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ORIGINAL ARTICLE

Antibiotic Resistance Pattern and Molecular Characterization of Resistant Genes in *Escherichia Coli* Isolated from Patients with Urinary Tract Infections

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- All authors were involved in drafting the manuscript or critically revising it for important intellect content.
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ABSTRACT

Background: The Urinary tract infection (UTI), is a critical factor in renal diseases and prevalent bacterial infection. Females have higher prevalence of UTI than males, which could be attributable to variety of physiochemical factors such as anatomic, hormonal and behavioral differences.
Objective: To evaluate the antibiotic susceptibility patterns of pathogenic bacteria and detect antibiotic-resistance genes in *Escherichia coli* isolated from UTI patients.
Method: This study was conducted from March to August 2024 at the Department of Biotechnology, Abdul Wali Khan University Mardan, in collaboration with the Department of Pathology, Khyber Teaching Hospital (KTH), Peshawar. Urine samples were collected from UTI patients attending the hospital. Pathogenic bacteria were isolated and identified, and antibiotic resistance genes (*Mcr-2, Mcr-3*, *Bla-TEM*, *AAc(3)IV*, *Bla-SHV*, *Bla-CTX-M*, *aadA*, *astA*, *fanA*, *estB*, *estA*, and *elt*) were detected in *E. coli* isolates. A total of 457 urine samples were collected during the study period.
Results: *E. coli* strains showed 75% resistance to norfloxacin and were multidrug resistant (MDR) with resistance to at least three antimicrobial agents. Several antibiotic-resistance genes, including *Mcr-2*, *Mcr-3*, *Bla-TEM*, *Bla-CTX-M*, *Bla-SHV*, *AAc(3)IV*, and *aadA*, were detected in the isolates. The predominant virulence gene, *estB*, exhibited a prevalence of 96.6% and was detected more frequently in female UTI patients (99.4%) compared to males (89.4%), showing a significant difference (P=0.0001). Both *astA* and *estB* genes were present in 99.4% of cases, with some isolates carrying 1-4 virulence genes. A significant difference in antibiotic-resistance genes between female and male UTI patients was observed.
Conclusion: The study highlights the high prevalence of multidrug-resistant *E. coli* and antibiotic-resistance genes among UTI patients in Peshawar, Pakistan. These findings emphasize the importance of monitoring antibiotic resistance patterns to support appropriate antimicrobial therapy for the effective management of UTIs.
Keywords: Urinary Tract Infections; *Escherichia coli*; Antibiotic Resistance; Drug Resistance, Microbial; Genotyping

INTRODUCTION: Urinary tract infection (UTI) is a multifactorial disease caused by several microorganisms, with the most common being pathogenic bacteria [1].The infection is considered one of the most common bacterial infections among people in domestic, community, hospital, and nosocomial settings, affecting males, females, and children [2]. The associated morbidity of these infections is high, and it is higher in males compared to females. The short distance between the urethra and anus makes women more prone to UTIs, facilitating contamination by bacteria from the feces [3]. Various variables contribute to the susceptibility of women to UTIs, among them urethral tone due to urine stasis and ureter vesical reflux [4]. UTIs are also prevalent in children, especially in infants and those up to 3 years of age [1].

Various bacteria have been reported as etiological agents of UTIs, including *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Proteus mirabilis*, *Proteus vulgaris*, *Staphylococcus aureus*, and *Pseudomonas* species [5]. Among these, *Escherichia coli* is considered the most prevalent uropathogen responsible for the majority of community-acquired UTIs [6]. Although UTIs and their epidemiology have been widely studied worldwide, limited data are available regarding the distribution of uropathogens and antibiotic resistance genes in *E. coli* isolates from patients in Khyber Pakhtunkhwa, Pakistan. Therefore, the present study was conducted to isolate and characterize uropathogenic bacteria and to investigate the antibiotic resistance genes in *E. coli* strains from UTI patients in this region.

Despite the various treatments for UTIs that were undertaken worldwide based on a wide range of scientific data [7], most of the causative agents of UTIs develop resistance to generally used antibiotics, thus posing a big challenge to effective therapeutic options. There is a growing concern worldwide about multidrug-resistant pathogens causing UTIs, including *E. coli* [8]. Consequently, the emergence of MDR isolates results in reduced antibiotic efficacy, leading to increased medical costs, mortality, and morbidity. This calls for analysis of the susceptibility profiles of different pathogens [9].

The widespread use of antibiotics has been linked to a growing incidence of antibiotic resistance among UTI pathogens worldwide [5]. Given the importance of hospitals in providing healthcare and the associated challenges such as prolonged hospital stays and increasing antibiotic resistance, it is crucial to gather accurate information on UTIs in Pakistan. Therefore, the current study aimed to describe the molecular epidemiology of *E. coli* strains isolated from UTI patients and develop new strategies for antibiotic sensitivity testing and therapeutics to combat the pathogenic organisms implicated in UTIs in the local population of this area.

METHOD: This research was approved by the Ethics and Research Committee of the Department of Pathology, Khyber Teaching Hospital, Peshawar, Pakistan, and the Department of Biotechnology, Abdul Wali Khan University Mardan (AWKUM), Pakistan (ERB Reference No: AWKUM/Biotech/2023; Approval Date: 15 March 2023). The research study was conducted at the Department of Biotechnology, Abdul Wali Khan University Mardan, in collaboration with the Department of Pathology, Khyber Teaching Hospital, Peshawar, Pakistan. This study was designed as a prospective observational study. The study aimed to isolate and characterize pathogenic bacteria from UTI patients and to identify antibiotic resistance genes in *E. coli* strains. A convenience sampling technique was used to collect urine samples from patients suspected of urinary tract infections. A total of 457 urine samples were collected during the study period. The study was conducted from March to August 2024.

UTI patients who visited the Department of Pathology, KTH, Peshawar, Pakistan, were included in the study. The patients were mainly from Peshawar and surrounding districts of Khyber Pakhtunkhwa (KPK), Pakistan, who attended KTH for diagnostic testing. Each participant was informed about the purpose of the study and provided written consent. A total of 344 patient urine samples (268 from females and 76 from males) were collected from the Department of Pathology, KTH, Peshawar, Pakistan. The study enrolled males and females between the ages of 18 and 60 years. Inclusion criteria included the provision of a midstream urine sample and the signing of the consent form. Only patients clinically suspected of urinary tract infection and confirmed by laboratory examination were included in the study. Patients who had taken any antibiotics in the last three months were excluded from the study. A demographic questionnaire was completed by the participants. The study primarily included outpatients presenting with symptoms of UTI, representing community-acquired infections rather than nosocomial infections. Volunteers diagnosed with UTI were asked to provide a midstream urine specimen in a sterile urine collection cup (BD, Woomed, South Africa) after informed consent. Following collection, the samples were stored at 4 °C for up to eight hours before being transported to the laboratory on ice for analysis.

All characteristics of the collected urine samples were noted, including color, flow rate, pH, and specific gravity. The colors varied from pale yellow to deep amber, indicating that some samples were in the normal state and others were indicative of disease status; in a few cases, there was even red coloration, which is due to a pigment called urochrome. In preparing the samples for further analysis, 5 ml of each sample was centrifuged at 3000 rpm at 4°C for 5 minutes in a tubular centrifuge made in Liuyang, China. The supernatant was discarded, and the pellet was kept for analysis. A drop or two of the solid elements were placed on well-cleaned and dried glass slides, after which it was visualized with a microscope (Olympus Binocular Microscope, Olympus Optical

Company Limited, Japan) in both 40x (low power) and 100x (high power) objectives. Each specimen was inoculated separately onto different agar media plates, such as MacConkey, Blood, and Cysteine-lactose Electrolyte Deficient (CLED) Agar.

The urine samples were streaked onto CLED Agar plates using a standard sterile loop calibrated to hold 0.01 ml for the semi-quantitative method. The plates were then incubated for 24 hours at 37°C. Physically distinct colonies from each plate were selected to obtain pure cultures and streaked onto EMB plates. Single colonies were chosen and sub-cultured again on nutrient agar plates. All plates were incubated at 37°C for 18 hours. After 24 hours, plates with no growth or colonies were further incubated for 48 hours. The positive samples were defined as those having a colony count above or equal to 10 CFU/ml. Isolation and confirmation of the isolates were carried out using standard microbiological techniques such as colonial morphology, Gram staining, growth on selective media, catalase and oxidase tests. The isolates were confirmed with API 10-S strip kit (Bio Merieux, Midrand, South Africa) according to the manufacturer's recommendations. *E. coli* ATCC 25922 (American Type Culture Collection) was used as a positive control.

Isolates were considered diseased-related bacteria according to their type, site of sampling, and either gram-positive or gram-negative. Diagnostic biochemical tests were conducted to identify the exact type of bacteria. All pure isolates were Gram-stained and further verified by using the API 10-S strip kit according to the manufacturer instructions. Additional tests for gram-positive bacteria included catalase, coagulase, optochin, and urease tests. Further tests for gram-negative bacteria included indole production, triple sugar iron agar utilization, citrate utilization, carboxylase production, oxidase reaction, and motility.

Urine samples for bacterial isolation were plated on MacConkey and Blood agar plates and incubated at 37°C for 24 hours. Profuse growth was seen on the plates, with a count equal to or greater than 10 per millilitre. Pure isolates were identified by form, morphology, cultural characteristics, and biochemical properties. Antibiotic susceptibility testing by the disc diffusion method was done according to the standards provided by the European Committee on Antimicrobial Susceptibility Testing [10]. Discs containing different antibiotics at specific concentrations were placed on Mueller-Hinton agar plates, and the diameter of inhibition zones was measured in millimeters and compared to standard diameter values [11]. The selection of antibiotics and their concentrations against the pathogenic bacteria obtained from UTI patients was done according to Kang et al, [9]. The minimum inhibitory concentrations (MICs) were determined using the EUCAST breakpoints version 10, with the MICs recorded immediately from the scale (µg/mL) where the edge of the inhibition ellipse intersected the MIC strip [12]. *E. coli* ATCC25922 was used as a positive control.

On nutrient agar plates, single colonies were selected, and genomic DNA was extracted from the selected colonies using the GeneJET Genomic DNA Purification Kit following the manufacturer's instructions (Thermo Scientific, Johannesburg, South Africa). The concentration and purity of the extracted genomic DNA were determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA). The DNA concentration was measured in nanograms per microliter (ng/µL), and the purity was assessed by comparing the optical density (OD) at 260 and 280 nanometers. The isolated DNA samples were loaded onto a 1% (w/v) agarose gel and subjected to electrophoresis at 100V for 60 minutes in 1X TAE buffer. Amplification products were visualized using the Bio-Rad Gel Doc XR+ molecular imager under UV transillumination (Bio-Rad, USA).

PCR assays were conducted to determine the prevalence of 12 virulence genes, including *Mcr-2*, *Mcr-3*, *Bla-TEM*, *AAc (3)IV*, *Bla-SHV*, *Bla-CTX-M*, *aadA*, *astA*, *fanA*, *estB*, *estA*, and *elt*, using specific primers [12]. The PCR reactions were performed in a 12 µL mixture, consisting of 1 µL of DNA, 1 µL (10 pmol) of each primer, 6 µL of green PCR master mix, and 3 µL of PCR water. The amplification of virulence genes was carried out in a Thermal Cycler (Eppendorf Mastercycler) using the previously described PCR conditions **(Table I)**. The PCR products were separated by electrophoresis on 1.8% agarose gels with a 100-bp DNA ladder as a size reference. The agarose gels stained with ethidium bromide were visualized and analyzed using a gel documentation system (Bio-Rad, USA).

Table 1: Primers and Annealing °C Used for Amplification of Antibiotic Resistance Genes IN *E. coli* from UTI patient.

Genes	Sequence (5' to 3')	Size	Annealing (°C)	Reference
Mcr-2	F: TGT TGC TTG TGC CGA TTG GA R: AGA TGG TAT TGT TGG TTG CTG	567	58	[20]

Mcr-3	F: TTG GCA CTG TAT TTT GCA TTT	542	50	[21]
	R: TTA ACG AAA TTG GCT GGA ACA			
Bla-TEM	F: CGCAGATAAATCACCACAATG	247	54	[12]
	R: GTCTATTTCGTTCATCCATA			
AAc(3)IV	F: TGCTGGTCCACAGCTCCTTC	653	59	[22]
	R: CGGATGCAGGAAGATCAA			
Bla-SHV	F: CACCACGATGCCATGTTCATCTGC	768	52	[12]
	R: TCGCCTGTGTATTATCTCCC			
Bla-CTX-M	F: ATGTGCAGCACCAGTAAAGT	545	54	[12]
	R: ACCGCGATATCGTTGGTGG			
aadA	F: GTGGATGGCGGCCTGAAGCC	525	68	[22]
	R: AATGCCCAGTCGGCAGCG			
astA	F: TCGGATGCCATCAACACAGT	125	62	[22]
	R: GTCGCGAGTGACGGCTTTGTAAG			
fanA	F: AATACTTGTTCAGGGAGAAA	230	55	[22]
	R: AACTTTGTGGTTAACTTCCT			
estB	F: TGCCTATGCATCTACACAAT	113	55	[22]
	R: CTCCAGCAGTACCATCTCTA			
estA	F: CAACTGAATCACTTGACTCTT	158	55	[22]
	R: TTAATAACATCCAGCACAGG			
elt	F: GGCGTTACTATCCTCTCTAT	172	55	[22]
	R: TGGTCTCGGTCAGATATGT			

Quality control methods were incorporated into all the laboratory work processes to ensure the reliability of the study findings. Shelf-life determination was done for all staining reagents, culture media, and antibiotic discs before use. Plates and antibiotic discs were stored under refrigeration at 2-8°C after preparation. All the laboratory methods were performed according to the pre-analytical, analytical, and post-analytical quality assurance stages.

GraphPad Prism 21 was used for cleaning, entry, and analysis of data. Most of the study variables were summarized using descriptive statistics in the form of frequency and percentage distribution tables for bacterial isolates, antimicrobial resistance patterns, and antibiotic resistance genes. The COR and 95% CI for affirmative responses to some parameters were calculated using logistic regression analysis. The level of statistical significance was set at a p-value less than 0.05.

RESULTS: This study was based on a collection of urine samples from 457 individuals. Out of the total isolates, 344 samples were positive for culture, indicating a UTI occurrence rate of 77.90%. Among the positive samples, 268 (77.90%) were from females and 76 (22.10%) were from male participants, with ages ranging from 5 to 81 years. The bacterial isolates included *E. coli* (268; 71.6%), *Enterobacter spp* (14; 4.06%), *Klebsiella* (27; 7.84%), *Staphylococcus aureus* (11; 3.19%), *Proteus spp* (9; 2.61%), *Staphylococcus saprophyticus* (7; 2.03%), and *Enterococcus spp* (8; 2.32%). The prevalence ratio of isolated bacteria showed that *E. coli* was more prevalent in females (192; 71.75%) compared to males (76; 28.35%). Among the uropathogenic bacteria, *Escherichia coli* was found to be the most frequent infection, with a higher prevalence in female patients compared to male patients **(Table 2)**.

Table 2: Total number of isolates and their prevalence based on gender.

No. of isolates	Total isolate	Female Patient (%)	Male Patient (%)	Isolate (%)
Gram Negative Bacteria				
E. coli	268	192 (71.64)	76 (28.35%)	77.90%
***Enterobacters*spp**	14	9 (5.22%)	5 (1.86%)	4.06%
Klebsiella	27	16 (10.07%)	11 (4.10%)	7.84%
Gram-Positive Bacteria				
Staphylococcus aureus	11	4 (4.10%)	7 (2.61%)	3.19%
***Proteus*spp**	9	8 (3.35%)	1 (0.37%)	2.61%
Staphylococcus saprophyticus	7	4 (2.61%)	3 (1.11%)	2.03%
Enterococci	8	5 (2.60%)	3 (37.5%)	2.32%

The antimicrobial resistance patterns of the isolates are shown in **(Table 3)**. Out of the total 344 isolates, all showed resistance to one or more of the 18 antimicrobial drugs tested. The most common resistances observed were to Norfloxacin (75%), Meropenem (64.9%), and Cefoxitin (58.2%). Norfloxacin exhibited the best efficacy against *E. coli*, with only 75% of the isolates being multidrug-resistant (MDR), meaning they were resistant to at least three different classes of antimicrobial drugs. The susceptibility profile of *E. coli* and *Klebsiella spp*. showed that none of the 18 antibiotics tested were 100% effective. *E. coli* isolates displayed 64.9% resistance to Meropenem (66.1% in females, 61.8% in males), 65.6% resistance to Ciprofloxacin (17.1% in females, 61.8% in males), but 97.1% susceptibility to Vancomycin. Vancomycin resistance was observed in 71.0% of male isolates and 65.6% of female isolates, while Norfloxacin resistance was observed in 75% of all isolates (85.4% in females, 48.6% in males). Ampicillin showed 78.3% susceptibility (77.6% in females, 80.2% in males), and Cefuroxime showed 76.4% susceptibility (73.9% in females, 82.8% in males) against *E. coli*. Additionally, a high degree of resistance was observed in *E. coli* isolates, with 85.4% showing resistance to Norfloxacin in females and 71.9% resistance to Vancomycin in males. The susceptibility rate of *E. coli* was 97.1% to Ciprofloxacin in female isolates and 85% to Levofloxacin in male UTI patients.

Table 3: Antimicrobial drug resistance patterns among 268 isolates of *E. coli* from UTI patients

Antibiotics	Female *E. coli* (n=192)			Male *E. coli* ((n=76)			Total (n=268)		
	R(%)	I(%)	S(%)	R(%)	I(%)	S(%)	R(%)	I(%)	S(%)
Piperacillin	71(36.9)	14(7.2)	107(55.7)	28(36.9)	4(5.2)	44(57.8)	(36.9)	(6.7)	(56.3)
Gentamycin	85(44.2)	23(11.9)	84(43.7)	16(21)	8(10.5)	52(68.4)	(37.6)	(11.5)	(50.7)
Meropenem	127(66.1)	5(2.6)	60(31.2)	47(61.8)	15(19.7)	14(18.2)	(64.9)	(7.4)	(27.6)
Ciprofloxacin	33(17.1)	7(3.6)	152(97.1)	47(61.8)	5(6.5)	24(31.5)	(29.8)	(4.4)	(65.6)
Vancomycin	31(16.1)	9(4.6)	152(1.6)	54(71.0)	4(5.2)	19(25)	(31.7)	(3.7)	(63.8)
Imipenem	50 (26)	10(5.2)	132(68.7)	40(25.6)	11(14.4)	25(32.8)	(33.5)	(7.8)	(58.5)

Norfloxacin	164(85.4)	3(1.5)	25(13)	37(48.6)	11(14.4)	28(36.8)	(75)	(5.2)	(19.7)
Amikacin	91(47.4)	11(5.7)	90(46.8)	40(52.6)	-	36(47.3)	(48.8)	(4.1)	(47.1)
Linezolid	70(36.4)	8(4.1)	129(67.1)	44(57.8)	11(14.4)	12(15.7)	(42.5)	(7)	(52.6)
Amoxicillin	76(39.5)	5(2.6)	111(57.8)	35(46.0)	4(5.2)	37(48.6)	(41.4)	(3.3)	(55.2)
Ampicillin	34(17.7)	10(5.2)	149(77.6)	7(9.2)	6(7.8)	61(80.2)	(15.7)	(5.9)	(78.3)
Cefepime	46(23.9)	4(2)	142(73.9)	34(44.7)	5(6.5)	37(48.6)	(29.8)	(3.3)	(66.7)
Cefoxitin	111(57.8)	9(4.6)	84(43.7)	45(59.2)	7(9.2)	24(31.5)	(58.2)	(5.9)	(40.2)
Ceftriaxone	65(33.8)	34(17.7)	93(48.4)	25(32.8)	6(7.8)	45(59.2)	(33.5)	(14.9)	(51.4)
Cefuroxime	37(19.2)	13(6.77)	142(73.9)	7(9.2)	7(9.2)	63(82.8)	(16.4)	(7.4)	(76.4)
Levofloxacin	51(26.5)	16(8.3)	125(65.1)	31(40.7)	7(9.2)	38(85)	(30.5)	(8.5)	(60.8)
Augmentin	69(35.9)	34(17.7)	89(46.3)	14(18.2)	5(6.5)	57(75)	(30.9)	(14.5)	(54.4)
Cefradine	100(52)	19(9.89)	73(38)	9(11.8)	4(5.2)	63(82.8)	(40.6)	(8.5)	(50.7)

The *estB* gene, a virulence determinant associated with adhesion, was detected in 96.6% (259 out of 268) of UTI isolates. Specifically, the detection rate of the *estB* gene was reported as 99.4% (191 out of 192) in female isolates and 89.4% (68 out of 76) in male UTI patients, with a significant *p-value* of 0.0001. The *Mcr-3* gene was present in 92.1% (247/268) of isolates, with 95.8% (184/192) in female isolates and 82.8% (63/76) in male UTI patients. Other detected genes included *Mcr-2* at 84.7%, *Bla-TEM* at 86.1%, *Bla-CTX-M* at 88.4%, *aadA* at 80.9%, *fanA* at 84.3%, and *estA* at 87.3% refer to **(Table 4)**. The *AAc(3) IV* gene was detected in 96.2% (258/292) of isolates, with 97.3% (187/192) in female isolates and 93.4% (71/76) in male isolates, showing no significant difference (*p-value* 0.1217). Similarly, the *Bla-CTX-M* gene was present in 88.4% (237/268) of isolates, with 90.6% (174/192) in female isolates and 82.8% (63/76) in male UTI patients, showing no significant difference. The most prevalent virulent gene, *estB*, was detected in 258 isolates (96.6%). Additionally, the prevalence of *astA* and *estB* genes was reported as 99.4% in the current study, with a total of 268 UTI isolates of *E. coli* containing the studied virulence genes, carrying one, two, three, and four virulence genes. Furthermore, the analysis of antibiotic resistance genes between female and male UTI patients showed a strong and significant association.

Table 4: Frequency of antibiotic resistance genes in *E. coli* from 268 UTI patients

Genes	(n=+ive) (%)	(n=-ive) (%)	Female (%)	Male (%)	Odds-R	Chi-sq	*P-value*
Mcr-2	227 (84.7)	41 (15.4)	178 (92.7)	49 (64.4)	20.61	56.13	0.0001
Mcr-3	247(92.1)	21(7.8)	184(95.8)	63(82.8)	4.746	12.62	0.0004
Bla-TEM	231(86.1)	37(13.8)	188(97.9)	43(56.5)	36.07	78.19	0.0001
AAc(3)IV	258(96.2)	10(3.7)	187(97.3)	71(93.4)	2.637	2.39	0.1217
Bla-SHV	178(66.4)	90(33.5)	107(55.7)	71(93.4)	0.080	34.68	0.0001
Bla-CTX-M	237(88.4)	31(11.5)	174(90.6)	63(82.8)	1.995	3.181	0.0745
aadA	217(80.9)	51(19.0)	183(95.3)	34(44.7)	25.12	90.39	0.0001

astA	243(90.6)	25(9.3)	191(99.4)	52(68.4)	88.15	62.09	0.0001
fanA	226(84.3)	42(15.6)	168(87.5)	58(76.3)	2.172	5.154	0.0232
estB	259(96.6)	9(3.3)	191(99.4)	68(89.4)	22.47	16.80	0.0001
estA	234(87.3)	34(12.6)	173(90.1)	61(80.2)	2.239	4.760	0.0291
elt	123(45.8)	145(54.1)	76(39.5)	47(61.8)	0.4043	10.86	0.0010

The statistical data represent the antibiotic resistance genes typing between male positive, negative, and female positive, negative samples among the group.

DISCUSSION: The UTI is considered the most common urogenital problem in the world, of which *E. coli* is the central causative agent [13]. Due to a variety of clinical factors, such as hormonal effects and anatomic abnormalities, females have higher rates of prevalence and incidence compared to males [1]. For this reason, urine samples from 344 UTI patients were analyzed for antibiotic susceptibility and antibiotic resistance genes in *E. coli*. In the present study, bacterial etiology was found in 75.27% (344/457) of patients. A total of 71.64% (192/268) of females and 28.35% (76/268) of males were infected with *E. coli*, which is consistent with the findings reported by Rehman et al. (2021) in Peshawar, Pakistan.

Klebsiella was the second most common pathogenic bacterium, accounting for 7.84% (27/344), consistent with the study by Nicolle, indicating that Klebsiella remains an important secondary cause of UTIs [14]. Gram-negative bacteria accounted for a higher percentage, 89.82%, compared to gram-positive bacteria, 10.17%, in these UTI patients, highlighting the dominance of gram-negative pathogens in urinary tract infections. The high frequency of *E. coli* in UTIs may be attributed to fecal contamination and the transmission of organisms from toilets to the urethra because poor hygienic conditions increase the susceptibility to various infections, especially in developing countries [15]. Surprisingly, our findings indicated a significant difference in the resistance pattern of *E. coli* compared to a previously reported low susceptibility, 5%, against Ciprofloxacin [12] ESBL production is a common resistance mechanism present in *E. coli*, which significantly contributes to treatment failure and limits antibiotic options [10].

We also observed a high degree of susceptibility in other gram-negative bacteria, including Pseudomonas and Klebsiella, which had a high sensitivity of 100% to Tazobactam/piperacillin but were highly resistant (100%) to ciprofloxacin. Our findings reflect a higher level of resistance compared to the study conducted by Marotta et al. (2020) [16]. Generally, antimicrobial resistance is considered one of the major health problems, especially in developing countries [5]. Multidrug-resistant infections are difficult to treat in countries like Iran, India, and Taiwan [17]. Antibiotic resistance is also found to be common in different regions, including Iran, suggesting that antimicrobial resistance is a widespread global health concern [18].

Among these, the most common genes detected in *E. coli* isolates were *estB*, 96.6%, followed by *Mcr-3*, 92.1%; *Mcr-2*, 84.7%; *Bla-TEM*, 86.1%; *Bla-CTX-M*, 88.4%; *aadA*, 80.9%; *fanA*, 84.3%; and *estA*, 87.3%, respectively. Ghenea et al, also found a high incidence of *CTX-M*, 57.7%; *TEM*, 20.3%; and *SHV*, 15.4%, which corroborated the present data [19]. The results of ESBL-positive isolates revealed a sharp rise of *CTX-M*, 97.4%, in comparison with UTI *Bla-CTX-M*, 88.4%, confirming the observations of the current investigation [9]. *CTX-M* gene prevalence was observed in higher rates among the studied *E. coli* isolates, 88.4%, supporting the present study's results [14]. Different studies have indicated that there are significant differences regarding the distribution of ESBL resistance genes among various geographical regions. These variations may be related to drug selection, antibiotic consumption rates, and differences in the timing when the isolate samples were collected. The most plausible reasons for such variation include self-medication and inappropriate utilization of antibiotics [16]. Urinary tract infections are among the most common infectious diseases globally. The resistance pattern of antibiotics varies in different geographical areas. The development of antibiotic resistance in *E. coli* is a critical threat to human health, emphasizing the need for continuous surveillance and rational antibiotic use.

CONCLUSION: In addition, the findings indicated that most of the prevailing pathogenic strains carried the genes responsible for resistance to the antibiotics. This study has gone further to show that some strains of *E. coli* are resistant to antimicrobial therapies and that the resistance genes in the UTI isolates are different between female and male *E. coli* strains, as well as the fact that some resistance genes are clearly linked to virulence genes. The *estB* gene was detected as the most prevalent virulence gene. Additionally, the analysis between female and male UTI patients revealed different significant associations of antibiotic resistance genes. The findings of this study may provide useful insights for clinicians to improve the management and treatment of UTI infections.

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