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MOLECULAR PROFILING AND ANTIMICROBIAL RESISTANCE PATTERNS OF EXTENDED-SPECTRUM BETA-LACTAMASE-PRODUCING ESCHERICHIA COLI ISOLATES FROM CLINICAL SAMPLES IN SOUTHWESTERN NIGERIA

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ABSTRACT

Escherichia coli, a Gram-negative bacterium, is a significant cause of infections, particularly when it produces extended-spectrum β-lactamases (ESBLs), which confer resistance to β-lactam antibiotics. This study investigated the prevalence and molecular characteristics of ESBL-producing E. coli isolates from selected hospitals in Southwestern Nigeria. A total of 105 clinical E. coli isolates were collected between August and November 2020. Identification was confirmed using biochemical tests, eosin methylene blue agar, and PCR with the TEcol primer. Antimicrobial susceptibility testing was conducted using the Kirby-Bauer disk diffusion method and interpreted following CLSI guidelines. Molecular analysis was performed to detect extended-spectrum β-lactamase genes (blaCTX-M, blaSHV, and blaTEM). Of the 105 isolates, 35 (33.3%) were confirmed as E. coli, with 60% harboring at least one ESBL gene. Specifically, 17.1% carried blaSHV, 37.1% blaTEM, and 40% blaCTX-M. All ESBL-producing isolates showed complete resistance to Amoxicillin/Clavulanic acid, Meropenem, Oxacillin, Ceftazidime, and Cefepime. Additionally, resistance to Imipenem, Chloramphenicol, Levofloxacin, Ciprofloxacin, and Cefoxitin ranged from 33.3% to 81%, highlighting a concerning level of multidrug resistance. These findings underscore the high burden of ESBL-producing E. coli in Southwestern Nigeria, particularly strains harboring blaCTX-M and blaTEM genes. The widespread resistance to both β-lactams and fluoroquinolones limits therapeutic options and poses a significant public health risk. This study emphasizes the urgent need for routine molecular surveillance, improved diagnostic practices, and robust antimicrobial stewardship programs to mitigate the spread and impact of multidrug-resistant E. coli infections.

Keywords: Molecular characterization, Extended-Spectrum Beta-Lactamases, Escherichia coli clinical isolates, Southwestern Nigeria

INTRODUCTION

Antibiotic resistance (AMR) is a significant global health issue that increases healthcare costs, worsens illness, raises the risk of death, and prolongs hospital stays for patients. Antimicrobial resistance has been known to be a threat to the prevention of and therapy against infectious diseases (Alabi et al., 2020, Tanko et al., 2020, Ayalneh et al., 2025). Bacteria develop resistance to antimicrobial agents through various mechanisms, the most common of which include: the production of enzymes that inactivate or modify antibiotics (for example, beta-lactamase enzymes that destroy the beta-lactam ring of penicillins and cephalosporins); alterations in the bacterial cell membrane that prevent antimicrobial uptake; modification of the antimicrobial target, rendering it unrecognizable or non-functional; and the development of alternative metabolic pathways that allow the bacteria to bypass the site of antimicrobial action (Cheesbrough, 2006).

Beta-lactam (β-lactam) antibiotics are a class of antimicrobial agents characterized by the presence of a β-lactam ring. They disrupt peptidoglycan biosynthesis by inactivating enzymes known as penicillin-binding proteins (PBPs) or transpeptidases, which are responsible for the transpeptidation of peptidoglycan precursors. This inhibition compromises cell wall integrity, ultimately leading to bacterial cell lysis. The most common mechanism of resistance to β-lactam antibiotics among clinically significant Gram-negative bacteria is the production of β-lactamase enzymes (Guiral Vilalta, 2018).

Extended-spectrum β-lactamases (ESBLs) are plasmid-mediated and the genes encoding these enzymes could be transferred easily among different bacteria (Bello et al., 2019, Egbule & Ejechi, 2021). Transmission of genes encoding ESBL enzymes can occur either by emerging bacterial clones or by horizontal gene transfer. In the latter case, plasmids containing resistance genes are transmitted between bacteria of the same and/or different species. (Brolund, 2014, Mohammed et al., 2024).

Also, ESBLs are a group of beta-lactamases that hydrolyse the extended-spectrum cephalosporins, the penicillins, and monobactams, but not cephamycin and carbapenems; they are as well inhibited by beta-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam. Carbapenems, which are last-resort beta-lactam antibiotics used to treat ESBL-producing bacterial pathogens, are increasingly becoming less effective against some of these bacteria. This is due to the emergence of more advanced beta-lactamase enzymes, known as carbapenemases, which include metallo beta-lactamases (MBLs). The rise of carbapenemases, including MBLs, which can break down carbapenems and other beta-lactam antibiotics, leading to resistance, poses a significant clinical and therapeutic challenge. (Egbule & Ejechi, 2021, Odewale et al., 2024, Etang et al., 2025).

Infectious diseases that became curable at the advent of antibiotics chemotherapy are again becoming killers of patients of all ages especially in developing countries. Extended-spectrum beta lactamases which are mainly

produced by E. coli render these antibiotics ineffective when used to treat infections caused by ESBL-producing organisms, consequently increasing cost of therapy, morbidity and mortality (Abba et al., 2019, Or et al., 2025). ESBLs have been reported to be plasmid encoded implying that these resistance determinants are found in our environment and can be transferred from one organism to another (Olowe et al., 2015, Or et al., 2025). The ongoing spread of ESBL resistance in Nigerian hospitals, especially to third generation cephalosporins and beta-lactams, has led to increased treatment failures with these drugs in developing countries. This situation continues to create challenges for patients, their families, and healthcare professionals (Thonda & Oluduro, 2017; Saleh et al., 2024). In the past years, there has been an increase in the incidence of extended spectrum beta lactamase producing infections because ESBLs gene is a world fast-growing threat for available antibiotics; its epidemiological effect and evaluation are still underestimated with low awareness (Thonda et al., 2018, Ayalneh et al., 2025) The emergence of the ESBLs is increasing in Nigeria; the prevalence of the isolates possessing these resistance enzymes concomitantly has caused serious problems for treatment and diagnosis (Thonda & Oluduro, 2017). According to the Investigation carried out by Thonda & Oluduro (2017), proliferation of beta-lactamase resistance among ESBL-producers may have been due to misuse of antibiotics, proliferation of resistant clones, transfer of resistance-carrying plasmids and inability to detect emerging phenotypes in developing countries; but most probably in the nearest future, if this irrational use is not stopped, infection with these Gram-negative bacteria increase the rate of resistant to drugs that are now sensitive, resulting in increase in morbidity and mortality.

In Europe and the United States, the number of bloodstream infections caused by ESBL-producing strains of the family Enterobacteriaceae is on the increase with the trend having a significant impact on mortality rates and hospital costs. This problem is even more pronounced in developing countries due to poverty and abuse of antibiotic use. There is widespread and significant prevalence of ESBL-producing Gram-negative bacteria in Nigeria. The preponderance of ESBL ranges from as low as 7.5% to as high as 82.3%. Yet, most laboratories and health care centers are unfamiliar with the significance of detecting ESBL-producing Gram-negative organisms. The prevalence of ESBL can vary from one geographical location to another, or from one health setting to another, and even for a given place over time (Olowe et al., 2014, Tanko et al., 2020).

In the southwest geopolitical zone of Nigeria, the prevalence of ESBL range from 7.5% to 76.9%; sources of ESBL isolates are mostly from clinical samples (61.5%); the genes blaTEM, blaCTX-M, blaSHV and blaOXA have been detected in this zone. The prevalence of ESBL-producing Gram-negative bacteria from north-central Nigeria range from 7.9% to 65%; Pseudomonas aeruginosa has the highest prevalence (65%) and Salmonella typhi has the lowest (7.9%) prevalence in this geographical zone. In Northeast Nigeria, the prevalence of ESBL-producing Gram-negative ranges from 16.7% to 82.3%. The prevalence of ESBL-producing Gram-negative bacteria from northwest Nigeria ranged from 12.8% to 41.2%; The prevalence of ESBL-producing Gram-negative bacteria from southeast Nigeria range from 8.1% to 74.3%. The prevalence of ESBL from south-south Nigeria ranges from 8.9% to 47.1% (Tanko et al., 2020, Mohammed et al., 2024).

Although ESBLs have been found in a large number of different Gram-negative bacteria species, it is more commonly found in E. coli and Klebsiella pneumoniae. The most frequent types of ESBL genes in Nigeria include blaTEM, blaCTX-M, and blaSHV; but there is a reported case of blaOXA-1. In most reports, the dominant variant is TEM, followed by SHV and CTX-M. These variants are also reported in many parts of the world and Africa. While there are a large number of studies on the detection of ESBL in Gram-negative bacteria in Nigeria, there are regions where the cases are still under-reported and the prevalence still unclear (Tanko et al., 2020).

There have been several studies on extended-spectrum beta lactamases in Nigeria, including Southwestern part of Nigeria; there have also been studies on the ESBLs among clinical isolates of Escherichia coli most especially phenotypically, but there is no recent study on the molecular characterization of extended-spectrum beta lactamases among clinical isolates of Escherichia coli in Southwestern Nigeria.

ESBL-producing bacteria such as E. coli are increasingly spreading globally creating a severe threat; their antimicrobial resistance development is multi-factorial, with antibiotic consumption being the major factor. Molecular testing for the presence of ESBLs will characterize and determine whether the antimicrobial resistance genes are present on transferable plasmids, transposons, or integrons, which can enhance easy spreading. The regular epidemiological assessments on the drug resistance patterns of the isolates and determination of the molecular resistance mechanisms can be useful for the empirical treatment of infections caused by ESBL-producing E. coli. Thus, investigating the molecular characterization of ESBLs in clinical isolates of E. coli from Southwestern Nigeria is a crucial step towards the determination of the molecular antimicrobial resistance mechanism; minimization of the spread of ESBL producing E. coli; selection of appropriate antibiotics for the treatment of infected patients; and determination of the appropriate preventive policy in hospitals to control further spreading of these resistant strains. Therefore, the aim of this study was to investigate the molecular characteristics of extended-spectrum beta-lactamases (ESBLs) in clinical Escherichia coli isolates from Southwestern Nigeria. The study focused on identifying E. coli isolates recovered from various clinical specimens, analyzing their antimicrobial resistance profiles, screening resistant strains for ESBL production, confirming ESBL-producing isolates, and detecting the presence of blaCTX, blaSHV and blaTEM genes in the confirmed ESBL-positive isolates.

MATERIALS AND METHODS

Study Area

The study was conducted at the following institutions: UNIOSUN Teaching Hospital in Osogbo, Osun State; OAU Teaching Hospital in Ile-Ife, Osun State; University College Hospital in Ibadan, Oyo State; and LAUTECH Teaching Hospital in Ogbomosho, Oyo State. These facilities are located in the South-West geopolitical zone of Nigeria, which is home to an estimated population of 50 million people.

Study Design, Inclusion and Exclusion Criteria

Escherichia coli isolates from urine, high vaginal swabs, stool, sputum, blood cultures, cerebrospinal fluid, wound, endocervical swab and catheter tip samples obtained from different patients were collected from the Medical Microbiology Laboratories of four hospitals located at Ibadan, Ogbomoso, Osogbo and Ile-Ife. All Escherichia coli isolates obtained from clinical samples from patients of all age-group, gender and ethnicity were included while Escherichia coli isolates obtained from non-clinical samples (such as animal samples, water samples, food samples, etc.) were excluded.

Ethical Considerations
The Ethics Review Committee of the Osun State Ministry of Health, Osogbo, Nigeria, granted ethical approval for this study

Sample Size Determination
The required sample size for this study was calculated using Cochran's formular, N = (Z2PQ)/ D2. Where Z = standard deviation (1.96), P = prevalence, D = degree of accuracy (0.05), and Q = 1 – P; while P = 25.9% (Odetoyin & Adewole, 2021). The total sample size calculated from the formula above was 295. The prevalence (P) value represents the proportion of clinical isolates of ESBL-producing E. coli identified in the study conducted by Odetoyin & Adewole (2021).

Data Collection
Between September and November 2020, a total of 105 Escherichia coli isolates were obtained from various clinical specimens (urine, high vaginal swabs, stool, sputum, blood cultures, cerebrospinal fluid, wound swabs, endocervical swabs, and catheter tips) collected from different patients. These samples were sourced from the Medical Microbiology Laboratories of four hospitals situated in Ibadan, Ogbomoso, Osogbo, and Ile-Ife.

E. coli Isolation and Identification
Clinical isolates of Escherichia coli were recovered from a variety of clinical specimens, as previously described, and initially cultured on Mueller-Hinton slants. Subsequent sub-culturing was performed on eosin methylene blue (EMB) agar, followed by incubation at 37°C for 24 hours. Presumptive E. coli isolates were identified based on phenotypic characteristics in accordance with Bergey's Manual of Determinative Bacteriology. Molecular identification was then conducted using the TEcol primer.

Antimicrobial Susceptibility Tests
Disk Diffusion: The Antibiotic susceptibility testing of the isolates were determined by the Kirby Bauer method (Bauer et al., 1966) and results were interpreted using Clinical and Laboratory Standards Institute (CLSI) criteria. Bacterial suspension of each isolate was prepared to match 0.5 MacFarland standards for antibiotic susceptibility as described by CLSI (CLSI, 2017). A sterile swab was dipped into the bacterial suspension and firmly rotated several times against the wall of the tubes to expel excess fluid. The swab was used to inoculate the entire surface of the plates ensuring to obtain uniform inoculation. The antibiotic discs Ceftazidime (30µg), Cefepime (30µg), Imipenem (10µg), Meropenem (10µg), Amoxycillin/Clavulanic Acid (30µg), Levofloxacin (5µg), Oxacillin (30µg), Ciprofloxacin (10µg), Chloramphenicol (30µg), and Cefoxitin (30µg)) were aseptically applied onto the surface of the inoculated agar plates. The antibiotic susceptibility of each isolate was carried out on Mueller Hinton agar (Oxoid, UK) and inoculated plates were incubated overnight in an incubator at 370C for 24 hours. E. coli ATCC 25922 was used as control. Antibiotic susceptibility test results were interpreted using CLSI criteria (CLSI, 2017), classifying them as resistant, intermediate, or sensitive. Isolates that were resistant to three or more antibiotic classes were identified as multidrug-resistant (MDR).

Phenotypic Detection of ESBLs in E. coli Isolates
The double disk synergy test was used to test for the production of ESBLs in the isolates. Discs containing Cefotaxime, Ceftazidime, and in the center the amoxicillin-clavulanic acid (20:10 µg) were utilized (Bradford, 2001). Isolates that exhibited enhanced susceptibility to one or more of the cephalosporin antibiotics (i.e. enhanced zone of inhibition) were selected as potential producers of ESBL.

DNA Extraction for E. coli
The DNA of the isolates was extracted by suspending 4-5 bacteria colonies in 300 µl of 1X TBE buffer in Eppendorf tubes appropriately labeled. The cells were boiled at 100oc for 10 minutes and were cooled rapidly on ice for 30 minutes. The cell lysate was centrifuged briefly at high speed (13, 400 rpm for 3 min), and the supernatant containing the genomic DNA was transferred into a fresh sterile Eppendorf tube. The extracted DNA was stored at -21°C until required for PCR. Previously extracted high-quality DNA sample of the E. coli ATCC 25922 strain was used as positive control to confirm successful DNA extraction. A reaction containing all reagents except the bacterial sample was used as negative control.

Strain Typing of the ESBL E. coli Isolates
Repetitive-PCR (Polymerase Chain Reaction) genomic fingerprinting method (Box PCR) was carried out on the ESBL-producing human strains to assess the relatedness of the different isolates as determined by the similarity of the patterns produced. A specific primer was used for this PCR. Biometra T1 Thermocycler PCR machine was used for the PCR. A volume of 1 µL of DNA templates was added to the reaction mixture (DyNAzyme TM II, PCR master mix-10 µL each, Primer (5')- 1 µL) and sterile distilled water was added to each tube to make a final volume of 20µL. DyNAzyme TMII PCR master mix (1.25 mL) contains 0.04 U/µL DyNAzyme TM II DNA polymerase, 20mM Tris-HCl (pH 8.8 at 25oC), 3 mM MgCl2, 100 mM KCl, Stabilizers and 400 µM of dNTPs. The reactions for the BOX PCR were run under the following conditions: An initial DNA denaturation at 950C for 1 minute followed by 30 cycles of DNA denaturation at 940C for 1 minute. At 530C for 1-minute annealing of specific primers occurred from which synthesis of DNA started. Extension of the DNA occurred at 650C for 8 minutes and a final extension of the DNA followed at 650C for 16 minutes (Mohapatra et al., 2007). Polymerase chain reaction products were analyzed on a 2% agarose gel. Polymerase chain reaction products were run for 100-120 volts for 24 hours. The agarose gel was then photographed under UV illumination and the picture was retrieved on the computer. The genetic relatedness of the ESBL E. coli isolates was determined by the similarity of the DNA band patterns obtained.

PCR Amplification for ESBLs
The ESBL-positive E. coli isolates by phenotypic testing were subjected to PCR amplification to screen for the blaTEM, blaCTXM, blaSHV genes. A known ESBL-producing bacterial strain (E. coli carrying ESBL genes) was used as positive control while a non-ESBL-producing bacterial strain was used as negative control.

Table 1: Primers Used for the Amplification of Genes

Primer(s)	Sequence 51-31	Product Size(bp)	Annealing Temperature(°C)
TEcol553	TGGGAAGCGAAAATCCTG	258	58
TEcol754	CAGTACAGGTAGACTTCTG		
TEMH F	CCCCGAAGAACGTTTTC	517	52
TEMH R	ATCAGCAATAAACCAGC		
CTX-M F	CGATGTGCAGTACCAGTAA	585	57
CTX-M R	TTAGTGACCAGAATCAGCGG		
SHV F	AGGATTGACTGCCTTTTTG	393	56
SHV R	ATTTGCTGATTTCGCTCG		

Gel Electrophoresis

At the completion of the amplification, PCR products were resolved on l.5% agarose gel prepared by dissolving 1.5g of agarose powder in 100 ml of 1X Tris-borate-EDTA (TBE) buffer solution inside a clean conical flask. The 1.5% agarose solution was heated in a microwave oven for 2-3 minutes and was observed for clarity which was an indication of complete dissolution. The mixture was then allowed to cool to about 500C after which 0.5 µl of ethidium bromide was then added. It was allowed to cool further and then poured into a tray sealed at both ends with support to form a mould with special combs placed in it to create wells. The comb was carefully removed after the gel had set and the plate was placed inside the electrophoresis tank which contained 1X TBE solution, 5µl of amplicon was mixed with 1µl of loading buffer and the mixture was loaded to the wells of the agarose gel. The power supply was adjusted to 100 volts for 25 minutes. For each run, a 100 base-pair molecular weight DNA standard (size marker) was used to determine the size of each PCR product. The DNA bands were then visualized with a short wave ultraviolet trans-illuminator and photographed using gene gel bio-imaging system. The PCR product was then analyzed. To ensure the specificity of amplification and identify possible nonspecific bands, PCR products were run on an agarose gel (1.5 – 2%) with a DNA ladder to check for correct band sizes.

3. 4 Statistical Analysis

Data underwent descriptive analysis and Chi-square testing to compare differences for categorical data by using SPSS 25 program. A p-value of <0.05 was considered significant, highlighting associations between variables and antibiotic resistance genes.

RESULTS AND DISCUSSION

Identification of Isolates

The cultural and biochemical characteristics of the bacterial isolates produced results that agreed with their identities. The clinical isolates were previously identified as E. coli at the Clinical Microbiology Departments of the Hospitals where they were collected. The clinical isolates were further identified by sub-culturing the isolates on EMB agar and by molecular detection of TEcol genes in the isolates, at the Department of Medical Laboratory Science, Ladoke Akintola University of Technology (LAUTECH), College of Health Sciences, Isale-Osun, Osogbo, Oyo State, Nigeria.

Table 2: Showing the Distribution of Isolates Confirmed for E. coli by EMB agar and TEcol gene

Sample	TEcol gene	EMB Agar
Aspirates	1	1
Blood culture	2	2
Catheter tip	0	1
Ear discharge	1	1
Endocervical Swab	1	2
High Vaginal Swab	1	2
Semen	0	1
Stool	8	14
Urine	16	24
Wound Swab	5	7
Total	35	55

Table 3 show the distribution of the samples from which the confirmed E. coli isolates were made by detection of TEcol genes. The highest percentage (45.7%) of the isolates were taken from urine, followed by those isolated from stool (22.9%), while isolates from aspirates, high vaginal swab, endocervical swab and ear discharge took only 2.9% of the total isolates used for the study.

Table 3: Showing the Different Samples from which E. coli was Confirmed with TEcol gene

Sample	Frequency	Percentage
Aspirates	1	2.9
Blood culture	2	5.7
Ear discharge	1	2.9
Endocervical Swab	1	2.9
High Vaginal Swab	1	2.9
Stool	8	22.9
Urine	16	45.7
Wound Swab	5	14.3
Total	35	100.0

Antimicrobial Susceptibility

The antimicrobial susceptibilities of the clinical E. coli isolates are shown in Table 4. Antibiotic susceptibility patterns of the E. coli isolates showed 100% resistivity to Oxacillin, Ceftazidime, Meropenem, and Amoxycillin/Clavulanic acid. The isolates showed a varying level of susceptibility to most of the antibiotics in the antibiotic susceptibility testing with Chloramphenicol having the highest prevalence of susceptibility (31.4%), followed by Imipenem with susceptibility rate of 28.6%.

Table 4: Antibiotic susceptibility pattern of isolates in the study population

	Resistance	Intermediate	Sensitive
C	19 (54.3)	5 (14.3)	11 (31.4)
IPM	14 (40)	11 (31.4)	10 (28.6)
CIP	25 (71.4)	8 (22.9)	2 (5.7)
OX	35 (100)	0 (0)	0 (0)
CAZ	35 (100)	0 (0)	0 (0)
LEV	22 (62.9)	4 (11.4)	9 (25.7)
FOX	28 (80)	2 (5.7)	5 (14.3)
MEM	35 (100)	0 (0)	0 (0)
AMC	35 (100)	0 (0)	0 (0)
FEP	34 (97.1)	1 (2.9)	0 (0)

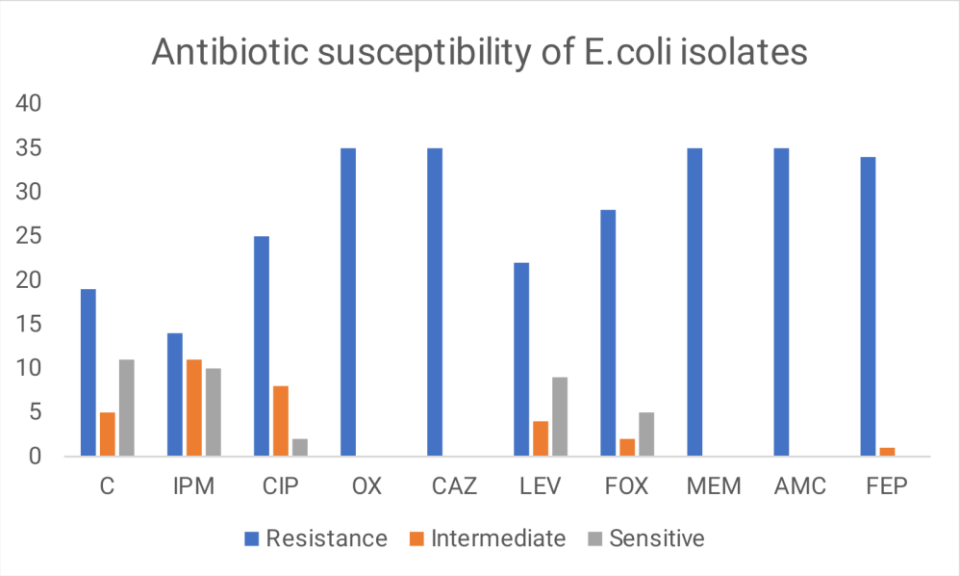

Figure 1: Antibiotic Susceptibility of E. coli Isolates

Extended Spectrum Beta-Lactamase Gene Characterization

BlaTEM, blaSHV and blaCTX-M genes were detected in all 33 of the 35 confirmed E. coli isolates.

Table 5 shows the number of isolates that were positive for each of the ESBL types detected with PCR during the study. CTX-M had 40% and TEMH types had 37.1% occurrences while the SHV was only found in 17.1% of the isolates.

Table 5: Showing the Number of Isolates Positive for ESBL genes

ESBL Type	Total Isolate Examined	No of Positives (%)
TEMH	35 (100)	13 (37.1)
CTX-M	35 (100)	14 (40)
SHV	35 (100)	6 (17.1)

Table 6 shows the Antibiotic resistance rate of the ESBLs-producing E. coli isolates, with 100% resistivity to Oxacillin, Ceftazidime, Meropenem, Cefepime and Amoxycillin/Clavulanic acid and Imipenem with the lowest resistivity.

Table 6: Antibiotic Resistance of ESBLs-Producing E. coli

Antibiotic	No. of Resistance/ No. of Tested	Resistance Tested (%)
C	10/21	47.6
IPM	7/21	33.3
CIP	15/21	71.4
OX	21/21	100
CAZ	21/21	100

LEV	13/21	61.9
FOX	17/21	81
MEM	21/21	100
AMC	21/21	100
FEP	21/21	100

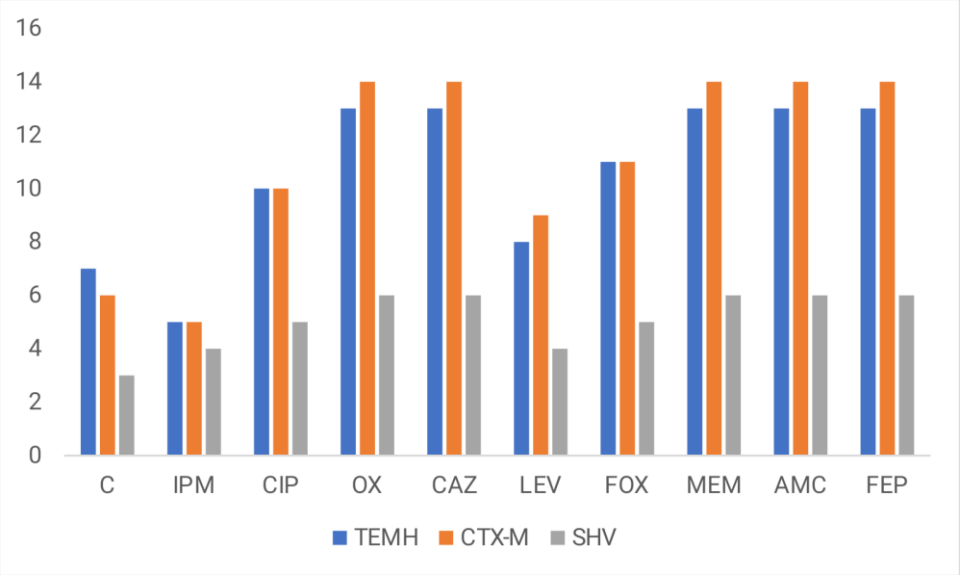

Figure 2: Antibiotics Resistance of ESBL-Producing Isolates

Table 7: Showing the Distribution of the ESBL genes in the Samples and their Association

Sample	TEMH gene	CTX-M gene	SHV gene
Aspirates	1	0	1
Blood culture	2	2	1
Ear discharge	0	1	0
Endocervical Swab	1	0	1
Stool	4	3	0
Urine	4	7	1
Wound Swab	1	1	2
Total	13	14	6
p-value	.240	.384	.021

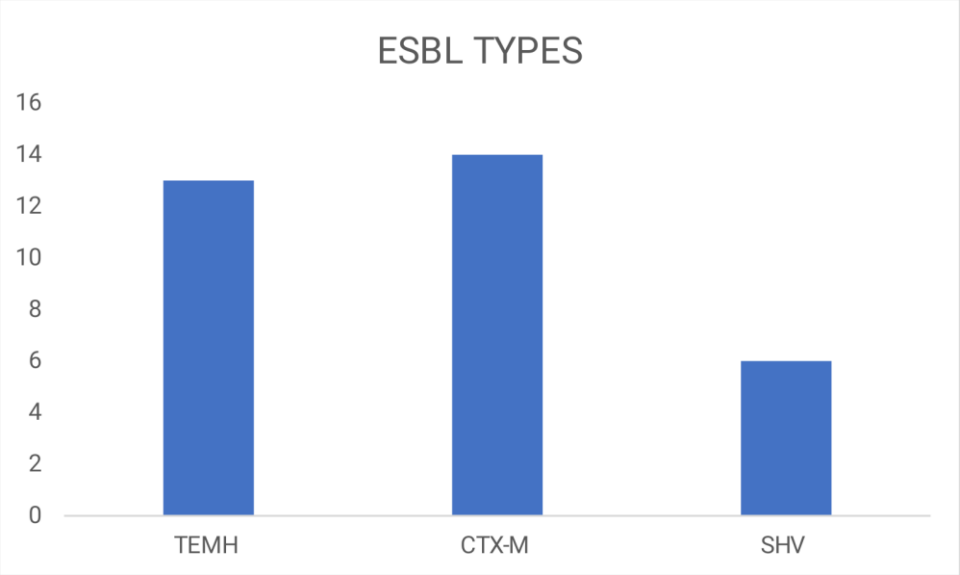

Figure 3: Distribution of ESBL in E. coli Positive Isolates

Discussion

Antimicrobial resistance among Escherichia coli is increasingly limiting available treatment options. ESBL-producing bacteria are of significant concern not only for infected individuals but also in the context of implementing appropriate infection control measures to prevent their spread. The global dissemination of ESBL-producing E. coli has been remarkably rapid, underscoring the urgent need for effective infection control strategies and continuous surveillance systems within communities. Several factors have been associated with the prevalence of ESBLs, including age over 60, previous antibiotic treatment, prolonged hospitalization, extensive use of antimicrobial agents, poor hygiene practices, immunosuppression, and diabetes. The growing prevalence of ESBLs is alarming, particularly as multidrug-resistant (MDR) pathogens are increasingly common in developing countries. This significantly restricts therapeutic options for managing severe infections, often leading to poor clinical outcomes. Accurate identification of β-lactamases is crucial for reliable epidemiological tracking and the effective management of antimicrobial resistance (Olowe et al., 2012, Tabar et al., 2016, Thonda et al., 2018, Hardany et al., 2019, Zhou et al., 2025).

In this study, we surveyed antimicrobial drug resistance and the ESBL gene detection in E. coli strains isolated from different clinical samples. Out of 105 E. coli isolates obtained from different clinical samples from some Medical Microbiology laboratories in the Southwest of Nigeria, a total number of 55 were confirmed E. coli by their production of green metallic sheen on EMB agar. However, only 35 (33.3%) proved to be truly E. coli isolates by molecular detection of TEcol genes using PCR technique. This indicates that conventional methods of identification of bacteria are not 100% reliable; though, they are the routine diagnostic techniques used in Medical Microbiology laboratories in developing countries. This implicates a high rate of misdiagnosis which isn't safe for patients, and could be one of the reasons for high prevalence of MDR bacteria both in the community and hospitals, as well as increase in therapeutic failures of the drugs administered. Hence, molecular method of identification is more reliable.

In this study, majority of the E. coli strains were found in urine samples which is in agreement with previous studies (Kader & Kumar, 2005, Fang et al., 2008, Ben Slama et al., 2011, Iroha et al., 2012, Inwezerua, 2014, Adeyemo et al., 2020, Saleh et al., 2024).

Antibiotic pressure is a major determinant of the emergence and dissemination of antibiotic-resistant organisms (Olowe et al., 2012, Sher et al., 2025). In this study, 94.3% of the E. coli isolates were discovered to have ESBL genes TEMH, CTX-M and SHV; which were found to be resistant to Ceftazidime (100%), Cefepime (100%), Imipenem (33.3%), Meropenem (100%), Amoxycillin/Clavulanic Acid (100%), Levofloxacin (61.9%), Oxacillin (100%), Ciprofloxacin (71.4%), Chloramphenicol (47.6%), and Cefoxitin (81%). TEMH, CTX-M and SHV were found to be highest in isolates resistant to Oxacillin, Ceftazidime, Meropenem, Amoxycillin/Clavulanic acid and Cefepime, which all had 100% resistivity. The presence of multidrug resistance in this study may be related to the dissemination of antibiotic resistance among hospital isolates of E. coli, as it is one of the main bacterial pathogens responsible for nosocomial infections especially in immunocompromised patients (Iliyasu et al., 2018). The high rate of multidrug-resistant pattern among Enterobacteriaceae strains carrying any beta-lactamase gene in this study may be attributed to high selection pressure because of self-medication, overuse and empirical use of third-generation Cephalosporins and Carbapenems without careful monitoring of resident multidrug-resistant isolates in hospital settings, particularly in the Intensive Care Unit (ICU), surgery ward and burn ward (Sadeghi et al., 2016, Mendes Pedro et al., 2025, Zhou et al., 2025).

The high resistance rates of ESBL-producing isolates of E. coli to Amoxycillin/Clavulanic Acid (100%), Meropenem (100%), Oxacillin (100%), Ceftazidime (100%), Cefepime (100%), Cefoxitin (81%) and Ciprofloxacin (71.4%) is in accordance to previous researches (Aibinu et al., 2004, Pdia et al., 2006, Olowe et al., 2008, Olowe et al., 2012, Inwezerua, 2014, Saleh et al., 2024). Contrary to the reports, the E. coli isolates had low susceptibility pattern to Imipenem (15.4%), Cefoxitin (7.7%) and Cefepime (0%) (Olowe et al., 2012, Inwezerua, 2014, Tabar et al., 2016, Thonda & Oluduro 2017, Akinbami et al., 2018, Iliyasu et al., 2018, Adekunle et al., 2019, Or et al., 2025, Zhou et al., 2025). The variation could be due to exposure of E. coli to various combinations of antibiotics. It is also attributable to widespread use of antibiotics for growth enhancement and animal prophylactic therapy, all of which creates a selective pressure which favours the emergence of antibiotic resistance bacteria (Olowe et al., 2015, Or et al., 2025).

Also, the observed differences in antibiotic resistivity and susceptibility might be due to the differences in the screening techniques at various centres of study. However, the observed prevalence of MDR indicates that the isolates might have been inadvertently exposed to these antimicrobials either from the clinics or agricultural products since E. coli can easily get exposed to the drugs used in animal husbandry and food industry through ingestion. Hence, controlled use of antimicrobials in both agricultural and clinical settings could be considered (Onanuga et al., 2019, Or et al., 2025).

The presence of an ESBL phenotype is a good marker of the MDR pattern and resistance to newer beta-lactam antibiotics. It is important to note that in this study, most of the isolates showed a MDR pattern. The resistance rate of Ceftazidime was 100%. The class-A ESBLs, TEM, SHV and CTX types, are the most widespread and clinically relevant worldwide (Arsalane et al., 2015, Ayalneh et al., 2025). The correct detection of ESBL producing microorganisms is a challenge for the laboratories, requiring not only phenotypic tests, but also genotypic tests for all genes associated with beta-lactamase production. ESBL producers are defined as isolates which test positive to at least one of the available phenotypic methods and contain at least a detectable ESBL gene (Olowe et al., 2015). Phenotypic identification of ESBL is based on the inhibition of enzymes by clavulanic acid (Gautam et al., 2019). The Health Protection Agency of the United Kingdom recommends testing Cefpodoxime or both Cefotaxime and Ceftazidime as a first screening test (Olowe et al., 2015). Study done by Olowe et al., (2015) revealed that the combination of the two latter drugs separated at 20 mm distance achieves 80% sensitivity to adequately detect ESBL production, meaning that only 20% of the isolates would need further testing.

In this study, CTX-M (40%) is the most predominant genotype of ESBL which is in accordance with previous studies done by Hassan & Abdalhamid (2014), Arsalane et al., (2015) and Parajuli et al., (2016), but contrary to the studies done by Olowe et al., (2012), Thonda & Oluduro (2017), Onanuga et al., (2019), Pandit et al., (2020) Khachab et al., (2025), and Zhou et al., (2025), Pearce et al., (2025). The wider spread of blaCTX-M is due to over-use of third generation Cephalosporins which has selected this strain. Some of the blaCTX-M types are also associated with mobile

genetic elements like class 1 integron element which contributes to its wider spread (Pokhrel et al., 2014, Zhou et al., 2025). CTX-M b-lactamases hydrolyze Cefotaxime very efficiently (Skočková et al., 2015, Ansari et al., 2025). CTX-M β-lactamases producing Enterobacteriaceae are typically resistant to quinolones, aminoglycosides and sulfonamides such as Ciprofloxacine, Gentamicin and Trimethoprim/Sulfamethoxazole (Olowe et al., 2015, Farzi et al., 2021).

TEMH is the second predominant ESBL genotype in this study; with approximately 37%. It has been reported previously that resistance to Oxyimino-Cephalosporins (for examples, Cefpodoxime and Ceftazidime), is caused mostly by TEM-type of ESBL (Ugwu et al., 2020, Ibrahim et al., 2023). This is most likely the reason for 100% resistivity of Ceftazidime observed in the ESBLs-producing E. coli in this study.

Moreover, coexistence of blaTEMH and blaCTX-M genes was detected in 28.6% of the strains while the coexistences of blaSHV and blaCTX-M genes, and blaTEMH and blaSHV genes were both detected in 14.2% and 14.2% of the strains respectively; this highlights the growing complexity of antibacterial resistance problems. The presence of more than one ESBL in a single isolate in this study is in accordance with previous studies done (Hassan & Abdalhamid, 2014, Arsalane et al., 2015, Parajuli et al., 2016, Ugwu et al., 2020, Farzi et al., 2021, Saleh et al., 2024, Zhou et al., 2025).

ESBLs-producers were significantly more resistant to augmentin, levofloxacin, gentamicin, ciprofloxacin, nalidixic acid, cefepime, ceftriaxone, ceftazidime and cefotaxime when compared with non- ESBLs producers. This widespread resistance may be due to the improper use of antibiotics in Nigeria, their easy accessibility, the lack of prescription regulations, and the absence of strict antibiotic policies in the country. (Odedoyin & Adewole et al., 2021). The prevalence of ESBL producers in our study area was 60%, which is in accordance with the study done by Ugwu et al., (2020) in the Southeast of Nigeria which was 60.34%. However, there is a drastic increase when compared to the studies done in the study area in the past; this is alarming and needs serious attention.

The prevalence of ESBL-producing E. coli in the study done by Mohammed et al., (2016) at NorthWest Nigeria was 23.8%; Giwa et al., (2018) also reported 50% ESBL-producing E. coli; Odetoyin & Adewole, (2021) reported a prevalence of 25.9%; Egbule & Ejechi (2021) also reported 42% ESBLs-producing E. coli. In the study done by Uyanga et al., (2020), the frequency of ESBL-producing E. coli was 19%; the isolates carried blaTEM, blaSHV and blaCTX-M-15 type ESBL genes; CTX-M-15 type ESBLs was confirmed in 42% of the isolates. Several studies from other parts of the world have shown different prevalence of blaCTXM-15 gene among E. coli isolates, including 84.7% (Chile), 98.8% (China), and 13.6% (Tanzania). Several studies from Europe and Asia have also reported that CTX-M gene is now replacing TEM and SVH genes as the commonest ESBL type in that part of the world (Uyanga et al., 2020). which aligns with previous studies showing blaCTX-M as the most widespread ESBL genotype. The observed variances in prevalence may be attributed to differences in study design and patient selection and differing patterns of antibiotic stewardship in the various centers. Moreover, geographical differences occur in clinical isolates which are also rapidly changing with time (Giwa et al., 2018)

Imipenem demonstrates high efficacy against ESBL-positive isolates, making it the recommended treatment for confirmed ESBL producers; though, widespread use of beta-lactam antibiotics, especially broad-spectrum and extended-spectrum beta-lactams, in both community and clinical infections has played a role in the rising prevalence of ESBL- and MBL-producing bacteria responsible for infections globally (Alabi et al., 2020; Mohammed et al., 2024). In the study done by Alabi et al., (2020), high percentage of E. coli relatively exhibited resistance to meropenem (11.3%), ertapenem (15%) and imipenem (18.8%); co-production of both ESBL and MBL was recorded 7.7% of E. coli. Co-production of ESBL and MBL signifies a serious public health threat, especially for the carbapenem resistance and thus requires continuous surveillance and strict restrictions in the use of extended-spectrum beta-lactams and carbapenems for community-based or nosocomial infections to prevent further increases and spread (Alabi et al., 2020). To analyze the mechanism(s) of resistance to carbapenems by MBL-producers, there might be need to screen of the isolates for lots of the MBL genes by PCR technique or to carry out whole genome sequencing; as isolates can resist carbapenems via different mechanisms, or possibly harbour the types of MBL genes not targeted (Alabi et al., 2020).

Antimicrobials are commonly used in animal farming for treating and preventing diseases, as well as to enhance animal growth. However, their excessive use leads to drug residues contaminating the environment, milk, and meat. This overuse also creates antimicrobial pressure, encouraging bacteria in the environment to develop resistance through mutations. Additionally, it promotes the survival of drug-resistant bacteria in areas where the antibiotic is present, making resistance more persistent (Srisrattakarn et al., 2025).

Food-producing animals are found to be a reservoir and risk factor for ESBL E. coli, especially in Cattle and poultry farms (Obuekwe & Ikediashi, 2021, Or et al., 2025). Cattle egrets (Bubulcus ibis), whistling ducks (Dendrocygna viduata) and cattle constitute important reservoirs of highly diverse extended spectrum β-lactamase producing E. coli with potentials of inter-species transmission, widespread and virulence (Fashae et al., 2021). Fashae et al., (2021) reported the predominance of CTX-M15 in wild birds and cattles in Ibadan, Nigeria, which is consistent with previous findings in chickens and humans (clinical and asymptomatic) in Nigeria. The study also marks the first report of the occurrence of CTX-M9 in Nigeria showing egret as a more prominent reservoir compared to cattle (Fashae et al., 2021).

Systemic review and meta-analysis done by Or et al., (2025) showed that there is a pooled prevalence of 26.6% ESBL-producing E. coli in broiler farms. The pooled prevalence of ESBL E. coli in broiler farms was almost identical between Asia; 33.0% and Europe; 32.8%, while Africa showed the lowest prevalence at 12.4%. There is a moderate prevalence of ESBL E. coli in broiler farms globally, and it has been fluctuating inconsistently in the last decade (Or et al., 2025).

Obuekwe & Ikediashi, (2021) detected TEM (40%,), OXA (60%) and SHV (80%) genes in isolates of ESBLs-producing Escherichia coli obtained from animal faeces in Edo State, while CTX-M gene (100%) was detected in all isolates (Obuekwe & Ikediashi, 2021).

The research done by Odewale et al., (2024) on Antibiotic susceptibility and detection of ESBLs on Automatic Teller Machines (ATMs) revealed the presence MDR bacteria and ESBL genes particularly blaTEM, blaSHV and blaCTX-M genes. The ATMs had microbial contaminants and have a role in the transmission of pathogenic microorganisms. E. coli isolated displayed resistance of over 40% to ceftazidime and resistance against third-generation cephalosporins (cefotaxime); high meropenem resistance was also discovered (Odewale et al., 2024).

Investigation done by Olajide et al., (2024) on "Presence and characterisation of extended spectrum beta-lactamase producing Gram-negative bacteria obtained from water in selected storage tanks" showed ESBL-producing E. coli and Citrobacter spp as the most prevalent isolates in storage tanks. The antibacterial susceptibility tests revealed high resistance of the ESBL-producing isolates to cefpodoxime, amoxicillin/clavulanate and low resistance to tetracycline (50.0%). This demonstrates the crucial role of the environment in spreading and distributing multidrug-resistant bacteria to various water sources, including those used for drinking and domestic purposes (Olajide et al., 2024).

Hence, it is crucial that all stakeholders in every sector work together to ensure the responsible use of antimicrobials in humans, animals, and agriculture, following the One Health approach (Srisrattakarn et al., 2025).

Co-existence of AmpC type enzymes in ESBL producers may alter the pores of the cell membranes, thereby reducing the affinity for β-lactamase inhibitors for enzymes such as TEM and SHV25. Production of different types of β-lactamases (TEM, SHV, CTX-M and OXA) by the same microorganism can lead to erroneous phenotypic conclusions (Gautam et al., 2019). According to Ugwu et al., (2020), the discrepancy in the percentage of phenotypic and genotypic β-lactamase confirmed producers might be because of co-expression of more than one ESBL genes in an organism. Occurrence of multiple ESBL type's combinations within the same organism has previously been reported to make phenotypic identification of the β-lactamases difficult and not reliable; it might also be that the genes detected by PCR are not effectively expressed phenotypically (Ugwu et al., 2020, Ansari et al., 2025). Krishnamurthy et al., (2013), Olowe et al., (2015), Adeyemo et al., 2020 and Ansari et al., (2025) in their studies, attributed it to lower sensitivity of the phenotypic method and the influence of environmental factors and maintained that the genotypic method has a 100% specificity and sensitivity as it uses specific PCR amplification of resistance genes. Olowe et al., (2015) stated that the differences in the result obtained using phenotypic and molecular methods may be due to other mechanisms of resistance other than ESBLs. Molecular detection and identification of beta-lactamases would be essential for a reliable epidemiological investigation of antimicrobial resistance (Arsalane et al., 2015, Akineden et al., 2025). Hence, phenotypic characterization of ESBLs in ESBLs-producing bacteria is not reliable, molecular characterization is.

Infections caused by ESBL-producing organisms are a global problem. Mobile genetic elements contained in the bacterial species are easily transferable to other organisms in the vicinity. Timely detection of the resistant strains along with their antimicrobial susceptibilities is very important for the effective management of infections caused by ESBLs-producing E. coli. However, limited facilities for detection and poor understanding of such bugs in the developing counties are responsible for global dissemination of such pathogens (Pandit et al., 2020).

The proliferation of beta-lactamase resistance among strains may increase the rate of resistance to drugs that are now sensitive, resulting in increase in morbidity and mortality (Thonda & Oluduro, 2017). Current therapy for strains of Enterobacteriaceae that express extended-spectrum beta-lactamases (ESBLs) is largely limited to broad-spectrum agents such as imipenem. However, the susceptibility of ESBL-producing E. coli to imipenem is already decreasing significantly, leading to an increase in therapeutic failures against strains that produce multiple beta-lactamases. As a result, therapeutic options for some of these organisms are becoming increasingly limited.

This study confirms that a high level of blaCTX-M-positive ESBL isolates is circulating in southwestern Nigeria. The trend of multidrug-resistant profiles associated with the recovery of the blaCTX-M gene is alarming. This underscores the need for early detection of MDR and extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli, as well as the establishment of an antimicrobial resistance surveillance network for Enterobacteriaceae to monitor trends and emerging resistance mechanisms in clinical isolates.

Considering the grave situation of antibiotic resistance in our country, it is high time all clinical laboratories began routinely and accurately detecting ESBLs. Numerous methods have been proposed for the detection of ESBLs in clinical isolates. Regardless of the detection method used, it is important to note that none of the phenotypic methods can identify every ESBL-producing isolate. Nevertheless, increased awareness of the ESBL issue among clinical microbiology laboratories and infection control personnel will improve the interpretation and application of these diagnostic tests. It is equally crucial to reinforce strict adherence to antibiotic stewardship and enforce infection prevention measures across all healthcare facilities to help curb the growing spread of multidrug-resistant bacteria.

CONCLUSION

This study highlights the rising prevalence of ESBL-producing E. coli harboring the CTX-M gene in Southwestern Nigeria, alongside the growing resistance of these strains to carbapenems, mirroring global trends. Therefore, it is imperative to urgently implement effective screening protocols for ESBL detection in clinical laboratories. Laboratory reports should explicitly state whether an isolate is a suspected or confirmed ESBL producer. Hospitals must strengthen infection control measures and establish robust antibiotic stewardship programs to limit the spread of resistant organisms. Furthermore, clear regulations governing antimicrobial use should be developed and strictly enforced, supported by a nationwide antimicrobial resistance surveillance system. Educating the public on the risks of improper antibiotic use is also essential for long-term containment efforts.

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