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ORIGINAL ARTICLE

Incidence of extended spectrum beta-lactamase (ESBL) producing *Escherichia coli* isolated from women with urinary tract infections in Jordan

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ABSTRACT

Background and Objectives: Urinary tract infections are one of the world's major health problems. In addition, clinical disorders may result from the presence of bacteria or fungi in urine. The aim of this study was to isolate *Escherichia coli (E. coli)* strains from midstream urine samples, and to determine molecular characterization of encoded Extended Spectrum Beta-Lactamase (ESBL) genes.
Materials and Methods: Collected urine samples were streaked on MacConkey, blood and EMB agar plates, then identifying *E. coli* isolates by using antibiotic susceptibility tests. ESBL production was measured using double disc diffusion. Furthermore, uniplex PCR was performed to identify two ESBL genes (bla_{CTX} and bla_{TEM}).
Results: Among 412 isolates, 198 (48.1%) were *E. coli* strains, followed by *Staphylococcus saprophyticus, Klebsiella* sp., *Serratia* sp., *Enterococci* sp. and *Proteus* sp. with frequency of 132 (32.0%), 51 (12.4%), 15 (3.6%), 10 (2.4%), and 6 (1.5%) respectively. Female participants who were between the ages of 40 and 49 years old, married, and pregnant were more likely to develop urinary tract infections (UTIs). *E. coli* species were present in 189 (95.5) of the recurrent UTIs. Regarding antimicrobial susceptibility testing of *E. coli* isolates, the highest percentage of resistance and susceptible rates were found for nalidixic acid (75.8%) and gentamicin (64.1%) respectively. Among the *E. coli* isolates, 25 (12.6%) were ESBL-producers. The bla_{CTX-M} gene was genetically confirmed in 20 (10.1%) of the isolates.
Conclusion: *E. coli* is the most common cause of UTI and ESBL production leads to increased resistance to common antibiotics and complicates treatment strategies.

Keywords: Antibiotic resistance; bla_{CTX-M} gene; *Escherichia coli*; Erythromycin; Extended-spectrum beta-lactamase; Urinary tract infections

INTRODUCTION

Urinary tract infections (UTIs) are clinical conditions caused by the presence of bacteria or fungi in the urine that can lead to severe infections of systemic organs, resulting in sepsis (1). UTIs are significant health issue that cause considerable morbidity, with symptoms ranging from asymptomatic bacteriuria

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to acute pyelonephritis (2), and also the financial burdens that are associated with their occurrence. Approximately, 2-3% of hospitalized patients develop UTIs during their hospital stay. Moreover, antibiotic misuse has led to the rise and spread of antibiotic resistance worldwide, endangering public health and increasing the risk of mortality (3). Approximately, 50% of the women experience UTI during their lifetime, and it is considered as the most common pregnancy complication with 20% reported prevalence among pregnant women (4). For example, the weight of the uterus can compress the ureters, delaying the passage of urine and causing partial emptying of the bladder, which can lead to the development of UTIs (5). Additionally, 70% of pregnant women have higher urine sugar levels, which encourages the growth of bacteria and further increases the risk of UTIs (6). On the other hand, males are less prone to develop UTIs because of the long urethra and the antimicrobial substances that are secreted from the prostatic glands. In neonates, occurrence of UTIs is about 1% and it is more common in male neonates than females, many of whom have congenital structural abnormalities (7).

Escherichia coli (E. coli) is the most common causative agent of UTIs, accounting for 70-80% of cases (8). *Klebsiella pneumoniae, Proteus, Acinetobacter, Staphylococcus saprophyticus,* Group B *Streptococcus*, and *Pseudomonas aeruginosa* are some of the other microorganisms that can cause UTIs. Several parts of the urinary system can become infected, causing bladder infection (cystitis), urethra infection (urethritis), ureters, and progressing to kidney infection (pyelonephritis), which leads to kidney scarring and may evolve to cause permanent renal damage causing hypertension or end-stage renal disease (3). Recently, a worldwide increase in antibiotic resistance among uropathogens has been noticed and has become a growing concern. As a result, the relationship between antibiotic resistance and bacteria is becoming increasingly complicated (9, 10). Ampicillin, ciprofloxacin, chloramphenicol, tetracycline, sulfamethoxazole, amoxicillin, nalidixic acid, sulfonamide, cotrimoxazole, and nitrofurantoin are the most commonly used antimicrobials for UTIs (11). Since the discovery of penicillin in 1923, β-lactam antibiotics have been the standard method of treatment for all human infectious bacterial diseases. However, many β lactam-resistant bacteria, on the other hand, have developed, which are able to produce enzymes capable of degrading and inactivating antibiotics (12). Extended-spectrum β –lactamases (ESBLs), Amp beta-lactamases, *Klebsiella pneumonia* carbapenemases, and Metallo B lactamases are all examples of β-lactamase enzymes (13). ESBLs are commonly classified into three types: CTX-M, SHV, and TEM (13). These enzymes are produced by gram-negative bacilli, and the isolation of ESBL-producing *E. coli* has occurred not only in the hospital setting but also in the community.

The main challenge with ESBL producing bacteria is that their encoded genes are constantly changing, and they have recently been shown to have genes conferring resistance to several non- β -lactam antibiotics which have resulted in limited medical options leading to high mortality rates (15). It is important to understand the risk factors for ESBL infection, as this can help to develop an effective infection control strategy by identifying patients who require empiric ESBL-targeted antimicrobial therapy (14). The most widely accepted risk factors for ESBL-producing *E. coli* infection are age, male sex, recent hospitalization, previous exposure to antibiotics, past catheterization experience, history of urogenital surgery, and recurring UTIs. Knowing these risk factors can help prevent the spread of infection and improve patient outcomes.

In Jordan, few studies have been performed to assess the prevalence of ESBL infection among UTI patients. A study by Hayajneh et al. (2015) (16), found that among the isolates tested, *E. coli* was the most common (70%). The incidence of ESBL producers was 43%, with the most common genetic type being lactam-15. Another study investigated the characteristics of ESBL among 165 urinary *E. coli* isolates. The prevalence of ESBL was found to be 50.3%. Molecularly, $bla_{\text{ctx-m}}$ either bla_{Tem} or both genes were (80.7%) out of 165 isolates, however the bla_{SHV} was not detected within isolates (16).

No published studies have assessed the risk factors for UTI caused by ESBL-producing *E. coli* in the Jordanian population. Considering this the present study is the first to identify both the risk factors for UTIs caused by ESBL-producing *E. coli* and the corresponding genes encoding ESBL. The current study aimed to isolate and characterize *E. coli* strains from urine samples of patients with UTI and determine the antimicrobial and antibiotic resistance profile of the isolated *E. coli* strains. Furthermore, the preva-

lence of ESBL-producing *E. coli* strains was evaluated, together with the molecular characterization of the genes encoded by these ESBL-producing *E. coli* strains.

MATERIALS AND METHODS

The study setting and ethical approval. A cross-sectional study was carried out to isolate and characterize *E. coli* strains from midstream-urine samples of women with UTI. Samples were collected from patients attending the PMLAB group, Alkhalidi Hospital, and Albasheer Governmental Hospital after obtaining consent for sample collection. This study was carried out from 1st of December 2023 to 30th of March 2024 and ethical approval was obtained from the Jordanian Ministry of Health. Patients whose urine cultures showed the presence of *E. coli* were interviewed about their demographics and use of antibiotics in the past three months. Medication and medical history were collected. After explaining the nature and the aim of the study, a consent form was obtained from each patient. Patients who did not want to participate in this study were excluded.

Inclusion and exclusion criteria. Woman patients with a positive *E. coli* urine culture who had a complex UTI diagnosis and symptoms consistent with UTI were included in this study. Patients who had an anatomical abnormality of the urinary tract system, an indwelling urinary catheter, or taking antibiotics at the time of sample collection, immunocompromised patients as well as unwilling patients to participate were excluded from the study.

Samples handling: samples receiving and storage. Specimens were collected from women attending the PMLAB group, Alkhalidi Hospital, and Albasheer Governmental Hospital who complained of urinary tract infections. They were directed to provide a morning midstream urine after washing the labia with soap and water. The specimens were transferred to the laboratory with a coolant pack. According to the standard procedure, the physical characteristics of the urine were evaluated such as sample color, odor, pH, volume, appearance, and specific gravity. For microscopic examination, samples were centrifuged at 2000 rounds per minute (rpm) for 5 minutes and the supernatants were discarded and sediments were mixed using a vortex. A drop was placed on a glass slide, covered with a coverslip, and examined at 40x magnification to investigate the presence of bacteria, epithelial cells, mucus, red blood cells (RBCs), and white blood cells (WBCs).

Each urine sample was streaked within fifteen to thirty minutes of submission on MacConkey, blood, and EMB agar plates using a loop and incubated aerobically for 24-48 hours at 37°C and the number of colonies was counted after the incubation. Cultures with a colony count of more than 10^5 cfu/ml were considered to be positive considering 10^5 cfu/ml as the cut-off value in the military hospital protocol according to IDSA guidelines (17). Growth of more than one bacterial species was considered contamination and the specimen was excluded. For short-term storage, samples were sub-cultured on nutrient agar and stored at 4°C. On the other hand, for long-term storage, four to five colonies of *E. coli* were inoculated in nutrient broth supplemented by glycerol and stored at -80°C (18).

McFarland turbidity standardization. An overnight culture from nutrient agar plates was suspended using the direct colony suspension method by selecting 5 pure colonies with an inoculation loop and transferring the growth to a tube of sterile saline to adjust its turbidity to 0.5 McFarland. Comparison was performed by comparing to a standard 0.5 McFarland turbidity solution.

Identification of isolated bacteria. A loop of urine was streaked on MacConkey agar plates and incubated at 37°C for 24 hours to obtain pure colonies. After the incubation period, MacConkey agar plates were examined for growth. A rose-pink colony indicates lactose fermentation while a pale translucent colony indicates no-lactose fermentation. Further Gram-staining was also performed (2).

Antimicrobial susceptibility testing. Thirteen antimicrobial agents were used for susceptibility testing of all *E. coli* isolates using the Kirby-Bauer method, purchased from Bioanalyse (Ankara, Turkey). The antibiotics used are listed in Table 1. Using the direct colony suspension technique, 3-4 colonies from an overnight pure single growth were dispersed in 5 mL of sterile physiological saline in a sterile tube. Turbidity was adjusted to 0.5 McFarland by comparison with a 0.5 McFarland turbidity standard solution. A

Table 1. Antibiotic discs were used for antibiotic susceptibility testing for *E. coli* isolates.

Antibiotic	Symbol	MIC (mm)	Concentration
Norfloxacin	NOR	17	10 µg
Oxacillin	OX	13	1 µg
Tigecycline	TGC	19	15 µg
Amikacin	AK	17	30 µg
Gentamicin	GM	15	10 µg
Rifampin	RF	20	5 µg
Levofloxacin	LEV	17	5 µg
Chloramphenicol	CL	18	30 µg
Ciprofloxacin	CIP	21	5 µg
Ampicillin	AM	29	10 µg
Cefazolin	CZ	18	30 µg
Sulphamethaxazole-Trimethoprim	SMX-TMP	17	10 µg
Nalidixic-acid	NA	15	5 µg

swab was streaked on the surface of Muller Hinton agar. Antimicrobial discs were then applied and the plates were incubated for 24 hours at 37°C, then the antibiotics were classified as resistant, intermediate, and sensitive by measuring the diameter of the inhibition zone using a ruler (19).

ESBL double disc synergy method. ESBL production was measured using the double disc diffusion method as recommended by CLSI (CLSI, 2011) (20). *E. coli* strains were incubated in Brain Heart Infusion broth (BHI) at 37°C for 24 h to reach an optical density of 0.5 McFarland (21). This suspension was streaked on MHA using a sterile cotton swab. Discs of ceftazidime CAZ (30 mg) and cefotaxime CTX (30 mg) both separately and in combination with ceftazidime/ clavulanic acid CZC (40 g) and with clavulanic acid, cefotaxime/ clavulanic acid CTC (40 kg) were placed on the surface of the bacteria on Muller Hinton agar. If there was an increase in the growth inhibitory zone around the ceftazidime or cefotaxime disc, the test was considered ESBL positive (22, 23).

Genotyping of ESBL-producing isolates. Genomic DNA was extracted using the Promega DNA Isolation Kit (Promega, USA) according to the manufacturer's instructions. The isolated DNA was stored at -20°C until further use (24).

PCR: primers. *E. coli* and ESBL primers were re-suspended by dissolving the lyophilised product in nuclease-free water to give a 100x stock primer concentration. To prepare the working primer concentration of IX, a given volume of stock was diluted with nuclease-free water. The forward primer for bla_{CTX} gene was F-5- GGGAGTAAAGTTAATACCTTTGCTC-3 (25 length bases) and the reverse primer was R-5-TTCCCGAAGGCACCAATC-3 (18 length bases) with amplicon size of 584.

And for bla_{TEM} (931 bp): F-TCCGCTCATGAGACAATAACC and R-TGGTCTGACAGTTACCAATGC (25).

PCR protocol. Uniplex PCR was performed to identify the *E. coli* species and find two of the ESBL genes (bla_{CTX-M} and TEM). Five sets of primers were used to type the ESBL genes in every phenotypically confirmed ESBL. Each PCR experiment contained 2 ul of bacterial template DNA, IX of forward and reverse primers (umol/mL or moles/mL) for each gene of interest, 12.5 ul of Go Tag Green Master Mix (Promega, USA) Master Mix, and 25 ul of nuclease-free water. A 25 ul PCR mixture was run through 35 cycles on a PCR thermocycler (MJ Research- INC, USA) as part of the uniplex PCR method. The PCR conditions employed in each cycle were as follows: denaturation for 20 seconds at 94°C, annealing for 20 seconds at 56°C or 60°C, and extension for 30 seconds at 72°C. The final extension was at 72°C for two min (26).

Initial denaturation at 94°C for 10 min was followed by 30 cycles of 94°C for 40 s, 60°C for 40 s, and 72°C for 1 min in the multiplex PCR reaction. The final extension step was conducted at 72°C for 7 min (27).

Gel electrophoresis. Red Safe Dye (iNtRON, Korea) and 2% agarose (Bio Basic Inc., Canada) were used to separate the PCR products. Two grams of agarose was added to100 milliliters of Tris base/borate/ EDTA (TBE) buffer (Carlo Erba Reagents, France). After 30 minutes of microwave cooking to solubilize the agarose, 10 ul of Red Safe Dye was added to a 2% agarose solution, which was then placed on an agarose gel electrophoresis tray and allowed to cool to room temperature. 10 microliters of the PCR product and 10 microliters of the ladder, a molecular weight marker, were added to each gel well. Electrophoresis (Biorad, USA) was run for one hour at 100 volts (28).

Statistical analysis. Statistical and data analyses

were performed to accomplish the objectives and analyze specific outcomes. All of the analyses were conducted using SPSS version 20 (IBM Corporation, Armonk, NY). Two-sided comparisons were used to determine significance, with a p-value < 0.05.

RESULTS

Demographic data. During four months (December 2023 - March 2024) urine samples were collected and examined from 412 females who complained of urinary tract infections. A total of 297 females were found to have urinary infections with colony counts above 10^5 CFU/ml. *E. coli* was the predominant infecting microorganism, followed by *Staphylococcus saprophyticus, Klebsiella* sp., *Serratia* sp., *Enterococci* sp., and *Proteus* sp. with frequencies of 198 (48.1%), 132 (32.0%), 51 (12.4%), 15 (3.6%), 10 (2.4%), and 6 (1.5%) respectively as Table 2 and Fig. 1 illustrate (p=0.05). The frequency of UTI among women showed no significant differences in UTI infections caused by *E. coli* and age groups. However, the relationship between age and female UTI caused by *E. coli* is shown in Table 3 in which five age groups were presented.

Table 2. Isolated causative microorganisms of UTIs in 412 females

Organism	Frequency	Percentage%
E. coli	198	48.1%
S. saprophyticus	132	32.0%
Klebsiella sp.	51	12.4%
Serratia sp.	15	3.6%
Proteus sp.	10	2.4%
Enterococci sp.	6	1.5%
Total	412	100%

P=0.05

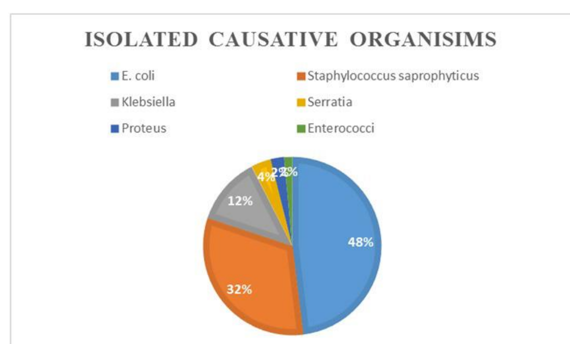

Fig. 1. Isolated causative microorganisms of UTIs in 412 females

***E. coli* species isolation and identification.** The identification of *E. coli* isolates was based on colony morphology and some biochemical tests. Regarding the shape and appearance of the colonies, on each of the nutrient, blood, MacConkey, and EMB agar, the colonies were round in shape and smooth in appearance. Regarding the colour of the colonies, in both nutrient and blood agar, the colonies were milky. While in MacConkey the colonies appeared in as pink and smooth. Whereas in EMB agar the colonies were shiny metallic green. For biochemical tests, an indole test was performed on all of the *E. coli* isolates, positive indole appeared as a red ring at the bottom of the tube and the negative Voges-Proskauer test appeared as yellow-brown. Hemolysin production was detected qualitatively in 49.2% of the *E. coli* isolates, the hemolysin production was detected by the presence of a clear zone of erythrocyte lysis on the blood agar (2).

Relation of some variables with *E. coli* infection. Table 4 shows the relationship between women whose culture result was positive and those who were married. Of the entire 412 patients, 310 were married and 102 were not. Similarly, out of the 310 married women, 197 (67.5%) had a positive culture result. Regarding the *E. coli* infection, out of 198 infected married women, 124 (62.6) were infected with *E. coli* and 74 (37.4) were not married. There was a significant difference between married infected and non-married women (P < 0.05).

UTIs were much more prevalent in non-pregnant women (65.8%) compared to pregnant women (34.2%). The rates were similar in non-pregnant and pregnant females infected with *E. coli* (Table 5).

The relationship between the number of pregnancies and the occurrence of *E. coli* in female UTIs is illustrated in Table 6. UTIs caused by *E. coli* were more frequent in women with 1-3 pregnancies. Significant differences were found between the different number of pregnancies and the incidence of *E. coli* infections (P< 0.05).

Recurrent female UTIs were observed in 187 (64.0%) of infected females and *E. coli* infection was found in 189 (95.5%) of recurrent cases (Table 7).

Antibiotic susceptibility test: disk diffusion

Table 3. Occurrence of *E. coli* to age group

Age group in years	Number of patients n= 412	No. (%) of infected patients n= 297	No. (%) of *E-coli*-infected patients n= 198
0-9	101	57 (56.4)	44 (43.6)
10-19	84	46 (54.8)	38 (45.2)
20-29	99	66 (66.7)	33 (33.3)
40-49	104	55 (52.8)	49 (47.1)
More than 50	24	17 (70.8)	7 (29.2)

P<0.05

Table 4. The correlation between *E. coli* infection occurrence and marital status

Marital status	Total number of pa-tients N= 412	No. (%) of infected patients N= 292	No. (%) of *E. coli*-infected patients N=198
Married	310	197 (67.5)	124 (62.6)
Non-married	102	95 (32.5)	74 (37.4)

P<0.05

Table 5. The correlation between *E. coli* infection occurrence and pregnancy

Females	No. (%) of infected patients N= 292	No. (%) of *E. coli*-infected patients N=198
Pregnant	100 (34.2)	101 (52.0)
Non-pregnant	192 (65.8)	97 (48.0)

Table 6. The correlation between *E. coli* infection occurrence and number of pregnancies

Females	No. (%) of infected pregnant patients N= 192	No. (%) of *E. coli*-infected pregnant patients N=101
1-3	72 (37.5)	43 (42.6)
3-5	53 (27.6)	36 (35.6)
More than 5	67 (34.9)	22 (21.8)

Table 7. Association between ***E. coli*** infection and UTIs recurrence

Status of infection	No. (%) of infected patients N= 292	No. (%) of *E. coli*-infected patients N= 198
Recurrent	187 (64.0)	189 (95.5)
Non-recurrent	105 (36.0)	9 (4.5)

method. All *E. coli* isolates were tested using the disc diffusion technique, and the findings were interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2024). The results for each antibiotic were classified as resistant, intermediate, or susceptible. The highest percentage resistant of *E. coli* to Nalidixic-acid antibiotic was (78.8%) and the highest percentage sensitive of *E. coli* to Gentamicin. was (64.1%) and Tigecycline was (59.6%) as shown in Table 8.

ESBL detection using double disc diffusion test. ESBL production was assessed in all genetically verified *E. coli* isolates using the CLSI recommended method of double disc diffusion (CLSI, 2019), even if the phenotypic screening test for ESBL was not performed. The test was considered positive when the growth-inhibitory zone around the cefotaxime or ceftazidime disc with clavulanic acid increased by 5 mm or more compared to the diameter surrounding the disc containing cefotaxime or ceftazidime alone (29). Among the *E. coli* isolates, 25(12.6%) were ESBL-producers.

Genotypic identification of ESBL-producing genes. Among the phenotypically confirmed *E. coli,* and the ESBL- producers confirmed by disc diffusion method, 20 (10.1%) of the *E. coli* isolates were positive in having bla_{CTX-M} gene. Fig. 2 shows a posi-

Table 8. Antibiotic susceptibility testing

Antibiotics	Disc content (µg)	Resistant	Intermediate	Sensitive
Norfloxacin	10 µg	96 (48.5%)	5 (2.6%)	97 (49.0%)
Oxacillin	1 µg	100 (50.5%)	1 (0.5%)	97 (49.0%)
Tigecycline	15 µg	80 (40.4%)	0 (0.0%)	118 (59.6%)
Amikacin	30 µg	90 (45.5%)	0 (0.0%)	108 (54.5%)
Gentamicin	10 µg	56 (28.3%)	15 (7.56%)	127 (64.1%)
Rifampin	5 µg	100 (50.5%)	6 (3.0%)	102 (51.5%)
Levofloxacin	5 µg	96 (48.5%)	7 (3.5%)	95 (48%)
Chloramphenicol	30 µg	70 (35.5%)	5 (2.5%)	123 (62.1%)
Ciprofloxacin	5 µg	77 (38.9%)	21 (10.6%)	100 (50.5%)
Ampicillin	10 µg	110 (55.6%)	7 (3.4%)	81 (41.0%)
Cefazolin	30 µg	100 (55.5%)	9 (4.5 %)	80 (40.4%)
Sulphamethaxa-zole-Trimethoprim	10 µg	108 (54.5%)	0 (0.0%)	90 (45.5%)
Nalidixic-acid	5 µg	150 (75.8%)	0 (0.0%)	48 (24.2%)

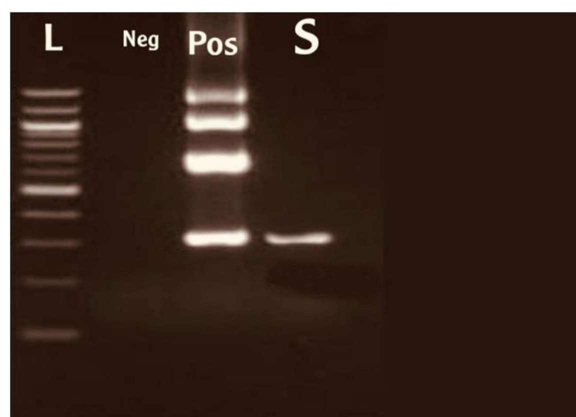

Fig. 2. Gel electrophoresis showing a positive *E. coli* strain that carries the bla_{CTX-M} gene. L: Ladder 100 bp, Neg: Negativetaive control (nuclease free water), Pos: Positive control (bla_{CTX-M} gene positive *E. coli* expected bands 500-600 bp), S: *E. coli* Sample.

tive *E. coli* strain that carry bla_{CTX-M} gene.

DISCUSSION

E. coli is the most common cause of UTI, accounting for 70-80% of cases. ESBL-producing *E. coli* are challenging because of their coding genes that are constantly changing and resistance to several β-lactam antibiotics which limits the treatment options. To our knowledge, little attention has been paid in Jordan to identify the risk factors and evaluate the prevalence of ESBL among UTIs-causing *E. coli* strains. Therefore, this study focused on the risk factors, phenotypic, and genotypic characterisation of ESBL-producing *E. coli* among patients with UTIs. The spread of ESBL genes among uropathogenic *E. coli* has made it more difficult to treat UTIs because of its resistance to many antibiotics like β-lactams. We should understand the virulence factors and resistance genes associated with ESBL-producing *E. coli* in UTIs patients especially females to create an effective treatment strategy and control the spread of antibiotic resistance.

This study has showed that *E. coli* was the predominant infecting microorganism in women with UTI, which may be due to the structure of the woman lower urinary tract and its proximity to the reproductive organs. Our results were in accordance with the results of a study performed by Khani et al. (2021) (30) who investigated the prevalence of ESBL-producing *E. coli* strains and found that *E. coli* was the most common microorganism that infect women. Another study by Mlugu et al. (2023) (31) investigated the microorganisms isolated from women with UTI and found that *E. coli* was the predominate species. Regarding the relationship between age and the occurrence of *E. coli* in women, the current study found that the age group of 40-49 years old was the most affected by UTIs. Some studies have looked at the risk factors for UTIs in women and found that this is the age at which most women are infected (31, 32). A strong association has been found between UTI infections and marital status, with married women having more UTIs than single women. This may be due to the anatomy of reproductive system in wom-

en and less hygienic procedures (33). Studies have also found that the highest percentage of UTIs occurs among married women in comparison with single women. Furthermore, married women were found to be more prone to develop UTIs (34, 35).

Pregnancy is a significant factor that contributes to UTIs, with 51.0% of pregnant women in this study having UTIs, which would be due to the pressure on the bladder (36). It was found that 68.5% of pregnant woman had developed UTIs. Regarding the number of pregnancies, women with1-3 pregnancies had the highest percentage of UTIs. ESBL production was observed in 25 (12.6%) of the women who had urinary tract infections at least one time in their lives. *E. coli* was the most resistant to erythromycin. Similar results were illustrated in other studies (37, 38) Another investigation by Chaudhry et al. (2014) (39) showed that 91% of *E .coli* isolates were resistant to erythromycin. In developed countries, these variations and disparities in antibiotic susceptibility profiles may be related to changes in hospital treatment procedures, sample population, sample size, infection control efforts, and level of education on glycemic control. The $bla_{\text{CTX-M}}$ gene was present in 20 (10.1%) of the *E. coli* isolates. These results are consistent with observations of many researchers who had studied the prevalence of some of the genes responsible for ESBL and found that the $bla_{\text{CTX-M}}$gene was the predominant gene among the isolates. This gene is known for its ability of causing therapeutic problems in ESBL *E. coli* infections (40).

CONCLUSION

In conclusion, our study has shown that *E. coli* is the most common cause of UTI and that ESBL production leads to increased resistance to common antibiotics and complicates treatment strategies. This study focused on understanding the virulence factors and resistance mechanisms associated with ESBL-producing *E. coli* in women UTIs. By understanding these factors, we can develop more effective treatment and infection control strategies to reduce the spread of antibiotic resistance and improve clinical outcomes. By analysing demographic data and clinical characteristics we observed a high prevalence of UTIs caused by *E. coli* in women especially in the age group of 40-49 years. In addition, marital status is an important factor in married females which shows a higher susceptibility to UTIs. However, further studies are needed to understand the mechanisms of pathogenesis and antibiotic resistance in ESBL-producing *E. coli* strains and their effects on the severity and recurrence of UTIs. This research is important to guide the development of targeted therapeutic interventions and public health initiatives to reduce the prevalence of ESBL-producing *E. coli* UTIs.

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