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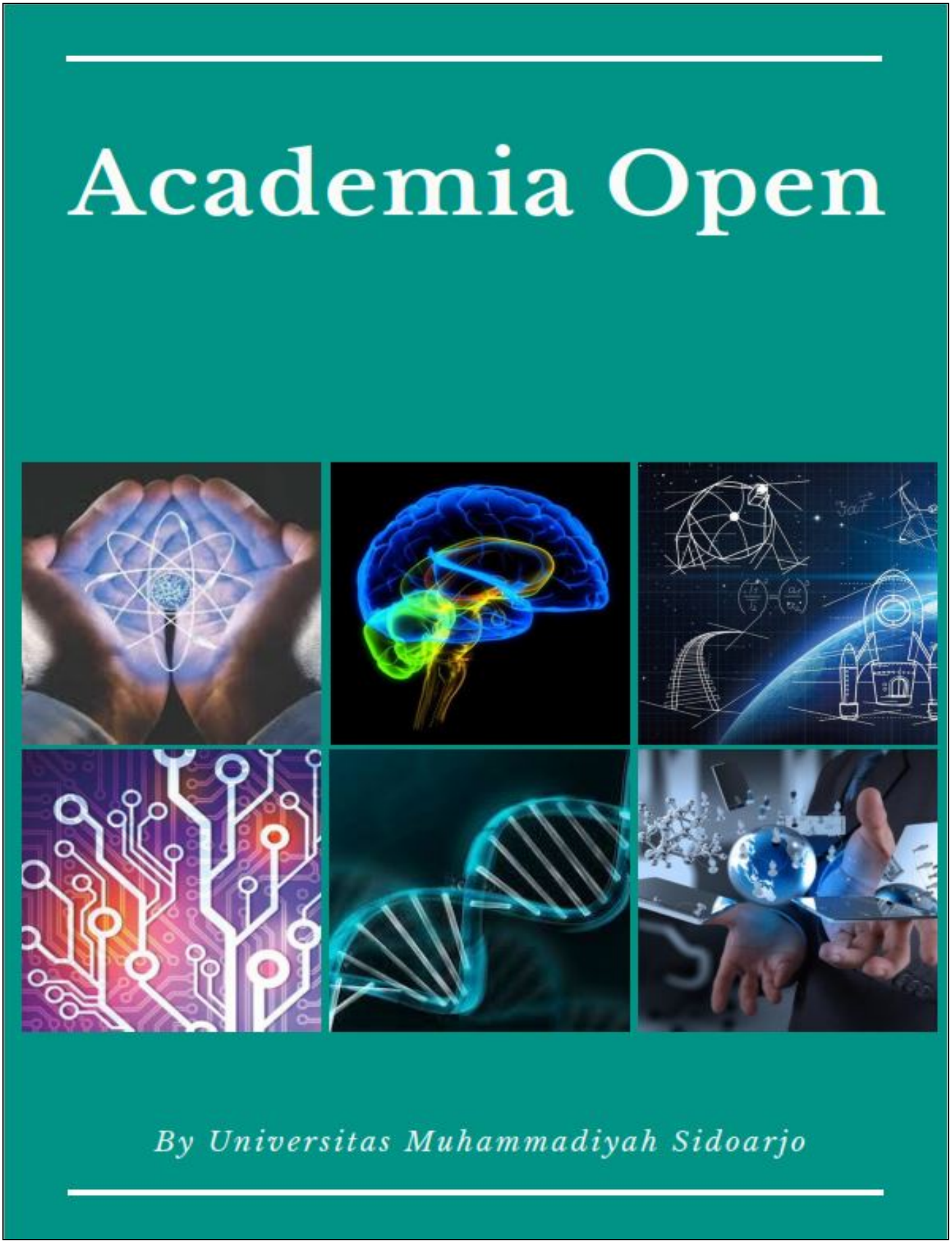

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E. coli Strains in Iraq Show High Antibiotic Resistance and Virulence Potential

Strain E. coli di Irak Menunjukkan Resistensi Antibiotik yang Tinggi dan Potensi Virulensi

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Abstract

General Background: Escherichia coli is a widely distributed species that includes strains ranging from highly pathogenic to harmless avirulent isolates, often found in the human gut. **Specific Background**: E. coli pathogenicity is determined by virulence factors like adhesins, invasins, toxins, and capsules, which are often transferred horizontally via phages and plasmids. **Knowledge Gap**: Despite advancements in understanding E. coli virulence, there is a need to further understand genetic factors contributing to urinary tract infections, especially in regions with high antibiotic resistance. **Aims**: The study investigated the prevalence of virulence genes and antibiotic resistance in E. coli strains from UTI patients at Al Hussien Teaching Hospital in Iraq. **Results**: The study reveals the presence of specific virulence genes in UPEC strains from a specific region, suggesting the potential involvement of the pap AH gene in UTI pathogenesis. **Novelty**: This study reports the prevalence of specific virulence genes in UPEC strains from a specific region, indicating the potential role of the pap AH gene in UTI pathogenesis. **Implications**: The study indicates that UPEC strains, characterized by high virulence factors like pap AH, are promising vaccine targets, necessitating further research for effective prevention strategies.

Highlights:

1. E. coli strains show high antibiotic resistance in UTI patients.
2. pap AH gene is crucial for UPEC virulence.
3. Virulence factors like pap AH are potential vaccine targets.

Keywords: E. coli, UTI, virulence factors, antibiotic resistance, pap AH gene

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Introduction

From infants to the elderly, urinary tract infections (UTIs) are among the most prevalent and widespread bacterial diseases in the world [1, 2]. A UTI is defined as having more than 105 bacteria per milliliter in the urine. In clinical terms, urinary tract infections (UTIs) are classified as either simple or complex cases based on the existence of structural or neurological abnormalities of the urinary system [3,4] as in Figure 3. Of all the bacteria, Escherichia coli, is the most common cause, responsible for about 80–90% of infections [5]. The urethra is the most common site of E. coli infection, which is followed by the bacteria's ascent to the bladder. The pathotype of extraintestinal pathogenic Escherichia coli that was originally identified from the urine of patients with urinary tract infections is known as uropathogenic Escherichia coli (UPEC). Normally, E. coli is present in the lower gastrointestinal tract of humans as a commensal flora. The term "UPEC" came up because they were different from those that were cultivated from stool specimens of healthy people and those that caused diarrhoea. The most significant indicator of pathogenicity in UPEC strains is their ability to adhere to host epithelial cells in the urinary tract, but they also have a large number of secreted (toxins, iron-acquisition systems) and structural (fimbriae, pili, flagella) virulence factors that are important to the pathogenesis [6] Figure 2. Escherichia coli is a Gram-negative bacillus that is a member of the Enterobacteriaceae family and is found in the human gastrointestinal system as a component of the microbiota. It rarely causes illness unless the host has weakened immune system, and it typically coexists peacefully with its host. Not many E. coli strains, though, have the ability to diverge from their commensal counterpart and become pathogenic. This means that some virulence characteristics that allow these strains to adapt to new environments and cause a wide range of diseases are acquired by them through plasmids, pathogenicity islands, or DNA horizontal transfer of transposons [7]. Complicated microbial populations encased in an extracellular polymeric substance (EPS) matrix that they self-produce are known as biofilms. Bacterial cells that are attached to both stationary surfaces and one another (living or inanimate) are found in biofilms [8]. Antibiotics, antiseptics, and disinfectants cannot reach bacterial cells living in this kind of sessile biofilm [9, 10]. Catheter-associated urinary tract infections (CAUTIs) are among the infections linked to indwelling medical devices that are caused by biofilm formation [11, 12]. One of the main contributing factors to the pathophysiology of CAUTIs is biofilm growth on the surface of a urinary catheter [13]. E. coli is one of the most prevalent species implicated in biofilm-related illnesses; it is especially crucial in urinary tract infections (UTIs), which can result in chronic infections [14]. The bacteria possess a variety of potential virulence factors, such as cytotoxic necrotising factor 1 (cnf1), A fimbrial adhesin (afa), S fimbriae (sfa), and pyelonephritis-associated pili (pap) [15]. The majority function as invasive and sticky elements that set off certain signalling pathways that inflame and harm the kidneys. There have been reports of their interventions in cases of cystitis, urethritis, and pyelonephritis [16. 17]. Since most isolates have substantial resistance to a variety of antimicrobial agent classes, including tetracyclines, penicillins, cephalosporins, aminoglycosides, fluoroquinolones, and macrolides, the advent of antimicrobial resistance highlights the significance of UPEC [18]. Consequently, the majority of treatment alternatives are ineffective, which raises the expense of hospital stays and therapy.

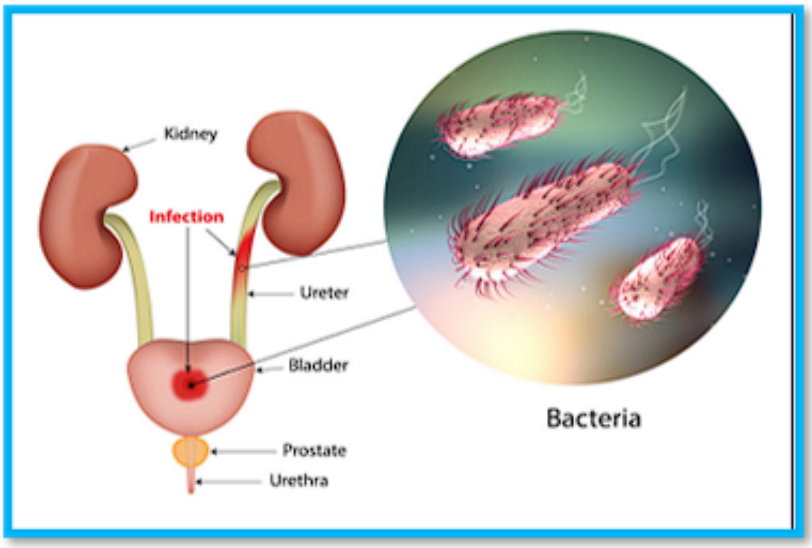

Figure 1. *Urinary Tract and Sites of bacterial infection.*

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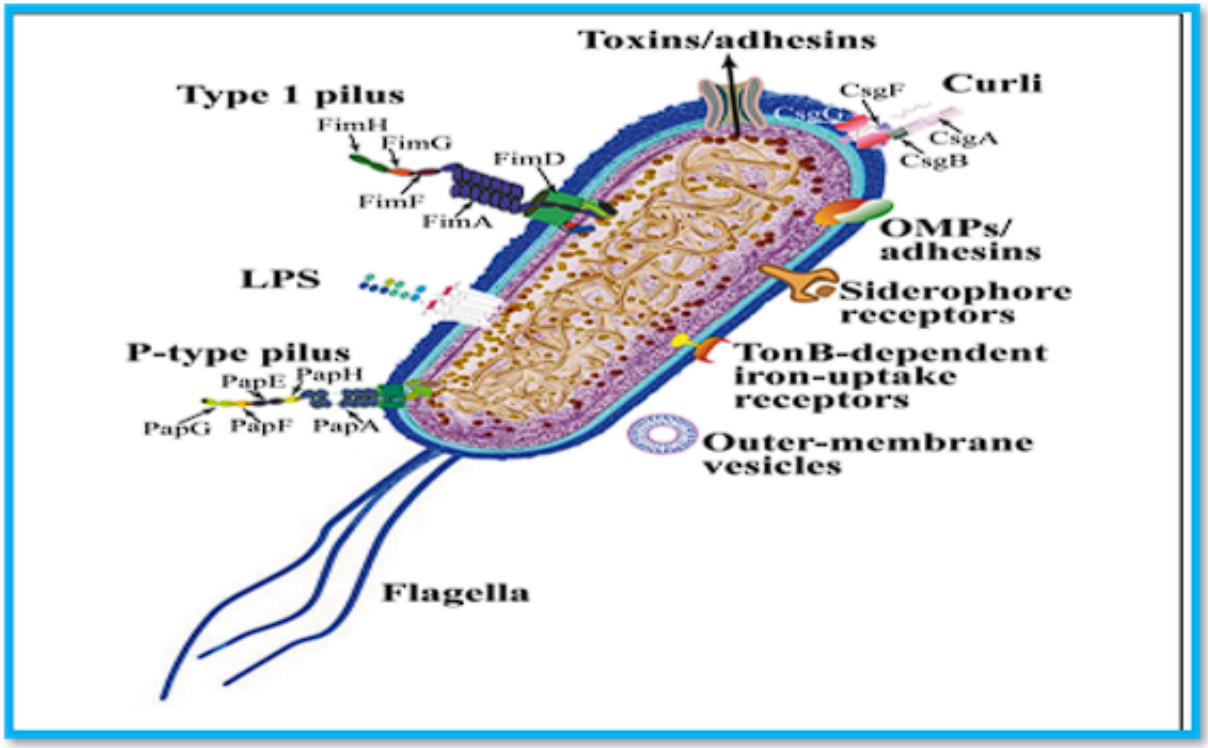

Figure 2. *Virulence Factors for E. coli*

Aim of the Study

The study's objective was to determine whether certain biofilm genes were present in E. coli by using the molecular technique of PCR

Methods

Urine Specimens

Between December 2023 and February 2024, fifty urine specimens were taken from patients suffering from urinary tract infections at Al-Hussein Teach Hospital in Thi-Qar, Iraq.

In order to minimise any microbiological and artifactual contaminations, midstream samples were collected in the morning and sent straight to the laboratory at 4°C in a sterile container [19].

E. coli Identification

The urine specimens were found to contain E. coli bacteria, as per the methodology developed by Bailey and Scott 19. Urine samples were stored at 37°C for 24 hours after being grown on nutrient agar (NA), MacConkey agar (MCA), and eosin-methylene blue (EMB) agar (Merck, Germany). In the MCA, the suspected colonies were identified as lactose-positive (pink in colour), while in the EMB agar medium, suspicious colonies were identified as green with metallic shine. The E. coli isolates were identified by Gramme staining, the Api20E test, and the Polymerase Chian Reaction (PCR) [19].

Antimicrobial Susceptibility Test

Using the disc diffusion method as described by references [20], the sensitivity of the isolated organisms to eight antibiotics was evaluated. The antimicrobials that were tested are from the cephems (ampicillin 10 mg, amoxicillin-clavulanate 20/10 mg, ceftazidime 30 mg, aztreonam 30 mg, gentamicin 10 mg, levofloxacin 5 mg, cefotaxime 30 mg, and trimethoprim 5 mg) classes, as well as the β-lactam, aminoglycoside, quinolones and fluoroquinolones, folate pathway inhibitors, and penicillin.

Polymerase Chain Reaction (PCR)

To identify the biofilm genes and E. coli molecules genetically, conventional PCR was employed to amplify the

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target DNA using certain primer pairs (Table 1&2).

Target Gene	Oligonucleotide Sequence (5’-3’)	Amplicon Size (bp.)	References
16sRNAE. coli	F: AGAGTTTGATCMTGGCT CAGR: CCGTCAATTCATTTGAGTTT	919 bp	[21]

Table 1. *Sequences, Size of Product, and Source for 16sRNA Primers*

Primer name	Sequences 5'-'3	TM	PCR product	Reference
papAH	F ATGGCAGTGGTGT CTTTTGGTGR CGTCC CACCATACGTGCTCT TC	61°C	720bp	[22]
saf	F CTCCGGAGAACTG GGTGCATCTTACR C GGAGGAGTAATTACA AACCTGGCA	71°C	410bp	[23]

Table 2. *Gene Reference, Product Size, and Primers Sequences for Biofilm Formation*

Preparation of PCR primers

In accordance with manufacturing instructions, the primers are prepared by dissolving the lyophilised primers in TE (Tris-EDTA) buffer to create a stock solution with a concentration of 100 pmole/Ml. Primer working solutions were made by diluting the stock solution with TE buffer and spinning down to an overnight temperature of 4°C. This resulted in a final working solution (10 pmole/Ml) for each primer.

The agrose gel electrophoresis method was used to analyse the multi-gene PCR results in the following steps:

1. 1% Using 1X TBE, agarose gel was made, dissolved in a water bath at 100 °C for 15 minutes, and then allowed to cool to 50–60 °C. Russell and Sambrook (2001)21.

2. The agarose gel solution was then mixed with 2µL of ethidium bromide dye.

3. After positioning the comb correctly, the tray was filled with agarose gel solution. The comb was then gently removed from the tray and 10µl of PCR product and 5ul of (1500 bp Ladder) were added to each comb well. The tray was then allowed to solidify for 15 minutes at room temperature.

4. After fixing the gel tray in the electrophoresis chamber, 1X TBE buffer was added to it. After that, electric current was run for 30 minutes at 110 volts and then for 60 minutes at 75 volts.

The PCR products were observed by the use of an ultraviolet transilluminator.

Conventional PCR detection reaction

PCR master mix reaction preparation

PCR master mix reaction was prepared according to company instructions (transgenbiotech inc.china) Table -3

as shown in link :- https://www.transgenbiotech.com/pcr_enzyme/easytaq_dna_polymerase.html

Component	Quantities
EasyTaq® DNA Polymerase	500 U×1
10×EasyTaq® Buffer and Mgcl2	1.2 ml×1
2.5 mM dNTPs	800 µl×1
6×DNA Loading Buffer	1 ml×1

Table 3. *Master mix contents*

Reaction Setup

Subsequently, the aforementioned PCR master mix reaction components are put into standard PCR tubes along with the Multiplex PCR as lyophilised materials that contain all additional components required for the PCR reaction, including Taq DNA Polymerase, dNTPs, and 6 mM MgCl2, pH 8.7. After that, the tube was centrifuged for three minutes using an Exispin vortex. then put into a thermocycler for multigene PCR.

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PCR Thermocycling Conditions

As indicated in Table-4, PCR thermocycler conditions for every gene were completed using a traditional PCR thermocycler equipment.

Component	50µl reaction
10 µM Forward Primer	0.5 µl
10 µM Reverse Primer	0.5 µl
Template DNA	Variable
Easy Taq 2X Master Mix with Standard Buffer	12.5 µl
Nuclease-free water	Variable
Total	25 µl

Table 4. *PCR Reaction*

No. of cycles cycle	Time	Temperature(˚C)	Step
1	3 min	94	Initial denaturation
35	30sec	94	Denaturation
	30 sec	Variables according to (primer's TM)	Annealing
	1 min	72	Extension
1	5 min	72	Final extension
-	∞	-4	Hold temperature

Table 5. *PCR Amplification Program for PCR*

Result and Discussion

According to the findings of the current study, E. coli is the primary cause of UTIs. Out of 50 samples that were collected, around 10 isolates of E. coli were found, which is consistent with several studies that found a high incidence of E. coli 24. The following were the findings of the test for antibiotic sensitivity: Beta-Lactam (amoxicillin-clavulanate) accounted for 80% of the resistance reported in this study, which was in line with another study25,26. The reason for this increased resistance could be that the majority of E. coli isolates contain beta-lactamase. The penicillins group, ampicillin, had 100% of the resistance; the percentage of ampicillin is similar to a previous study25. The antibiotic aztreonam belonged to the monobactams group, whereas the resistance percentage to ceftazidime varied among the cephems group, where it was ceftazidime 70% and cefotaxime 60%,27,28,29, which corresponded with ceftazidime results in the current study. Our analysis disagreed with the study that was published 28 .Additionally, 50% of the UPEC isolates had gentamicin resistance, according to the data. This result was in line with Al-Taai24. Furthermore, 30% of the antibiotics were levofloxacin, which is a member of the quinolone and fluoroquinolone groups; the resistance percentage was established at 25%, while 50% of the group is composed of trimethoprim.DNA was extracted from 10 bacterial isolates, including E. coli, using a genomic DNA minikit, according to 30.Similar outcomes were also seen when the DNA samples were subjected to gel electrophoresis analysis, as Figure 3 illustrates. Here, DNA bands indicative of purified DNA samples were found. Ten isolates of E. coli were subjected to molecular identification using specific primers for the PCR amplification of 16S rRNA; all of the isolates produced positive results. Figure 4.

The existence and activity of a few potential virulence factors determine how severe an infection is. The UPEC adherence into the renal epithelial cells is necessary for the initiation of infection. Among the key components of UPEC adherence to the renal epithelium and the ensuing damage are fibrinal factors (afa, sfa, and pap) 31, 32. PCR was performed on each of these isolates to identify the presence of the Pap AH and saf genes. The papAH gene was looked at for each of them. The findings revealed that 10 (100%) of these isolates had these genes, despite the fact that six of them carried saf genes. Figures 5 and 6. Several investigations, including ones conducted in Mexico and India 33, 34, showed outcomes that were similar to ours.According to the current study, there is a correlation between having adherence genes and antibiotic resistance. It also found that the highly resistant isolates contain both genes, which clarifies the crucial role these genes play in resistance.The low number of samples obtained, the absence of molecular analysis of antibiotic resistance genes, and the absence of urine sample assessment of healthy paediatric children as a control group were the limitations of the current investigation.

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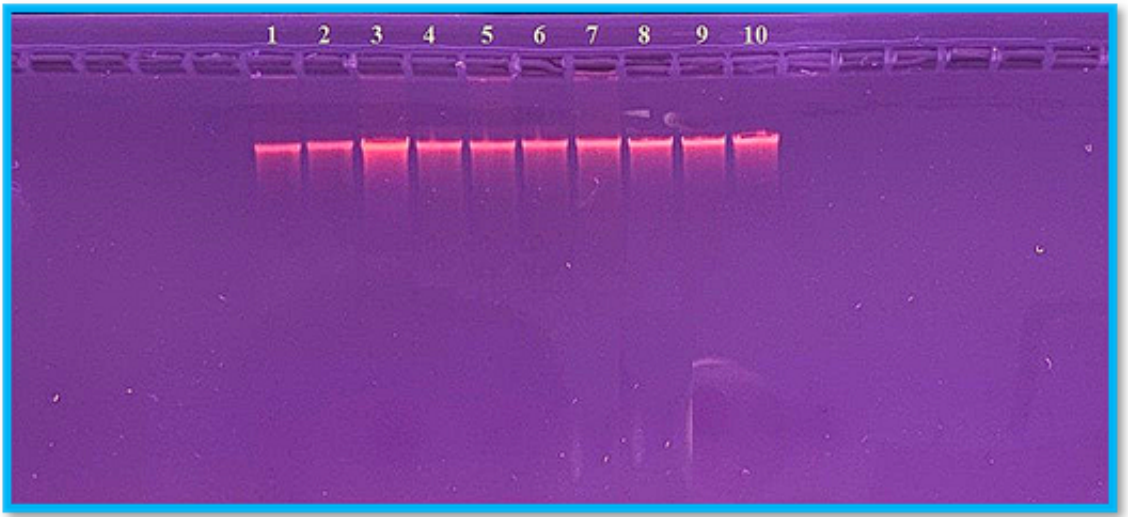

Figure 3. *Agarose gel electrophoresis stained with Ethidium, then exposed to UV Bromide dye for photography, revealed (1–10) positive results for DNA extraction, shown by clear bands that stand for DNA molecules that were extracted from bacteria.*

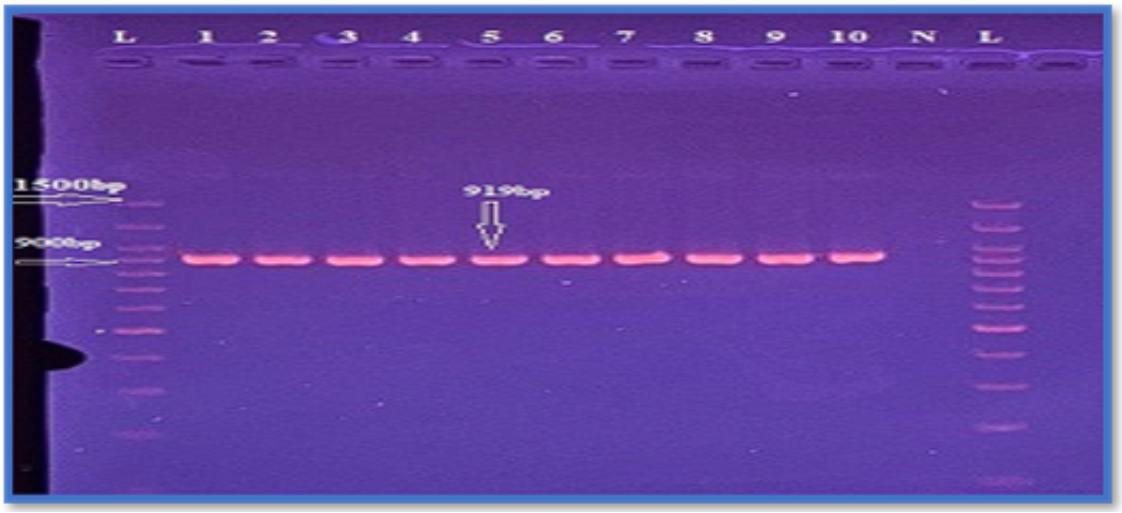

Figure 4. *The amplicon size (of 919 bp) of the 16SrRNA primer PCR assays was determined by Agarose gel electrophoresis examination of the PCR results from ten PCR assays conducted at 57°C. The primer was diluted with 1.5% Agarose for 15 minutes at 110 voltage and then decreased to 75 volts for 60 minutes. Gels were stained with ethidium bromide and exposed to ultraviolet light for photography. Lane L showed the DNA ladder (1500-100 bp), Lanes 1–10 indicated positive results, and Lane (N) showed the negative control.*

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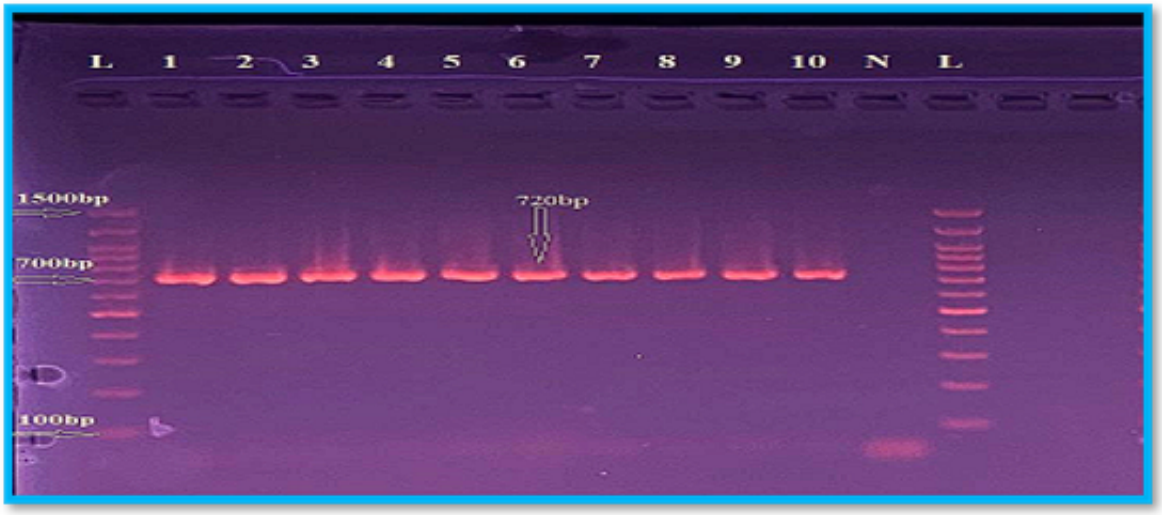

Figure 5. *The amplicon size (720 bp) of the primer TM at 61°C was determined by Agarose gel electrophoresis examination of the PCR products of ten PCR assays using the papAH primer. The voltage was initially set at 110 for 15 minutes, and after that, it was dropped to 75 volts for 60 minutes. Gels were stained with ethidium bromide and exposed to ultraviolet light for photography. Lane L showed the DNA ladder (1500-100 bp), Lanes 1–10 indicated positive results, and Lane (N) showed the negative control.*

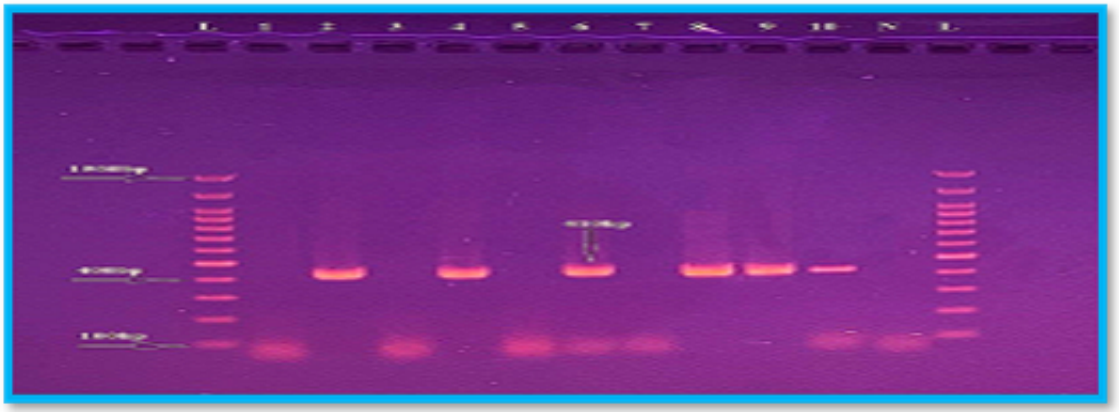

Figure 6. *The amplicon size (410 bp) of the saf primer PCR assays was determined by Agarose gel electrophoresis analysis of the PCR results. The primer was used at 71°C, with an initial voltage of 110 for 15 minutes, and a subsequent voltage of 75 volts for 60 minutes. Lanes L: DNA ladder (1500-100)bp, Lanes 2, 4, 6, 8, 9, and 10) represented positive results, Lanes 1, 3, and 5 represented negative results, and Lane (N) represented negative control. Gels were stained with ethidium bromide and photographed under UV light.*

Conclusion

We hypothesize that the increased frequency of the pap and sfa genes may be the cause of the patients' UTIs. High virulence factor prevalence can help UPEC strains colonize the urogenital tract more easily, which increases the bacteria's ability to adhere to target cells and make them more harmful. Therefore, these genes may be the focus of a vaccination designed to prevent E. coli infections. To ascertain the pathophysiology of UTIs and to discover the virulence factors of E. coli strains that cause them, more research is necessary.

References

1. . D. Tambekar, D. Dhanorkar, S. Gulhane, V. Khandelwal, and M. Dudhane, “Antibacterial Susceptibility of Some Urinary Tract Pathogens to Commonly Used Antibiotics,” African Journal of Biotechnology, 2006. [Online]. Available: https://www.ajol.info/index.php/ajb/article/view/43162
2. . H. Garrec, L. Drieux-Rouzet, J.-L. Golmard, V. Jarlier, and J. Robert, “Comparison of Nine Phenotypic Methods for Detection of Extended-Spectrum β-Lactamase Production by Enterobacteriaceae,” Journal of Clinical Microbiology, vol. 49, no. 3, pp. 1048–1057, Mar. 2011, doi: 10.1128/jcm.02130-10.

3. . K. Naber, “Improved Classification of Urinary Tract Infection: Future Considerations,” Academia.edu, Mar. 2024. [Online]. Available: https://www.academia.edu/115819659/Improved_Classification_of_Urinary_Tract_Infection_Future_Considerations
4. . M. M. Zacchè and I. Giarenis, “Therapies in Early Development for the Treatment of Urinary Tract Inflammation,” Expert Opinion on Investigational Drugs, vol. 25, no. 5, pp. 531–540, Mar. 2016, doi: 10.1517/13543784.2016.1161024.
5. . R. Kucheria, P. Dasgupta, S. H. Sacks, M. S. Khan, and N. S. Sheerin, “Urinary Tract Infections: New Insights into a Common Problem,” Postgraduate Medical Journal, vol. 81, no. 952, pp. 83–86, Feb. 2005, doi: 10.1136/pgmj.2004.023036.
6. . K. Y. Loh and N. Sivalingam, “Urinary Tract Infections in Pregnancy,” PubMed, Aug. 31, 2007. [Online]. Available: https://pubmed.ncbi.nlm.nih.gov/25606081/
7. . J. Bien, O. Sokolova, and P. Bozko, “Role of Uropathogenic Escherichia coli Virulence Factors in Development of Urinary Tract Infection and Kidney Damage,” International Journal of Nephrology, vol. 2012, pp. 1–15, Jan. 2012, doi: 10.1155/2012/681473.
8. . M. Jamal et al., “Bacterial Biofilm and Associated Infections,” Journal of the Chinese Medical Association, vol. 81, no. 1, pp. 7–11, Jan. 2018, doi: 10.1016/j.jcma.2017.07.012.
9. . K. Jefferson, “What Drives Bacteria to Produce a Biofilm?,” FEMS Microbiology Letters, vol. 236, no. 2, pp. 163–173, Jul. 2004, doi: 10.1016/j.femsle.2004.06.005.
10. . A. L. S. D. Santos et al., “What Are the Advantages of Living in a Community? A Microbial Biofilm Perspective!,” Memórias Do Instituto Oswaldo Cruz, vol. 113, no. 9, Jul. 2018, doi: 10.1590/0074-02760180212.
11. . A. Reisner et al., “Type 1 Fimbriae Contribute to Catheter-Associated Urinary Tract Infections Caused by Escherichia coli,” Journal of Bacteriology, vol. 196, no. 5, pp. 931–939, Dec. 2013, doi: 10.1128/JB.00985-13.
12. . G. Sharma et al., “Escherichia coli Biofilm: Development and Therapeutic Strategies,” Journal of Applied Microbiology, vol. 121, no. 2, pp. 309–319, Mar. 2016, doi: 10.1111/jam.13078.
13. . B. W. Trautner and R. O. Darouiche, “Role of Biofilm in Catheter-Associated Urinary Tract Infection,” American Journal of Infection Control, vol. 32, no. 3, pp. 177–183, May 2004, doi: 10.1016/j.ajic.2003.08.005.
14. . V. Ballén, V. Cepas, C. Ratia, Y. Gabasa, and S. M. Soto, “Clinical Escherichia coli: From Biofilm Formation to New Antibiofilm Strategies,” Microorganisms, vol. 10, no. 6, p. 1103, May 2022, doi: 10.3390/microorganisms10061103.
15. . J. Bien, O. Sokolova, and P. Bozko, “Role of Uropathogenic Escherichia coli Virulence Factors in Development of Urinary Tract Infection and Kidney Damage,” International Journal of Nephrology, vol. 2012, pp. 1–15, Jan. 2012, doi: 10.1155/2012/681473.
16. . C. Shah, R. Baral, B. Bartaula, and L. B. Shrestha, “Virulence Factors of Uropathogenic Escherichia coli (UPEC) and Correlation with Antimicrobial Resistance,” BMC Microbiology, vol. 19, no. 1, Sep. 2019, doi: 10.1186/s12866-019-1587-3.
17. . B. R. Dadi, T. Abebe, L. Zhang, A. Mihret, W. Abebe, and W. Amogne, “Distribution of Virulence Genes and Phylogenetics of Uropathogenic Escherichia coli Among Urinary Tract Infection Patients in Addis Ababa, Ethiopia,” BMC Infectious Diseases, vol. 20, no. 1, Feb. 2020, doi: 10.1186/s12879-020-4844-z.
18. . B. Kot, “Antibiotic Resistance Among Uropathogenic Escherichia coli,” Polish Journal of Microbiology, vol. 68, no. 4, pp. 403–415, Dec. 2019, doi: 10.33073/pjm-2019-048.
19. . T. Hameed, A. A. Nafeesah, S. Chishti, M. A. Shaalan, and K. A. Fakeeh, “Community-Acquired Urinary Tract Infections in Children: Resistance Patterns of Uropathogens in a Tertiary Care Center in Saudi Arabia,” International Journal of Pediatrics and Adolescent Medicine, vol. 6, no. 2, pp. 51–54, Jun. 2019, doi: 10.1016/j.ijpam.2019.02.010.
20. . R. M. Humphries et al., “CLSI Methods Development and Standardization Working Group Best Practices for Evaluation of Antimicrobial Susceptibility Tests,” Journal of Clinical Microbiology, vol. 56, no. 4, Apr. 2018, doi: 10.1128/jcm.01934-17.
21. . H. Momtaz et al., “Uropathogenic Escherichia coli in Iran: Serogroup Distributions, Virulence Factors and Antimicrobial Resistance Properties,” Annals of Clinical Microbiology and Antimicrobials, vol. 12, no. 1, p. 8, Jan. 2013, doi: 10.1186/1476-0711-12-8.
22. . J. R. Johnson and A. L. Stell, “Extended Virulence Genotypes of Escherichia coli Strains from Patients with Urosepsis in Relation to Phylogeny and Host Compromise,” The Journal of Infectious Diseases, vol. 181, no. 1, pp. 261–272, Jan. 2000, doi: 10.1086/315217.
23. . C. L. Bouguenec, M. Archambaud, and A. Labigne, “Rapid and Specific Detection of the Pap, Afa, and Sfa Adhesin-Encoding Operons in Uropathogenic Escherichia coli Strains by Polymerase Chain Reaction,” Journal of Clinical Microbiology, vol. 30, no. 5, pp. 1189–1193, May 1992, doi: 10.1128/jcm.30.5.1189-1193.1992.
24. . H. R. Mohammed Yasir Q., “Antibiotic Resistance Patterns and Adhesion Ability of Uropathogenic Escherichia coli in Children,” Iraqi Journal of Genetics and Epigenetics, Nov. 12, 2018. [Online]. Available: https://jige.uobaghdad.edu.iq/index.php/IJB/article/view/30
25. . . S. K. Al-Alak, M. R. Ali, and S. H. Authman, “Isolation of Multi-Antibiotic Resistant Escherichia coli from Urinary Tract Infection and the Detection of PapC and FimH Virulence Genes by Polymerase Chain Reaction,” ResearchGate, Feb. 2014. [Online]. Available: https://www.researchgate.net/publication/309589477_Isolation_of_Multi_Antibiotics_Resistance_Escherichia_coli_from_urinary_tract_infection_and_the_Detection_of_PapC_and_fimH_virulence_genes_by_Polymerase_chain_reaction_Technique_Isolation_of_Multi_Ant
26. . M. A. K. Shakhatreh, S. F. Swedan, M. A. Al-Odat, and O. F. Khabour, “Uropathogenic Escherichia coli

(UPEC) in Jordan: Prevalence of Urovirulence Genes and Antibiotic Resistance," Journal of King Saud University - Science, vol. 31, no. 4, pp. 648–652, Oct. 2019, doi: 10.1016/j.jksus.2018.03.009.
27. . M. A. Rezaee, V. Sheikhalizadeh, and A. Hasani, "Detection of Integrons Among Multi-Drug Resistant (MDR) Escherichia coli Strains Isolated from Clinical Specimens in Northern West of Iran," Brazilian Journal of Microbiology, vol. 42, no. 4, pp. 909–915, Oct. 2011, doi: 10.1590/s1517-83820110004000010.
28. . H. A. Ghaffoori and A. A. Suleiman, "Isolation, Identification and Antibiotic Resistance Profile Distribution of Clinical E. coli in Iraqi Patients," Genius Journals, May 31, 2022. [Online]. Available: https://geniusjournals.org/index.php/emrp/article/view/1528
29. . M. R. Green and J. Sambrook, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York, 2012. [Online]. Available: https://www.scirp.org/reference/referencespapers?referenceid=2822110
30. . N. Alfuraiji, A. Al-Hamami, M. Ibrahim, H. K. Rajab, and B. W. Hussain, "Uropathogenic Escherichia coli Virulence Characteristics and Antimicrobial Resistance Amongst Pediatric Urinary Tract Infections," Journal of Medicine and Life, vol. 15, no. 5, pp. 650–654, May 2022, doi: 10.25122/jml-2021-0148.
31. . P. Lüthje and A. Brauner, "Virulence Factors of Uropathogenic E. coli and Their Interaction with the Host," Advances in Microbial Physiology, vol. 63, pp. 337–372, Jan. 2014, doi: 10.1016/bs.ampbs.2014.08.006.
32. . S. Mittal, M. Sharma, and U. Chaudhary, "Study of Virulence Factors of Uropathogenic Escherichia coli and Its Antibiotic Susceptibility Pattern," Indian Journal of Pathology and Microbiology, vol. 57, no. 1, pp. 61–65, Jan. 2014, doi: 10.4103/0377-4929.130899.
33. . M. G. Ballesteros-Monrreal et al., "Virulence and Resistance Determinants of Uropathogenic Escherichia coli Strains Isolated from Pregnant and Non-Pregnant Women from Two States in Mexico," Infection and Drug Resistance, vol. 13, pp. 295–310, Jan. 2020, doi: 10.2147/idr.s226215.

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