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ORIGINAL STUDY

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Molecular Detection of Virulence Genes and Antibiotic Resistance in Uropathogenic *Escherichia Coli*: Focus on Babylon Province, Iraq

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Abstract

Background: Urinary tract infections (UTIs) affect individuals of all ages, with uropathogenic *Escherichia coli* (UPEC) being their primary causative agent. The accumulation rate of antimicrobial resistance poses a major public health challenge, underscoring the need for genetic monitoring of resistant strains.

Objectives: This study aimed to find virulence genes and antibiotic resistance in UPEC.

Materials and Methods: 110 patients, including 65 females and 45 males, aged 15 to 40 years, were screened for UPEC at Al-Hilla city between January and March 2025. Urine samples were collected to identify *E. coli* and to analyze associated virulence factors.

Results: Biochemical tests confirmed UPEC isolates ($n = 25$, 22.7%). Females represented 60% ($n = 15$), while males represented 40% ($n = 10$) accounted for UPEC that indicated higher susceptibility in females. Polymerase chain reaction was employed to identify virulence genes, including *Sat*, *usp*, *hlyA*, and *cnf*, and all isolates carried the 16S rRNA gene for strain identification. The *Sat* and *usp* genes were detected in 96% of the isolates, while the *hlyA* gene was present in 92%. None of the isolates harbored the *cnf* gene.

Conclusion: Although our study included only 25 isolates, antimicrobial resistance in *UPEC* is a global issue, and our findings represent only the tip of the iceberg, highlighting the need for larger-scale surveillance studies.

Keywords: Urinary tract infections, Uropathogenic *Escherichia coli*, Virulence genes, Antimicrobial resistance, *hlyA*, *Sat*

1. Introduction

Urinary tract infections (UTIs) are globally distributed diseases caused by *Escherichia coli*, particularly its uropathogenic strains [1, 2]. These infections represent a major public health challenge and are classified as uncomplicated or complicated [3]. Uropathogenic *E. coli* (UPEC) is the primary cause of both lower UTIs (e.g., cystitis) and upper UTIs (e.g., pyelonephritis), particularly in females due to anatomical predisposition [4]. UPEC exhibits remarkable adaptability in the urinary tract, utilizing diverse virulence mechanisms including adhesion, invasion, biofilm formation, intracellular survival, and immune evasion to disseminate [5].

Specifically, adhesins such as P fimbriae and type 1 fimbriae enable attachment to uroepithelial cells, facilitating subsequent invasion and the formation of intracellular bacterial communities, which promote immune evasion and recurrence [6]. Biofilm formation further shields bacteria from antibiotics and host defenses [7, 8]. Uncomplicated UTIs are common in otherwise healthy women with normal anatomy,

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while complicated UTIs occur in patients with risk factors like catheter use, anatomical anomalies, or chronic illnesses such as diabetes mellitus or immunosuppression [9, 10]. UPEC produces several toxins and virulence proteins, such as secreted autotransporter toxin (*Sat*), hemolysin A (*hlyA*), cytotoxic necrotizing factor 1 (*cnf1*), and uropathogenic-specific protein (*usp*), which enhance its ability to damage host tissue and persist within the urinary tract [11]. In conclusion, UTIs represent a major global health issue, predominantly caused by UPEC.

The rising prevalence of multidrug-resistant (MDR) UPEC strains is a major concern, as they exhibit resistance to first-line agents such as β-lactams, fluoroquinolones, and trimethoprim-sulfamethoxazole, thereby limiting treatment options. The rise of MDR UPEC highlights the urgency for novel antimicrobial agents, alternative treatment strategies, and the development of preventive tools like vaccines [12]. Without such innovations, UPEC-related UTIs, especially in high-risk populations, may result in more severe outcomes, including chronic kidney infections, sepsis, and long-term health complications. This study was aimed at investigating the occurrence of virulence factors and antimicrobial resistance of UPEC of an Iraqi community.

2. Materials and methods

2.1. The study design

A cross-sectional study was conducted at Al-Hilla Teaching Hospital between January and March 2025, enrolling 110 patients (65 females and 45 males) aged 15–40 years. Immunocompromised individuals, those receiving antibiotic therapy, and non-consenting patients were excluded.

2.2. Isolation and identification of bacteria

Urine samples ($n = 110$) were collected from 65 female and 45 male patients aged between 15 and 40 years, all with suspected UTIs. These samples were promptly sent to the Microbiology and Antimicrobial Substances Research Unit in the Department of Microbiology at Al-Hillah Teaching Hospital for further analysis. Initially, the specimens were cultured on blood agar and MacConkey agar for primary isolation, where presumptive identification of *E. coli* colonies was performed. The isolates were then subcultured onto mannitol agar and incubated at 37 °C for 24 hours to promote bacterial growth and isolation. *E. coli* detection was conducted using standard microbiological and biochemical methods, including inoculation on mannitol agar. Further characterization of the isolates was done based on colony morphology, blood agar pigmentation, and biochemical tests.

Standard biochemical tests covering indole generation, methyl red, Voges-Proskauer, citrate use (IMViC), and urease testing came next. In summary, bacteria known as *E. coli* are isolated with a positive reaction for indole and methyl red, and a negative reaction to Voges-Proskauer, citrate, and urease tests.

2.3. Antibiotic susceptibility assessment

The antibiotics used in susceptibility testing were levofloxacin, amoxicillin-clavulanic acid, cefepime, amikacin, and ampicillin, which are prescribed routinely in our hospital. Every *E. coli* sample grew on nutrient agar at 37 °C overnight. Two to three colonies were then cultured in Muller-Hinton broth and left overnight at 37 °C. The bacterial suspension was injected into Muller-Hinton agar plates with cotton swabs the next day. The disk diffusion method was performed following Clinical and Laboratory Standards Institute ([13]) standards; however, exact zone diameters were not recorded. Results are reported qualitatively as susceptible, intermediate, or resistant. Strains resistant to at least one agent in three or more antimicrobial classes were classified as MDR.

2.4. DNA extraction

Approximately 300μL of lysis solution (Sigma-Aldrich, USA) and 20μL of universal sorbent (Sigma-Aldrich, USA) were added to pre-labeled Eppendorf tubes corresponding to each sample. Then, 100μL of the sample was added using aerosol-safe filter tips. Tubes were firmly closed, briefly mixed on a vortex, and incubated at 65 °C for 5 minutes, followed by a short vortex and 2-minute incubation at room temperature. Tubes were centrifuged at 10,000 rpm for 30 seconds, and the supernatant was carefully removed without disturbing the pellet. Next, 1mL of washing buffer solution (Qiagen, Germany) was added, and the sorbent was fully resuspended by vigorous vortexing. Tubes were uncapped and incubated at 65 °C for 5–10 minutes to dry. For DNA elution, 65μL of Tris-EDTA buffer was added, vortexed to resuspend the sorbent, and incubated again at 65 °C for 5 minutes. Finally, tubes were centrifuged at 12,000rpm for 1 minute, and the DNA-containing supernatant was carefully collected and stored at –20 °C for downstream applications (*vide infra*).

2.5. The detection of PCR for genes associated with virulence

Specific oligonucleotide primers selected from published sequences were used to detect virulence genes

Table 1. PCR primer sequences, amplicon sizes, and annealing temperatures for the detection of uropathogenic E. coli virulence genes and 16S Rrna.

Gene name	Gene	Gene sequence	Amplicon size	Tm (°C)	Reference
Alpha haemolysin	*hlyA*	F: AACAAGGATAAGCACTGTTCTGGCT R: ACCATATAAGCGGTCATTCCCGTCA	577	61	[14]
Cytotoxic necrotizing factor	*cnf*	F: AAGATGGAGTTTCCTATGCAGGAG R: TGGAGTTTCCTATGCAGGAG	498	58	[15]
Secreted autotransporter toxin	*Sat*	F: TATCACGCAATGCCAATGTT R: GACCCGGCGTTACAGTTTTA	393	63	[16]
Uropathogenic-specific protein	*usp*	F: ACATTCACGGCAAGCCTCAG R: GCGAGTTCCTGGTGAAAGC	448	62	[16]
16s rRNA	*16s rRNA*	F: CATGCCGCGTGTATGAAGAA R: CGGGTAACGTCAATGAGCAAA	100	59	[16]

(Table 1). Traditional PCR targeting the 16S rRNA gene with species-specific primers was performed to confirm *E. coli* isolates (Table 1). PCR reactions were prepared in a 25μL volume, containing 5μL PCR Master Mix, 1μL of forward and reverse primers, 2μL DNA template, and nuclease-free water to volume. Amplifications were carried out on a SimpliAmp™ Thermal Cycler (Applied Biosystems, USA) with an initial denaturation at 95 °C for 5min, followed by 30 cycles of 94 °C for 1min, annealing for 1min (see Table 1 for melting temperatures), and 72 °C for 30s, with a final extension at 72 °C for 5min. PCR products were resolved on 1.5% agarose gels stained with SYBR Safe (Invitrogen, USA) and visualized under UV using a trans-illuminator to confirm successful amplification.

2.6. *Gel electrophoresis*

PCR products were analyzed using 2% agarose gel electrophoresis containing 0.5 μg/mL ethidium bromide. Samples mixed with loading dye were loaded into wells alongside a 100 bp or 1 kb DNA ladder. Electrophoresis was run at 100 V for 40 minutes using a Wix Electrophoresis Power Supply. Bands were visualized under UV light, photographed, and com pared with expected sizes to conrm amplication of target virulence genes Figs. 2 to 5 (*vide infra*).

2.7. *Statistical analysis*

All frequency data were analyzed using Pearson's chi-squared test, with Fisher's exact test applied when appropriate. Statistical analyses were performed using SPSS version 22, and results are presented primarily as percentages. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. *Isolation and identification of E. coli*

Patients ($n = 110$) clinically diagnosed with UTIs provided urine samples. Following normal aseptic procedures to minimize contamination, *E. coli*-positive cases were distributed across various age groups. The distribution of *E. coli* positive cases across age groups showed a statistically significant difference ($p = 0.005$). Among participants aged 15–20 years, 15 were positive; in the 21–30 age group, 45 were positive; and in the 31–40 age group, 35 were positive.

Table 2. Antimicrobial resistance rates of uropathogenic E. coli isolates isolated from urinary tract infection.

Antibiotic	No. of resistant isolates	% of Resistance
Ampicillin	20	80%
Amoxicillin-Clavulanic acid	11	44%
Levofloxacin	5	20%
Amikacin	4	16%
Multidrug resistant	4	16%
Fully susceptible isolates	3	12%

3.2. *Antimicrobial susceptibility test of the UPEC*

The antibiotic susceptibility of UPEC isolates was assessed using representatives from several classes of antibiotics, including Fluoroquinolone (levofloxacin), β-lactam (Aminopenicillin) + β-lactamase inhibitor (amoxicillin–clavulanic acid), β-lactam (4th-generation cephalosporin) (cefepime), Aminoglycoside (amikacin), and β-lactam (Aminopenicillin) (ampicillin; Table 2). In this regard, UPEC isolates showed the highest resistance to ampicillin, followed by amoxicillin–clavulanic acid, levofloxacin, and amikacin. Especially, a subset of isolates was identified as MDR, exhibiting resistance to three or more antibiotics, while others remained sensitive to all tested antibiotics.

3.3. *Virulence gene prevalence in E. coli isolates*

PCR amplification of the 16S rRNA gene confirmed that all UPEC strains isolated from UTIs were *E. coli*. Fig. 1 shows all isolates positive for the *E. coli*-specific 16S rRNA gene. Table 3 summarizes the frequency of virulence genes among these isolates. In this context,

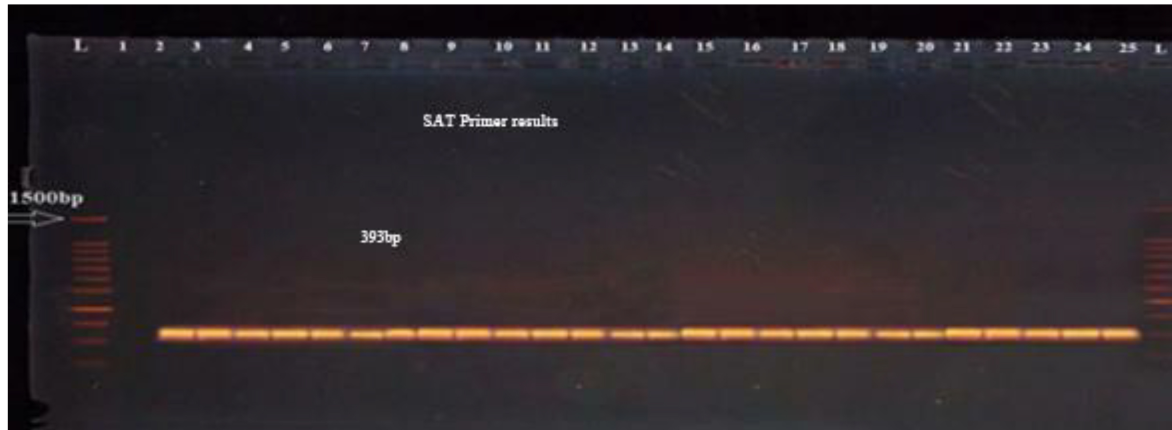

Fig. 1. Gel electrophoresis of PCR product of sat primer (393 bp) gene, L = ladder (100–1500), annealing temperature (Tm) of 63 °C, 1% agarose gel, starting at 100 volts for 10 minutes and then reduced to 70 volts for 60 minutes, the 2–25 represent the positive results in samples for this gene while 1 isolate negative result.

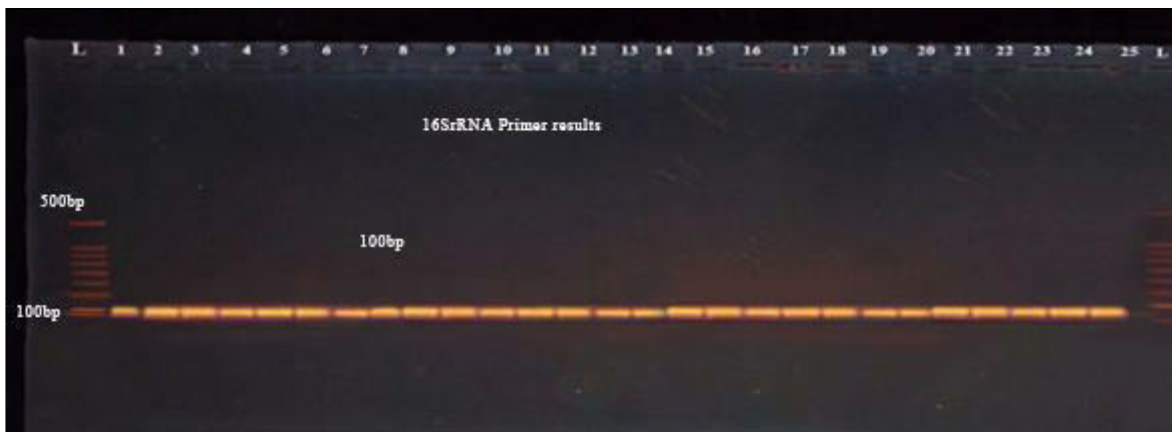

Fig. 2. Gel electrophoresis of PCR product of 16S rRNA primer (L = ladder (100–1500), annealing temperature (Tm) of 59 °C, 1% agarose gel, starting at 100 volts for 10 minutes and then reduced to 70 volts for 60 minutes, the 1–25 represent the positive results in samples for this gene.

Table 3. Prevalence of 16S rRNA and virulence genes in uropathogenic Escherichia coli isolates.

Gene	No. of isolates	(%)
16S rRNA	25	100%
Sat	24	96%
usp	24	96%
hlyA	23	92%
cnf	0	0%

Sat and *usp* were detected in all isolates, *hlyA* was also highly prevalent, while *cnf* was not detected in any isolates.

4. Discussion

The present study analyzed the prevalence of UPEC across different age groups, demonstrating significant variation. The most frequent cases were individuals aged 21–30 years, followed by the 31–40 years age group, with the least frequent cases were 15–20 years age group. This distribution pattern is consistent with earlier reports [17, 18], our findings added region-specific evidence from Iraq and confirmed that young adults, particularly females, are at greater risk of UTIs due to anatomical and hormonal factors. Such age-related epidemiological profiling provides valuable insight into population groups most susceptible to UPEC infections, thereby guiding preventive and clinical strategies.

Another contribution of this study is the assessment of antimicrobial resistance among UPEC isolates. The results revealed alarmingly high resistance rates to ampicillin and amoxicillin-clavulanic acid, consistent with global findings [19, 20], but particularly concerning in this local context given the heavy reliance on these antibiotics for empirical therapy (e.g., [21, 22]). An array of studies from different parts of the globe reported high resistance rates of *E.coli* strains to

Fig. 3. Gel electrophoresis of the PCR product of cnf primer. A single band at 498 bp was observed (L = ladder (100–1500), annealing temperature (Tm) of 58 °C, 1% agarose gel, starting at 100 volts for 10 minutes and then reduced to 70 volts for 60 minutes, the 1–25 represent the Negative results in samples for this gene.

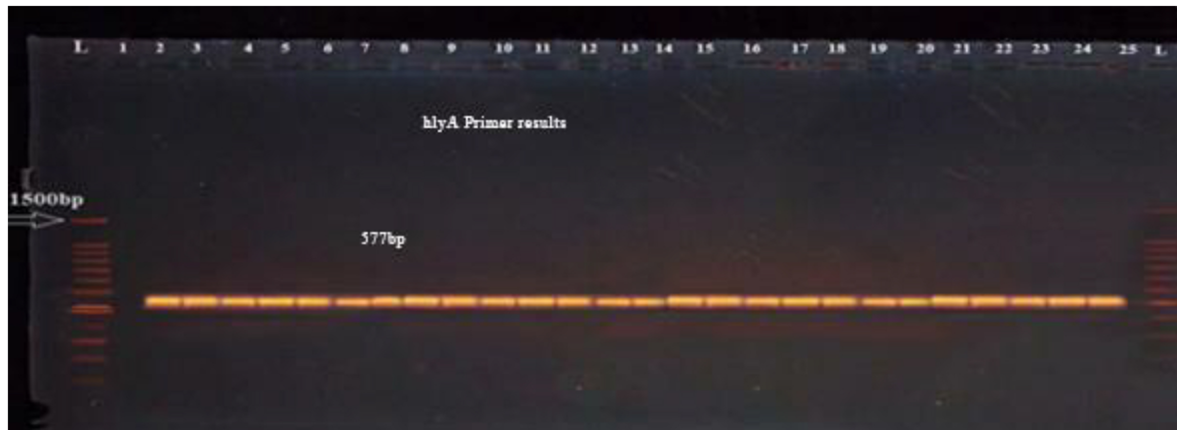

Fig. 4. Gel electrophoresis of the PCR product of hlyA primer. A single band at 577 bp was observed (L = ladder (100–1500), annealing temperature (Tm) of 61 °C, 1% agarose gel, starting at 100 volts for 10 minutes and then reduced to 70 volts for 60 minutes, the 2–24 represents of the positive results in samples for this gene while 1 and 25 isolates represent the negative results.

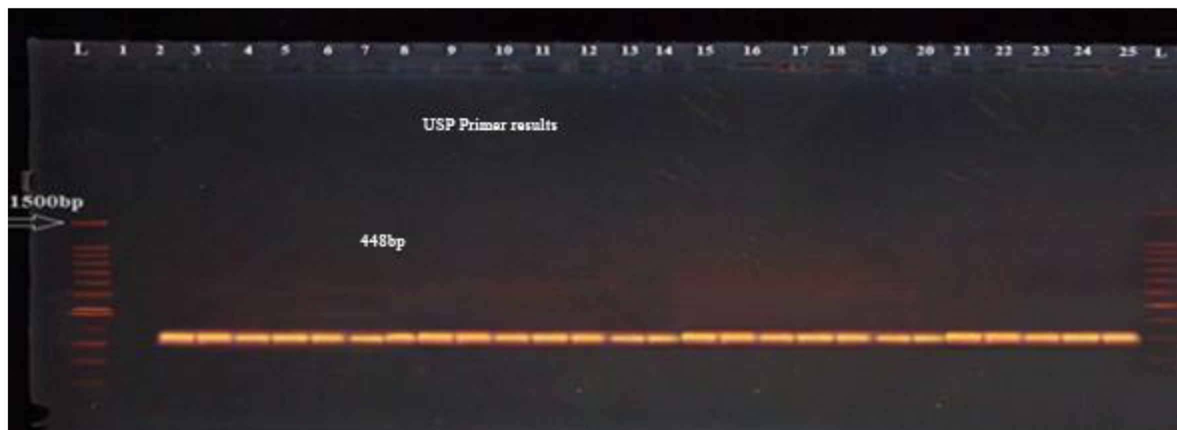

Fig. 5. Gel electrophoresis of the PCR product of usp primer. A single band at 448 bp was observed (L = ladder (100–1500), annealing temperature (Tm) of 62 °C, 1% agarose gel, starting at 100 volts for 10 minutes and then reduced to 70 volts for 60 minutes, the 2–25 represents of the positive results in samples for this gene and 1 isolate represents the negative results.

ampicillin and amoxicillin-clavulanic acid [23, 24]. The lowest resistance rates to amikacin and cefepime indicate that these drugs may still be viable options for treatment in complicated or resistant cases, supporting earlier findings [25, 26]. Importantly, the detection of MDR strains in 16% of isolates and the finding that only 12% of isolates were fully susceptible highlight an emerging regional resistance trend that mirrors global concerns [27, 28]. Therefore, the occurrence of antibiotic-resistant UPEC around the globe is a medical issue and needs investigations and novel policies, such as vaccine design for highly virulent UPEC.

The molecular profiling of virulence genes represents an additional aspect of this research. The remarkably high prevalence of *Sat*, *usp* and *hlyA*, demonstrates that cytotoxicity and host tissue damage are dominant pathogenic mechanisms in local strains, consistent with earlier studies [29, 30]. In this line, *Sat* belongs to the *E. coli* Class-1 of serine protease autotransporters, which lead to tissue damage and break complement molecules and are dubbed for their roles to fulminate into sepsis (for a review see [31]. The dominant prevalence of *Sat* in UPEC must be considered as a serious clinical warning because it is coincident with antimicrobial resistance, since *Sat* exerts cytopathic effects on the urinary system [31]. Tissue damage caused by *Sat* may diminish antibiotic penetration, worsening treatment outcomes. Similar to *Sat*, *usp* showed an identical frequency of occurrence among UPEC in the present study. In this regard, *usp* gene was detected in 98.2% of UPEC isolates from females with UTIs in Iraq, indicating that *usp* contributes to virulence through host DNA damage and cancer risk [32, 33]. The abovementioned virulence gene, *hlyA,* was more prevalent among UPEC isolates. Another study showed that the prevalence of *hlyA,* responsible for the secretion of hemolysin toxins by *E. coli,* is higher in cases of UTIs than in cases of diarrhea in some Iraqi communities [34]. In essence, *HlyA* is a pore-forming toxin that disrupts host cell membranes, causing cell lysis and the release of iron and other nutrients essential for bacterial growth. By hemolysis and damaging leukocytes and renal proximal tubular epithelial cells, HlyA causes cytotoxicity, inflammation, and tissue damage, thereby facilitating bacterial invasiveness and pathogenicity [24]. Interestingly, the absence of *cnf* in all isolates may reflect regional strain variation or differences in virulence gene expression, as noted previously [35, 36]. Future precise resistome and virulome investigations are required to dig deeper into the resistance mechanisms and virulence profiles of UPEC in Iraq.

Finally, the use of PCR amplification and gel electrophoresis provided precise molecular confirmation of these virulence genes (393 bp for *Sat*, 448 bp for *usp*, and 100 bp for *16S rRNA*), validating the robustness of the diagnostic approach. Collectively, these findings contribute novel epidemiological and molecular insights into the virulence and resistance of UPEC strains in this region, reinforcing the importance of routine surveillance and molecular diagnostics in the management of UTIs.

5. Conclusion

This study provides the first comprehensive report on virulence determinants and antibiotic resistance in UPEC strains isolated from clinical centers across Al-Hilla Teaching Hospital, Iraq. The findings reveal that most UPEC isolates exhibit resistance to clinically prescribed antibiotics, with particularly high resistance to β-lactam (ampicillin). Moreover, this study demonstrated UPEC strains were equipped with highly virulent factors that intensify their pathogenesis. This study highlights the limited therapeutic options available for such infections. To achieve effective control of UTIs caused by resistant and virulent UPEC strains, alternative therapeutic strategies like vaccine design must be considered, despite potential challenges related to cost and patient compliance.

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Conflict of interest

There was no conflict of interest during the preparation of this article.

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki. Verbal consent was obtained from all participants, and the study was approved by the Committee on Publication Ethics, College of Medicine, University of Babylon, Iraq (Approval No. 395-4/1/2025), in accordance with international ethical standards.

Author contributions

- **Conceptualization:** Lara Hashem Abdzaid and Jwan Ahmed Ali
- **Methodology:** Lara Hashem Abdzaid, Tiba Habeeb Saifi, and Jwan Ahmed Ali
- **Software:** Tiba Habeeb Saifi, Jwan Ahmed Ali, and Layth Jasim Mohammed
- **Validation:** Layth Jasim Mohammed and Isaac Karimi
- **Formal analysis:** Lara Hashem Abdzaid and Tiba Habeeb Saifi
- **Investigation:** Lara Hashem Abdzaid, Tiba Habeeb Saifi, and Jwan Ahmed Ali
- **Resources:** Layth Jasim Mohammed and Isaac Karimi
- **Data Curation:** Isaac Karimi and Layth Jasim Mohammed
- **Visualization:** Lara Hashem Abdzaid, Layth Jasim Mohammed, and Isaac Karimi
- **Writing Original Draft:** Lara Hashem Abdzaid, Tiba Habeeb Saifi, Jwan Ahmed Ali and Layth Jasim Mohammed
- **Writing Review & Editing:** Layth Jasim Mohammed and Isaac Karimi
- **Supervision:** Lara Hashem Abdzaid and Jwan Ahmed Ali

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