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Molecular Detection of Selected Extended-Spectrum Beta-Lactamase Resistant Genes in Escherichia coli Isolated from Cancer Patients with Urinary Tract Infections in Abuja

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Urinary tract infections (UTIs) are among the most common infections in cancer patients and are frequently complicated by antimicrobial resistance, particularly due to extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli. This study aimed to determine the prevalence, antimicrobial resistance patterns, and selected molecular characteristics of ESBL-producing E. coli isolated from cancer patients with UTIs at the National Hospital Abuja, Nigeria. A total of 162 urine samples were collected and analyzed using standard microbiological methods. Isolates were identified as E. coli, followed by antibiotic susceptibility testing in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. Phenotypic detection of ESBL production was performed using antibiotic susceptibility testing and the VITEK 2 Compact system, while molecular detection of selected ESBL genes was carried out using polymerase chain reaction (PCR). Out of 162 samples, 18 (11.2%) E. coli isolates were recovered. A higher occurrence was observed in males (15.0%) compared to females (9.8%), and among patients aged ≥70 years (21.4%). The isolates exhibited high resistance to ampicillin (100%), cefuroxime (83.3%), trimethoprim-sulfamethoxazole (77.7%), and ceftriaxone (66.6%), while showing complete susceptibility to amikacin and meropenem (100%). Multiple antibiotic resistance (MAR) indices ranged from 0.5 to 0.9, with 0.8 being the most frequent. Of the 18 isolates, 8 (44.4%) were confirmed as ESBL producers. Among these ESBL-producing isolates, blaSHV, blaCTX-M-4, and blaCTX-M genes were detected in 62.5%, 50.0%, and 37.5% of isolates, respectively, while blaTEM was not detected. In conclusion, there is a notable presence of multidrug-resistant and ESBL-producing E. coli among cancer patients with UTIs in the study area, posing a significant therapeutic challenge. These findings highlight the need for routine surveillance, antimicrobial stewardship, and the integration of molecular diagnostics in clinical settings, and clinicians are encouraged to adopt evidence-based antibiotic therapy and strengthen infection control practices to limit the spread of resistant strains.

Keywords: Urinary tract infections, *Escherichia coli*, extended-spectrum beta-lactamase (ESBL), antimicrobial resistance, polymerase chain reaction (PCR)

Introduction

Cancer remains a leading cause of morbidity and mortality worldwide, with a disproportionately higher burden in low- and middle-income countries (World Health Organization [WHO], 2023). Advances in cancer treatment, including chemotherapy, radiotherapy, and targeted therapies, have significantly improved patient survival. However, these therapeutic interventions are often associated with immunosuppression, which increases patients' susceptibility to opportunistic infections (Kamboj & Sepkowitz, 2021). Immunocompromised cancer patients are therefore at a higher risk of developing infections that are more severe, recurrent, and difficult to treat.

Urinary tract infections (UTIs) are among the most common infections affecting cancer patients and are associated with increased morbidity, prolonged hospital stay, and higher healthcare costs (Medina & Castillo-Pino, 2019). UTIs occur when microorganisms colonize and multiply within the urinary tract, often originating from the gastrointestinal flora, with risk factors such as catheterization, hospitalization, and weakened immunity contributing significantly. Among uropathogens, Escherichia coli is the most frequently implicated organism, responsible for the majority of UTIs (Asmare *et al*., 2024). In cancer patients, infections caused by E. coli are particularly concerning due to impaired host defences and increasing antimicrobial resistance.

Despite increasing reports of extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli in Nigeria and sub-Saharan Africa, there remains limited data specifically focusing on immunocompromised populations such as cancer patients, who are at a higher risk of infection and adverse clinical outcomes. Most existing studies in the region have largely emphasized general patient populations, with less attention given to the unique resistance dynamics and molecular characteristics of isolates obtained from oncology settings. Furthermore, while ESBL genes such as blaCTX-M and blaSHV have been widely reported, there is still insufficient localized data on their distribution and co-occurrence patterns in clinical isolates from tertiary healthcare institutions in Abuja.

In addition, variations in antimicrobial resistance profiles across different regions and healthcare settings highlight the need for context-specific data to guide empirical therapy. The integration of phenotypic and molecular approaches in this study provides a more comprehensive understanding of ESBL-mediated resistance among uropathogenic E. coli in cancer patients. Therefore, this study aims to determine the prevalence, antimicrobial resistance patterns, and selected ESBL genes (blaCTX-M, blaCTX-M-4, blaSHV, and blaTEM) among E. coli isolates from cancer patients with urinary tract infections at the National Hospital Abuja, thereby contributing localized molecular epidemiological data to inform clinical management and antimicrobial stewardship in this high-risk population.

Materials and Methods

Materials

All culture media used in this study, including Cysteine Lactose Electrolyte Deficient (CLED) agar, Mueller–Hinton agar (MHA), Luria–Bertani (LB) broth, peptone water, and 5% sheep blood agar, were obtained from Oxoid Ltd. (UK). Reagents included Gram staining reagents (crystal violet, Lugol's iodine, acetone, and neutral red), Kovac's reagent (Oxoid, UK), and normal saline.

Equipment utilized included an autoclave (Certoclav, UK), incubator (Quincy Lab Inc., USA), hot air oven (Memmert, Germany), microscope (Leica, USA), vortex mixer, analytical weighing balance (Bio-Rad, UK), and the VITEK 2 Compact system (bioMérieux, France). Additional consumables included sterile Petri dishes, test tubes, pipettes, swab sticks, universal containers, and disposable loops. Primers targeting ESBL genes (blaTEM, blaCTX-M, blaSHV, and blaCTX-M-4) were used as previously described (Ibrahim *et al*., 2022; Raseala *et al*., 2020).

Study area and design

This cross-sectional study was conducted at the National Hospital, Abuja, Nigeria, a major referral center located in the Federal Capital Territory (Abuja Master Plan, 2020).

Ethical approval

Ethical approval was obtained from the Research Ethics Committee of the National Hospital, Abuja.

Sample size determination

The sample size was calculated using Fisher's formula:

$$n = \frac{Z^2 P(1-P)}{d^2}$$

where Z = 1.96 at 95% confidence level, P = 0.12 (estimated prevalence), and d = 0.05 (margin of error), yielding a minimum sample size of 162. A total of 162 urine samples were therefore collected and analyzed in this study to meet the calculated requirement.

Sample collection and processing

Midstream urine samples (5 mL) were aseptically collected from consenting cancer patients into sterile containers and processed within one hour. Samples were cultured using a calibrated loop (0.001 mL) on CLED and blood agar, followed by incubation at 37°C for 18–24 hours. Significant bacteriuria was defined as $\geq 10^5$ CFU/mL (Hooton *et al*., 2021).

Isolation and identification of *escherichia coli*

Isolates were identified based on colony morphology, Gram staining, and biochemical tests including indole and citrate utilization (Cheesbrough, 2006). Confirmation was performed using the VITEK 2 Compact system (bioMérieux, France).

Antimicrobial susceptibility testing and ESBL detection

Antimicrobial susceptibility testing was performed using the VITEK 2 Compact system (bioMérieux, France) and interpreted in accordance with Clinical and Laboratory Standards Institute (CLSI, 2023) guidelines. ESBL production was phenotypically detected using the same system. Quality control for antimicrobial susceptibility testing was ensured using standard reference strains, including Escherichia coli ATCC 25922 as a negative control for ESBL production and quality assurance of antibiotic susceptibility results. All procedures were conducted in accordance with CLSI recommendations to ensure accuracy and reproducibility.

The Multiple Antibiotic Resistance (MAR) index was calculated as:

$$\text{MAR Index} = \frac{\text{Number of antibiotics resisted}}{\text{Total number of antibiotics tested}}$$

(Krumperman, 1983; Nkene *et al*., 2019).

DNA extraction and quantification

Genomic DNA was extracted from confirmed E. coli isolates using the alkaline lysis method (Abimiku *et al*., 2016). DNA concentration was determined using a NanoDrop spectrophotometer.

Molecular detection of ESBL genes

Multiplex polymerase chain reaction (PCR) was performed to detect blaTEM, blaCTX-M, blaSHV, and blaCTX-M-4 genes. Amplification conditions included initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation (94°C for 1 minute), annealing (55°C for 30 seconds), and extension (68°C for 30 seconds), with a final extension at 68°C for 7 minutes. PCR products were resolved on 1% agarose gel and visualized under ultraviolet light.

To ensure reliability of the molecular assay, appropriate quality control measures were implemented. Known ESBL-producing E. coli strains were used as positive controls for target gene amplification, while nuclease-free water served as a negative control to detect contamination. All PCR reactions were performed in duplicate, and only reproducible bands were considered valid. DNA extraction quality and concentration were verified prior to amplification using a NanoDrop spectrophotometer.

Results

Isolation and identification of *Escherichia coli*

A total of 162 urine samples were analyzed, from which 18 (11.2%) Escherichia coli isolates were obtained. The isolates were identified based on cultural, morphological, and biochemical characteristics, including yellowish colonies on CLED agar, translucent colonies on blood agar, Gram-negative rod shape, indole positivity, methyl red positivity, and citrate negativity. These findings are consistent with standard identification criteria for E. coli. See Table 1 in the appendix.

Prevalence of *Escherichia coli*

The overall prevalence of E. coli was 11.2%. A higher prevalence was observed in males (15.0%) compared to females (9.8%), although this difference was not statistically significant ($\chi^2 = 0.5828$, $p = 0.4452$). Age-related distribution showed the highest prevalence among patients aged ≥70 years (21.4%), while the lowest prevalence was observed in the 31–40 age group (4.3%), with no statistically significant association ($p = 0.1808$).

Table 2: Occurrence of *Escherichia coli* isolated from cancer patients with urinary tract infection in relation to gender

Gender	No. Sample	No. isolated	Percentage (%)
Male	40	6	15.0
Female	122	12	9.8
Total	162	18	11.1

KEY: X2 = Chi-square; *X*2 = 0.5828, *P-value* = 0.4452

Antimicrobial susceptibility pattern

The isolates demonstrated high resistance to ampicillin (100%), cefuroxime (83.3%), trimethoprim-sulfamethoxazole (77.7%), and ceftriaxone (66.6%). Moderate resistance was observed for ciprofloxacin and ampicillin-sulbactam (55.5%), while lower resistance rates were recorded for gentamicin (33.3%), nitrofurantoin (27.7%), and piperacillin/tazobactam (16.6%). In contrast, all isolates were fully susceptible to amikacin and meropenem (100%).

Table 3: Antibiotics susceptibility of *Escherichia coli* isolated from cancer patients with urinary tract infection

Antibiotics	Disc Content(µg)	**No. (%) Resistance (n= 18)**	**No. (%)** Susceptible **(n= 18)**
Amikacin (AK)	30	0 (0.0)	18 (100)
Gentamicin (CN)	10	6(33.3)	12(66.6)
Meropenem (MEM)	5	0(0.0)	18(100)
Piperocillin/ Tazobactam (TZP)	30	3(16.6)	15(83.3)
Nitrofuratoin (F)	30	5(27.7)	13(72.2)
Cefuroxime (CXM)	30	15(83.3)	3(16.6)
Ceftriazone (CRO)	30	12(66.6)	6(33.3)
Amoxicillin-Clavulanic acid (AMC)	30	9(50.0)	9(50.0)
Ampicillin (AMP)	30	18(100.0)	0(0.0)
Trimethoprim sulfamethoxazole (SXT)	30	14(77.7)	4(22.2)
Ciprofloxacin (CIP)	5	10(55.5)	8(44.4)
Ampicillin sulbactam (SAM)	30	10(55.5)	8(44.4)
Chloramphenicol (C)	25	7 (38,8)	11(61.1)

Antibiotic resistance profile and MAR index

All isolates exhibited multidrug resistance, with MAR indices ranging from 0.5 to 0.9. The most frequent MAR index was 0.8 (27.7%), followed by 0.5 and 0.7 (22.2% each), indicating exposure to high-risk environments with significant antibiotic pressure. The most common resistance profile included CRO–CXM–AMC–AMP–C–SXT–CIP–SAM (16.6%).

Table 4: Antibiotic Resistance Profile of *Escherichia coli* isolated from cancer patients with urinary tract infection

Antibiotic resistance profile	Frequency (%) (n=18)
CRO- CXM-AMC- C- CIP	1(5.5)
CRO- TZP- AMC- AMP- SXT	1(5.5)
CRO- CXM- CN- AMP- SXT	2(11.1)
CRO- CXM- AMC- AMP- CN- F	2(11.1)
CRO- CXM- AMC- AMP- CIP- SXT	2(11.1)
CRO- CXM- AMC- AMP- TZP- SXT	1(5.5)
CRO- CXM- AMC- AMP- C- CN- CIP	1(5.5)
CRO- CXM- AMC- CN- AMP- F- SXT	2(11.1)
TZP- CXM- AMC- AMP- SXT- SAM- C	1(5.5)
CRO- CXM- AMC- AMP- SXT- C- CN- F	2(11.1)
CRO- CXM- AMC- AMP- C- SXT- CIP- SAM	3(16.6)
CXM- AMC- AMP- C- CN- SXT-CIP- SAM- F	2(11.1)

Table 5: Multiple Antibiotics Resistance (MAR) Index of *Escherichia coli* isolated from cancer patients with urinary tract infection

No. of antibiotic resistance to (a)	No. of antibiotics tested (b)	MAR index (a/b)	Frequency (%) (n=18)
10	10	1.0	0 (0.0)
9	10	0.9	2 (11.1)
8	10	0.8	5 (27.7)
7	10	0.7	4 (22.2)
6	10	0.6	3 (16.6)
5	10	0.5	4 (22.2)
4	10	0.4	0 (0.0)
3	10	0.3	0 (0.0)
2	10	0.2	0 (0.0)
1	10	0.1	0 (0.0)

Phenotypic and molecular detection of ESBL

Out of the 18 E. coli isolates, 8 (44.4%) were confirmed as ESBL producers, while 10 (55.6%) were non-ESBL producers. Molecular analysis of the ESBL-producing isolates revealed the presence of blaSHV in 62.5% (5/8), blaCTX-M-4 in 50.0% (4/8), and blaCTX-M in 37.5% (3/8), while blaTEM was not detected in any isolate.

Table 6: Phenotypic Detection of Extended Spectrum B-Lactamase Production in Cephalosporin Resistant *Escherichia coli* isolated from cancer patients with urinary tract infection

ESBL	No. (%) isolated
Production	8 (44.4)
Non-production	10 (40.0)
Total	18 (11.2)

KEY: % = Percent

Occurrence of extended spectrum β -lactamase resistance genes

The occurrence of ESBL resistance genes from E. coli producing ESBL is as shown in Table 7. Out of 8 isolates 3(37.5%) were detected to encode blaCTX-M genes, 4 (50.0 %) were detected to encode blaCTX-M-4 genes, 5 (62.5%) were detected to encode blaSHV genes and none was detected to encode for blaTEM as shown in agarose gel electrophoresis in Plate 4.1 to 4.3 respectively.

Table 7: Occurrence of Extended Spectrum Beta-Lactamase Resistance Genes in *Escherichia coli* isolated from cancer patients with urinary tract infection

ESBL genes	*Escherichia coli*	
	No. isolates	No. (%) detected
blaCTX-M	8	3(37.5)
blaCTX-M-4	8	4(50.0)
blaSHV	8	5(62.5)
BlaTEM	8	0(0.00)

KEY: SHV: Sulphydryl variable, TEM: Temoneira, CTX-M: Cefotaximase –munchen, bla: beta- lactamase

Discussion

Urinary tract infections (UTIs) remain a significant clinical concern among cancer patients due to their immunocompromised status, which predisposes them to opportunistic infections. In this study, the prevalence of Escherichia coli was 11.2%, confirming its role as a key uropathogen in this population. This finding aligns with recent reports identifying E. coli as the leading cause of UTIs globally (Asmare *et al*., 2024; Murray *et al*., 2022). However, the prevalence observed in this study is lower than some reports from other regions, which may reflect differences in study populations, healthcare settings, and infection control practices.

Although a higher prevalence of E. coli was observed among male patients (15.0%) compared to females (9.8%), this difference was not statistically significant. Similarly, the higher occurrence observed among patients aged ≥70 years did not demonstrate a statistically significant association. These findings should therefore be interpreted with caution, as they do not establish a definitive relationship between gender or age and infection risk within this study population. Comparable studies have also reported variable associations between demographic factors and UTI prevalence, often influenced by clinical conditions such as catheterization and hospitalization rather than inherent demographic predisposition (Medina & Castillo-Pino, 2019; Shawon & Paul, 2024).

The antimicrobial resistance profile observed in this study is of considerable clinical concern. High resistance rates to commonly used antibiotics such as ampicillin, cefuroxime, trimethoprim-sulfamethoxazole, and ceftriaxone were identified. While this study does not assess temporal trends, these findings are consistent with recent global and regional reports indicating a high burden of antimicrobial resistance among uropathogenic E. coli isolates (Murray et al., 2022; Mahajan et al., 2024). The observed resistance patterns may therefore reflect the broader challenge of antimicrobial misuse and limited stewardship in many healthcare settings, rather than a trend demonstrated within this study.

Conversely, the complete susceptibility of isolates to amikacin and meropenem supports their continued effectiveness against multidrug-resistant E. coli. This observation is consistent with recent studies reporting preserved activity of carbapenems and certain aminoglycosides against ESBL-producing organisms (Montelin *et al*., 2024). However, reliance on these agents should be approached cautiously, as increased use may contribute to the emergence of further resistance.

All isolates in this study exhibited multidrug resistance, with MAR indices ≥0.5, suggesting exposure to environments with substantial antibiotic pressure. High MAR indices have been associated with high-risk sources such as hospital environments, where antibiotic use is frequent (Nkene *et al*., 2019). These findings underscore the importance of strengthening antimicrobial stewardship and infection control practices in clinical settings.

The prevalence of ESBL-producing E. coli in this study was 44.4%, indicating a substantial burden of β-lactam resistance among isolates. This finding is comparable to recent reports highlighting the increasing global distribution of ESBL-producing Enterobacterales, particularly in healthcare-associated infections (Montelin *et al*., 2024; Kalu *et al*., 2025). Molecular analysis revealed the presence of blaSHV, blaCTX-M-4, and blaCTX-M genes, supporting existing evidence that CTX-M-type enzymes are among the most распространённые ESBL determinants worldwide (Kalu et al., 2025). The absence of blaTEM in this study may reflect regional variation in gene distribution or differences in selective pressure within the study setting.

Overall, the findings of this study demonstrate a significant burden of multidrug-resistant and ESBL-producing E. coli among cancer patients with UTIs in Abuja. While the study is limited to a single center and does not evaluate temporal trends, it provides important localized data on antimicrobial resistance patterns and ESBL gene distribution in a high-risk population. These findings reinforce the need for continuous surveillance, rational antibiotic use, and integration of molecular diagnostics to guide effective clinical management and reduce the spread of resistant pathogens.

Conclusion

This study demonstrated a prevalence of 11.2% *Escherichia coli* among cancer patients with urinary tract infections at the study site, with the isolates exhibiting high levels of antimicrobial resistance to commonly used antibiotics, including ampicillin, cefuroxime, trimethoprim-sulfamethoxazole, and ceftriaxone. In contrast, complete susceptibility to amikacin and meropenem was observed, indicating their continued effectiveness for the management of complicated infections within this setting. A substantial proportion of the isolates (44.4%) were confirmed as extended-spectrum β-lactamase (ESBL) producers, with the detection of blaSHV, blaCTX-M-4, and blaCTX-M genes, highlighting the molecular basis of resistance among these strains.

These findings indicated a notable burden of multidrug-resistant and ESBL-producing E. coli in this high-risk population, which may complicate treatment outcomes and limit therapeutic options. However, this study does not assess temporal trends, and therefore the results should be interpreted within the context of existing literature reporting widespread antimicrobial resistance among uropathogens in similar settings. The findings have important implications for clinical practice and public health. They underscore the need for continuous antimicrobial resistance surveillance, strengthened antimicrobial stewardship programs, and the integration of molecular diagnostic approaches in routine clinical management. These measures are essential to guide evidence-based therapy, reduce inappropriate antibiotic use, and limit the spread of resistant pathogens, particularly among vulnerable populations such as cancer patients.

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Table 1: Cultural, morphological and biochemical characteristics of *Escherichia coli* isolated from cancer patients with urinary tract infection

Cultural characteristics	Morphological characteristics		Biochemical characteristics								Inference
	Gram stain	Morphology	Ind	Mr	Vp	Ct	Lac	Glu	Gal	Suc	
Pinkish colonies o MCA and greenis metallic shee colonies on EMl agar	-	Rod shape	+	+	-	-	+	+	+	+	*E. coli*

KEY: MCA= Mac Conkey Agar; EMB= Eosin Methylene blue; - =Negative; + =positive; Ind =Indole; Mr = Methyl Red; Vp =Voges Proskauer; Ct =Citrate; Lac=Lactose; Glu = Glucose; Gal =Galactose; Suc=Sucrose.