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https://doi.org/10.7616/gaijihs.v1.i19

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PREVALENCE, ANTIMICROBIAL SUSCEPTIBILITY PATTERNS, AND MOLECULAR CHARACTERIZATION OF ESBL-PRODUCING ESCHERICHIA COLI AMONG PATIENTS WITH URINARY TRACT INFECTIONS IN EDO STATE, NIGERIA

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ARTICLE INFO

Received Date: 12th March, 2026
Accepted Date: 10th April, 2026
Published Date: 12th April, 2026

Citation: Olley, M. & Osaiyuwu, O.C (2026) Prevalence, Antimicrobial Susceptibility Patterns, and Molecular Characterization of Esbl-Producing Escherichia Coli Among Patients with Urinary Tract Infections in Edo State, Nig.: GAIJHS; Vol.1, Issues I, 27-44 April ,2026

ABSTRACT

Background: Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* has emerged as a significant public health concern due to its role in antimicrobial resistance and treatment failure in urinary tract infections (UTIs). Continuous surveillance of resistance patterns and molecular characterization of ESBL genes are essential for effective infection control and antimicrobial stewardship.
Aim: This study investigated the prevalence, antimicrobial susceptibility patterns, and molecular characteristics of ESBL-producing *Escherichia coli* isolated from urine samples of patients with UTIs attending Igbinedion University Teaching Hospital, Okada, Edo State, Nigeria.
Methods: A cross-sectional study was conducted between April and June 2025. Four hundred midstream urine samples were collected from patients presenting with symptoms of UTIs. Samples were cultured on Cystine Lactose Electrolyte Deficient (CLED) agar, and bacterial isolates were identified using standard microbiological methods. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method following Clinical and Laboratory Standards Institute (CLSI) guidelines. ESBL production was detected phenotypically, while polymerase chain reaction (PCR) was used to confirm *E. coli* isolates and detect ESBL genes (blaCTX-M, blaTEM, and blaSHV).
Results: Of the 400 urine samples analyzed, 160 (40.0%) yielded significant bacterial growth. *Escherichia coli* accounted for 39 (24.4%) of the isolates. Phenotypic ESBL production was detected in 19 (48.7%) isolates, while ESBL genes were identified in 16 (84.2%) of these isolates by PCR. The predominant ESBL gene was blaCTX-M (68.8%), followed by co-occurrence of blaCTX-M and blaTEM (18.8%), while blaTEM alone was detected in 12.5% of isolates. No blaSHV-positive isolate was detected. Antimicrobial susceptibility testing revealed that amikacin exhibited the highest activity against *E. coli* isolates (64.1% susceptible). High resistance rates were observed for cotrimoxazole (89.7%), amoxicillin-clavulanate (74.4%), ciprofloxacin (53.8%), aztreonam (53.8%), gentamicin (53.8%), ceftriaxone (51.3%), and ceftazidime (51.3%). Multidrug resistance and extensive drug resistance were observed in 27 (69.2%) and 10 (25.6%) isolates, respectively.
Conclusion: This study demonstrated a high prevalence of multidrug-resistant and ESBL-producing *E. coli* among patients with UTIs in Okada, Nigeria, with blaCTX-M identified as the predominant ESBL gene. The high resistance rates to commonly prescribed antibiotics underscore the need for routine antimicrobial resistance surveillance, molecular monitoring of resistance determinants, and strengthened antimicrobial stewardship programs to curb the spread of resistant uropathogens.
Keywords: *Escherichia coli*, urinary tract infection, extended-spectrum β-lactamase, antimicrobial resistance, *blaCTX-M*, multidrug resistance, Nigeria.

https://doi.org/10.7616/gaijihs.v1.i19

INTRODUCTION

Antimicrobial resistance (AMR) has emerged as one of the most critical global health threats in the 21st century, undermining the effectiveness of antibiotics that once served as the foundation for treating infectious diseases (World Health Organization [WHO], 2023). Extended-spectrum β-lactamases (ESBLs) are bacterial enzymes capable of degrading and neutralizing a wide range of β-lactam antibiotics, including penicillins, cephalosporins, and monobactams, although carbapenems generally remain effective (Bush & Bradford, 2020). These enzymes are commonly produced by members of the Enterobacterales, particularly *Escherichia coli* and *Klebsiella pneumoniae*, and are of significant concern in both clinical and community settings (Castanheira et al., 2021; Yassara et al., 2025).

Infections caused by ESBL-producing bacteria are associated with increased severity of illness, higher mortality rates, prolonged hospitalization, elevated treatment costs, and limited therapeutic options (Bush & Bradford, 2020; Abay et al., 2025; Mustafa et al., 2025). ESBL-producing *E. coli* is a major cause of infections such as urinary tract infections (UTIs), bloodstream infections, and pneumonia, particularly among hospitalized and immunocompromised individuals (Tamma et al., 2021; Qibtiyah et al., 2025). Urinary tract infections remain among the most common bacterial infections worldwide, but the emergence of multidrug-resistant *E. coli* has complicated treatment strategies and, in severe cases, resulted in treatment failure (Flores-Mireles et al., 2015; Mancuso et al., 2023; Dao et al., 2026).

The occurrence of ESBL-producing *E. coli* varies across geographical regions but is particularly pronounced in developing countries such as Nigeria. Research conducted in a Nigerian hospital revealed that 35% of *E. coli* isolates produced ESBLs, highlighting the growing challenge of antimicrobial resistance in clinical practice (Nwafia et al., 2019; Alabi et al., 2025; Olaitan et al., 2025). These resistant strains are frequently associated with risk factors such as previous antibiotic use, recent surgical procedures, prolonged hospital stays, intensive care unit admissions, and the use of invasive medical devices (Karanika et al., 2016). In community settings, ESBL-producing bacteria may also be transmitted through zoonotic routes, including direct contact with livestock and consumption of contaminated food products (Ewers et al., 2012).

The spread of ESBL-producing bacteria is further exacerbated by inappropriate antibiotic use, inadequate infection prevention and control measures, and weak antimicrobial resistance surveillance systems (WHO, 2023). Consequently, there have been global calls for strengthened antimicrobial stewardship programs and improved resistance monitoring to mitigate the growing burden of AMR (Centers for Disease Control and Prevention [CDC], 2023; WHO, 2023). Continuous surveillance of resistance patterns is essential not only for guiding effective treatment but also for preventing the dissemination of resistant strains within healthcare facilities and the wider community (Prestinaci et al., 2015).

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Therefore, this study aimed to determine the prevalence, antimicrobial susceptibility pattern, and molecular characteristics of ESBL-producing *Escherichia coli* isolated from patients with urinary tract infections attending Igbinedion University Teaching Hospital, Okada, Edo State, Nigeria.

METHODS

Study Area

This work was Carried out in Igbinedion University Okada Ovia North East Local Government of Edo state. Okada is a rural area and local government headquarters of Ovia North East Local Government area of Edo state

Ethical Permission

Ethical approval for this study was obtained from the Ethics Committee of the Igbinedion University Teaching Hospital, Okada

Study Design

This research design was a cross- sectional study, conducted between April and June, 2025, at the Medical Laboratory Science Department, Igbinedion University Okada, Edo State, Nigeria. A non- repetitive mid-stream urine sample were collected from participants presenting with symptoms of urinary tract infections..

Specimen Collection

A total of 400 urine samples were collected from both male and female patients of varying age groups, drawn from multiple clinics and wards including the Antenatal Clinic, General Outpatient Department (GOPD), Gynecology Ward, Female and Male Medical Wards.

Bacterial culture

The urine samples were inoculated aseptically onto Cystine Lactose Electrolyte Deficient (CLED) agar; plates were incubated at 37 degrees Celcius overnight aerobically. Isolates were subjected to standard biochemical tests for identification (Cheesebrough, 2006). Molecular confirmation of *Escherichia coli* isolates was subsequently performed using polymerase chain reaction (PCR) targeting the *uidA* gene as previously described by Tille, (2022)..

Antimicrobial susceptibility testing

The antimicrobial susceptibility of *E. coli* isolates to 12 antimicrobial agents was assessed using the Kirby-Bauer disc diffusion method. The antimicrobial included: ciprofloxacin (CIP, 5 µg), amoxicillin-clavulanate (AUG, 30 µg), cefepime (CPM, 30 µg), cefotaxime (CTX, 25 µg), ceftazidime (CXM, 30 µg), ceftriaxone (CTR, 30 µg), gentamicin (GEN, 10 µg), amikacin (AMK, 30 µg), cotrimoxazole (25 µg), nitrofurantoin (NIT, 30 µg), levofloxacin (LBC, 5 µg), and aztreonam (AT, 30 µg). The plates were incubated at 37°C for 24 hours following Clinical and Laboratory Standards Institute (CLSI) guidelines. The diameters of inhibition zones were measured and interpreted according to CLSI standards. The sensitivity tests were standardised using E. coli NCTC no. 10418) (CLSI, 2024; Tille, 2022).

Data Analysis

All data obtained was analyzed using SPSS 20 package. Descriptive statistics including frequencies and percentages were used to summarize the data. The chi test was employed to

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test the hypotheses, and a P-value of less than 0.05 was considered statistically significant.

RESULTS

Table 1 shows the age distribution of *Escherichia coli* and ESBL-producing *E. coli* isolates. The highest proportion of *E. coli* isolates was recovered from patients aged 61–70 years (16.7%), followed by those aged 31–40 years (13.5%) and 21–30 years (12.1%). ESBL-producing *E. coli* was most frequently detected among patients aged 61–70 years (8.3%). However, the distribution of both *E. coli* isolates (χ^2 = 9.279, p = 0.158) and ESBL-producing *E. coli* isolates (χ^2 = 8.130, p = 0.229) across age groups was not statistically significant.

Table 2 indicate the Molecular characterization of the 16 ESBL-producing *E. coli* strains, the *bla*CTX-M gene was the predominant ESBL determinant, detected in 11 (68.8%) isolates. The *bla*TEM gene alone was detected in 2 (12.5%) isolates, while co-occurrence of *bla*CTX-M and *bla*TEM was observed in 3 (18.8%) isolates. No isolate harboured *bla*SHV either alone or in combination with other ESBL genes. These findings indicate that *bla*CTX-M is the dominant ESBL genotype among urinary *E. coli* isolates in this study population.

Table 3 shows the susceptibility profile. Amikacin demonstrated the highest susceptibility rate among the tested antibiotics, with 64.1% of *E. coli* isolates being sensitive. Cotrimoxazole exhibited the highest resistance rate (89.7%), followed by amoxicillin-clavulanate (74.4%). Resistance to fluoroquinolones (ciprofloxacin and levofloxacin) and third- and fourth-generation cephalosporins (ceftriaxone, ceftazidime, cefotaxime, and cefepime) was also high, ranging from 43.6% to 53.8%, indicating a substantial burden of multidrug resistance among the isolates.

Table 4 shows the classification of antimicrobial resistance among the 39 *E. coli* isolates revealed that multidrug resistance (MDR) was the predominant phenotype, occurring in 26 (66.7%) isolates. Extensively drug-resistant (XDR) isolates accounted for 10 (25.6%), while 2 (5.1%) isolates were non-drug resistant (NDR). Only 1 (2.6%) isolate exhibited pandrug resistance (PDR). The observed distribution of resistance phenotypes was not statistically significant (χ^2 = 7.35, p = 0.149).

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Table 1. Age Distribution of *Escherichia coli* and ESBL-Producing *Escherichia coli* Isolated from Urine Samples of Patients Attending Igbinedion University Teaching Hospital, Okada

Age Group (Years)	**Number Examined (n)**	***E. coli* Isolates n (%)**	**ESBL-Producing *E. coli* n (%)**
0–10	40	1 (2.5)	1 (2.5)
11–20	48	2 (4.2)	0 (0.0)
21–30	91	11 (12.1)	1 (1.1)
31–40	74	10 (13.5)	4 (5.4)
41–50	54	4 (7.4)	3 (5.6)
51–60	45	3 (6.7)	3 (6.7)
61–70	48	8 (16.7)	4 (8.3)
Total	**400**	**39 (9.75)**	**16 (4.0)**
Variable	**χ^2**	**p-value**	**Significance**
Distribution of *E. coli* across age groups	9.279	0.158	Not Significant
Distribution of ESBL-producing *E. coli* across age groups	8.130	0.229	Not Significant

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Table 2. Distribution of ESBL Genes among ESBL-Producing *Escherichia coli* Isolates Recovered from Urine Samples of Patients Attending Igbinedion University Teaching Hospital, Okada (n = 16)

ESBL Gene Profile	**Frequency, n (%)**
*bla*SHV	0 (0.0)
*bla*CTX-M	11 (68.8)
*bla*TEM	2 (12.5)
*bla*SHV + *bla*CTX-M	0 (0.0)
*bla*SHV + *bla*TEM	0 (0.0)
*bla*CTX-M + *bla*TEM	3 (18.8)
*bla*SHV + *bla*CTX-M + *bla*TEM	0 (0.0)
Total	**16 (100.0)**

Table 3. Antimicrobial Susceptibility Profile of *Escherichia coli* Isolates from Urine Samples Obtained from Patients Attending Igbinedion University Teaching Hospital, Okada (n = 39)

Antimicrobial Agent	**Disc Content (µg)**	**Sensitive n (%)**	**Intermediate n (%)**	**Resistant n (%)**
Amikacin	30	25 (64.1)	6 (15.4)	8 (20.5)
Levofloxacin	10	13 (33.3)	9 (23.1)	17 (43.6)
Ciprofloxacin	10	12 (30.8)	6 (15.4)	21 (53.8)
Ceftriaxone	30	15 (38.5)	4 (10.3)	20 (51.3)
Gentamicin	10	9 (23.1)	9 (23.1)	21 (53.8)
Cefepime	30	5 (12.8)	15 (38.5)	19 (48.7)
Aztreonam	30	7 (17.9)	11 (28.2)	21 (53.8)
Nitrofurantoin	30	15 (38.5)	10 (25.6)	14 (35.9)

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Antimicrobial Agent	Disc Content (µg)	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Cotrimoxazole	25	1 (2.6)	3 (7.7)	35 (89.7)
Amoxicillin-Clavulanate (Augmentin)	30	7 (17.9)	3 (7.7)	29 (74.4)
Cefotaxime	30	8 (20.5)	12 (30.8)	19 (48.7)
Ceftazidime	30	10 (25.6)	9 (23.1)	20 (51.3)

Table 4. Classification of Antimicrobial Resistance among *Escherichia coli* Isolates Recovered from Urine Samples of Patients Attending Igbinedion University Teaching Hospital, Okada (n = 39)

Class of Antimicrobial Resistance	Frequency, n (%)	χ^2 and P-value
NDR (Non-Drug Resistant)	2 (5.1)	
XDR (Extensively Drug Resistant)	10 (25.6)	$\chi^2 = 7.35$
MDR (Multidrug Resistant)	26 (66.7)	P = 0.149
PDR (Pandrug Resistant)	1 (2.6)	(P > 0.05)
Total	**39 (100.0)**	

Footnote

NDR = Susceptible to all antimicrobial classes tested. **MDR** = Resistance to at least one agent in three or more antimicrobial classes. **XDR** = Resistance to all but one or two antimicrobial classes. **PDR** = Resistance to all antimicrobial agents in all classes tested.

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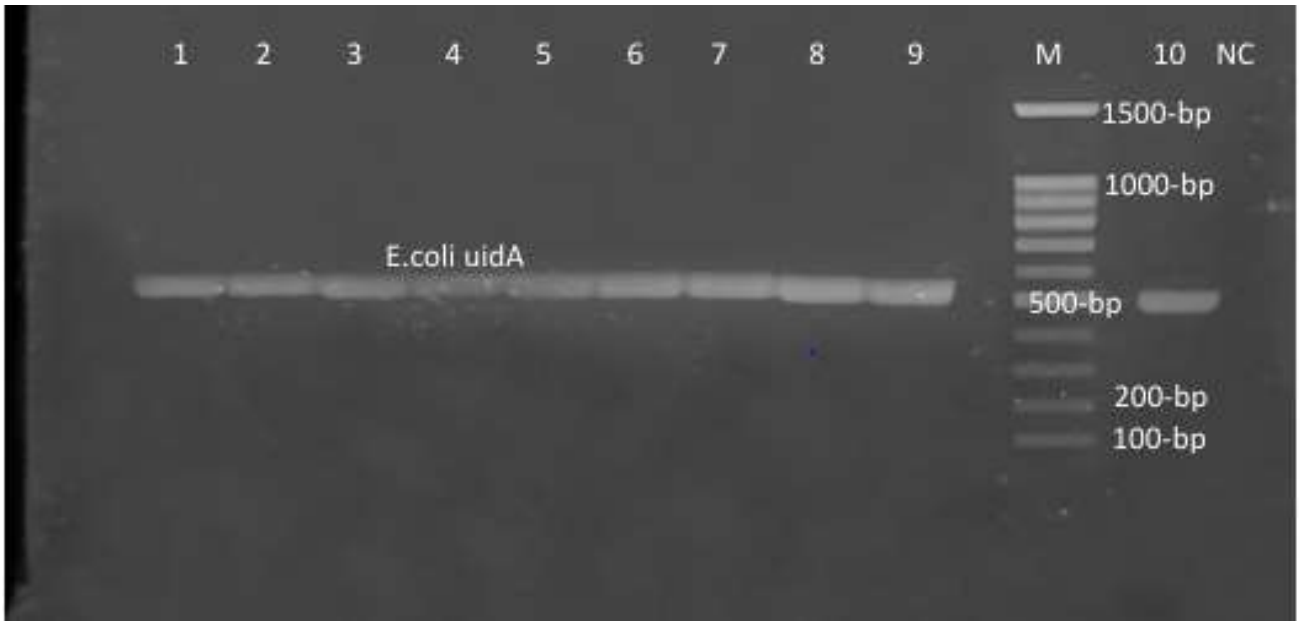



Figure 1:Agarose gel electrophoresis (2%) showing the typical amplion of the uidA gene product of E. coli isolates

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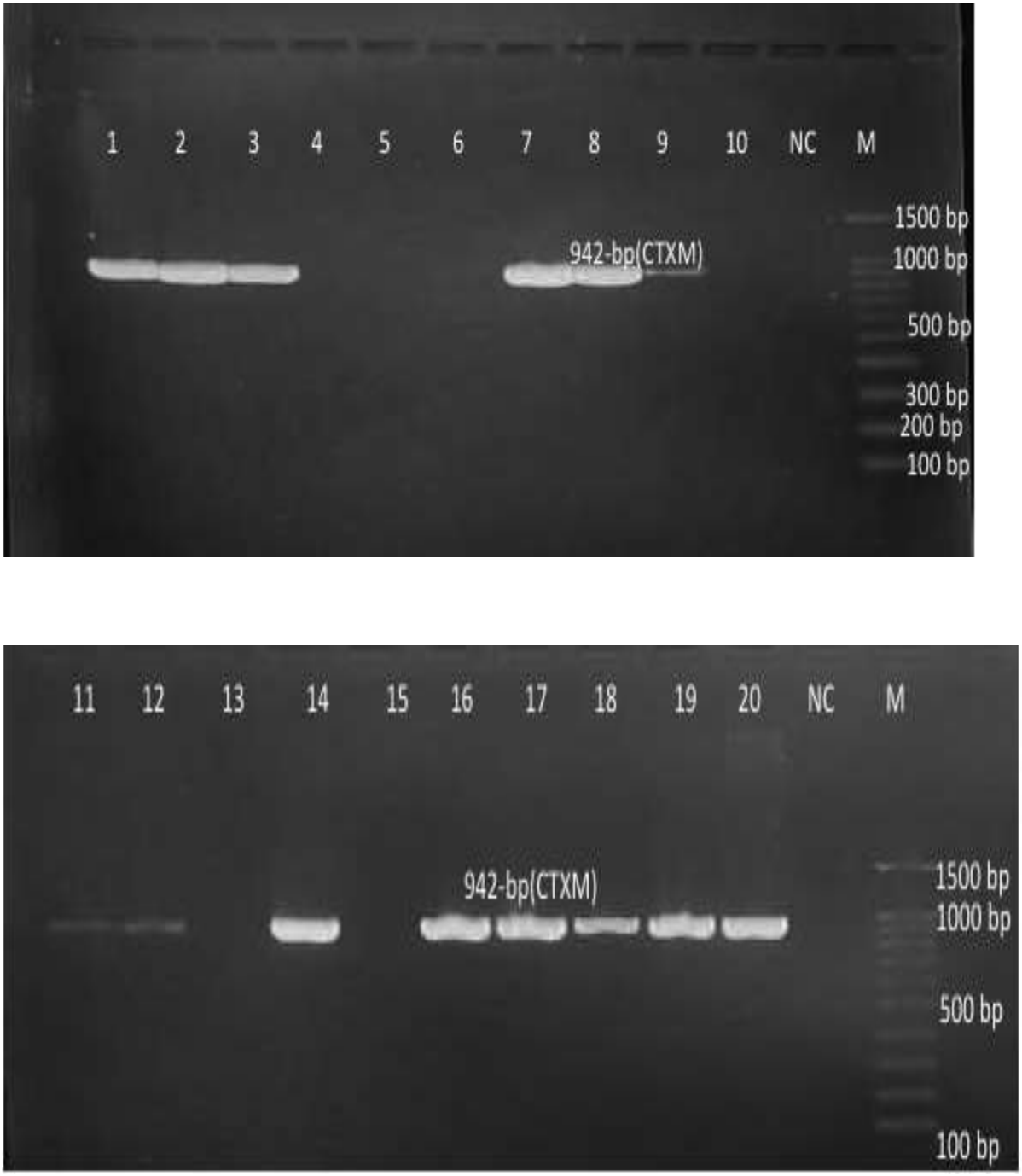

Figure 2: ESBL genes (blaCTX-M) using PCR amplification of target resistance genes.

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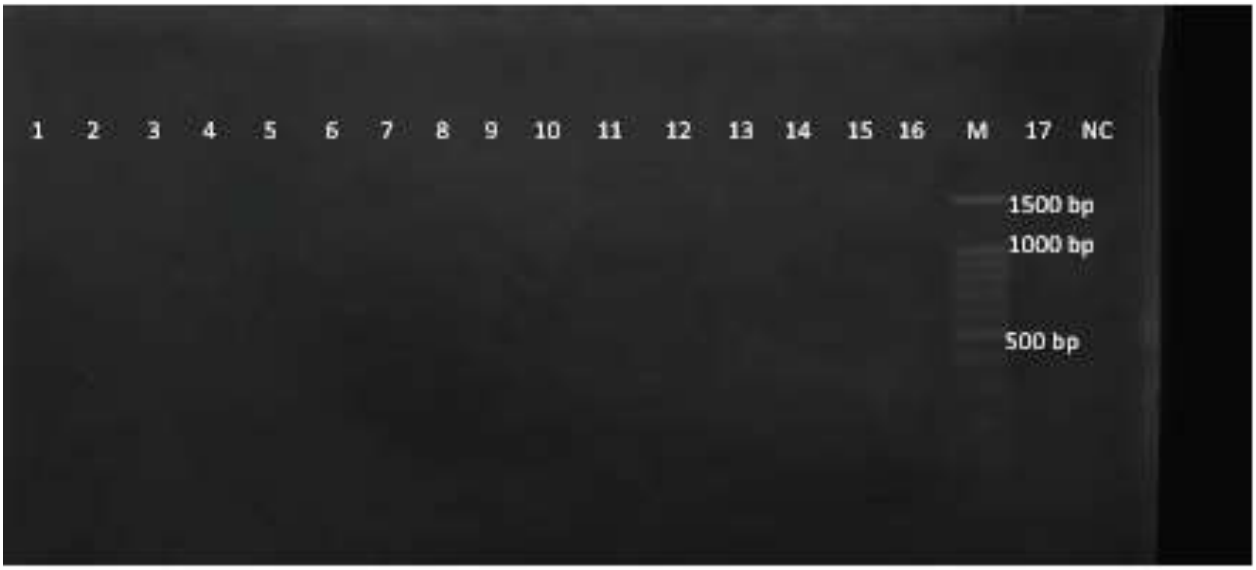
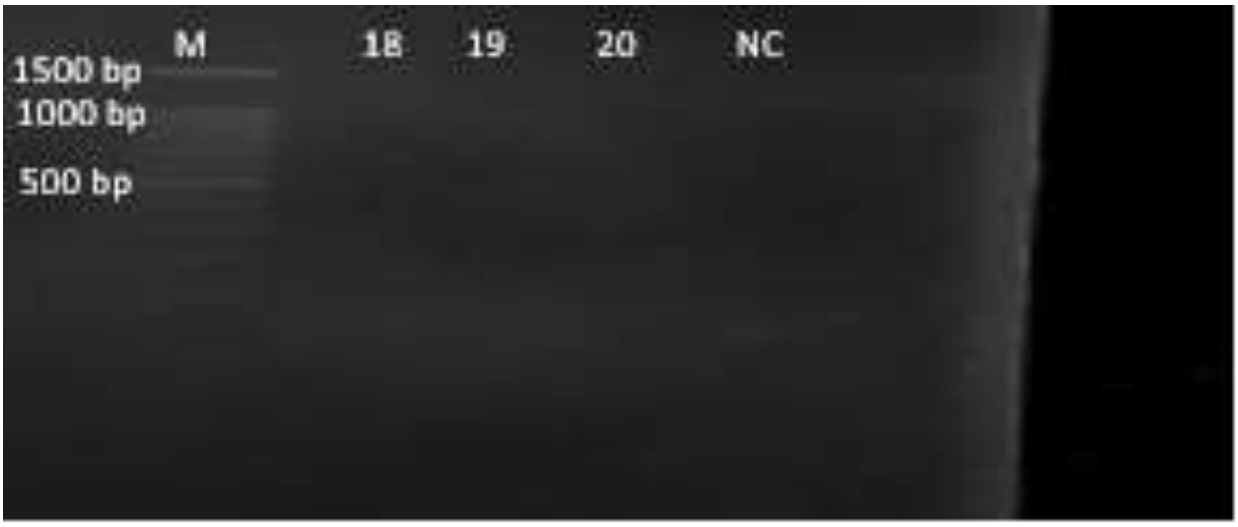


blaSHV



blaSHV

Figure 3: Detection of ESBL genes (blaSHV) using PCR amplification of target resistance genes.

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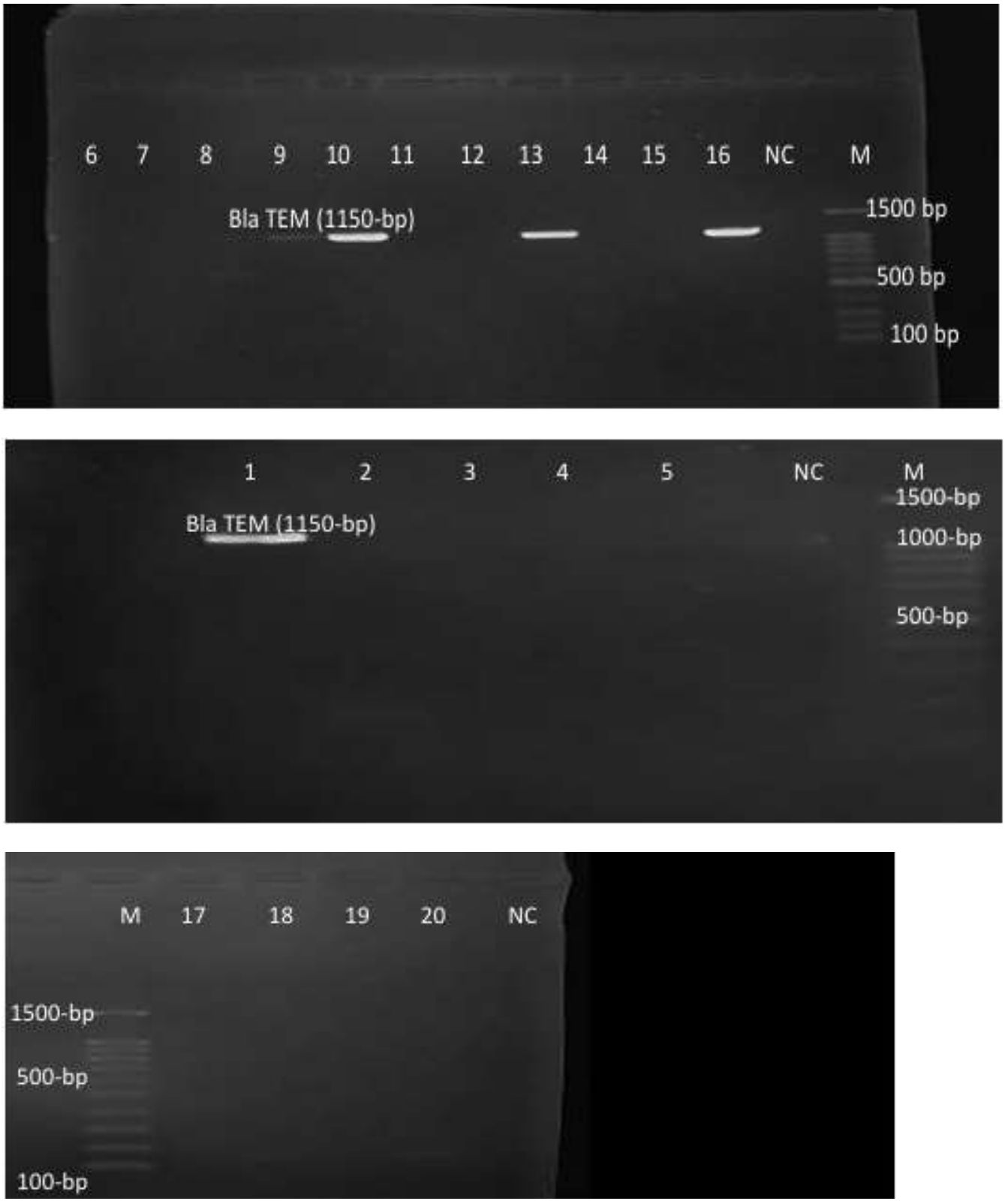

Figure 3: Detection of ESBL genes (blaTEM) using PCR amplification of target resistance genes.

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Discussion

Antimicrobial resistance (AMR) has become one of the most significant public health challenges worldwide, compromising the effectiveness of antibiotics and increasing the burden of infectious diseases. Among the major drivers of AMR is the emergence of extended-spectrum β-lactamase (ESBL)-producing bacteria, particularly Escherichia coli, which is a leading cause of urinary tract infections (UTIs) globally (Al-Hasso & Mohialdeen, 2023; Bush & Bradford, 2020).

In the present study, Escherichia coli was isolated from 39 (9.75%) of the 400 urine samples examined from patients attending Igbinedion University Teaching Hospital (IUTH), Okada. Phenotypic detection identified ESBL production in 19 (48.7%) of the E. coli isolates, while molecular characterization confirmed ESBL-associated genes in 16 isolates (84.2% of phenotypically positive isolates). The prevalence of ESBL-producing E. coli observed in this study demonstrates the continuing circulation of resistant *E. coli* strains within the study population. Similar findings have been reported in Nigeria by Mofolorunsho et al. (2021), who documented ESBL production among uropathogenic E. coli isolates, and by Tanko et al. (2020), who highlighted the widespread occurrence of ESBL-producing Enterobacteriaceae across the country. The presence of these resistant strains may be associated with indiscriminate antibiotic use, self-medication, and inadequate antimicrobial stewardship practices, which are common challenges in many developing countries.

The age distribution of E. coli isolates showed that the highest prevalence occurred among individuals aged 61–70 years (16.7%), followed by those aged 31–40 years (13.5%) and 21–30 years (12.1%). Similarly, ESBL-positive E. coli isolates were more frequently detected among older adults, particularly within the 61–70-year age group (8.3%). Although the observed differences were not statistically significant ($p > 0.05$), the findings suggest an increased susceptibility of older individuals to infections caused by resistant organisms. This observation is consistent with reports by Omar et al. (2024), who identified age as an important factor associated with ESBL-producing E. coli infections. Elderly patients often have underlying medical conditions, repeated healthcare exposure, prolonged antibiotic use, and compromised immunity, all of which may predispose them to colonization and infection by resistant bacteria.

The antimicrobial susceptibility profile of E. coli isolates revealed varying levels of resistance to the antibiotics tested. Amikacin demonstrated the highest susceptibility rate (64.1%), indicating that it remains one of the most effective agents against uropathogenic E. coli in the study area. Moderate susceptibility was observed with nitrofurantoin and ceftriaxone, while high resistance rates were recorded against cotrimoxazole (89.7%), amoxicillin-clavulanate (74.4%), ciprofloxacin (53.8%), gentamicin (53.8%), aztreonam (53.8%), ceftriaxone (51.3%), and ceftazidime (51.3%). These findings are comparable with those reported by Anueyiagu et al. (2024) and Haruna et al. (2024), who documented increasing resistance of uropathogenic bacteria to commonly prescribed antibiotics in Nigeria.

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The extremely high resistance observed against cotrimoxazole and amoxicillin-clavulanate may be attributable to their widespread availability, frequent empirical use, and misuse within the community. Likewise, the elevated resistance to fluoroquinolones such as ciprofloxacin and levofloxacin raises concerns because these agents are often recommended for the treatment of complicated urinary tract infections. The retained activity of amikacin suggests that it may remain a useful therapeutic option for severe infections caused by multidrug-resistant E. coli, although its use should be guided by susceptibility testing and antimicrobial stewardship principles.

Analysis of antimicrobial resistance patterns showed that multidrug resistance (MDR) was the predominant resistance phenotype, occurring in 26 (66.7%) of the isolates. Extensive drug resistance (XDR) was detected in 10 (25.6%) isolates, while pan-drug resistance (PDR) was identified in one isolate (2.6%). Only two isolates (5.1%) were classified as non-drug resistant (NDR). Although the distribution was not statistically significant ($p > 0.05$), the high proportion of MDR isolates is clinically significant and reflects the growing burden of antimicrobial resistance among uropathogenic E. coli. Similar trends have been documented in Nigeria and other developing countries, where multidrug-resistant Enterobacterales continue to complicate treatment outcomes and increase healthcare costs (Ogbolu et al., 2018; Prestinaci et al., 2015).

Molecular characterization of ESBL-positive E. coli isolates revealed that blaCTX-M was the predominant ESBL gene, occurring in 68.75% of isolates, followed by the combined blaCTX-M/blaTEM genotype (18.75%) and blaTEM alone (12.5%). None of the isolates harbored blaSHV either alone or in combination with other genes. The predominance of blaCTX-M observed in this study is consistent with global and regional trends, where CTX-M enzymes have become the most widespread ESBL family among Enterobacterales (Bush & Bradford, 2020). Similar findings were reported by Saka et al. (2020), who identified blaCTX-M as the most prevalent ESBL gene among multidrug-resistant E. coli isolates in Nigeria.

The absence of blaSHV in the present study contrasts with reports from some regions where SHV-type ESBLs remain common. Such variations may be attributed to geographical differences in antimicrobial usage patterns, circulating plasmids, bacterial clones, and local infection control practices. The detection of combined blaCTX-M and blaTEM genes in some isolates further suggests the existence of plasmid-mediated co-transfer of resistance determinants, which facilitates the rapid dissemination of multidrug resistance among bacterial populations.

Overall, the findings of this study demonstrate the emergence and persistence of ESBL-producing and multidrug-resistant E. coli among patients attending Igbinedion University Teaching Hospital, Okada. The predominance of blaCTX-M and the high resistance rates observed against commonly used antibiotics underscore the need for routine antimicrobial susceptibility testing, molecular surveillance, strict antibiotic stewardship programs, and strengthened infection prevention and control measures to limit the spread of resistant pathogens within healthcare facilities and the community.

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Conclusion

In conclusion, this study shows that *Escherichia coli* remains an important uropathogen among patients attending Igbinedion University Teaching Hospital (IUTH), Okada, with a notable proportion producing ESBL enzymes. The presence of ESBL-producing and multidrug-resistant strains limits the effectiveness of commonly used antibiotics, particularly cephalosporins and fluoroquinolones, and poses a significant clinical challenge.

The predominance of ESBL genes, especially blaCTX-M, highlights the ongoing spread of plasmid-mediated resistance. These findings emphasize the need for routine antimicrobial susceptibility testing, strict antibiotic stewardship, and improved infection prevention and control practices to reduce the spread of resistant *E. coli* in both hospital and community settings.

Limitations

This study was limited to a single tertiary health facility (Igbinedion University Teaching Hospital, Okada), which may restrict the generalizability of the findings to other healthcare settings and communities.

The molecular analysis focused on selected ESBL genes (blaTEM, blaSHV, and blaCTX-M), which may not represent the full range of resistance genes present in the isolates. Other ESBL or carbapenemase genes could have been missed.

Finally, resource constraints limited the study to a relatively small number of ESBL-confirmed isolates for molecular characterization, which may affect the broader interpretation of gene distribution.

Recommendations

The followings are the recommendations based on the research findings:

Strengthen antimicrobial stewardship programs in Igbinedion University Teaching Hospital, Okada, to promote the rational use of antibiotics and reduce the emergence and spread of ESBL-producing *Escherichia coli*.

Enhance routine surveillance and laboratory capacity for antimicrobial resistance, including regular screening for ESBL genes, to guide effective empirical treatment and improve infection control practices.

Conflict of interest declaration

The authors declare that there are no conflicts of interest regarding the publication of this study. The research was conducted independently, and no financial or personal relationships influenced the design, execution, analysis, or reporting of the findings.

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Funding: This research is not funded.
Acknowledgments
Not applicable.
Conflicts of Interest

The authors declare no conflict of interest.
Authenticity
This manuscript is an original work