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Original Article

Study of Some Virulence Factors and Resistance Patterns in Extended Spectrum β-Lactamases-Producing Uropathogenic *Escherichia coli*

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Abstract

Background: The *Enterobacteriaceae* family is mostly responsible for infections of urinary tract, which are among the greatest prevalent conditions developed by bacteria in people, including *Escherichia coli*. Extended-spectrum *β*-lactamases (ESBLs)-producing *Escherichia. Coli* are a leading source of community and hospital-acquired infections globally. These bacteria are resistant to both non-*β*-lactam and *β*-lactam antibiotics. The capacity of uropathogenic *Escherichia coli* strains to result in urinary tract infection is associated with the formation of several virulence factors. **Aim**: This study was performed to isolate and determine ESBLs-producing uropathogenic*Escherichia coli* then find out the patterns and changes in the antibiotic resistance profile. **Methods**: The time range for this cross-sectional study was October 2024-January 2025, including 155 midurine samples collected from outpatients at Al-Najaf hospitals. Microscopy and macroscopic tests were performed for identifying *E. coli* isolates. Phenotypic recognition of ESBLs using the double disc synergy test (DDST), then molecular detection by multiplex polymerase chain reaction of virulence factors. **Results**: The distribution of 155 isolates showed 78 (50.3%) were UPEC strains, and the other uropathogenic bacteria were 77 (49.7%). The phenotypic method showed that 39 (50%) reported using the DDST were ESBLs-producers. Results of antibiotic susceptibility in this study among 39 ESBLs-producing UPEC isolates revealed that Meropenem had the highest susceptibility, Fosfomycin, Chloramphenicol, and Nitrofurantoin at 36 (92.3%), 38 (97.4%), 37 (94.8%), and 36 (92.3%), respectively. The results revealed that only one gene out of the five types of virulence factors, the*pap* gene, was observed in 12 (60%) of the isolates. The remaining factors were absent *fimH, sfa, pic, aer* (0.0%). **Conclusion**: Most of the ESBLs-producing UPEC isolates have the *pap* gene, while other virulence factor genes *(sfa, fimH, aer,* and *pic*) were not encoded in the 20 UPEC isolates.

Keywords: *E. Coli; Esbls; Pap Gene; UPEC; UTI*

Introduction

The most prevalent bacterial infections in humans, urinary tract infections (UTIs), they're frequently triggered by members of the *Enterobacteriaceae* family. The most common cause of UTIs among them is *Escherichia coli* (*E. coli*) (Zagaglia*et al*., 2022). UTIs may be mild, such as urethritis and cystitis, or can be severe, such as septic shock, bacteremia, and pyelonephritis in terms of their clinical manifestations, origin, and disease progression (Yang *et al*., 2022). The capacity of uropathogenic *E. coli* (UPEC) strains to induce UTIs is linked with the formation of several virulence factors. This involves the processes of biofilm formation, adhesive proteins, toxins, and iron acquisition mechanisms. Certain virulence factors are located in chromosomal regions called pathogenicity islands (PAIs). Besides, antibiotic resistance (Firoozeh *et al*., 2022). In areas where inadequate hygienic practices and poor sanitation are prevalent, ESBLs-producing *E. coli* has been identified as a major multidrug resistance (MDR) bacterium linked to serious community- and hospital-acquired infections globally(Mahmud *et al*., 2020). Furthermore, genes conferring resistance to other

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antibiotic families, involving aminoglycosides, sulfonamides, and fluoroquinolones, are often found in plasmids generating ESBLs. The range of antibiotics that are efficient against *Enterobacteriaceae* that produce ESBLs, involving Fluoroquinolones, Aminoglycosides, Tetracyclines, and Trimethoprim/sulfamethoxazole, is limited because these microorganisms frequently show resistance to antimicrobial agents other than those that are broken down by ESBLs (Shoaib *et al*., 2025). Quickly, effective treatment of infections brought on by organisms that produce ESBLs is often ineffective and linked to a high death rate. Hence, in addition to identifying their virulence factors, this study was performed to isolate and determine ESBLs-producing UPEC then find out the patterns and changes in the antibiotic resistance profile.

Materials and Methods

The time range for this cross-sectional study was October 2024–January 2025, including 155 midurine samples collected at random from outpatients at Al-Najaf hospitals: Al-Sadr-Medical City, Al-Furat Hospital, Al-Sajjad Hospital, and Al-Hakim General Hospital. Some patients do not have symptoms, while others have symptoms such as fever, lower abdominal pain, especially in females, burning during urination, and itching. The specimens were quickly brought to the microbiological lab and examined. Microscopic and macroscopic examination utilizing usual microbiological protocols (Trinh & Lee, 2022). The inoculation was done on nutrient agar, macConkey, eosin methylene blue (Himedia, India), and chrome agar (ChromAgar™ ECC, France) using the standard loop technique, and the incubation was at 37 ̊C for 24 hrs. In addition to the API 20E profile, biochemical assays such as oxidase and catalase were performed.

Phenotypic Detection of ESBL

Double Disc Synergy Test (DDST)

Bacteria from the inoculum (0.5 McFarland-turbidity) were applied over Mueller-Hinton agar. A disc of amoxicillin/clavulanate (30 µg) was placed 30mm from Aztreonam (AT), Cefotaxime (CTX), Ceftriaxone (CRX), and Ceftazidime (CAZ) discs. Each disk had a content of 30µg in the test. A "phantom zone" indicates positive ESBL production results (Das *et al*., 2023).

Antimicrobial Susceptibility Testing (AST)

All the UPEC isolates were evaluated for antimicrobial susceptibility using the Kirby-Bauer disc diffusion test. Antibiotics used involved Nitrofurantoin (F-300µg), Piperacillin (PRL-100U), Ciprofloxacin (CIP-5µg), Chloramphenicol (C-30µg), Amoxicillin/clavulanic acid (AMC-30µg), Aztreonam (AT-30µg), Azithromycin (AZM-15µg), Fosfomycin (FO-200µg), Meropenem (MEM-10µg), Minocycline (MI-30µg), Gentamicin (GEN-10µg), Trimethoprim-Sulfamethoxazole (SXT-25µg), and Ceftazidime (CAZ-30µg) (Bioanalyse, Turkey). The quality control strain for AST was *E. coli* ATCC 25922 (University of Kufa).

Multiplex PCR for Detection Virulence Factors-encoding Genes

Escherichia coli isolates that showed ESBL enzyme production by the phenotypic method were examined for virulence factor presence. Using multiplex PCR (M-PCR), the presence of genes encoding aerobactin (*aer* gene), S family adhesions (*sfa* gene), pyelonephritis-associated pili (*pap* genes), type 1 fimbriae (*fimH* gene), and pyelonephritis isolates compared (*pic* gene). Was detected in 20 isolates of ESBL-producing UPEC and the primers listed in (Table 1). The DNA was extracted using the Wizard Miniprep DNA Kit (Promega, USA). M-PCR amplification techniques were performed in an overall volume of 50µL of a combination that contained PCR buffer 10×, 250µM of each of the deoxynucleoside triphosphates, 1.5mM of $MgCl_2$, 0.5µM of each of the specific primers, 1.5U of *Taq* polymerase (Sigma), and 5µL of template DNA, and 0.5µM of each of the specific primers of virulence genes. 32 cycle of a denaturation-phase for 30s at 94°C, annealing of the primers for 30s at 54°C, and the extension for 60s at 72°C comprised the amplification conditions. The extension phase was adjusted up for three more seconds every cycle, followed by a final extension phase of five minutes at 72°C. The PCR outcomes were assessed using 1.5% agarose gel electrophoresis. The gel was then

stained with ethidium bromide and captured on camera. The thermocycler (Biometra, Germany) used to cycle the M-PCR combination was programmed using the technique outlined by Bahalo *et al*., 2013.

***Table 1**: Primers Used In M-PCR to Identify Virulence Factor-Encoding Genes*

Target Gene	Primer-Sequence		Amplicon Size (bp)	Virulence factors	Ref.
Pap	R	5′ATATCCTTTCTGCAGGGATGCAA3′	328	Pyelonephritis associated pili	(Bahalo *et al*., 2013)
	F	5′GACGGTGTACTGCAGGGTGTC3′			
sfa	R	5′CGGAGGAGTAATTACAAACCTGC3′	410	S fimbriae	
	F	5′CTCCGGAGAACTGGGTGCATCT3′			
fimH	R	5′TTGCGTACCAGCATTAGCAATGTCC3′	465	Type1 fimbriae	
	F	5′AACAGCGATGATTTCCAGTTTGTGTG3′			
aer	R	5′ACCCGTCTGCAAATCATGGAT3′	269	Aerobactin	
	F	5′AAACCTGGCTTACGCAACTGT3′			
pic	R	5′GTGTACCGCTCAGGGTGATT3′	352	pyelonephritis isolates-compared	
	F	5′GGAAGTGACAGGGCATTTGT3′			

M-PCR-Multiplex-Polymerase Chain Reaction; F - Forward; R-Reverse; bp- Base Pair; A- Adenosine; T-Thymidine; C- Cytidine; G-Guanosine

Data Analysis

Through the URL https://www.statskingdom.com/310GoodnessChi.html, the findings were examined using chi-square, *P*-value, and percentages for analysis. A P-value of less than 0.05 was used to define statistical significance for each test.

Ethical Approval

The Iraqi Ministry of Health accepted this study under Ethics Approval No. 33394.

Results

Bacterial Distribution

The distribution of 155 isolates showed 78/155 (50.3%) were UPEC strains and the other uropathogenic bacteria were 77/155 (49.7%) (Figure 1).

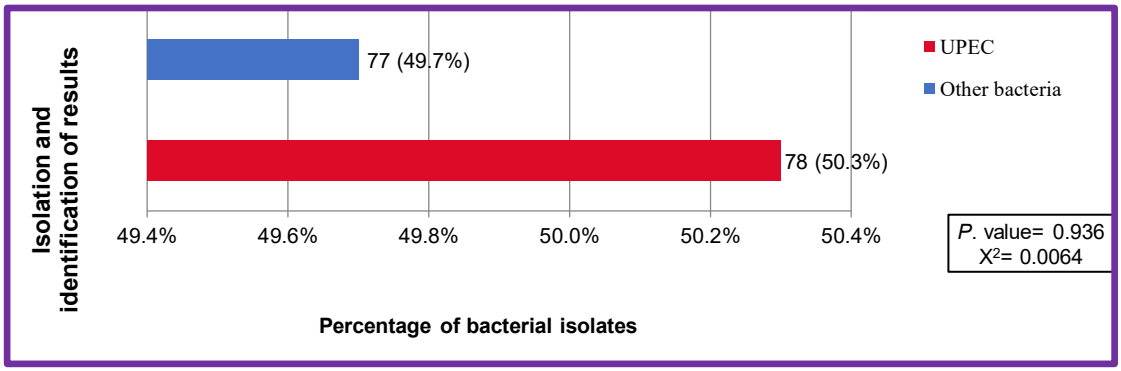

***Figure 1:** Percentages of Bacterial Distribution from Collected Isolates*

Prevalence of ESBLs-Producing Escherichia coli

39 out of 78 (50%) reported utilizing the DDST, Figures (2,3)

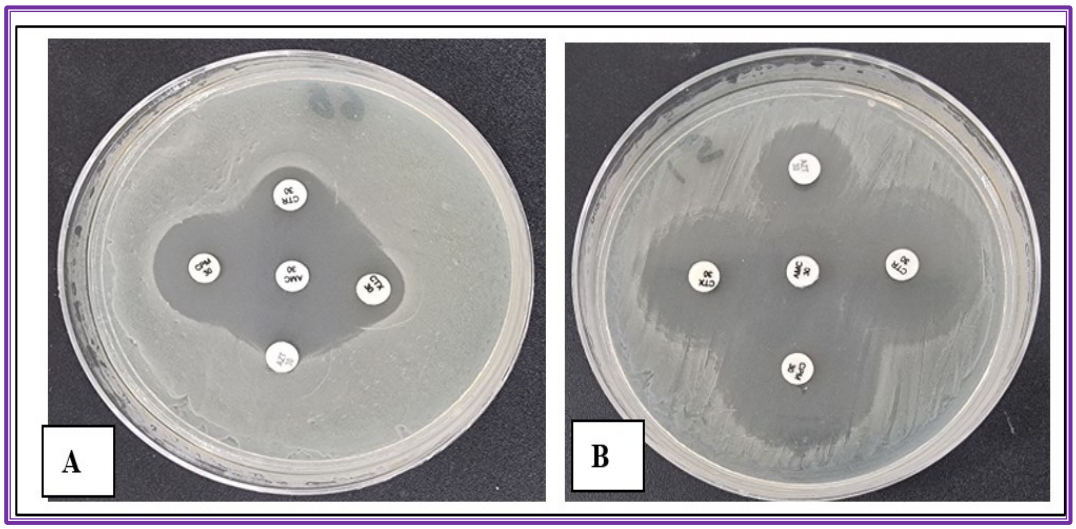

***Figure 2**: Phenotypic Method (DDST) for Detecting Esbls Enzymes*

Figure 2(A) shows positive results for ESBLs-producing. (B) Shows negative results for ESBLs-producing.

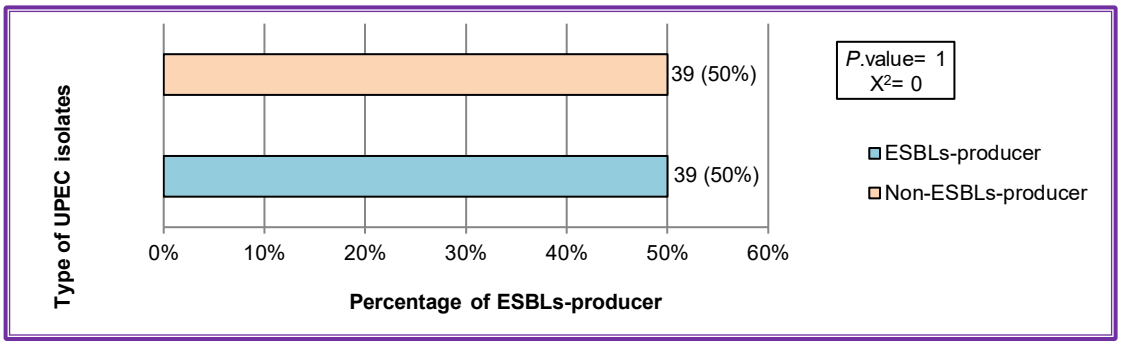

***Figure 3:** Prevalence of ESBLs-producers among 78 UPEC Isolates*

*Antibiotic Susceptibility Profile of ESBLs-Producing Uropathogenic Escherichia co**li***

This study shows that of *β*-lactam antimicrobial agents like Ceftazidime had resistance at 36/39 (92.3), while Meropenem had of susceptibility at the same percentage 36/39 (92.3). Meanwhile, amongst non-*β*-lactam antimicrobial agents, the resistance was noticed in Trimethoprim-Sulfamethoxazole and Azithromycin at 20 (51.2%), whereas Fosfomycin showed the highest level of susceptibility, Chloramphenicol, and Nitrofurantoin (38 (97.4%), 37 (94.8%), and 36 (92.3%), respectively) (Table 2).

***Table 2:** Antibiotic Susceptibility Testing of 39 Esbls-Producing UPEC Isolates Using Disc Diffusion Method*

Main Class/ Subclass Of Antibiotics		**Antibiotic Agent**	**N= 39 of *E. coli* Isolates no. (%)**			**X^2**	***P.* Value**
			R	**I**	**S**		
β-lactams	Monobactams	Aztreonam	18 (46.1)	0 (0.0)	21 (53.8)	19.8	< 0.0001
	β-lactams/*β*-lactamase inhibitor combinations	Amoxicillin-clavulanate	18 (46.1)	7 (17.9)	14 (35.8)	4.7	0.092 NS
	Pencillin	Piperacillin	28 (71.7)	2 (5.1)	9 (23)	27.8	< 0.0001
	Cephempemes	Ceftazidime	36 (92.3)	2 (5.1)	1 (2.5)	61	< 0.0001
	Carbapenems	Meropenem	1 (2.5)	2 (5.1)	36 (92.3)	61	< 0.0001
β-lac	Aminoglycosides	Gentamicin	9 (23)	0 (0.0)	30 (76.9)	36.4	< 0.0001

	Fosfomycin	Fosfomycin	0 (0.0)	1 (2.5)	38 (97.4)	72.1	< 0.0001
	Macrolides	Azithromycin	20 (51.2)	0 (0.0)	19 (48.7)	19.5	< 0.0001
	Tetracycline	Minocycline	17 (43.5)	8 (20.5)	14 (35.8)	3.23	0.19 NS
	Nitrofurans	Nitrofurantoin	1 (2.5)	2 (5.1)	36 (92.3)	61	< 0.0001
	Phenicols	Chloramphenicol	1 (2.5)	1 (2.5)	37 (94.8)	66.4	< 0.0001
	Folate pathway antagonists	Trimethoprim-Sulfamethoxazole	20 (51.2)	13 (33.3)	6 (15.3)	7.5	0.023 NS
	Fluoroquinolones	Ciprofloxacin	19 (48.7)	2 (5.1)	18 (46.1)	14	0.0009

S- Sensitive; R- Resistance; I- Intermediate; X^2- Chi square test statistic; P-value < 0.0001- Extremely significant difference; NS- No significant difference

Virulence Factor-encoding Genes Detection in ESBLs-Producing Uropathogenic Escherichia coli

In order to determine the frequent virulence factors in these isolates, 20 isolates that produced ESBLs were examined in this study. Five virulence factor genes, *aer*, *sfa*, *pap*, *fimH*, and *pic* were chosen. The findings showed that 12/20 (60%) of the ESBLs-UPEC isolates had the *pap* gene a single gene of the five types, while *sfa*, *aer, fimH*, and *pic* (0.0%) (Figure 4) (Table 3).

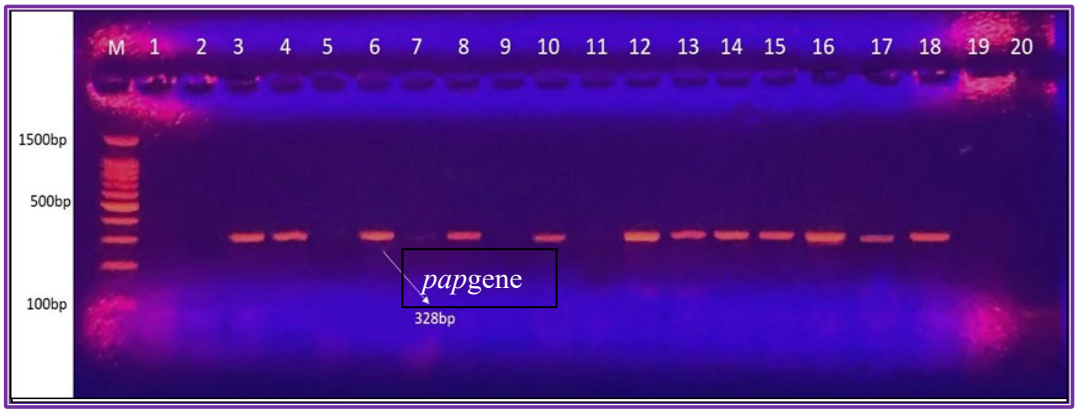

Figure 4: *The multiplex PCR product of virulence factor-encoding genes among 20 UPEC isolate. Lane M: 100bp ladder; Lane 1-20: PCR products; Lane 1, 2, 5, 7, 9, 11, 19, 20 shows a negative result; Lane 3, 4, 6, 8, 10, 12-18 shows positive results (bands of pap gene 328 bp).*

Table 3: *Distribution of Genes Encoding Virulence Factors among 20 UPEC Isolates*

Virulence Factors Type	N (%) of Isolate Gave Negative Results	N (%) of Isolate Gave Positive Results	*P.* Value	X^2
sfa	20 (100)	0 (0.0)	<0.0001***	20
aer	20 (100)	0 (0.0)	<0.0001***	20
pap	8 (40)	12 (60)	0.37 NS	0.8
fimH	20 (100)	0 (0.0)	<0.0001***	20
pic	20 (100)	0 (0.0)	<0.0001***	20

*sfa- S fimbriae; aer- aerobactin; pap- polynephritis-associated pilli; fimH- type1 fimbriae; pic- polynephritis isolates compared; P. Value - probability value; ***- Extremely significant difference; NS- No significant difference, X^2 -Chi square test.*

Discussion

Among urological and renal disorders, UTIs are the most common. UTIs are known to be the most common urological and renal problem. It is often associated with morbidity in both outpatient and inpatient settings (Mancuso *et al*., 2023). *E. coli* was identified from urine samples the most often in the present study, with 78 (50.3%) isolates. According to the same studies, the most frequent bacteria identified in UTIs were UPEC strains (Altayb*et al*., 2021; Ahmed &Hawezy, 2023). In Iraq, a further study by Al-Gasha'a*et al*. (2020) revealed that 33.3% of the isolates were UPEC. Furthermore, UPEC isolates were the most common pathogen in UTI patients, with corresponding percentages of 47% and 51.5%, according to two other studies conducted in Al-Najaf, Iraq (Mhana & Aljanaby, 2023; Al-

Janabi & Aljanaby, 2024). The distal gut microbiota is the source of the UPEC strain, which accounts for about 90% of UTI globally (Rahman *et al*., 2022). Urinary tract disorders are caused by the UPEC strain's ability to infiltrate uroepithelial cells and create intracellular bacterial populations via a variety of factors associated with virulence, including toxins, surface polysaccharides, adhesive proteins, flagella, and iron-acquisition systems (Quan *et al*., 2021).

The present study's UPEC isolates were all examined using a phenotypic approach, which revealed that some isolates were able to resist the ESBLs. 39 out of 78 (50%) reported utilizing the DDST, which was considered very sensitive, Figures (2,3).

Amro *et al*. (2022) revealed that the DDST readability and ease make it the most popular method, it is a trustworthy method for detecting ESBLs enzymes. The DDST's sensitivity ranges from 77-89 % across many studies (Meena *et al*., 2021; Muthukrishnan *et al*., 2025). Another study stated that DDST was had an 84.3% sensitivity rate and 71.9% specificity rate (Al-Tamimi *et al*., 2022).

According to the findings of the present study, 50% of the isolates are capable of producing these enzymes. According to the findings, which were roughly in line with the results stated by Hasan & Ibrahim (2022); Ehsan *et al*. (2023), who observed that 64% and 48% of isolates had ESBLs, respectively. According to Barzegar *et al*. (2022), 46.6% of the strains obtained were UPEC isolates that produced ESBLs (Barzegar *et al*., 2022). Numerous variables, including infection severity, hospital/ward, species, patient demographic, and geographic location, influence the proportion of ESBLs-positive isolates, which varies significantly from study to study (Alsamawi *et al*., 2022). According to the findings in Iraqi governorates, in Babylon Province was 72% (Hussein & Alwash, 2022). While in Baghdad city, 72.7% (Hussein, 2025). In Al-Najaf, it was 23.7% Alkhudhairy and Alshammari (2019). In Karbala, 23.5% (Hanoon *et al*., 2022). In the Sulaimani province, it was 59.3% (Abubaker & Anoar, 2022).According to Gharavi *et al*. (2021), it was 35.7% in Iran. The prevalence of ESBLs was shown to be 38% in other countries, such as Sri Lanka (Perera *et al*., 2022), and 25% in Nigeria (Iseghohi *et al*., 2020).

Patients using high doses of third-generation cephalosporins may have the highest percentage of ESBLs among the UPEC isolates found in this study, patients who took medication without a prescription or physician's supervision, and widespread antibiotic abuse by both patients and physicians. Al-khikani *et al*. (2020) reported that the same factors, together with a lack of personal knowledge, were shown to be responsible for an insufficient antibiotic course for eliminating the pathogen in order to increase infection cure rates and avoid resistance or treatment failures. The spread of bacterial infections that result in ESBLs is likely induced by many factors, such as population density, geographic location, sanitation, and antibiotic usage, according to Castanheira *et al*. (2021).

This study confirmed that of *β*-lactam antimicrobial agents, Ceftazidime had the greatest degree of resistance at 36/39 (92.3), while Meropenem had the greatest degree of susceptibility at the same percentage 36/39 (92.3). Meanwhile, amongst non-*β*-lactam antimicrobial agents, the highest resistance was noticed in Trimethoprim-Sulfamethoxazole and Azithromycin at 20 (51.2%), whereas Fosfomycin showed the highest level of susceptibility, Chloramphenicol, and Nitrofurantoin 38 (97.4%), 37 (94.8%), and 36 (92.3%), respectively (Table 2).

Different strains' genomes and variations in antibiotic use cause different forms of antibiotic resistance worldwide, and variations in the accessibility of novel broad-spectrum antibiotics (Muteeb*et al*., 2023). Genes that encode ESBLs are often located on plasmids or transposons with other resistance genes. They may thus spread quickly and cause resistance against a number of antimicrobial agents, such as Fluoroquinolone, Aminoglycosides, Sulphonamides-trimethoprim, and Chloramphenicol. According to the findings of this study, particularly strongly efficient antibiotics with high susceptibility were Fosfomycin 97.4%, Chloramphenicol (97.4%, 94.8%, respectively), and Nitrofurantoin and Meropenem (92.3%) this results also showed in study by Başkan *et al*., (2026). These medications are excellent first-line treatments because of their low resistance percentages, particularly for severe infections caused by UPEC isolates that produce ESBLs. Fosfomycin works well for mild, acute

cystitis. Overall, combined treatment is more effective than monotherapy (Husna *et al*., 2023), and since it isn't suggested to treat UTIs, it could potentially be used to treat UTIs again because of its effectiveness in overcoming the infection, even if it is brought on by MDR bacteria that develop ESBLs (Hosoi *et al*., 2024).

Although Chloramphenicol is an old antibiotic, its low frequency or lack of resistance is primarily caused by its side effects, including aplastic anemia, and its use is restricted, particularly in pregnant women and children. A relatively low level of resistance is also a result of its use in animals and plants (Alkhudhairy& Alshammari, 2019).

Meropenem is an effective antibiotic that works to kill both Gram-positive and Gram-negative bacteria (GNB). It works well as a final option to treat bacterial infections. Due to the fact that it is administered intravenously, it has been categorized as a costly antibiotic.

Nitrofurantoin is now an excellent first-line antibacterial medication since UPEC strains have very little resistance to it, this result is consistent with Alwashaish *et al*. (2026). It could work better than Fosfomycin to treat UTIs in pregnant women (Ari *et al*., 2023). Subsequently, the sensitivity of the moderately effective antibiotics in this study was found to be 48.7% for Ciprofloxacin, 46.1% for Aztreonam, and 23% for Gentamicin. In certain cases, these antibiotics could be beneficial. An inadequate option for treatment for these isolates is suggested by the high susceptibility, whereas Ceftazidime was useless at 92.3%, Azithromycin was 51.2%, Amoxicillin-clavulanate and Piperacillin were 71.7%, Trimethoprim-sulfamethoxazole was 51.2%, and Minocycline was 43.5%.

A study in Al-Najaf demonstrated that the most effective antibiotics were Fosfomycin and Meropenem (0% resistant), Trimethoprim (11.1%), Nitrofurantoin (5.6%), and. Certain antibiotics, such as Ceftazidime 88.9% and Aztreonam (100% resistance), were ineffective (Alkhudhairy& Alshammari, 2019). A study conducted in Baghdad found that Amikacin and Meropenem were the two most efficient antibiotics, while Ticarcillin and Co-Trimoxazole were among the least effective, while Chloramphenicol and Nitrofurantoin were only moderately effective (Mohammed *et al*., 2022). In Karbala, Iraq, resistance to the most effective antibiotics: Chloramphenicol at 5%, Amikacin at 7%, Nitrofurantoin at 8% (Al Her *et al*., 2025). In Saudi Arabia revealed that Amikacin 93.3–100%, Meropenem 95–99%, and Nitrofurantoin 81–91% were the antibiotics that exhibited the greatest sensitivity. Comparatively, the Cephalosporin, Ampicillin, and Aztreonam groups showed total resistance to ESBLs-producing UPEC isolates, whereas the Ciprofloxacin and Cotrimoxazole groups exhibited resistance percentages ranging from 56%-74% and 45%-53%, respectively (Alghamdi *et al*., 2023). In Iran, Meropenem 98.2% and Amikacin 90.7% were the most effective antibiotics, and Ampicillin 11% showed the lowest percentage of susceptibility (Esfahani *et al*., 2023).

Uropathogenic *Escherichia coli* bacteria use several kinds of strategies to colonize host cells and create communities, which trigger UTIs. Virulence factors are one of these strategies; they might stand for the motility of bacteria, adhesion to host cell surface receptors, or a dysfunction of the host's defensive system. In order to determine the frequent virulence factors in these isolates. The findings showed that 12/20 (60%) of the ESBLs-UPEC isolates had the *pap* gene, which encodes P fimbriae, while *sfa*, *aer, fimH*, and *pic* (0.0%) were the other genes that were not present (Figure 4) (Table 3).

Out of the five categories of virulence factors, the *pap* gene was the only one that appeared in 60% of the isolates, according to the study's findings. UPEC strains acquire some of their virulence factors from other bacteria, and depending on their importance, they may lose some of them (Whelan *et al*., 2023).

The observed frequency may result from the negative relationship between type 1 fimbriae expression and P fimbriae expression, indicating a direct association between pathogenesis-related genes (Isidro-Coxca *et al*., 2024), all of these factors are critical for these bacteria and have a key role in infection, persistence, and recurrence. These findings are also similar to previous studies (Helmy *et al*., 2023 ; Abbas *et al*., 2025), which found that a frequently prevalent gene, the *pap* gene, was highly

related to complicated UTIs; meanwhile, another study reported a low *pap* frequency of 8.2% (Ghavidel *et al*., 2020).

Despite the potential importance of the *fimH* gene in UTIs, this investigation discovered that none of the isolates had it. This result might be explained by a lack of understanding of the timing of the production of these genes during UTI. Additional studies indicated similar findings (Zhou *et al*., 2023), whereas one other study observed a high percentage of *fimH* at 63% (Osman *et al*., 2024) and (El Halfawy *et al*., 2026). This difference may be due to the different in number of samples(Ghafer *et al*., 2022).

This study revealed that the isolates did not have S fimbriae. This is due to the fact that S fimbriae's expression, like that of other fimbriae species, is significantly impacted by temperature, osmolality, and environmental factors (Whelan *et al*., 2023). A study additionally indicates that S fimbriate is more closely connected with sepsis and meningitis as it may be the source of the transfer of UPEC infections from UT into the bloodstream, resulting in bacteremia (Whelan *et al*., 2023). This study's findings are consistent with other studies (Ghavidel *et al*., 2020; Fonseca-Martínez *et al*., 2023).

This study demonstrated that none of the isolates had the *aer*-gene. This might be because this factor is one of numerous mechanisms that bacteria utilize to get iron. There are other gene types, including *iroN*, *fyuA*, and *iutA* (Fonseca-Martínez *et al*., 2023). It is possible that bacteria obtain iron by mechanisms other than aerobactin. Alternatively, this finding might be attributed to the fact that only a few kinds of PAIs include the *aer* gene (Mohammadzadeh *et al*., 2019). On the other hand, this gene may not have a role in the progression of UTIs. Related studies indicate that the *aer* gene is low in UPEC isolates (Tayh *et al*., 2021; Ahmed, 2021). While Dadi *et al*. (2020) discovered that the high percentage of the *aer* gene in their findings might be related to UPEC isolates adopting aerobactin as the primary route of iron uptake (Dadi *et al*., 2020). The *pic* gene encodes an autotransporter protein that is present in many GNB, including *E. coli*. It has a crucial role in colonization during infection (Göksel & AkçeliK, 2021). There have been relatively few studies on this gene in UPEC strains. According to the findings of this study, the *pic* gene is not detected in UPEC isolates. Similar study indicate a low percentage of this gene in UPEC isolates (Emami., 2022).

Limitations

One of the primary limitations of this study was the small sample size. Also, genetic testing was limited to a few genes due to time constraints and the high cost of materials and the primers used in PCR technology and DNA extraction.

Future Scope

Future research should expand in geographical scope and sample diversity, as well as diversify testing for antibiotic susceptibility to other antibiotics and determine their resistance levels and whether it can be used to get rid of UTIs.

Conclusion

The majority of the isolates comprise UPEC bacteria. Fosfomycin, Chloramphenicol, Nitrofurantoin, and Meropenem have been determined to be the highest effective antibiotics among the UPEC testedisolates. Most of the ESBLs-producing UPEC isolates have the *pap* gene, while other virulence factor genes (*sfa*, *fimH*, *aer*, and *pic*) were not encoded in the 20 UPEC isolates. This study helps in understanding the effectiveness of certain antibiotics, as well as identifying the prevalence of virulence factors in UPEC bacteria.

Conflict of Interest

Authors of this study reveal not any conflicts of interest.

Acknowledgement

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