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Serine Protease Autotransporters Genes Distribution and their Association with Multiple Antibiotic Resistance of Uropathogenic *Escherichia coli*

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Abstract

Background. One of the characteristic features of Uropathogenic *Escherichia coli* (UPEC) is their secretion of serine protease autotransporters of Enterobacteriaceae (SPATEs). **Aim.** To investigate the prevalence of serine protease autotransporter genes in *E. coli* and their correlation with antibiotic susceptibility patterns. **Methods.** A total of 500 urine specimens were collected from patients with UTIs to isolate UPEC. An antibiotic sensitivity test was performed using the Kirby-Bauer technique and molecular detection of virulence genes was performed using polymerase chain reaction (PCR). **Results.** 100 *E. coli* isolates were obtained. The results of the antibiotic sensitivity assessment showed that piperacillin (80%); and ampicillin (92%) have the highest resistance rate while meropenem (1%); amikacin (2%); nitrofurantoin (2%); and fosfomycin (5%) have the lowest resistance rate. The prevalence of serine protease autotransporters genes was *vat* 58.3%, *sat* 36.6%, *pic* 30% , *tagB* and *tagC* 18.3%. **Conclusion.** At least one serine protease autotransporter gene was identified in 68.3% of *E. coli* isolates. There was also a significant correlation between resistance to some antibiotics and the presence of virulence genes in the UPEC isolates.

Keywords: Serine protease autotransporters of Enterobacteriaceae (SPATEs), Uropathogenic *Escherichia coli* (UPEC), Urinary tract infections (UTIs).

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Introduction

Urinary tract infections (UTI) are one of the most prevalent diseases worldwide, incurring significant social and medical costs related to hospitalization (1,2). Uropathogenic Escherichia coli (UPEC) is the predominant uro-pathogen associated with UTI development. UPEC are strains of Escherichia coli that deviate from their commensal role as intestinal flora, proliferate and remain in the urinary tract, and display a variety of virulence factors and mechanisms that enable them to cause infections and diseases in the urinary tract system. These E. coli strains are frequently associated with UTIs and are therefore referred to as UPEC. (3)

Autotransporters are secreted proteins that serve multiple functions and are produced by various Gram-negative bacteria. Serine protease autotransporters of Enterobacteriaceae (SPATEs) are essential for the survival and virulence of pathogens and play a significant role in both intestinal and extraintestinal infections. The type Va secretion system facilitates the transport of these proteases to the external environment, where they operate as proteases that exhibit a range of substrate specificities and biological roles such as adherence and cytotoxicity (4).

Over the past few decades, there has been a growing incidence of antimicrobial resistance in bacteria responsible for UTI, which has emerged as a significant public health issue (5). E. coli generally acquire antimicrobial resistance genes via mobile genetic elements. A significant number of mobile elements that encode resistance, especially plasmids, are exchanged among various members of Enterobacteriaceae, thereby facilitating the dissemination of resistance genes. Mobile genetic elements can encode virulence factors, and there may be an interaction between virulence and antimicrobial resistance. (6,7) .

This study aimed to investigate the prevalence of serine protease autotransporter genes in E. coli, and their correlation with the pattern of antibiotic susceptibility.

Materials and methods

Bacterial sample collection

This study involved the collection of 500 clinical midstream urine specimens in clean, fully labeled screw-capped containers from patients of various ages with UTIs, conducted from October 2023 to December 2023. Bacterial isolates were initially diagnosed using traditional techniques, followed by confirmation via molecular methods using polymerase chain reaction (PCR).

Identification of bacterial isolates

A loopful of each urine specimen following the delivery of specimens to the laboratory was cultured on MacConkey agar using the streaking method; after incubation at 37°C for 24 h, a single colony from the suspected UPEC was sub-cultured on selective and differential culture media, including Eosin Methylene Blue (EMB) agar and chromogenic agar. The presence of *E. coli* was confirmed by PCR using primer pairs targeting *uidA* gene.

Antibiotic susceptibility test

The Kirby-Bauer method was employed to determine the antibiotic sensitivity of *E. coli* on Mueller-Hinton agar. Eighteen antibiotic discs were used to assess the susceptibility of the isolated UPEC, as most of these antibiotics are frequently prescribed by physicians for UTI patients. Approximately three growing colonies on Brain Heart Infusion agar, aged 18 to 24 h, were transferred to a tube containing 5 mL of normal saline and thoroughly mixed. Subsequently, the bacterial suspension was evenly distributed across the surface of dishes containing Mueller-Hinton agar using a sterile cotton swab. Antibiotic disks were positioned on the surface of the cultured plate using sterilized forceps, ensuring equal spacing, and the discs were gently pressed down. After an 18-hour incubation period at 35°C, the inhibition zones of each antibiotic disk were measured with a metric ruler, and the results were documented as resistant, sensitive, or intermediate based on the Clinical and Laboratory Standards Institute (CLSI) breakpoints, 2024.

Genomic DNA extraction and determination of concentration

Sixty of the isolated UPEC strains were chosen for molecular identification based on the resistance rates of *E. coli* to the 18 antibiotics used in the study. Genomic DNA was extracted using a commercial extraction kit (Favorgen Genomic DNA Extraction Kit) provided by Favorgen Biotech (Ping Tung, Taiwan). Following DNA extraction, the concentration of genomic DNA was measured using spectrophotometer, which was then used for PCR amplification.

Detection of serine protease autotransporters virulence genes in E. coli

This study utilized Conventional PCR to detect five virulence genes in *E. coli* isolates obtained from patients with urinary tract infections. This study utilized forward and reverse primers for six genes, as detailed in Table 1. The *uidA* primer was specifically employed to amplify the target *uidA* gene, acting as a species-specific primer for *E. coli*. Five additional primers were used to identify the distribution of the five virulence-associated genes (SPATEs): *pic, sat, vat, tagB,* and *tagC*.

PCR amplification and gel electrophoresis

The PCR amplification reaction was carried out in a total volume of 20µl which contained 10µl of the master mix, 1.5µl of each primer (forward and reverse), 3µl of DNA genome, and 4 µl of PCR-free nuclease water. The amplification conditions used are listed in Table 1. Following amplification, the PCR products were run on gel electrophoresis using 1.5% agarose gel for 45 min and compared with a 100 bp ladder (Promega). Following the run, DNA bands on the agarose gel were visualized using ultraviolet light

Results

Isolation and identification

A total of 100 *E. coli* isolates were examined in this study. Of all the patient groups, females (80 specimens , 80%) were the most affected group of patients in comparison to males (20 specimens , 20%), as shown in Table 2. The participants in this study were aged between 15 and ≥65 years old. A total of 100 bacterial isolates from *E. coli* were obtained by culturing in various media. MacConkey agar identified lactose-fermenting pink colonies of *E. coli*, whereas EMB agar was used to assess the ability of each isolate to produce green metallic sheen colonies. Chromogenic agar confirmed the presence of *E. coli*, yielding pink colonies

Table 1: PCR conditions and genes specific primers.

Target gene	Primer sequence (5´-3´)	Amplicon size (bp)	PCR program run	Reference
uidA	F: ATGGAATTTCGCCGATTTTGC R: ATTGTTTGCCTCCCTGCTGC	166	95C° for 4 mins 95C° for 40 sec 59.5C° for 40sec 72C° for 40 sec 72C° for 5 mins 30 cycles	(8)
pic	F: ACTGGATCTTAAGGCTCAGGAT R: GACTTAATGTCACTGTTCAGCG	572	95C° for 4 mins 95C° for 40 sec 58.3C° for 30sec 72C° for 40 sec 72C° for 5 mins 30 cycles	(9)
sat	F: TCAGAAGCTCAGCGAATCATTG R:CCATTATCACCAGTAAAACGCACC	930	95C° for 4 mins 95C° for 40 sec 59.5C° for 30sec 72C° for 40 sec 72C° for 5 mins 30 cycles	
vat	F: AACGGTTGGTGGCAACAATCC R: AGCCCTGTAGAATGGCGAGTA	420	95C° for 4 mins 95C° for 40 sec 61.7C° for 30sec 72C° for 40 sec 72C° for 5 mins 30 cycles	
tagB	F: TAAGTGTGGGAGACGGAACC R: AATAGCCGACCATTTTGCTG	436	95C° for 4 mins 95C° for 40 sec 61C° for 30 sec 72C° for 40 sec 72C° for 5 mins 30 cycles	This study
tagC	F: CGGGTTCAGATTGGAAAGAA R: TAACCGTCAGGGTACGGAAG	430	95C° for 4 mins 95C° for 40 sec 61C° for 30 sec 72C° for 40 sec 72C° for 5 mins 30 cycles	This study

Table (2): Sample distribution according to age groups and gender.

Age group	Gender		p-value	Total
	Females	Males		
	No.(%)			
15-24	22(91.7%)	2(8.3%)	**0.0001****	24(24%)
25-34	16(84.2%)	3(15.8%)	**0.0001****	19(19%)
35-44	19(90.5%)	2(9.5%)	**0.0001****	21(21%)
45-54	11(78.6%)	3(21.4%)	**0.0001****	14(14%)
55-64	7(70%)	3(30%)	**0.206**	10(10%)
65≤	5(41.7%)	7(58.3%)	**0.564**	12(12%)
p-value	**0.004****	**0.392**		**0.106**
Total	80(80%)	20(20%)		100(100%)

** Refer to Significant difference at p≤0.01.
* Refer to Significant difference at the p≤0.05

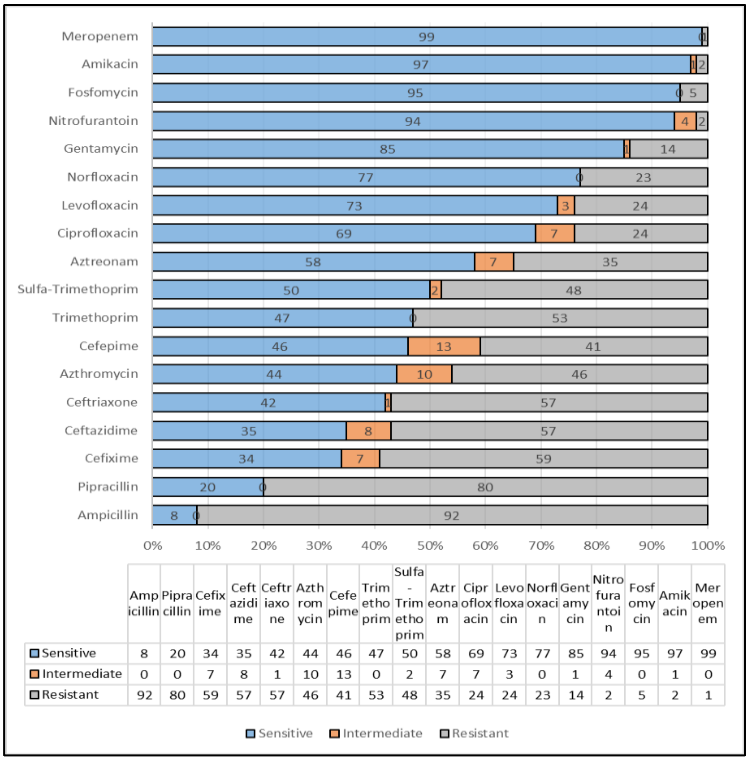
Antibiotic sensitivity test:

It has been found that 64% of the isolates examined in this study were considered resistant to multiple antibiotic classes, including MDR (63%) and XDR (1%). This study showed that multidrug resistance to three, four, five, six, seven, and eight antimicrobial classes was observed in 21%, 16%, 7%, 12%, 7%, and 1% of *E. coli* isolates, respectively (Table 3). However, resistance to one and two classes was noted in 17% and 14% of patients, respectively. Resistance to the three classes was the most common and was observed in 21% of the isolates.

Among the UPEC isolates tested in this study, resistance rates were as follow as shown in Figure (1): Ampicillin (92%), Piperacillin (80%) Ceftriaxone (57%), Ceftazidime (57%), Cefixime (59%), Cefepime (41%), Aztreonam (35%) Meropenem (1%) Ciprofloxacin (24%), Norfloxacin (23%), Levofloxacin (24%), Gentamicin (14%), Amikacin (2%), Nitrofurantoin (2%), Trimethoprim (53%) Trimethoprim–sulfamethoxazole (48%), Fosfomycin (5%) , and Azithromycin (46%) .

Table (3): Antibiotic resistance and their percentages in UPEC isolates.

Sensitivity of isolates		No. of isolates	Percent
Sensitive to all antibiotic classes		5	**5.00%**
Non MDR	One class resistant	17	**17.00%**
	Two classes resistant	14	**14.00%**
MDR	Three classes resistant	21	**21.00%**
	Four classes resistant	16	**16.00%**
	Five classes resistant	7	**7.00%**
	Six classes resistant	12	**12.00%**
	Seven classes resistant	7	**7.00%**
XDR	Eight classes resistant	1	**1.00%**



	Ampicillin	Pipracillin	Cefixime	Ceftazidime	Ceftriaxone	Azthromycin	Cefepime	Trimethoprim	Sulfa-Trimethoprim	Aztreonam	Ciprofloxacin	Levofloxacin	Norfloxacin	Gentamycin	Nitrofurantoin	Fosfomycin	Amikacin	Meropenem
▢ Sensitive	8	20	34	35	42	44	46	47	50	58	69	73	77	85	94	95	97	99
▢ Intermediate	0	0	7	8	1	10	13	0	2	7	7	3	0	1	4	0	1	0
▢ Resistant	92	80	59	57	57	46	41	53	48	35	24	24	23	14	2	5	2	1

Figure 1: Antibiotic sensitivity results in UPEC isolates.(meropenem (MEM), amikacin (AK), fosfomycin (FO), nitrofurantoin (NIT), gentamycin (CN), norfloxacin (NOR), levofloxacin LE), ciprofloxacin (CIP), aztreonam (AT), sulfa-trimethoprim (COT), trimethoprim (TR), cefepime (CPM), azthromycin (AZM), ceftriaxone (CTR), ceftazidime (CAZ), cefixime (CFM), pipracillin (PRL), ampicillin (AMP).)

PCR Analysis

From a total of 100 UPEC isolates, 60 were selected for PCR amplification, and 96.6% (58/60) of *E. coli* were successfully amplified and produced a single band of the target *uidA* gene as a species-specific gene with a molecular weight of 166 bp, as shown in Figure (2).

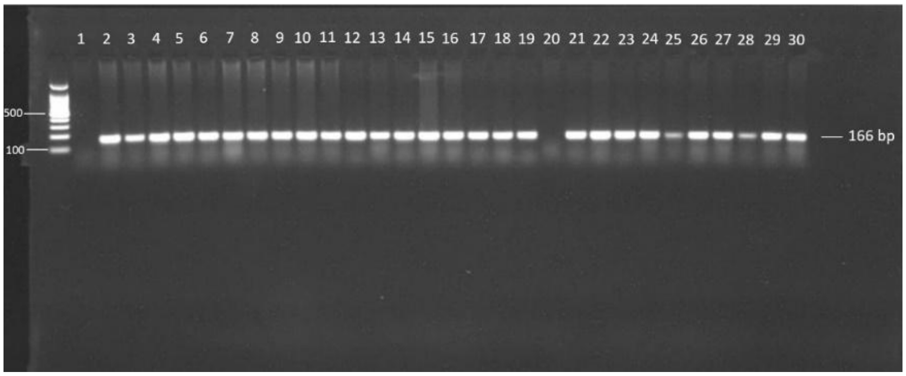


Figure 2: The PCR amplification of *uidA* (166 pb) in UPEC. Gel electrophoresis of amplified gene in agarose 1.5%, 80 V/cm for 45 min., stained with safe red dye and visualized on a UV transilluminator. In the presence of 100 bp DNA ladder. Lane (1-30): Amplicons of *uidA* gene.

The selected 60 isolates of *E. coli* were subjected to the determination of the genes related to serine protease autotransporters and their distribution among UPEC patients (Figure 3). The most common virulence gene among UPEC isolates was *vat,* which was detected in 58.3% (35/60) of the isolates. The second most common virulence gene was *sat,* which was detected in 36.6% (22/60) of the isolates. The prevalence of *pic* gene was 30% (18/60). The lowest prevalence of *tagB* and *tagC* was 18.3% (11/60), According to this study, only 68.3% (41/60) of isolates had at least one of the five virulence genes.

Association between antimicrobial resistance and SPATEs genes

Furthermore, additional investigations were conducted to determine whether the serine protease auto-transporter virulence genes have a relationship with increasing antibiotic-resistant genes, which found a significant positive correlation between piperacillin, ampicillin, ceftriaxone, cefixime, and ceftazidime with the presence of virulence genes in the UPEC isolates ($p < 0.01$), while Trimethoprim and Azithromycin showed a significant correlation at ($p < 0.05$). Table (4)

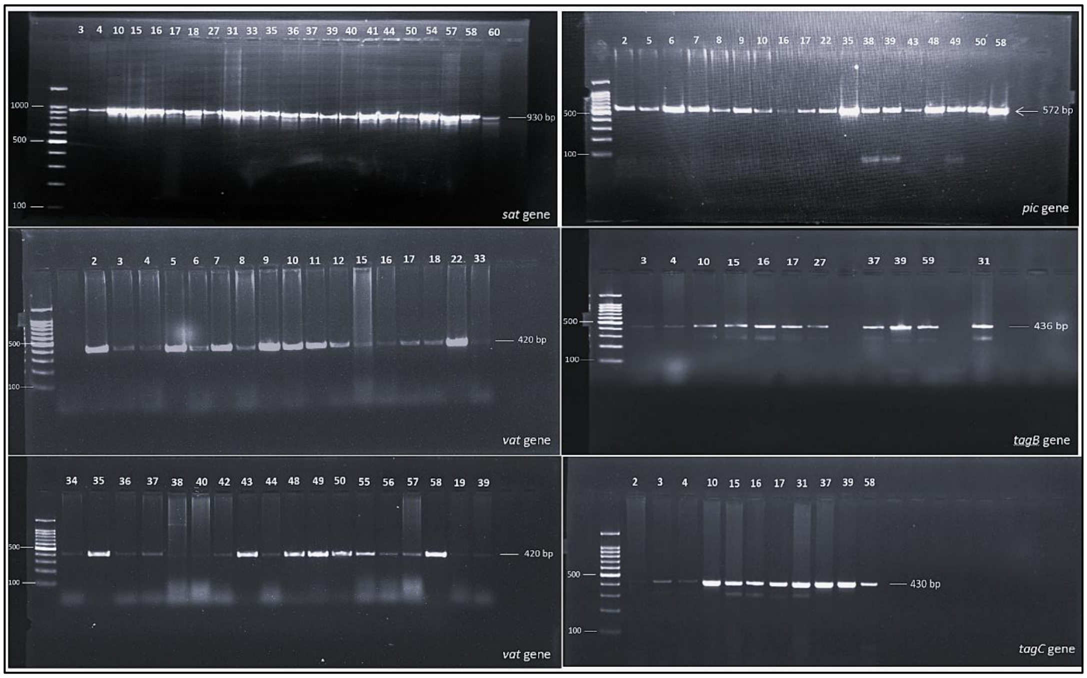


Figure 3: The PCR amplification of SPATEs genes for *E. coli*. PCR amplification of *sat* (930 bp), *vat* (420 bp), *pic* (572 bp) , *tagB* (436 bp) and *tagC* (430 bp) in UPEC. Gel electrophoresis of amplified gene agarose 1.5%, 80 V/cm for 45 min., stained with safe red dye and visualized on a UV transilluminator. In the presence of 100 bp DNA ladder.

Association among SPATEs genes in UPEC isolates

After pairwise comparisons of SPATEs genes and their interactions utilizing Spearman's correlation coefficient, there were highly significant positive correlations between *pic* and *vat* (r = 0.553, p value < 0.000), *sat* and *vat* (r = 0.292, p value < 0.02), *sat* and both t*agB* and *tagC* (r = 0.533, p value < 0.000), *vat* and *tagC* (r = 0.313, p value < 0.015), and *tagB* and *tagC* (r = 0.777, p value < 0.000).

Table (4): Association between antimicrobial resistance and virulence genes

Antibiotics resistance (No=60) (%)	**Virulence genes (No=60) (%)**					**p-value**
	pic 18(30%)	***sat*** 22(36.6%)	***vat*** 35(58.3%)	***tagB*** 11(18.3%)	***tagC*** 11(18.3%)	
Pipracillin 56(93.3%)	18(32.1%)	21(37.5%)	33(58.9%)	11(19.6%)	11(19.6%)	**0.002****
Ampicillin 60(100%)	18(30%)	22(36.6%)	35(58.3%)	11(18.3%)	11(18.3%)	**0.0001****
Ceftriaxone 50(83.3%)	16(32%)	20(40%)	30(60%)	10(20%)	10(20%)	**0.003****
Cefixime 51(85%)	17(33.3%)	19(37.2%)	31(60.7%)	11(21.5%)	11(21.5%)	**0.004****
Ceftazidime 50(83.3%)	17(34%)	20(40%)	32(64%)	11(22%)	11(22%)	**0.003****
Cefepime 39(65%)	14(35.8%)	15(38.4%)	24(61.5%)	10(25.6%)	10(25.6%)	**0.061**
Gentamycin 14(23.3%)	5(35.7%)	6(42.8%)	7(50%)	6(42.8%)	5(35.7%)	**0.975**
Amikacin 2(3.3%)	1(50%)	0	1(50%)	0	0	**1.000**
Ciprofloxacin 23(38.3%)	5(21.7%)	9(39.1%)	9(39.1%)	10(43.4%)	8(34.7%)	**0.772**
Norfloxacin 22(36.6%)	4(18.1%)	9(40.9%)	8(36.6%)	10(45.4%)	8(36.6%)	**0.615**
Levofloxacin 23(38.3%)	5(21.7%)	9(39.1%)	9(39.1%)	10(43.4%)	8(34.7%)	**0.772**
Aztreonam 35(58.3%)	12(34.2%)	12(34.2%)	20(57.1%)	10(28.5%)	10(28.5%)	**0.251**
Nitrofurantoin 2(3.3%)	0	1(50%)	0	1(50%)	1(50%)	**1.000**
Meropenem 1(1.6%)	1(100%)	1(100%)	1(100%)	1(100%)	1(100%)	**1.000**
Fosfomycin 4(6.6%)	2(50%)	0	2(50%)	0	0	**0.931**
Trimethoprim 46(76.6%)	13(28.2%)	18(39.1%)	26(56.5%)	11(23.9%)	10(21.7%)	**0.025***
Sulfa-Trimethoprim 41(68.3%)	10(24.3%)	16(39%)	21(51.2%)	10(24.3%)	9(21.9%)	**0.088**
Azthromycin 41(68.3%)	12(29.2%)	14(34.1%)	24(58.5%)	9(21.9%)	9(21.9%)	**0.024***
p-value	**0.0001****	**0.0001****	**0001**	**0.008****	**0.009****	

**** Refer to Significant difference at p≤0.01. * Refer to Significant difference at the p≤0.05.**

Discussion

A higher frequency of UTI among females is commonly reported in this study, this is mainly due to a shorter female urethra which can facilitate bacterial movement from the urethral opening to the bladder. Colonization of the vaginal introitus by gastrointestinal pathogens may increase the risk of urinary tract infiltration (10). The development of high rates of antibiotic resistance and MDR phenotypes in bacteria associated with UTIs has become a significant public health issue globally (11). The increase in MDR strains observed in this study might have resulted from various factors, such as excessive prescription of antibiotics or self-medication without proper antibiotic sensitivity testing. Additionally, careless use of antibiotics contributes to the emergence of MDR, leading to the spread of antibiotic-resistant pathogens in clinical settings (12), a similar study by Al-Hasnawy *et. al*. (13) found that MDR (88.09%) and XDR (11.9%).

Resistance rates of ampicillin (92%) and piperacillin (80%) showed the highest resistance compared to other classes of antibiotics. These findings are consistent with those of previous studies (14–16). Penicillins are not effective against *E. coli*; thus, based on the results obtained, these antibiotics should be avoided in the treatment of UTIs. Ceftriaxone (57%), ceftazidime (57%), and cefixime (59%) showed higher resistance to UPEC than cefepime (41%) in the same group of cephalosporins, which is similar to other findings (14,17–19). This could be attributed to the overuse of expanded spectrum cephalosporins in clinical setting (20,21). In the case of monobactams, including aztreonam, the resistance rate in this study was (35%) which is higher than that of Khalaf and Flayyih (22), which is (10%) and less than that of Hasan and Ibrahim (23), which is (45%). The resistance of UPEC to β-lactam antibiotics is mainly attributed to the production of β-lactamase enzymes which break down the essential β-lactam ring, thereby reducing the effectiveness of these antibiotics. The main type of β-lactamase generated by UPEC is CTX-M-type extended-spectrum β-lactamase (ESBL), which is capable of hydrolyzing third-generation cephalosporins, monobactams, and other β-lactams. (24,25). Thus, the restricted use of these antibiotics could be beneficial in preventing or reducing the development or spread of multidrug-resistant gram-negative bacteria (26).

Carbapenem antibiotics showed the lowest resistance among the UPEC bacteria, and this category of antibiotics is currently widely used for treating complex microbial infections. (27) . In the current study, meropenem (1%) showed the greatest effectiveness against uropathogenic isolates and was among the most suitable medications for UTIs, which is consistent with the findings of Jassim Mohammed *et. al*. (28). Different studies found that the resistance rate to this antibiotic was higher (8.3%) (29).

Fluoroquinolones have been utilized to treat *E. coli* infections, as they remain effective against urinary tract infections. In the current study, the resistance patterns of the UPEC isolates to the following fluoroquinolones were analyzed: ciprofloxacin (24%), norfloxacin (23%), and levofloxacin (24%). This finding is in line with those of other studied (28,30,31).

The results of the present study for the aminoglycoside antibiotics gentamicin (14%) and amikacin (2%) were consistent with those of Al-Sa'ady *et al*. and Khalaf and Flayyih (22,32). *E. coli* isolates demonstrate a minimal level of resistance to the antibiotic nitrofurantoin (2%), making this medication a preferred option for treating UTIs in patients. The results of this study are consistent with those of previous studies (33,34). Another study found that there was increasing resistance to this antibiotic (35).

In this study, 53% and 48% of the isolates showed resistance to trimethoprim and trimethoprim–sulfamethoxazole respectively, which is consistent with the results of other studies (36,37). These two antibiotics are suggested as the first options for treating UTIs. (38). This study also found that *E. coli* was resistant to fosfomycin (5%) which is the same as that reported by Seok *et. al*. (19). The resistance mechanism to fosfomycin may include decreased permeability to this antibiotic, whereas drug inactivation can occur through the acquisition of *fos* genes, primarily via plasmids, leading to fosfomycin resistance (39).

Resistance to azithromycin was 46%, which is different from those reported by Al-Khfaji *et al*. (40) and Ali and Al-Dahmoshi (41) which showed resistance of 28.6% and 10%, respectively.

The prevalence of *uidA* 96.6% (58/60) of *E. coli* are similar to the study performed by other researchers (42,43). Whereas distribution of genes related to serine protease autotransporters among UPEC patients, The most common virulence gene was *vat,* which was detected in 58.3% (35/60) of the isolates. One study found that the distribution of this gene was 68% (44), while another study found a different result, which was 27% (45). The second most common virulence gene was *sat* 36.6% (22/60) of the isolates, similar to the study by Freire *et al*. (46) with a distribution of 34.2%. The prevalence of *pic* gene was 30% (18/60), which is in line with the 31% reported by Heimer *et al*. (47) . The lowest prevalence of *tagB* and *tagC* was 18.3% (11/60), which is similar to the result of Habouria *et al*. (48) who found that the distribution was 10%.

Ethical approval

Before being included in the study, every patient's proper permission was obtained. The study protocol subject information, and consent form were reviewed and approved by a local ethics committee according to document number CSEC/1023/0089 on October 28, 2023.

Conclusion

E. coli develops resistance to the most commonly used antibiotics, and an increase in MDR strains was observed in this study. 64% of the isolates examined in this study were considered resistant to multiple classes of antibiotic, including MDR (63%) and XDR (1%). Therefore, continuous surveillance of antimicrobial resistance in UPEC is essential to avoid treatment failures, enhance strategies to reduce the rate of antibiotic-resistant organisms, and provide optimal treatment for patients with UTI. The prevalence of *uidA* gene among the tested *E. coli* isolates was 96.6% (58/60) and at least one of the five serine protease autotransporter virulence-related genes was identified in 68.3% (41/60) of *E. coli* isolates. There was a significant correlation between resistance to some antibiotics, including piperacillin, ampicillin, ceftriaxone, cefixime, ceftazidime, trimethoprim, and azithromycin, and the presence of these virulence genes in the UPEC isolates. In addition, there were highly significant positive correlations among some serine protease autotransporter virulence-related genes.

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