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Investigation of Some Virulence Genes of Uropathogenic *Escherichia coli* isolated from patients with Urinary Tract Infection in Mosul City, Iraq

Fanar Dawas Mahmood [1], Safaa Asaed Mohammed [1] and Khansaa Basem Fadhil [2,*]

[1] *Directorate General of Education of Nineveh.*
[2] *Northern Technical University.*

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Abstract

Approximately 150 million people are infected (UTIs) affect up to 150 million people worldwide, and *Escherichia coli* (*E. coli*) is the leading cause of these infections globally. This study investigated the morphological, biochemical, and antibiotic susceptibility profiles of these bacteria using the VETIK2 system. The study also identified the virulence and antiviral gene profiles of *E. coli* strains identified in the GenBank database and discovered a new gene (FamsAh LC842012.1) that is a major cause of UTIs in patients in Mosul, Iraq.

Materials and Methods: 37 urine samples were collected from Al-Hayat International Hospital in Mosul from September 1 to November 31, 2025. Bacterial isolation was done by MacConkey, Eosin Methylene Blue (EMB) and blood agar. Confirmation was done by Gram staining, IMViC tests, catalase and urease tests, TSI (Triple Sugar Iron) agar reactions, and 16S rRNA sequencing. Antimicrobial susceptibility tests were conducted by the Vitek-2 system with 15 antibiotics. Virulence genes were detected by PCR in a reference strain registered as a new isolate (FaMsAh LC842012.1) in NCBI (National Center for Biotechnology Information).

Results: Out of 37 uropathogens, *E. coli* (14, 37.8%) was the most commonly identified organism. All the isolates were Gram-negative bacilli. They showed pink colonies on MacConkey agar and a metallic green sheen on EMB agar, and an IMViC profile of (++−−). Molecular identification through 16S rRNA, identified the isolates as *E. coli*. Most isolates showed extensive multidrug resistance (MDR Uropathogenic *E. coli*) among 9 of the 15 antibiotics tested, including amoxicillin-clavulanate, piperacillin-tazobactam, and several cephalosporins. However, the isolates were susceptible to the carbapenems (imipenem, meropenem) and to gentamicin, fosfomycin, nitrofurantoin and trimethoprim-sulfamethoxazole. The positive PCR revealed the presence of all virulence factors, (fimH, stx1, eae, hlyA), and then, the first hybrid UPEC/STEC/EPEC of its kind.

Conclusions: The first emerging strain of multidrug-resistant Uropathogenic *E. coli* (UPEC) with the unprecedented presence of virulence genes associated with diarrheal disease was identified in Iraq. Monitoring the emerging UPEC strains in Iraq is of utmost importance.

Keywords: Uropathogenic *E. coli*; UTIs; Virulence genes; Biochemical Tests; (UPEC); Morphology

1. Introduction

Every year, around 150 million people suffer from urinary tract infections (UTIs), making them one of the top bacterial infections in the world[1]. Most of the pathogens that cause these infections are Uropathogenic *E. coli* (UPEC). UPEC is responsible for 80% of community-acquired UTI, and still a significant amount of hospital-acquired UTIs[2]. UPEC are

* Corresponding author: Fanar Dawas Mahmood

pathogens with a lot of complex and specialized virulence factors. Some of these factors include adhesins like P-fimbriae, hemolysin, and other systems to help them capture iron. These factors help UPEC transition from intestinal flora to the urinary tract which is a nutrient-poor environment[3].

With the rise in global antimicrobial resistance (AMR), the treatment of UTIs has changed a lot. The first line treatments that are used are trimethoprim-sulfamethoxazole, fluoroquinolones, and beta-lactams. UPEC has been observed to have decreased susceptibility to these standard treatment options[4]. UPEC infections caused by Extended-Spectrum Beta-Lactamase (ESBL) *E. coli* infections are the most concerning. The CTX-M-15 variant is the most prevalent worldwide and is linked to the multidrug-resistant (MDR) clone ST131[5].

Uropathogenic *E. coli* (UPEC) obtains the ability to cause disease through the acquiring of specific virulence factors which allow for colonization, destructive tissue, and the ability to hide from the immune system. The fimH gene is an example of a gene that enhances UPEC pathogenicity. The fimH gene is coding for one type of type 1 adhesin which is able to make an association to specific receptors at the level of the bladder uroepithelium. This association is one of the first steps in establishing an infection of the urinary tract[6]. The hlyA gene is also correlated with enhancing the virulence factor of UPEC. The hlyA gene is responsible for producing alpha-hemolysin. The alpha-hemolysin disrupts the integument of the host causing the host to leak nutrients that can lead to host tissue destruction[7]. The stx1 (shiga toxin) genes and eae (intimin) genes, while more typically linked to enterohemorrhagic strains, have been discovered in uropathogenic isolates. The stx1 and eae genes are suggestive of a more pronounced ability to induce host cellular destruction and a close association to the supportive tissue of the host. These genes, in aggregate, are able to alter the nature of a commensal *E. coli* to a UPEC that can bypass the host system of defenses and cause relapsing infections[8].

Beta-lactam antibiotics are not the only class of antibiotics where the resistance mechanisms are fully understood. The resistance mechanisms to fluoroquinolones have also been well studied. In most of the regions of the world fluoroquinolone resistance is high due to mutation of the nodes of the ergosome operation at the point of fumarylacetoacetate hydrolase (gyrA) and parC (QRDRs)[9]. The co-location of antibiotic resistance genes on the mobile genetic elements (MGEs) of different antibiotic classes, including plasmids of the IncF type, is Rapid development of multi-drug resistance (MDR) where only the last line antibiotics like colistin and carbapenems remain effective[10]. The discovery of genes of carbapenemases (especially, the enzyme variants blaNDM and blaKPC) in uropathogenic strains is one of the directly concerning signs of the uropathogenic beta-lactam Resistance mechanisms. The resistance mechanisms related to the UPEC create the maximum beta-lactam resistance crisis[11].

The most recent and useful molecular techniques in microbiology are rapid tests and sequencing (WGS) of the whole genome of the infectious microbe. Rapid tests detect the genes of antibiotic resistance, and therefore the phenotypic antibiotic resistance patterns of the microbe the resistance mechanisms of which are in the plasmids (MHRA) phenotypic resistance patterns. This occurs much faster than the 48 to 72 hours that are normally required for conventional tests[12]. Therefore, rapid tests and sequencing techniques (WGS) of the whole genome allow 'precision medicine' to be applicable to the management of infectious diseases. This means that patients' clinical outcome is improved substantially, and the rapid tests and sequencing techniques (WGS) of the whole genome also reduce the selective pressure (SP), which drives the further development (exacerbation) of antibiotic resistance mechanisms[13].

Surveillance at both the local and global scales is warranted for the ongoing changes in antimicrobial susceptibility of UPEC. The phenomenon of antimicrobial resistance occurs due to various mechanisms and understanding these is vital in the formulating of new treatment approaches to combat the infection as well as in the designing of effective strategies to prevent the dissemination of multi-drug resistant (MDR) uropathogens from a public health perspective.

2. Materials and Method

2.1. Ethical Considerations

On August 10, 2025, the local ethics committee of the College of Science/ University of Mosul, examined and approved the study's protocol (reference code: SREC-25-3-7).

2.2. Study Design

The study involved collecting 37 samples from Al-Hayat International Hospital laboratories from patients of both sexes with urinary tract infections in sterilized screw cap containers . in a period between September 1st and November 31st, 2025.

2.3. Culture of samples

After preparing the culture media according to the manufacturer's instructions, which included MacConkey agar, blood agar, and EMB as a differential medium, the samples were directly cultured using a streaking method onto the culture media suitable for bacterial growth. The plates were incubated upside down at 37°C for 18-24 hours.

The isolated and purified colonies were then transferred to MacConkey's medium ,EMB and blood agar, and the plates were incubated again for 24 hours at 37°C until use for diagnostic testing.

2.4. Bacterial Isolation and Identification

According to the diagnostic procedures recommended by [14, 15], the isolation and identification of *E. coli* associated with patients under study were performed as follows:

2.5. Microscopic and morphological examination

Identification of the bacterial isolates was performed through a series of standardized biochemical and morphological tests. Initially, Gram staining was conducted using the classic four-step method (crystal violet, Gram's iodine, 95% ethanol, and safranin) to determine cellular morphology and wall characteristics.

2.6. Cultural characteristics

To assess lactose fermentation, isolates were inoculated onto MacConkey agar and monitored for the production of pink colonies. Differential primary identification was further supported by culturing on Eosin Methylene Blue (EMB) agar, where the presence of a metallic green sheen was recorded as a presumptive marker for *E. coli*.

2.7. Biochemical Tests

The physiological profile of the Enterobacteriaceae was established using the IMViC series, comprising Indole production (Kovac's reagent), Methyl Red and Voges-Proskauer tests for metabolic pathways, and Citrate utilization on Simmons Citrate slants. Additionally, enzymatic activity was evaluated via the Catalase test using 3% and the Urease test on Christensen's urea medium to detect ammonia production. Finally, the Triple Sugar Iron (TSI) test was employed to characterize carbohydrate fermentation patterns (glucose, lactose, and sucrose) alongside gas and hydrogen sulfide production, providing a comprehensive phenotypic fingerprint of the uropathogenic strains.

2.8. Diagnosis by Vitek-2

The diagnosis of all of the isolates was further confirmed via the Vitek-2 compact system(Biomerioux, France), using specific GN Card and according to the manufacturer instruction.

2.9. DNA Extraction

A commercial DNA extraction kit (Geneaid, Taiwan), was used to extract DNA from all isolates of *E. coli* under study. The bacteria were cultured on MacKonkey agar and then incubated at 37 °C for 18-24 hour. The extraction steps were then followed according to the manufacturer's instructions. After completion of the extraction process, the extracted DNA were frozen at -20 °C until use. Purity was measured using a NanoDrop Spectrophotometer (BioDrop, England), [16].

2.10. Bacterial diagnosis through 16s rRNA

All *E. coli* isolates were undergone 16s rRNA detection using PCR technique to verify their identity. Pure bacterial isolates were cultured aerobically in MacKonkey agar(HiMedia, India) for 24 hours of agitation at 200 rpm and 30 °C. Centrifugation was used to harvest cells (12500× g, 10 min). DNA samples were prepared as guided by the Presto Mini gDNA Bacteria Kit from Geneaid Biotech Ltd. Amplification of 16S rRNA was employed using the bacterial genome as a template and the following primers: Forward primer 5'- AGAGTTTGATCMTGGCTCAG -3' and Reverse primer 5'- AAGGAGGTGATCCARCCGCA -3' [17]. The thermocycling conditions were one cycle of 6 minutes at 95 °C, 35 cycles of 45 seconds at 95 °C, 60 seconds at 55 °C, and 60 seconds at 72 °C, and a final extension of 5 minutes at 72 °C. Gel electrophoresis was used to examine the results using 2% agarose in 0.5× of TBE buffer. Gene band visualization was done using UV illumination.

For nucleotide sequence analysis, the resulting DNA bands were extracted and purified using the extraction kit provided by Geneaid Biotech Ltd. The resulting DNA samples, with the primers used, were sequenced and analyzed using Genetic Analyzer 3130 (Hitachi Co., Ltd., Japan). Nucleotide sequences were aligned with recorded sequences deposited in the NCBI database, and the results were analyzed using BLAST.

2.11. Antimicrobial Susceptibility Testing (AST)

Testing for antimicrobial susceptibility was carried out against multiple antimicrobial classes included: Amoxicillin/Clavulanic Acid, Piperacillin/Tazobactam, Cefazolin, Cefuroxime, Cefuroxime Axetil, Ceftazidime, Ceftriaxone, Cefepime, Imipenem, Meropenem, Gentamicin, Ciprofloxacin, Fosfomycin,Nitrofurantoin, and Trimethoprim/Sulfamethoxazole, using Vitek-2 system, according to the instruction. Interpretation of the results was done according to the CLSI, 2025 guidelines [18].

2.12. Molecular Investigation

A single *E. coli* isolate which was registered in NCBI as a new strain was molecularly investigated for the presence of different virulence genes included: *fimH, stx1, eae, and hlyA* genes, using traditional PCR technique. Amplification of studied genes was employed using the bacterial genome as a template and the primers shown in (Table 1), and according to what was describes by [17]. The thermocycling conditions were one cycle of 6 minutes at 95 °C, 35 cycles of 45 seconds at 95 °C, 60 seconds at 55 °C, and 60 seconds at 72 °C, and a final extension of 5 minutes at 72 °C. Gel electrophoresis was used to examine the results using 2% agarose in 0.5× of TBE buffer. Genes bands visualization was done using UV illumination

Table 1 Primers sequences of the studied virulence genes *fimH, stx1, eae, and hlyA*

Gene	Primer	Size
fimH	F" TGCAGAACGGATAAGCCGTGG R" GCAGTCACCTGCCCTCCGGTA	508 bp
stx1	F" AAATCGCCATTCGTTGACTACTTCT R" TGCCATTCTGGCAACTCGCGATGCA	370 bp
Eae	F" GTGGCGAATACTGGCGAGACT R" CCCCATTCTTTTTCACCGTCG	861 bp
hlyA	F" AGCGTACGTTCCGCTGGCAA R" ACCCGCTGCAGCTTTTGTTCCT	696 bp

3. Results

3.1. Bacterial Isolation

The primary isolation results showed the predominance of *E. coli* with 14 isolates, following by, Proteus 8 isolates, Pseudomonas 6 isolates, Klebsiella 5 isolates, and and finally Staphylococcus type 4 isolates.

3.2. Gram Staining and Colonial Morphology

Gram staining of the uropathogenic isolates revealed short, plump, pink-staining rods arranged singly or in pairs, consistent with the Gram-negative bacillary morphology characteristic of *E. coli*.

3.3. Cultural Characteristics

One of the first selective and differential media used in the testing of the Isolate on MacConkey Agar gave the following results as bright pink to deep rose colonies formed during the first 24 hours of incubation at a constant temperature of 37 °C. This color change was due to vigorous lactose fermentation. This also results in a drop of the local pH which activates the neutral red indicator of the agar, confirming a positive lactose fernenter phenotype for the isolates. Furthermore, in a Eosin Methylene Blue Agar parallel inoculation, a hallmark characteristic for *E. coli* was observed. This was characterized as having a distinct metallic green and black center.

3.4. IMViC Tests

The IMViC tests for *E. coli* showed a profile of (+ + - -). The Indole test showed a red cherry ring which indicates the presence of tryptophanase. The Methyl Red test was positive which indicates that fermentation had occurred and the end products were stable acids. There was no acetoin production, thus the Voges-Proskauer test was negative and kept

with the metabolic pathway of *E. coli*. The Slants of Simmons Citrate agar remained green confirming that the isolates could not utilize citrate as a source of carbon due to the absence of citrate permease in *E. coli*.

3.5. Testing for Catalase and Urease

Enzymatic profiling results supported the identification. The Catalase test was positive, exhibiting strong and immediate effervescence upon direct contact with the colony and 3% hydrogen peroxide. However, the Christensen urea medium inoculated with the isolates remained yellow throughout the incubation period, signifying a negative Urease test.

3.6. Triple Sugar Iron (TSI) Agar

The Triple Sugar Iron (TSI) test revealed an Acid/Acid (A/A) reaction, with both slant and butt turning yellow, indicating glucose, lactose, and sucrose fermentation. Gas production was observed as agar displacement and bubbles, typical of *E. coli*. No blackening indicated the absence of hydrogen sulfide (H_2S), distinguishing the isolates from H_2S-positive Enterobacteriaceae like Salmonella and Proteus. This TSI profile confirmed the identity of the uropathogenic strains as *E. coli*.

Table 2 Cultural and Biochemical characteristics of the studied *E. coli* isolates

Test	Result	Observation
Gram Stain	Negative bacilli	Pink rods
MacConkey Agar	Lactose fermenter	Pink colonies
EMB Agar	Presumptive *E. coli*	Metallic green sheen
Indole	+ Positive	Cherry-red ring (Kovac's)
Methyl Red	+ Positive	Red coloration (pH ↓)
Voges-Proskauer	− Negative	No acetoin produced
Citrate	− Negative	No growth; medium stays green
Catalase	+ Positive	Vigorous effervescence
Urease	− Negative	Medium remains yellow
TSI	A/A, Gas (+), H_2S (−)	Yellow slant & butt, gas, no blackening

3.7. Molecular Identification

Baaed on 16s rRNA detecting results, the identity of all 14 *E. coli* isolates was confirmed, while the sequencing results shown the apperance of a new isolate which was recorded in NCBI under the reference number FaMsAh LC842012.1.

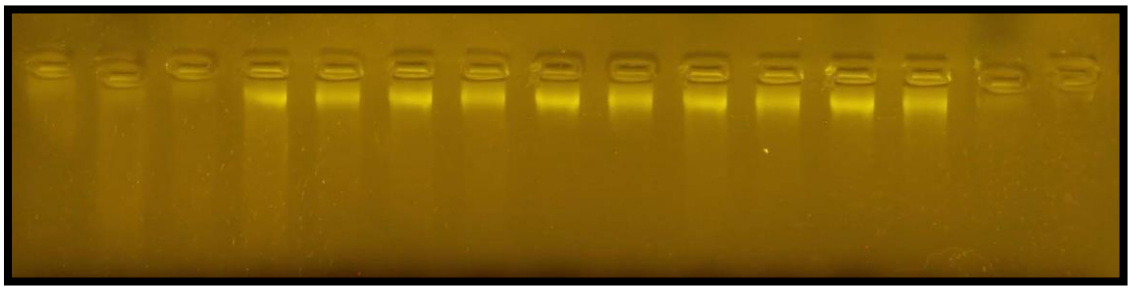
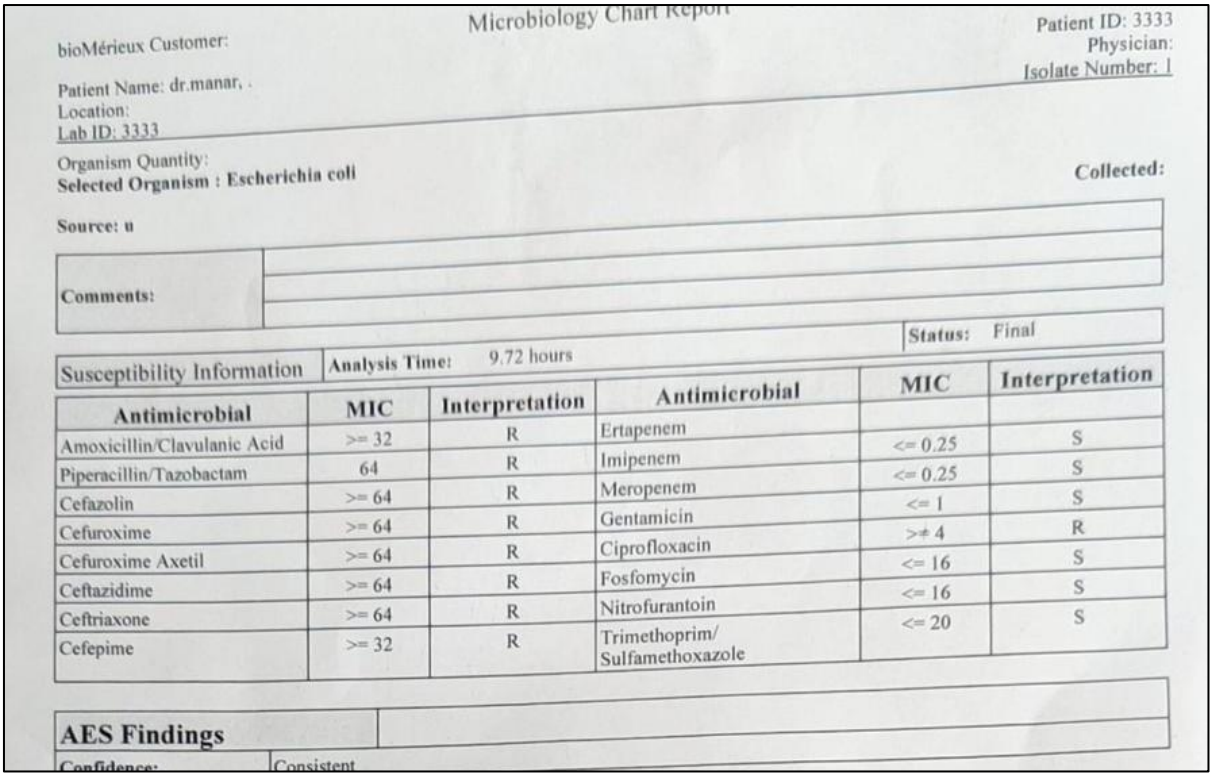


Figure 1 The genome extracted from the bacteria under study, separated by 1% agarose gel

3.8. Antibiotic Susceptibility test results

The studied isolate shown resistance to 9 types of used antibiotics out of 15 as shown in Figure 2.



Microbiology Chart Report

bioMérieux Customer:

Patient Name: dr.manar, .
Location:
Lab ID: 3333

Patient ID: 3333
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Escherichia coli

Collected:

Source: u

Comments:

Susceptibility Information | **Analysis Time:** 9.72 hours | **Status:** Final

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Amoxicillin/Clavulanic Acid	>= 32	R	Ertapenem	<= 0.25	S
Piperacillin/Tazobactam	64	R	Imipenem	<= 0.25	S
Cefazolin	>= 64	R	Meropenem	<= 1	S
Cefuroxime	>= 64	R	Gentamicin	>= 4	R
Cefuroxime Axetil	>= 64	R	Ciprofloxacin	<= 16	S
Ceftazidime	>= 64	R	Fosfomycin	<= 16	S
Ceftriaxone	>= 64	R	Nitrofurantoin	<= 20	S
Cefepime	>= 32	R	Trimethoprim/ Sulfamethoxazole		

AES Findings

Confidence: Consistent

Figure 2 The sensitivity of *E. coli* bacteria to antibiotics using the Vitec2 system

3.9. Virulence Genes Investigation

The amplification results of the studied genes through PCR technique showed that the isolate studied possessed all the studied genes, as shown in figure .

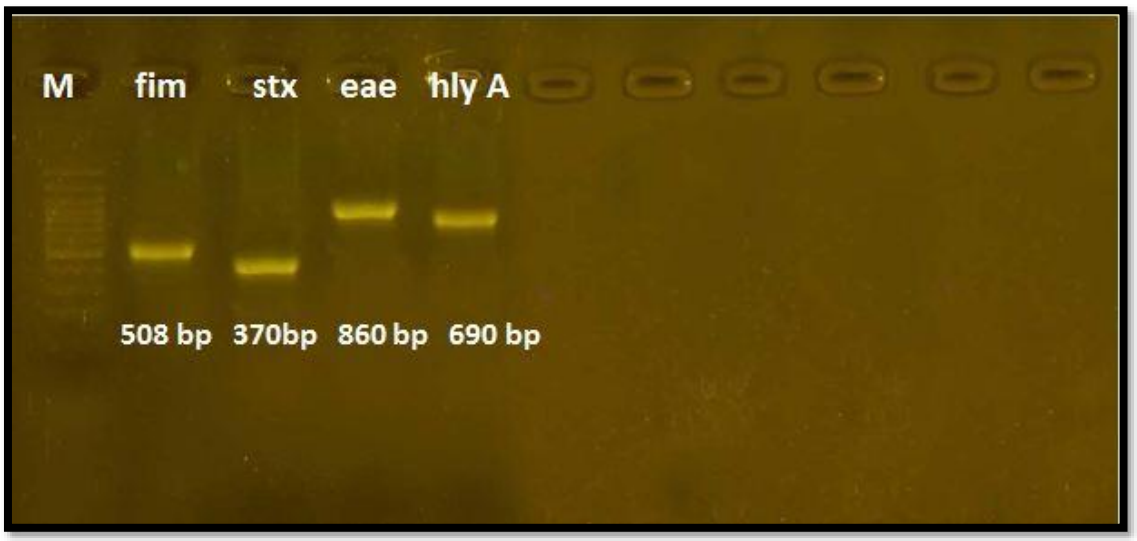


Figure 3 The PCR reaction product for the virulence genes of the bacteria under study, separated by 2% agarose gel

4. Discussion

A total of 37 clinical isolates were obtained from urine samples, distributed as follows: *E. coli* 14 37.8%, Proteus spp. 8 21.6%, Pseudomonas spp. 6 16.2%, Klebsiella spp. 5 13.5%, Staphylococcus spp. 4 10.8%. *E. coli* was the most predominant isolate (37.8%), which is consistent with its recognized role as the leading uropathogen.

This finding strongly aligns with global epidemiological data. A 2024 cross-sectional study from Pakistan [19], reported a UPEC prevalence of 61.8% among 110 urine samples from community-acquired UTI patients, with *E. coli* being by far the dominant organism among all isolated uropathogens. A 2025 retrospective study from Lebanon [20], similarly confirmed *E. coli* as the primary causative agent in female UTI patients, representing the majority of positive urine cultures across all age groups. A 2025 systematic review in Lancet Infectious Diseases [21], reinforces this, noting *E. coli* as the globally dominant uropathogen in both community and hospital settings, representing 50–80% of all UTI isolates depending on the geographic region. The presence of Klebsiella, Proteus, and Pseudomonas alongside *E. coli* in the study also matches the typical polymicrobial landscape described in recent UTI surveillance studies.

The isolate showed extensive multidrug resistance to 9 out of 15 antibiotics including amoxicillin-clavulanic acid, piperacillin-tazobactam, and multiple cephalosporins, consistent with the high MDR rates (60–87.8%) reported in Iraqi UPEC studies from Baghdad and Ramadi [22, 23], while remaining susceptible to carbapenems (imipenem, meropenem), gentamicin, fosfomycin, nitrofurantoin, and trimethoprim-sulfamethoxazole—a resistance pattern aligning with regional antibiograms showing preserved carbapenem activity against MDR UPEC in Iraq [24, 25].

Notably, PCR analysis revealed 100% prevalence of *fimH*, *stx1*, *eae*, and *hlyA* virulence genes—a combination significantly exceeding recent Iraqi studies which reported *fimH* at 85.3% in Ramadi and 95.3% in Baghdad, and *hlyA* at only 21.9% in Ramadi [22, 23]. This gene is essential for bladder epithelial adhesion and is considered a hallmark of UPEC pathogenicity. The near-universal presence in Iraqi isolates aligns with global patterns where *fimH* is detected in 86–100% of UPEC strains [26]. *hlyA* (Alpha-hemolysin): our 100% prevalence is markedly higher than the 21.9% reported in Ramadi [22], and the 15.8% in the Colombian study. This cytotoxin damages host cell membranes and promotes tissue invasion[7]. The significantly higher rate in our isolates suggests enhanced virulence potential and may indicate selection pressure favoring hemolytic strains in our patient population.

The simultaneous presence of *stx1* (Shiga toxin) and *eae* (intimin) genes is particularly remarkable, as these DEC markers were detected in 0% of 452 UPEC strains in a Brazilian study and represent an extremely rare hybrid UPEC/STEC/EPEC pathotype not previously documented in Iraq [27]. While a study on Minced Meat in Iraq included 7 *E. coli* Isolates showed that All isolates were containing hly A gene, 6 isolates were containing the virulence gene *Stx1*, 5 isolates containing were *eae* A gene and 4 isolates were containing Stx2 gene[28]. The *stx1* and *eae* genes are primarily associated with Shiga toxin-producing *E. coli* (STEC) and enteropathogenic *E. coli* (EPEC), not typically Uropathogenic *E. coli* (UPEC). *stx1* encodes Shiga toxin 1, causing cell death, while *eae* encodes intimin for adhesion, potentially contributing to severe, albeit rare, STEC-related urinary tract infections or hybrid strain pathogenesis [29].

5. Conclusion and Recommendations

This study identified *E. coli* as the predominant uropathogen (37.8%) in Mosul, with a novel strain (FaMsAh LC842012.1) exhibiting unprecedented 100% carriage of fimH, stx1, eae, and hlyA virulence genes—a rare hybrid UPEC/STEC/EPEC pathotype. The isolates displayed extensive multidrug resistance (9/15 antibiotics) while remaining susceptible to carbapenems, gentamicin, fosfomycin, nitrofurantoin, and trimethoprim-sulfamethoxazole. Recommendations: (1) Implement continuous molecular surveillance using whole-genome sequencing to monitor hybrid strain dissemination; (2) Reserve carbapenems for severe infections given preserved susceptibility; (3) Consider nitrofurantoin or fosfomycin as first-line options for uncomplicated UTIs; (4) Establish screening protocols for Shiga toxin production given stx1 carriage and potential hemolytic-uremic syndrome risk; (5) Enhance infection control measures to prevent nosocomial transmission of this high-virulence hybrid str

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