Genotyping of Iraqi Uropathogenic *Escherichia coli* with Adhesion Genes *FimH* and *PapGII* by Multiplex PCR

Ghofran Khudhair Ismail, Lina Abdulameer S. Alsaadi
Department of Biology, College of Sciences, Diyala University, Diyala, Iraq.

Article Info

Article history:

Received 18,12, 2024
Revised 22, 07, 2025
Accepted 20,10, 2025
Published 30, 04,2026

Keywords:

Antibiotics Resistance,
E.coli,
FimH,
papGII,
Genotyping,
Multiplex PCR,
UTIs.

ABSTRACT

Escherichia coli is the most common causative agent of urinary tract infections (UTIs). Important virulence factors are the *FimH* and *PapGII* adhesion genes. The current work sought to ascertain the prevalence of the *fimH* and *papGII* genes, to delineate fimbrial adhesin gene (FAG) patterns and genetic diversity of these isolates by the utilization of the *FimH* gene. Out of 223 positive urine samples, the UPEC strains were confirmed through the standard microbiological and biochemical tests, the antimicrobial susceptibility test, and molecular screening of the adhesion genes (*fimH*, *papGII*) were done by Multiplex PCR. The current study showed that fifty-three E. coli isolates scored the highest resistance to ampicillin (94.3%), amoxicillin-clavulanate (92.5%), cefotaxim (90.6%), Piperacillin-tazobactam (79.2%), Piperacillin (73.6%), Ceftriaxone (75.5%), and ceftazidime (92.5%). Resistance patterns showed that 22 (41.5%), 29(54.7%) and 2 (3.8%) of the isolates multi drug resistant MDR, extensively drug resistant (XDR) and multi drug sensitive (MDS), respectively. Results by multiplex PCR showed that all 20 isolates 100% have the *fimH* gene, followed by *papGII* gene 55% (11/20) isolates. The conducted sequencing reactions indicated the accurate identity of the investigated samples, which were found to be attributed to *Escherichia coli*. The alignment of nucleic acid sequences of the five isolates with the reference sequences revealed the presence of fifteen nucleic acid variants. The tree suggested that the samples under investigation were located in four separate and neighboring phylogenetic locations within a single, different phylogenetic tree. The acquired data strongly support the hypothesis that adhesion genes *FimH* and *PapGII* are associated with virulence factors of *E.coli*.

Corresponding Author:

Lina Abdulameer S. Alsaadi
Department of Biology, College of Sciences,
University of Diyala, Baqubah City, Diyala Governorate, Iraq.

Email: linaabdulameer@uodiyala.edu.iq

1. INTRODUCTION

Urinary tract infections (UTIs) rank among the most prevalent bacterial infections, particularly affecting women and older persons, resulting in a substantial worldwide healthcare expenditure burden [1]. The elevated morbidity of urinary tract infections (UTIs), particularly in women of reproductive age, along with rising antibiotic resistance to frequently prescribed UTI medications, substantially escalates healthcare expenditures for this ailment. The swift rise of multidrug-resistant (MDR) microorganisms undeniably represents a significant worldwide health issue [2]. Pathogenic *Escherichia coli* is classified as a paramount group of bacteria necessitating the rapid advancement of novel antibiotics and innovative therapeutic approaches. *Escherichia coli* is a Gram-negative, facultative anaerobic, rod-shaped bacteria. Uropathogenic *E. coli* (UPEC) represents a considerable health risk among deleterious extraintestinal Enterobacteriaceae strains. UPEC is regarded as the primary etiological agent of urinary tract infections (UTIs), which rank as the second most often diagnosed infectious disease in humans globally [3]. UPEC is responsible for 40–50% of nosocomial urinary tract infections and 75–85% of community-acquired urinary tract infections [4]. Polycystic ovarian syndrome (PCOS) is a prevalent endocrine condition impacting 4–20% of women of reproductive age globally, contingent upon the diagnostic criteria employed [5]. The origin of PCOS remains uncertain; however, factors such as obesity, ovarian follicle maturation, insulin sensitivity, and chronic systemic inflammation have been suggested as potential explanations [6].

Women with Polycystic Ovary Syndrome (PCOS) exhibit increased vulnerability to infections relative to those without the illness [7]. Escherichia coli has various fimbriae types, primarily type I and type III (type P), which facilitate adhesion to the surface receptors of host cells. Johnson et al. elucidated the function of the pap operon in adhesion and pathogenicity, particularly highlighting the papG gene, which encodes the tip of this fimbrial type, recognized as a crucial attachment point [8]. The *fimH* operon encodes the initial kind of fimbriae, distinguished by the superior quality of receptors on the urinary epithelium. Hojati et al. established the significance of the *fimH* gene in the pathogenicity of UPEC bacteria, indicating that the *fimH* gene encodes the tip of fimbriae and is essential for the colonization of *E. coli* in the urinary tract [9]. This study aimed to ascertain the prevalence of the *fimH* and *papGII* genes in uropathogenic *E. coli*, given the extensive distribution of these pathogens capable of infecting and colonizing the urinary epithelium, as well as the significant genetic diversity of *E. coli* globally and specifically in Iraq. The research focused on elucidating fimbrial adhesin gene (FAG) patterns and the molecular diversity of UPEC strains isolated from Iraqi women in Diyala Province.

2. METHOD

2.1. ISOLATION AND IDENTIFICATION OF *ESCHERICHIA COLI*

The study included 250 Iraqi women samples, were collected from women ages ranging 18 to 65 years old, Patients were selected from the Infertility, Al Batool Educational Hospital, Public Health Laboratoried Baquba City, and Baqubah General Hospital and have been investigated in this study for the collection of urine samples from patients. This study extended from December 2023 to February 2024. Two groups (PCOS with UTI and UTI without PCOS). Urine was collected midstream in a disposable sterile screw urine cap, labeled with the patient's ID, and kept in a cooling icebox until it was transported to the laboratory for diagnosis. 50 µl of each sample were cultured on Blood agar and MacConkey agar (Himedia/India) and placed in an incubator at 37 oC for 24 hours. After that, morphological (gram staining) and biochemical tests were performed to confirm that it was *E. coli* bacteria, the Vitek 2 compact system was used.

Table 1. Primers Oligonucleotide Sequences Used in This Study.

Amplified gene	Initial denaturation	No. of cycle	Denaturation	Annealing	Elongation	Final extension
fimH	95°C/ 5min	35	94°C/ 45sec	63°C/45sec	72°C/45sec	72°C/7min
papGII	95°C/ 5min	35	94°C/ 45sec	63°C/60sec	72°C/45sec	72°C/7min

2.2. ANTIBIOTIC SENSETIVITY TEST (AST)

The Kirby-Bauer method was used to evaluate the isolates' sensitivity to a few chosen antibiotics in accordance with Vandepitte [10]. Until the turbidity of MacFarland's standard (1.5 x 108 cells/mL) was reached, three to five colonies grown on nutrient agar were transferred to tubes containing normal saline. The isolate was interpreted as either sensitive, intermediate, or resistant to particular antibiotics including of aminoglycosides ((Amikacin (AK), Gentamicin (CN) , penicillins (Ampicillin AMP), Piperacillin(PRL) , carbapenems (Imipenem (IPM) , Meropenem (MEM) , tetracyclines (Tetracycline (TE) , fluoroquinolones (Ciprofloxacin (CIP), Levofloxacin (LEV), cephalosporins (Cefriaxone (CRO) , Cefoxitins (FOX) ,Ceftazidime (CAZ) , Cefotaxime (CTX), (Aztreionam (ATM), Amoxicillin-Calvulanic acid(AMC) , Piperacillin-Tazobactam (TPZ), (Trimethoprim (TMP) by comparing the inhibition zone with the standards set by CLSI [11].

2.3. DNA EXTRACTION

Among fifty-three *E. coli* isolates, only 20 MDR and XDR isolates have been used for genotyping study. The genomic DNA was isolated from bacterial growth following the ZR fungal/yeast/bacterial DNA MiniPrep protocol according to manufacturers' instruction (ZYMO) from the USA.

2.4 PCR AMPLIFICATION

PCR tests were performed on only 19 XDR isolates using primers of *FimH* and *PapGII* genes Table 1. Final volume for PCR mixture was 25 µl (12.5 of Master Mix 2x, 3 µl template DNA, 1 µl primers for each forward and reverse primer , amplicons of 508 bp (*FimH* -F and *FimH* -R), 190 bp (*PapGII* -F and *PapGII* R), finally, 5.5 µl nuclease free water) in uniplex PCR Eppendorf tubes but amount changed in multiplex PCR, mixed briefly via vortex then been placed in thermocycler polymerase chain reaction. The program used for each multiplex PCR mixture was illustrated in the Table 2. All PCR products were analyzed by agarose gels (1.5%) electrophoresis and then stained with ethidium bromide**.**

Table 2. Amplification program of primer

Amplified gene	Initial denaturation	No. of cycle	Denaturation	Annealing	Elongation	Final extension
fimH	95°C/ 5min	35	94°C/ 45sec	63°C/45sec	72°C/45sec	72°C/7min
papGII	95°C/ 5min	35	94°C/ 45sec	63°C/60sec	72°C/45sec	72°C/7min

2.5 DNA SEQUENCING OF PCR AMPLICONS *FIMH* GENE

In current study, a single PCR fragment that covered a portion of the coding regions of the *Escherichia coli* fimbrial protein was chosen. The patterns of genetic diversity in bacteria were evaluated using the genetic variations of this locus. A total of five isolates (assigned E3, E11, E12, E14, TO E19) were included in the present study. These isolates were screened to partially amplify the *FimH* sequences. With regard to the amplicons of 508 bp were sent for sequencing (Macrogen Research, Seoul, Korea). The sequences obtained were compared with those deposited in the NCBI database using the BLAST program .

2.6. INTERPRETATION OF SEQUENCING DATA

The results of the targeted fragments' PCR sequencing were aligned, modified, and compared to their corresponding sequences in the reference database using BioEdit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA). The different variations found in each sample that was sequenced were recorded using PCR amplicons and their locations throughout the corresponding genome. The found nucleic acids were cataloged in PCR amplicons together with their corresponding positions in the genome that was used as a reference. Using SnapGene Viewer version 4.0.4 .every variant found in the bacterial sequences was annotated. Each genomic sequence that was studied was assigned a unique accession number when it was submitted to NCBI.

2.7. CONVERSION OF NUCLEIC ACID VARIANTS INTO AMINO ACID residues

Using the BioEdit suite, we aligned the sequencing results of the PCR products with their corresponding sequences in the reference database, modified them, and then evaluated them. The PCR amplicons and their respective positions within the reference genome were used to catalog the changes found in each sample that was sequenced. Protein Data Bank was used to acquire the amino acid sequences of the particular proteins. Using the Expasy translate suite, we transformed all nucleic acid sequences from the samples we looked at to their corresponding amino acid sequences so we could assess how the identified variations in the DNA affected the encoded protein).

2.8. DEPOSITION OF SEQUENCES TO GENBANK

The sequences that were evaluated and analyzed were then uploaded to the NCBI BankIt site in accordance with the instructions given by [13]. The sequences that were evaluated were assigned a unique GenBank entry number after being submitted as nucleic acid sequences to the National Center for Biotechnology Information (NCBI).

2.9 COMPREHENSIVE PHYLOGENETIC TREE CONSTRUCTION

A detailed comprehensive tree was created in the current study following the neighbour-joining strategy. The identified variants were compared with their adjacent homologous reference sequences utilizing the NCBI-BLASTn service [14]. A comprehensive inclusive tree, incorporating the observed variant, was constructed using the neighbor-joining method and shown as a circular cladogram with the iTOL suite [15].

ETHICAL CLEARANCE

The Declaration of Helsinki provides the basis for the ethical standards that were followed throughout the investigation. The study was conducted with the patients' verbal and analytical consent prior to subject recruitment. The research approval number 4689, dated September 23, 2023, indicates that the Diyala University, College of Sciences, Department of Biology evaluated and approved the study protocol, subject information, and consent form.

STATISTICAL ANALYSIS

Nominal and ordinal data were characterized by frequency and percentages. The disparities in percentages were assessed using the Chi-square test at a significance level of $P \leq 0.05$. SPSS version 22.0 was utilized for the examination of current data.

3. RESULTS AND DISCUSSION

Results of the conducted study showed that the most participants had E. coli (21%), another bacteria (68%), and no bacterial growth (11%), with significant differences ($P<0.05$) (Table 3).

Table 3: Frequency and percentages of bacterial growth

Bacteria	N	%	P value
E. coli	53	21%	P<0.01**
Another bacteria	170	68%	
No growth	27	11%	
Total	250	100%	

3.2 FREQUENCY AND PERCENTAGES OF *E. COLI* POSITIVITY AMONG STUDY GROUPS

Results of present research showed the positivity of *E. coli* was highest in UTI infected woman with PCOS (32.60%), following UTI infected pregnant woman without PCOS (23.07%), and then infected UTI non-pregnant woman without polycystic ovarian syndrome (PCOS) (19.76%) with significant different (*P*<0.05). In contrast, the differences among positivity of *E. coli* within study groups was no significant (*P*<0.05) (Table 4).

Table 4. Frequency and percentages of *E. coli* positivity among study groups

Study groups	Total positive	*E.coli*	%	P value
UTI infected non- pregnant woman without PCOS	86	17	19.76%	*P*<0.001***
UTI infected pregnant woman without PCOS	91	21	23.07%	*P*<0.001***
UTI infected woman with PCOS	46	15	32.60%	*P*<0.001***
Total	223	53	23.76%	*P*<0.001***
P value		*P*>0.05		

UTIs are a leading cause of morbidity and high healthcare expenses in individuals of all ages. Young and sexually active women are more likely to be affected, but other factors also contribute. Populations at risk include the elderly and those using genitourinary aids or catheters. UTI is a significant public health concern that affects millions of people each year. Results of conducted study showed the most participants have growth 53 (21%) were caused by *E. coli*. This is consistent with the findings of [16] an Iraqi study who found that among Gram-negative isolates, 20% were identified as *E. coli. Escherichia coli* is the primary bacterial agent responsible for urinary tract infections, as evidenced by the current study, which corroborates several investigations indicating a significant prevalence of *E. coli* in Duhok city and Babylon city, Iraq [17,18,19]. The results showed that *E. coli* was present UTI infected pregnant woman without PCOS (23.07%), and then infected UTI non- pregnant woman without PCOS (19.76%) with significant different (*P*<0.05). result of current study agrees with Simba *et al.* (2022) was reported in Kenya at 23.5% [20]. Other study in Baghdad, Iraq reported that *E. coli* is the most common uropathogen in PCOS with UTI 42.8% and 44.4% for UTI without PCOS [21]. A study by Tahir (2022) found a substantial (p < 0.05) incidence of urinary tract infections (UTIs) among pregnant women compared to non-pregnant women, with the highest percentage of patients in the age bracket of 36-45 years. Among pregnant women, identified gram-negative bacteria included *E. coli*, which accounted for 40.8% [22]. Results of present research showed the positivity of *E. coli* was highest in UTI infected woman with PCOS (32.60%), The current study's findings corresponds with what was found by El-beaty and Altaii, (2024) that *E. coli* isolates 22(36.67%) were the most common isolates in PCOS women [23].

3.3 ANTIBIOTIC RESISTANCE PATTERN

Fifty-three *E. coli* isolates were evaluated for resistance to 16 antibiotic discs from various classes of antibiotics using the disc diffusion method, revealing differing levels of resistance among the isolates. Results of current study showed the *E. coli* isolates scored highest resistance to AM (94.3%), AMC (92.5%),CTX (90.6%),TPZ (79.2%), PRL (73.6%), CRO (75.5%), and CAZ (92.5%). Based on the sensitivty, current study showed the IMP and MEM scored highest efficiency (69.81 %) and (84.9%) against *E. coli* isolates. The differences among antibiotic resistance against *E. coli* isolates were significant (p<0.05) (Figure 1).

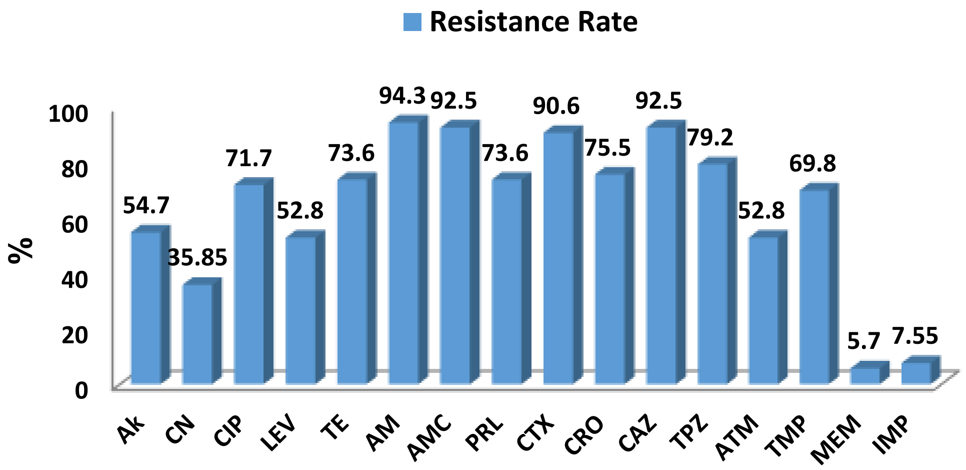

Figure 1. Antibiotic Susceptibility profile of *E. coli* . AK: amikacin; CN: Gentamicin; CIP: Ciprofoloxacin; LVE: Levofloxacin; TE : Tetracyclin ; AMP Ampicillin: AMC Amoxicillin-Calvulanic acid: PRL: Piperacillin; CTX: Cefotaxime; CRO: Ceftriaxone; CAZ: ceftazidime; TZP: Piperacillin -tazobactam; ATM Aztreionam; TMP Trimethoprim: MEM: meropenem; IPM: Imipenem.

The results of the current study indicated that the *E. coli* isolates exhibited the highest resistance to the penicillin group, with ampicillin resistance at 94.3%. This percentage aligns with a prior study [19]. Additionally, 92.5% resistance was observed for Amoxicillin - Clavulanic acid, consistent with findings from another study [21]. The increased resistance may be attributed to the majority of *E. coli* isolates producing beta-lactamase enzymes. The resistance percentages within the cephalosporin group varied, with ceftazidime at 92.5% and cefotaxime at 90.6%. The findings of the current study align with those reported by Abdullah et al. (2024). The results demonstrated differing degrees of resistance across the isolates. Resistance rates were notably high for ampicillin (92.6%), amoxicillin (93.6%), ceftriaxone (88.9%), and ciprofloxacin (82.4%). Meropenem had a resistance rate of 8.2%, whereas imipenem demonstrated a resistance rate of 12.4%. [24]. The current study concurs with [25] regarding Al-Diwaniya, Iraq, indicating that *E. coli* had a strong resistance profile to ampicillin (97.9%) and ceftriaxone (81.3%), while demonstrating significant susceptibility to meropenem (97.9%) and amikacin (97.6%). While current study agrees with Abdulrahman [25] in Al-Diwaniya, Iraq the higher resistance profile of *E. coli* was to ampicillin (97.9%) and ceftriaxone (81.3%) while was highly susceptible to meropenem (97.9%) and amikacin (97.6%.).

In this investigation, antibiotic susceptibility testing of *E. coli* isolates showed that 22 (41.5%), 29(54.7%) and 2 (3.8%) of the isolates multi drug resistant MDR, extensively drug resistant (XDR) and multi drug sensitive (MDS), respectively (Figure 2).

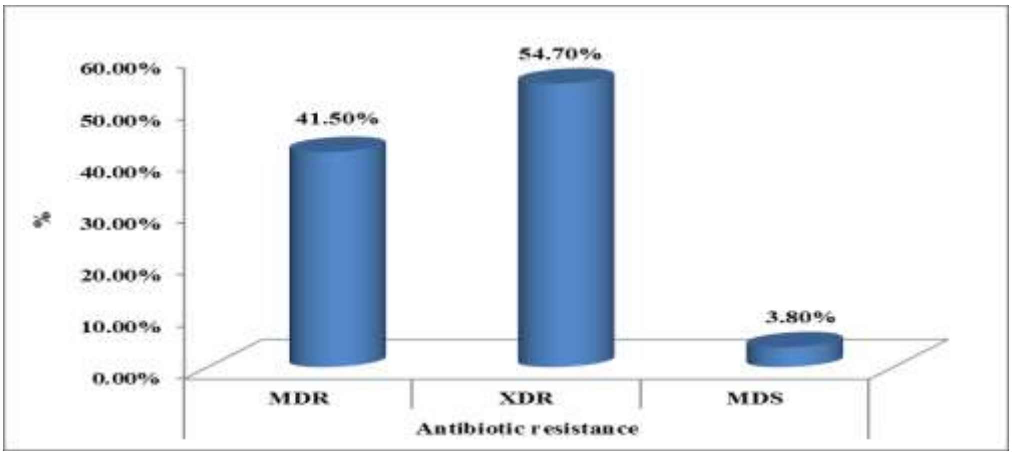

Figure 2. Multidrug resistance pattern of *Escherichia coli*

In this study were showed the IMP and MEM scored highest efficiency (69.81 %) and (84.9%) against E. *coli* isolates. The result agree with study by [26] showed the highest levels of sensitivity were found for Meropenem (97.3%) and Imipenem (95.6%).

3.4 MOLECULAR INVESTIGATION OF THE FIMBRIAE GENES *FIMH* AND *PAPGII*

In the current study, the result of fimbriae genes *fimH* and *papGII* **genes** were detected in extensively drug resistance and multidrug resistance *E. coli* (20) selected isolates using specific primers.

Results by multiplex PCR technique showed that all 20 isolates 100% have the *fimH* gene figure, followed by *papGII* when the rates were 55% (11/20) isolates, while 9 strains (45%) of *E. coli* did not contain the *papGII* gene Figure 3.

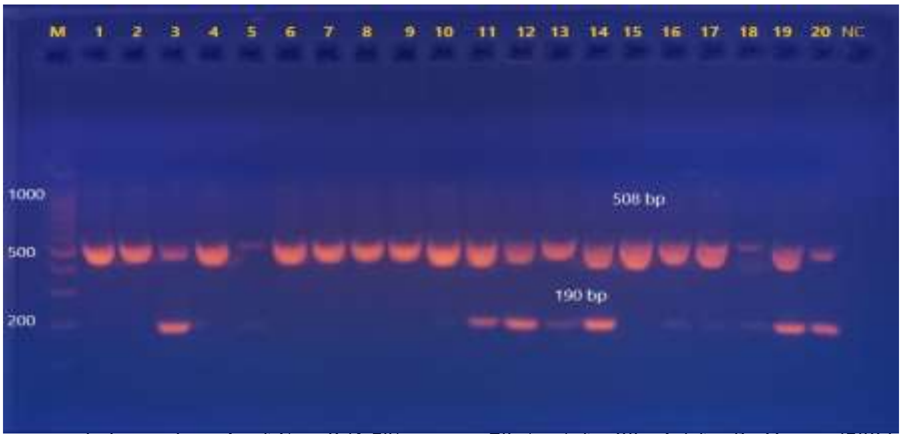

Figure 3. Agarose gel electrophoresis of *E .coli* (1.5% agarose,70v/cm^2 for 90 min) for *fimH* gene (508 bp amplicon) and *papGII* gene (190 bp amplicon) respectively. Lane M, represent M100bp DNA Ladder, lanes 3,5, 11-14 and 16-20 (+ +) , lanes1,2 ,4 and 6-10 (+ -) , lane N.C. (Negative control).

Multiplex PCR analysis revealed that all 20 isolates, representing 100%, possess the *fimH* gene.The genotypic identification of genes encoding adherence factors in the current investigation closely aligns with previous findings concerning the adhesion factor gene *fimH*, [19, 27, 28], hence affirming the extensive prevalence of virulence genes in patients with urinary tract infections. The predominance of the *fimH* gene in *E. coli* isolates contradicts the findings of study [29], which reported a frequency of 60 cases (51.7%) among UTI patients. The current study contradicts [12], which stated that molecular tests identified the *FimH* gene in 52 isolates at a rate of 82.50% and the *PapGII* gene in 19 isolates at a rate of 30.15%. The present study observed that its findings align with those of other worldwide studies, which have established that the *fimH* gene is a significant virulence factor in UPEC, irrespective of geographical distribution.

3.5 SEQUENCING OF PCR AMPLICONS *FIMH*

The current study includes five isolates designated as E3, E11, E12, E14, and E19. The isolates were tested to partially amplify the *FimH* sequences. Concerning the amplicons measuring 506 bp. The NCBI BLASTn engine demonstrated around 99% sequence homology between the sequenced samples and the reference target sequences of *Escherichia coli*. By contrasting the observed nucleic acid sequences of the examined samples with the obtained nucleic acid sequences (GenBank acc. CP025573.1).

Table 5. The observed mutation pattern in the PCR products of the *FimH* amplicons of *Escherichia coli* was compared with the corresponding NCBI reference sequences (GenBank acc. no. CP025573.1).

No.	Sample	Native	Allele	Position in PCR fragment	Variant summary in PCR amplicons	Position in the protein	Variant summary in protein
1.	E11,E14,E19	G	C	60	60G>C	138Gly (138G)	Silent p.138G=
2.	E11	G	A	61	61G>A	139Gly (139G)	Missense p.139G>R
3.	E11,E14,E19	A	G	63	63A>G	139Gly (139G)	Silent p.139G=
4.	E11,E14,E19	A	T	78	78A>T	143A (143Ala)	Silent p.143A=
5.	E3,E14,E19	C	T	182	182C>T	178P (178Por)	Missense p.178P>L
6.	E12	C	T	195	195C>T	182C (182Cys)	Silent p.182C=
7.	E14,E19	G	A	209	209G>A	187R (187Arg)	Missense p.187R>H
8.	E12	C	T	226	226C>T	193L (193Leu)	Silent p.193L=
9.	E11	C	T	237	237C>T	196Y (196Tyr)	Silent p.196Y=
10.	E11	T	C	240	240T>C	197P (197Pro)	Silent p.197P=
11.	E11	A	G	246	246A>G	199S (199Ser)	Silent p.199S=
12.	E14,E19	G	A	252	252G>A	201P (201Pro)	Silent p.201P=
13.	E11	A	T	296	296A>T	216Y (2116Tyr)	Missense p.216Y>F
14.	E11	A	G	329	329A>G	227N (227Asn)	Missense p.227N>S
15.	E11,E14	C	A	363	363C>A	238P (238Pro)	Silent p.238P=

The sequencing results indicated the presence of fifteen nucleic acid variants (60G>C, 61G>A, 63A>G, 78A>T, 182C>T, 195C>T, 209G>A, 226C>T, 237C>T, 240T>C, 246A>G, 252G>A, 296A>T, 329A>G, 363C>A) that were variably distributed in E3 – E19 samples compared with the reference sequences of *Escherichia coli* (GenBank acc. no. CP025573.1) Table 5.

The investigated nucleic acid sequences were converted to their corresponding positions in the major capsid protein. All nucleic acid sequences of the investigated sample were translated to their corresponding amino acid sequences using the Expasy translate suite. Amino acid alignment of these amino acid sequences with the reference sequences showed that the investigated 506 bp amplicons consisted of 168 amino acid sequences in the entire amino acid sequences in the fimbrial protein. The covered amino acid residues were started from the 118^{th} position until 285^{th} position in the entire fimbrial protein. Results indicated that ten of these variants were given silent effects (p.138G=, p.139G=, p.143A=, p.182C=, p.193L=, p.196Y=, p.197P=, p.199S=, p.201P=, p.238P=). Whereas five of them were given missense impact (p.139G>R, p.178P>L, p.187R>H, p.216Y>F, p.227N>S) on the fimbrial protein Table 5. All the investigated genomic sequences were deposited in the NCBI web server, and unique accession numbers were obtained for all analyzed sequences. GenBank PQ273408, PQ273409, PQ273410, PQ273411, and PQ273412 were deposited in NBCI to represent the E3, E11, E12, E14, to E19 samples, respectively.

The evolutionary tree produced in this work is displayed in a circular cladogram (Figure 4). Each variant emphasizes a unique evolutionary distribution of the incorporated sequences. The samples we examined were aligned with other relevant sequences to constitute the sequences presently featured in the cladogram. The comprehensive tree included a total of thirty-four aligned nucleic acid sequences. In the constructed cladogram, the samples were grouped into two phylogenetic clades within the genus *Escherichia*. In the clade-1, all our investigated samples are incorporated, while the other clade is an outgroup clade to assess the main phylogenetic positioning of this clade in comparison with the other strains of the same species. It was found that both clades were found to be positioned away from each other.

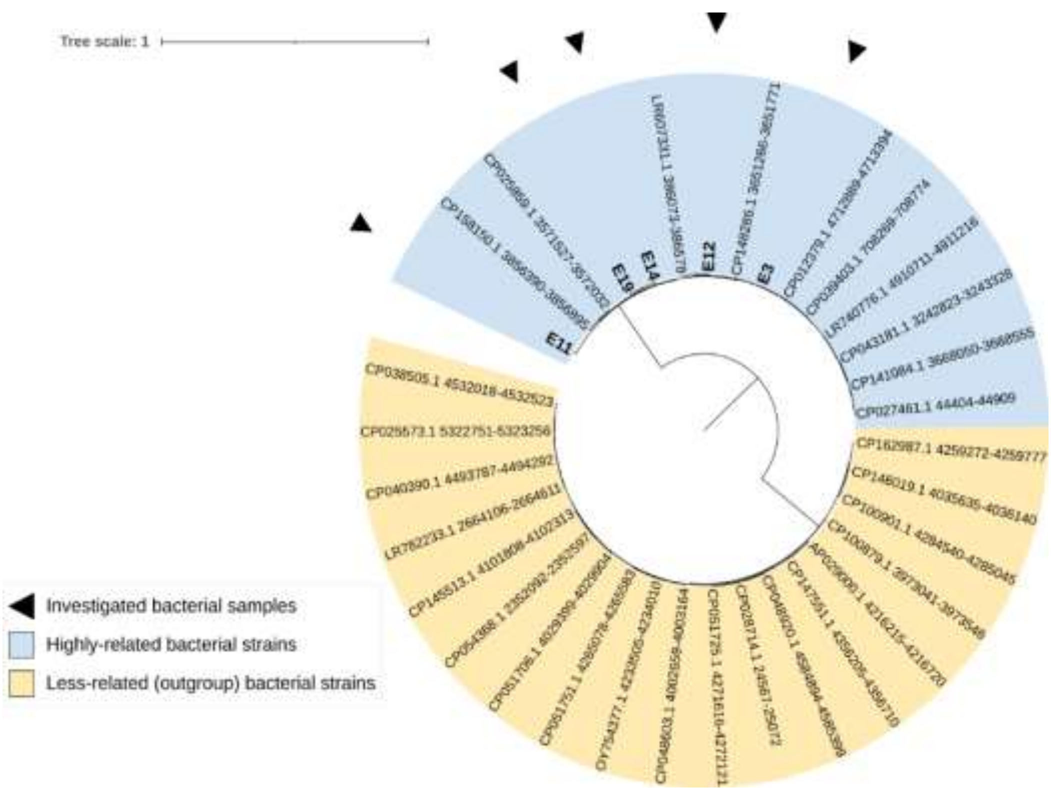

Figure 4. The detailed circular cladogram phylogenetic tree of FimH genomic segments for five *Escherichia coli* samples.

The black triangle denotes the examined bacterial sequences. All the specified numbers correspond to the GenBank accession number of each respective species. The figures at the upper section of the tree indicate the degree of scale range among the organisms categorized within the comprehensive tree. The letter "E" denotes the code of the examined isolates. The present analysis of this phylogenetic tree has corroborated the sequencing reactions, since it elucidates the neighbor-joining-based placement of the sequences being examined. This location precisely represents the relationships among the sequences, validating the veracity of the sequencing data and the evolutionary distances between the samples.

4. CONCLUSION

The results showed the wide spread of *fimH* and *papG II* genes in most Iraqi UPEC. The samples were positioned close to different Asian–European strains, suggesting that the *FimH* gene used was highly capable of determining the true source from where the samples were obtained. This study discovered that *FimH* sequences had the corresponding capacity to identify the *Escherichia coli* sequences under analysis in the patients under investigation. A clear biological diversity has been observed at the employed *FimH* locus across the examined samples for the indicated species. It is possible to find the phylogenetic distributions of the different kinds of bacterial sources by using this genetic locus.

REFERENCES

[1] Whelan, Shane, Brigid Lucey, and Karen Finn. "Uropathogenic *Escherichia coli* (UPEC)-associated urinary tract infections: the molecular basis for challenges to effective treatment." *Microorganisms* 11, no. 9 (2023): 2169.DOI: https://doi.org/10.3390/microorganisms11092169

[2] Medina, Martha, and Edgardo Castillo-Pino. "An introduction to the epidemiology and burden of urinary tract infections." *Therapeutic advances in urology* 11 (2019): 1756287219832172.DOI: https://doi.org/10.1177/1756287219832172

[3] Rozwadowski, Marcin, and Damian Gawel. "Molecular factors and mechanisms driving multidrug resistance in uropathogenic *Escherichia coli*—an update." *Genes* 13, no. 8 (2022): 1397.DOI: https://doi.org/10.3390/genes13081397

[4] Terlizzi, Maria E., Giorgio Gribaudo, and Massimo E. Maffei. "UroPathogenic *Escherichia coli* (UPEC) infections: virulence factors, bladder responses, antibiotic, and non-antibiotic antimicrobial strategies." *Frontiers in microbiology* 8 (2017): 1566. https://doi.org/10.3389/fmicb.2017.01566

[5] Deswal, R., Narwal, V., Dang, A., & Pundir, C. S. The prevalence of polycystic ovary syndrome: a brief systematic review. *Journal of human reproductive sciences*, 2020, 13.4: 261-271. https://doi.org/10.4103/jhrs.JHRS_95_18

[6] Ibáñez L, Oberfield SE, Witchel S, Auchus RJ, Chang RJ, Codner E, Dabadghao P, Darendeliler F, Elbarbary NS, Gambineri A, Garcia Rudaz C. An international consortium update: pathophysiology, diagnosis, and treatment of polycystic ovarian syndrome in adolescence. Hormone research in paediatrics. 2017 Dec 12;88(6):371-95. https://doi.org/10.1159/000479371

[7] Juber NF, Abdulle A, Ahmad A, AlAnouti F, Loney T, Idaghdour Y, Valles Y, Ali R. Associations between Polycystic Ovary Syndrome (PCOS) and Antibiotic Use: Results from the UAEHFS. Antibiotics. 2024 Apr 26;13(5):397.https://doi.org/10.3390/antibiotics13050397

[8] Johnson JR, Stell AL. Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. The Journal of infectious diseases. (2000) Jan 1;181(1):261-72. https://doi.org/10.1086/315217

[9] Hojati Z, Zamanzad B, Hashemzadeh M, Molaie R, Gholipour A. The FimH gene in uropathogenic *Escherichia coli* strains isolated from patients with urinary tract infection. Jundishapur journal of microbiology. (2015) Feb;8(2). doi: 10.5812/jjm.17520

[10] Vandepitte J. Basic laboratory procedures in clinical bacteriology. World Health Organization; (2003). Dec 31.

[11] Clinical and laboratory standards institute (CLSI). Performance standards for antimicrobial susceptibility testing 33rd ed. CLSI supplement M100(ISBN 978-1-68440-170-3. Clinical and laboratory standards institute,USA,(2023). https://doi.org/10.3390/biomedicines11112936

[12] Sayed N, Salman H, Tahan ZS. Serotyping of Syrian Uropathogenic *Escherichia coli* with Adhesion Genes *FimH* & *PapGII*. Iraqi Journal of Science. (2024). Apr 30:1895-904. **DOI:** https://doi.org/10.24996/ijs.2024.65.4.10

[13] Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. GenBank. Nucleic acids research. (2016) Nov 28;45(D1):D37-42. **https://doi.org/10.1093/nar/gks1195**

[14] Zhang Z, Schwartz S, Wagner L, Miller W. A greedy algorithm for aligning DNA sequences. Journal of Computational biology. (2000). Feb 1;7(1-2):203-14. **https://doi.org/10.1089/10665270050081478**

[15] Letunic I, Bork P. Interactive Tree Of Life (iTOL) v4: recent updates and new developments. Nucleic acids research. (2019). Jul 2;47(W1):W256-9. **https://doi.org/10.1093/nar/gkz239**

[16] Mouhammed, K., Gdoura, R. Study of the Genomic Characterization of Antibiotic-Resistant *Escherichia Coli* Isolated From Iraqi Patients with Urinary Tract Infections. Indian J Microbiol **64**, 457–466 :2024. https://doi.org/10.1007/s12088-023-01123-

[17] Albazaz RI, Yassin NA. Prevalence and molecular detection of virulence genes among multidrug resistant *Escherichia coli* from human clinical samples and poultry in Duhok city, Iraq. Med J Babylon, 2024;21:S81-7. DOI: 10.4103/._204_23

[18] Shuwaili, Iman Neamah Hussein Al and Al-maliky, Haydar Khamis . "Bacterial isolates associated with Urinary tract infection in Pregnant Women in North of Thiqar," *Maaen Journal for Medical Sciences*: (2024).Vol. 3 : Iss. 2 ,. https://doi.org/10.55810/2789-9136.1047

[19] Abbood DH, Obaid Alwan ZH. Molecular detection of genes encoding for adhesion factors in biofilm formation among uropathogenic *Escherichia coli* isolates. Med J Babylon 2023;20:258-63. DOI: 10.4103/MJBL.MJBL_233_22

[20] Simba, SM, Omwenga, EO, and Musyoki, SK. Prevalence of *E. coli* as a causative agent of urinary tract infections and its drug susceptibility patterns among pregnant mothers seeking medicare at Kisii teaching and referral hospital, Kenya. Int J Community Med Public Heal . 2022: 9:1161–9. doi: 10.18203/2394-6040.ijcmph20220671

[21] Ibraheem RS, Hussain SS, khalawe Tektook N. Biofilm Formation And Antibiotic Resistance For *E. Coli* Isolated From Urinary Tract Infections From Iraqi Women With The Polycystic Ovarian Syndrome. Journal of Pharmaceutical Negative Results. 2023 Mar 17:3194-201. https://doi.org/10.47750/pnr.2023.14.03.400

[22] Tahir PH. Prevalence of Urinary Tract Infections Caused by Some Gram-Negative Bacteria Among Pregnant Women in Kirkuk Province – Iraq. Microbiol Infect Dis. 2022; 6(2): 1-4 https://doi.org/10.1038/ki.1994.370

[23] El-beaty WA, Altaii HA. DETECTION OF GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA FROM IRAQI WOMEN INFECTED WITH POLYCYSTIC OVARY SYNDROME. InObstetrics and Gynaecology Forum 2024 Jun 19 (Vol. 34, No. 3s, pp. 1976-1989). https://doi.org.obstetricsandgynaecologyforum.com /618

[24] Abdullah HM, Haleem B, Tariq M, Akhlaq S, Manawar S, Afzal MI. Unraveling the resistance puzzle: Antibiotic susceptibility and genetic insights into extra-intestinal *E. coli* Strains in UTIs. The Professional Medical Journal. 2024 May 31;31(06):899-906.46- https://doi.org/10.29309/TPMJ/2024.31.06.7871

[25] Alfetlawi B, Jasim A. Determining the Prevalence of Upper and Lower Urinary Tract Infections' Pathogens and Their Antibiotic Susceptibility Profile for Adult Patients in Al-Diwaniya, Iraq (Conference Paper). Iraqi Journal of Pharmaceutical Sciences 2022;31(Suppl.):86-91.48 https://doi.org/10.31351/vol31issSuppl.pp86-91

[26] Abdulrahman IS. Antimicrobial susceptibility pattern of pathogenic bacteria causing urinary tract infections at Azadi Hospital in Duhok City\Kurdistan Region of Iraq. Science Journal of University of Zakho. 2018 Jun 30;6(2):46-50.54- https://doi.org/10.25271/2018.6.2.435

[27] Aljebory IS, Mohammad KA. Molecular Detection of Some Virulence Genes of *Escherichia coli* Isolated from UTI Patients in Kirkuk City, Iraq. Journal of Global Pharma Technology. (2019);11(3):349-55. DOI: www.jgpt.co.in

[28] Allami M, Bahreini M, Sharifmoghadam MR. Antibiotic resistance, phylogenetic typing, and virulence genes profile analysis of uropathogenic *Escherichia coli* isolated from patients in southern Iraq. Journal of Applied Genetics. (2022). May;63(2):401-12. **https://doi.org/10.1007/s13353-022-00683-2**

[29] Qadir HA, Abdulla AB, Abduljabbar HN. Molecular Study of Virulence Factors of *Escherichia coli* Isolated from Patient with urinary tract infection in Wasit Province. *Al-Kut Univ. College Journal*. (2018);3(2):39-50. **https://doi.org/10.1063/5.0182049**

BIOGRAPHIES OF AUTHORS

	**Dr. Lina Abdulameer S. Alsaadi** is the Associate Professor at the Department of Biology, College of Sciences, University of Diyala. Republic of Iraq Ministry of Higher Education and Scientific Research, Iraq. She got Doctoral degree of Microbiology / Microbial Genetics, University of Diyala, Iraq in 2020. She interests in Community diseases, Antibiotic resistant bacteria in Diyala Hospitals, Biosynthesis of nanoparticles and their applications. She can be contacted at email: linaabdulameer@uodiyala.edu.iq .
	**Ghofran Khudhair Ismail** is a Master's student at College of Science, University of Diyala, Iraq. She received the B.Sc. degree in Biology science from the University of Diyala in Iraq She can be contacted at email: scibioms222304@uodiyala.edu.iq