been found to have 4 distinct variants: *pap*GI, *pap*GII, *pap*GIII, and *pap*GIV. Although *pap*GII is linked to pyelonephritis in adult women and children, acute prostatitis in males, and invasive infections that can result in bacteremia in UTI patients, it has little impact in the pathophysiology of UTI and related clinical syndromes. While *pap*GIV's role and frequency remain undetermined, papGIII has been associated to cystitis in adults and children.[18]

This research showed that the presence of the *sfa* 9 gene is significantly correlated with biofilm formation in UPEC. Mannose-resistant S pili were categorized into *sfa*-associated pili, F1C-pili (Foc) and S/F1C-associated pili (Sfr). These ad-hesins share a high degree of homology but differ in their receptor specificity; S-pili are expressed primarily by *E. coli* (NMEC) strains that cause sepsis (SEPEC) and neonatal meningitis, but can also occur in UPEC strains that cause increased UTI.[19] F1C fimbriae, part of the S fimbriae superfamily, are found in about 30% of UPEC strains. The adhesive virulence factor S fimbriae has been identified in UPEC. In addition, the binding ability of S fimbriae with sialic acid molecules has caused urosepsis.[20] In this study, a high percentage of UPEC was found to form slime on CRA media. Most UPEC were known to be positive for the *pap* 7 gene and *sfa* 9 gene. This study demonstrated high prevalence of *pap* and *sfa* genes related to the ability to form biofilms. These results were different from several previous studies. Based on previous research8 which has reported that. The weakness of this study is that researchers did not differentiate the types of UTI caused by UPEC. Another research showed that the results of biofilm formation tests with microtiter plates showed the ability to form strong, medium, and weak biofilms in 32%, 33%, and 24% of isolates, respectively.[21] The most common virulence gene was *fim*A (74%) followed by *hly*F (68%), *pap*A (44%), *pap*C (32%), *iro*N (26%), and *cnf* (20%).

The biofilm formation of UPEC isolates was (33.33%) which was strong biofilm, (61.90%) was medium biofilm, and (4.76%) was weak biofilm. The *sfa* and *afa* genes were present at frequencies of 80.95% and 14.28%, respectively. At least one adhesive gene was present in most isolates.[22] A total of 81 (72.32%) of 112 *E. coli* strains isolated from UTI samples were positive for biofilm development (22.2% strong, 33.3% moderate, and 44.4% weak). The *afa* and *sfa* genes were detected in 29.4% and 49.1% of the isolates, respectively. The *afa* and *sfa* genes have a significant correlation with strong biofilm formation in UPEC.[23] Variation in *E. coli* virulence genes is caused by differences in the isolation of UPEC strains in different geographic regions. However, resistance to beta-lactam antimicrobials and cephalosporins is very common in strains with better biofilm-forming abilities.[24]

The application of genetic modification techniques is a new way to reduce the virulence of pathogenic bacteria. By utilizing technologies for gene editing such as the clustered regularly interspaced short palindromic repeats (CRISPR)-associated (CRISPR-Cas) system, it is possible to alter the genetic makeup of pathogen biofilms, which over time can reduce their virulence.[25] Future research should prioritize advanced models, clinical validation, and multidisciplinary collaboration to refine therapeutic strategies, advances

Print ISSN: 2527-4384, Online ISSN: 2527-3442
DOI: 10.21705/mcbs.v9i2.589

therapeutic and innovative development that improves clinical outcomes and patient care.[26]

The resistance of UPEC to various antibiotics and other virulence factors associated with biofilms such as surface proteins that bind to receptors on eukaryotic cells surface or extracellular matrix proteins are adhesion factors, was not assessed. Future research should focus on evaluating UPEC resistance to a arrange of antibiotics and identifying additional genes involved in biofilm formation in UPEC.

## Conclusion

An increase in the expression of *pap* and *sfa* genes, which are associated with the ability to form biofilms, could potentially serve as as a diagnostic markers for biofilm formation or as a targets for vaccines and management strategies in patients with biofilm-associated infections.

## Acknowledgment

We would like to thank the Institute of Tropical Disease, Universitas Airlangga, Surabaya, for their valuable insights and contributions to this study. We also thank Universitas Wijaya Kusuma Surabaya for providing funding through Research Scheme No. 108/LPPM/UWKS/IV/2023.

## Authors' Contributions

RP contributed to the conceptualization, data curation, formal analysis, methodology, validation, visualization, and writing of the original draft, as well as the review and editing of the manuscript. SLU contributed to the conceptualization, investigation, methodology, validation, and writing of the original draft, as well as the review and editing of the manuscript.

## References

1. Al Lawati H, Blair BM, Larnard J. Urinary tract infections: Core curriculum 2024. Am J Kidney Dis. 2024; 83(1): 90-100.
2. StatPearls [Internet]. Treasure Island: StatPearls Publishing; ©2024. Complicated Urinary Tract Infections [update 2024 October 7; cited 2024 Nov 4]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK436013/
3. Zakaria AS, Edward EA, Mohamed NM. Pathogenicity islands in uropathogenic escherichia coli clinical isolate of the globally disseminated O25:H4-ST131 pandemic clonal lineage: First report from Egypt. Antibiotics. 2022; 11(11): 1620. doi: 10.3390/antibiotics11111620.
4. Puspitasari D, Rusli EA, Husada D, Kartina L. Escherichia coli and Klebsiella pneumonia as the most common bacteria causing catheter associated urinary tract infection. Mol Cell Biomed Sci. 2021; 5(3): 121-6.
5. Purbowati R, Pratiwi VM, Masfufatun M, Tania POA, Khumaeni A. Antibacterial and antibiofilm effects of gold and silver nanoparticles against the uropathogenic Escherichia coli by scanning electron microscopy (SEM) analysis. HLS. 2023; 11(2): 2-18.
6. Green HD, Horn GTV, Williams T, Eberly A, Morales GH, Mann R, *et al*. Intra-strain colony biofilm heterogeneity in uropathogenic Escherichia coli and the effect of the NlpI lipoprotein. Biofilm. 2024; 8: 100214. doi: 10.1016/j.bioflm.2024.100214.
7. Saharan BS, Beniwal N, Duhan JS. From formulation to function: A detailed review of microbial biofilms and their polymer-based extracellular substances. The Microbe. 2024; 5: 100194. doi: 10.1016/j.microb.2024.100194
8. 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.
9. Kadry AA, Al-Kashef NM, El-Ganiny AM. Distribution of genes encoding adhesins and biofilm formation capacity among Uropathogenic Escherichia coli isolates in relation to the antimicrobial resistance. Afr Health Sci. 2020; 20(1): 238-47.
10. Boroumand M, Sharifi A, Manzouri L, Khoramrooz SS, Khosravani SA. Evaluation of pap and sfa genes relative frequency P and S fimbriae encoding of uropathogenic Escherichia coli Isolated from hospitals and medical laboratories; Yasuj City, Southwest Iran. Iran Red Crescent Med J. 2024; 21(8): 1-8.
11. Gadar K, McCarthy RR. Using next generation antimicrobials to target the mechanisms of infection. NPJ Antimicrob Resist. 2023; 1(1): 11. doi: 10.1038/s44259-023-00011-6.
12. Kurniawan A, Basri AH, Amalia AN, Setiani FT, Tyarini IA, Nugraheni N. Identifikasi gen Pap C dan Fim A pada bakteri Escherichia coli penghasil ESBL sebagai faktor adhesi pada infeksi saluran kemih. Media Informasi Penelitian Kabupaten Semarang. 2023; 5(1): 80-5.
13. Purbowati R, Utami SL, Raharjo D, Masfufatun M. Detection of csg and lux genes in biofilm-forming uropathogenic Escherichia coli associated with urinary tract infections. J Multidiscip Appl Nat Sci. 2024; 5(1): 1-9.
14. Purbowati R. Kemampuan pembentukan slime pada Staphylococcus epidermidis, Staphylococcus aureus, MRSA dan Escherichia coli. Jurnal Florea. 2017; 4(2): 1-10.
15. Mori S, Yamada A, Kawai K. Evaluation of the biofilm detection capacity of the Congo Red Agar method for bovine mastitis-causing bacteria. Jpn J Vet Res; 2024; 71(3): 109-16
16. Basnet A, Tamang B, Shrestha MR, Shrestha LB, Rai JR, Maharjan R, *et al*. Assessment of four in vitro phenotypic biofilm detection methods in relation to antimicrobial resistance in aerobic clinical bacterial isolates. Plos One. 2023; 18(11): e0294646. doi: 10.1371/journal.pone.0294646.
17. Gahlot DK, Taheri N, MacIntyre S. Diversity in genetic regulation of bacterial fimbriae assembled by the chaperone usher pathway. Int J Mol Sci. 2023; 24(161): 1-18.
18. Golpasand T, Keshvari M, Behzadi P. Distribution of chaperone-usher fimbriae and curli fimbriae among uropathogenic Escherichia coli. BMC Microbiol. 2024; 24(1): 344. doi: 10.1186/s12866-024-03472-5.
19. Makarova MA, Александровна MM, Matveeva ZN, Николаевна MЗ, Kaftyreva LA, Алексеевна КЛ. An integrative approach to assessing the pathogenic potential of Escherichia coli strains isolated from urine. Journal of microbiology, epidemiology and

immunobiology. 2024; 101(1): 72-9.

20. Zhou Y, Zhou Z, Zheng L, Gong Z, Li Y, Jin Y, *et al*. Urinary tract infections caused by uropathogenic Escherichia coli: Mechanisms of infection and treatment options. Int J Mol Sci. 2023; 24(13): 10537. doi: 10.3390/ijms241310537.
21. Memar MY, Vosughi M, Rahbar Saadat Y, Ardalan M, Yekani M, Niknafs B, *et al*. Virulence genes and antibiotic susceptibility patterns of Escherichia coli isolated from nosocomial urinary tract infections in the northwest of Iran during 2022-2023: A cross-sectional study. Health Sci Rep. 2024; 7(11): e70149. doi: 10.1002/hsr2.70149.
22. Khalid AN, Abu-Resha RA. Expression of sfa and afa genes in uropathogenic Escherichia coli under probiotic effect. Al-Rafidain J Med Sci. 2024; 7(2): 66-71.
23. Goodarzi R, Yousefimashouf R, Sedighi I, Moradi A, Nouri F, Taheri M. Detection of adhesion encoding genes, antibacterial susceptibility test and biofilm formation of uropathogenic Escherichia coli Isolated from urinary tract infections in children. J Adv Med Biomed Res. 2022; 30(138): 47-53.
24. Ramírez Castillo FY, Guerrero Barrera AL, Harel J, Avelar González FJ, Vogeleer P, Arreola Guerra JM, et al. Biofilm formation by Escherichia coli isolated from urinary tract infections from Aguascalientes, Mexico. Microorganisms. 2023; 11(12): 2858. doi: 10.3390/microorganisms11122858.
25. Niu H, Gu J, Zhang Y. Bacterial persisters: Molecular mechanisms and therapeutic development. Sig Transduct Target Ther. 2024; 9(1): 174. doi: 10.1038/s41392-024-01866-5.
26. Wang D, Wang L, Liu Q, Zhao Y. Virulence factors in biofilm formation and therapeutic strategies for Staphylococcus aureus: A review. Animals and Zoonoses. 2024; doi: 10.1016/j.azn.2024.11.003.