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Phenotypic and Genotypic Detection of Extended Spectrum Beta-Lactamase Producing Escherichia coli in Wound Infections Among Patients Attending Federal Teaching Hospital, Ido-Ekiti

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Abstract

Wound infections caused by Extended-Spectrum Beta-Lactamase (ESBL)-producing bacteria remain a major threat to patient recovery and clinical management in hospital settings. This study was therefore conducted between January and July,2026, to determine the phenotypic detection and molecular characterization of blaCTX-M gene among *Escherichia coli* isolated from wound swab samples in Federal Teaching Hospital, Ido-Ekiti, Ekiti State. Wound swab samples were collected from ninety-five patients presenting with clinical wound infections. The samples were processed for isolation and identification of *Escherichia coli* using standard microbiological methods. Phenotypic screening for ESBL production was performed using the Double Disk Synergy Test (DDST) according to Clinical and Laboratory Standards Institute (CLSI) guidelines, and the confirmed ESBL-producing isolates were analyzed using molecular techniques (Polymerase Chain Reaction). The demographics of the patients were obtained using a well-structured questionnaire, and the data generated were analyzed with SPSS version 26.0. Out of ninety-five wound swab samples tested, 8 (8.42%) were identified as *Escherichia coli*, with the highest occurrence rate in the age group 60 years and above (20.83%), showing a statistically significant association with age (p value = 0.0301), while no statistically significant association was observed with sex (p value = 0.8310). Gentamicin exhibited lowest resistance to *Escherichia coli* 1(12.50%) with resistance rate ranging from 12.50% to 75%. Among the 8 recovered *Escherichia coli* isolates, phenotypic screening confirmed ESBL production in 5 (62.50%) of the cases. The molecular characterization of the phenotypically confirmed ESBL-producing isolates revealed the presence of the blaCTX-M gene in 3 (60.00%) samples, exhibiting a distinct molecular weight band size of 585bp. However, phenotypic screening methods could be a reliable and accessible means of routinely identifying ESBL-producing *Escherichia coli*, most especially when CLSI guidelines are strictly followed. Nevertheless, molecular technique could be a more reliable option which will prevent false positive or negative results and ensure accurate surveillance of antimicrobial resistance genes in clinical settings.

INTRODUCTION

The emergence and dissemination of antimicrobial resistance (AMR) represent one of the most significant global public health threats of the 21st century. Among the primary resistance mechanisms, the production of extended-spectrum beta-lactamases (ESBLs) by Gram-negative bacteria, particularly members of the Enterobacteriaceae family, poses a critical challenge to effective clinical therapy. Beta-lactam antibiotics, including penicillins, cephalosporins, and monobactams, have historically been the cornerstone of treatment for serious bacterial infections. However, the selective pressure exerted by the widespread and often inappropriate use of these agents has driven the evolution and horizontal spread of ESBL genes, rendering many frontline antibiotics ineffective. ESBLs are enzymes that hydrolyze the beta-lactam ring of these antibiotics, conferring resistance to oxyimino-cephalosporins (e.g., cefotaxime, ceftazidime) and aztreonam, while remaining susceptible to inhibition by clavulanic acid [1].

Escherichia coli, a commensal inhabitant of the gastrointestinal tract, is also a leading cause of both community-acquired and hospital-acquired infections. Its pathogenic potential is vast, ranging from

urinary tract infections and bacteremia to gastrointestinal illnesses and wound infections. In the context of wound care, *Escherichia coli* is a frequently isolated pathogen, particularly in surgical site infections, traumatic wounds, and diabetic foot ulcers. The presence of *Escherichia coli* in a wound signifies a breach in the skin's protective barrier and can lead to delayed healing, local tissue destruction, and systemic complications such as sepsis. The management of these infections is becoming increasingly complex due to the rising prevalence of multidrug-resistant (MDR) strains, especially those producing ESBLs [2].

The genotypic diversity of ESBLs is considerable, with the TEM, SHV, and CTX-M families being the most clinically relevant globally. Over the past two decades, the CTX-M type enzymes have emerged as the predominant ESBL type worldwide, largely supplanting TEM and SHV variants in many regions [3]. The blaCTX-M genes are often located on mobile genetic elements such as plasmids, transposons, and integrons, facilitating their rapid dissemination among bacterial populations. The CTX-M-15 variant, in particular, has been associated with the globally successful and highly virulent *Escherichia coli* sequence type ST131 lineage, underscoring the convergence of resistance and pathogenicity [4]. In the Nigerian healthcare setting, several studies have documented a high prevalence of ESBL-producing *Escherichia coli* from various clinical specimens, with CTX-M being the most frequently detected genotype [5]. This trend is alarming, as it severely limits therapeutic options, often forcing clinicians to resort to last-resort antibiotics like carbapenems, which are expensive and may be unavailable in resource-limited settings.

METHODOLOGY

Study Design

This research employed a cross-sectional descriptive design. Wound swab samples was collected from both inpatients and outpatients diagnosed with wound infections at the Federal Teaching Hospital, Ido-Ekiti. The samples were processed for the isolation, identification, and characterization of *Escherichia coli*. Phenotypic screening for ESBL production was done, followed by genotypic detection of ESBL genes using polymerase chain reaction (PCR) techniques [6, 7].

Isolation and identification of bacteria from the wound samples

Wound swabs were inoculated onto MacConkey agar and Chocolate agar using the streak plate method and incubated aerobically at 37°C for 18–24 hours. Pure bacterial colonies were obtained after incubation. Colony size, colour, odour, shape, consistency, opacity, elevation and edge were recorded. Isolates underwent Gram staining and selected biochemical tests, including catalase, indole, citrate utilization, oxidase, urease, motility and triple sugar iron (TSI) reactions. Presumptive identification followed standard microbiological procedures described in the project [8].

Antibiotic Susceptibility Testing of Escherichia coli Isolated from Wound Samples

Antibiotic susceptibility testing (AST) was performed to determine the antimicrobial susceptibility patterns of the confirmed *Escherichia coli* isolates using the Kirby-Bauer disk diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2024) using antibiotics procured from Oxiod to ensure quality and consistency. Antibiotics used include Amoxicillin-Clavulanate (20/10 µg), Cefotaxime (30 µg), Ceftazidime (30 µg), Imipenem (10 µg), Meropenem (10 µg), Gentamicin (10 µg), Amikacin (30 µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg) and were incubated at 37°C for 18–24 hours. Following incubation, the diameters of the zones of inhibition were measured in millimeters using a calibrated ruler. Results were interpreted as Sensitive (S), Intermediate (I), or Resistant (R) according to CLSI 2025 [9] breakpoints.

Phenotypic Detection of ESBL Production in Escherichia coli Isolated from Wound Samples

The double disk synergy test was performed as a screening method for ESBL production, as recommended by CLSI 2020 [10]. An amoxicillin-clavulanic acid (30 µg) disk was placed at the centre of the plate. Disks of cefotaxime (30 µg) and ceftazidime (30 µg) were placed at a distance of 15–20 mm (centre to centre) from the amoxicillin-clavulanic acid disk. The plates were incubated at 37°C for 18–24 hours. ESBL production was interpreted as positive when the inhibition zone of either cefotaxime or ceftazidime was enhanced or distorted towards the amoxicillin-clavulanic acid disk, forming a characteristic "keyhole" or "champagne cork" shape [11]. This synergy occurs because clavulanic acid inhibits the ESBL enzyme, allowing the cephalosporin to exert its antibacterial effect. Quality control was performed using appropriate reference strains in accordance with CLSI 2025 [9] recommendations.

DNA Isolation and Amplification

Isolation of DNA using Cetyl trimetyl Ammonium Bromide (CTAB) method was done according to [12]. Conventional PCR was used for identifying the presence of the gene using specific forward primer (CTX-M-F: 5'-CGCTTTGCGATGTGCAG-3') and reverse primer (CTX-M-R: 5'-ACCGCGATATCGT TGGT-3') which were designed to bind to the conserved regions flanking the blaCTX-M gene being the most prevalent used globally. The extracted DNA is mixed with a master mix containing Taq polymerase, dNTPs (deoxynucleotide triphosphates), primers, and buffer. The thermal cycler goes through initial denaturation, followed by cycles of denaturation, annealing, and extension. The products of amplification are visualized to confirm the presence of the gene. The PCR products are run on an agarose gel containing a fluorescent dye (ethidium bromide). An electric current separates the DNA fragments by size. The gel is examined under UV light. A positive result is confirmed if a visible band appears at the expected base-pair size when compared to a DNA molecular weight ladder.

Ethical Consideration, Adherence and Statistical Analysis

The ethical clearance for this research was given by the Human Research and Ethics Committee, Federal Teaching Hospital, Ido-Ekiti and the College of Medicine Research Ethics Committee, Federal University,

Oye-Ekiti after due processes, before the commencement of this research. All methods used in this research were carried out in accordance with relevant guidelines and regulations of the ethics committees.

The data obtained were entered and analyzed using SPSS version 26.0. Chi-square (χ^2) tests was calculated and a p-value < 0.05 was considered statistically significant.

RESULT

Demographic Characteristics of Study Participants

A total of 95 patients with wound infections were evaluated (Table 1). Males constituted 58.95% (56/95) of the study population, while females accounted for 41.05% (39/95). The distribution by sex was not statistically significant ($\chi^2 = 3.042$, df = 1, p = 0.0811). Participants aged 20–39 years constituted the largest age group (30.53%), followed by those aged 40–59 years (29.47%), ≥ 60 years (25.26%), and 1–19 years (14.74%). The distribution across age groups was also not statistically significant ($\chi^2 = 5.926$, df = 3, p = 0.1153)

Table 1
Overall Demographic Characteristics of Patients with Wound Infection (n = 95)

Sex	**Frequency n(%)**	**χ^2**	**df**	**P value**
Male	56(58.95)	3.042	1	0.0811
Female	39(41.05)			
Total	95(100)			
Age Group (years)				
1–19	14(14.74)	5.926	3	0.1153
20–39	29(30.53)			
40–59	28(29.47)			
60 and above	24(25.26)			
Total	95(100)			

Isolation Rates of Bacterial Pathogens

Staphylococcus aureus was the most frequently isolated organism, accounting for 31.58% (30/95) of the samples, followed by *Pseudomonas aeruginosa* (14.74%, 14/95), *Klebsiella aerogenes* (9.47%, 9/95),

Escherichia coli (8.42%, 8/95), *Proteus vulgaris* (4.21%, 4/95), and *Proteus mirabilis* (1.05%, 1/95). No bacterial growth was observed in 30.53% (29/95) of the samples (Table 2). The distribution of bacterial isolation outcomes differed significantly ($\chi^2 = 59.663$, df = 6, $p < 0.001$).

Table 2
Isolation Rate of Bacteria from Wound Samples

Isolated Organisms	Frequency n(%)	χ^2	df	P value
Staphylococcus aureus	30(31.58)	59.663	6	< 0.001
Pseudomonas aeruginosa	14(14.74)			
Klebsiella aerogenes	9(9.47)			
Escherichia coli	8(8.42)			
Proteus vulgaris	4(4.21)			
Proteus mirabilis	1(1.05)			
No Growth	29(30.53)			
Total	95(100)			

Prevalence of Escherichia coli

Escherichia coli was isolated from 8.42% (8/95) of the wound samples, compared with 61.05% (58/95) for other bacterial isolates and 30.53% (29/95) with no bacterial growth (Table 3). This distribution was statistically significant ($\chi^2 = 41.558$, df = 2, $p < 0.001$).

Table 3
Prevalence of *Escherichia coli* in isolates from Wound Samples

Isolated Bacteria	Frequency n(%)	χ^2	df	P value
Escherichia coli	8(8.42)	41.558	2	< 0.001
Other Bacteria	58(61.05)			
No Growth	29(30.53)			
Total	95(100)			

Association Between Demographic Characteristics and Escherichia coli Isolation

Among male participants, *Escherichia coli* was isolated from 5 of 56 samples (8.93%), compared with 3 of 39 samples (7.69%) among females (Table 4). There was no statistically significant association between sex and E. coli isolation ($\chi^2 = 0.046$, df = 1, $p = 0.8310$). Fisher's exact test also showed no significant association ($p = 1.0000$).

The isolation of *Escherichia coli* varied across age groups. No *Escherichia coli* isolates were recovered from participants aged 1–19 years or 20–39 years. Three isolates were recovered from participants aged 40–59 years (10.71%), while five were recovered from those aged ≥ 60 years (20.83%). The association between age group and *Escherichia coli* isolation was statistically significant ($\chi^2 = 8.940$, df = 3, p = 0.0301).

Table 4
Isolation of *Escherichia coli* from Wound Samples in Relation to Demographics

Demographic Variable	**Number Examined n(%) n = 95**	**Number of Positive n(%) n = 8**	**Isolation Rate (%)**	**χ^2**	**df**	**P value**
Sex						
Male	56(58.95)	5(62.50)	8.93	0.046	1	0.8310
Female	39(41.05)	3(37.50)	7.69			
Total	95(100)	8(100)				
Age Group (years)						
1–19	14(14.74)	0(0)	0.00			
20–39	29(30.53)	0(0)	0.00			
40–59	28(29.47)	3(37.50)	10.71	8.940	3	0.0301
60 and above	24(25.26)	5(62.50)	20.83			
Total	95(100)	8(100)				

Antimicrobial Susceptibility

The eight *Escherichia coli* isolates demonstrated varying antimicrobial susceptibility patterns (Table 5). All isolates were susceptible to amikacin (100%), while 87.50% were susceptible to gentamicin. Imipenem, meropenem and ciprofloxacin each showed 50.00% susceptibility. Resistance was highest to levofloxacin (75.00%), followed by cefotaxime and ceftazidime (62.50% each).

Table 5
Antimicrobial susceptibility pattern of *Escherichia coli* isolates from wound samples

Antimicrobial agent	Resistant isolates / tested	Resistance (%)
Cefotaxime (30 µg)	5/8	62.50
Ceftazidime (30 µg)	5/8	62.50
Imipenem (10 µg)	4/8	50
Meropenem (10 µg)	4/8	50
Gentamicin (10 µg)	1/8	12.5
Amikacin (10 µg)	0/8	0
Ciprofloxacin (5 µg)	4/8	50
Levofloxacin (5 µg)	6/8	75

Phenotypic ESBL Detection

Phenotypic screening using the Double Disk Synergy Test identified ESBL production in 5 of the 8 *Escherichia coli* isolates (62.50%), while 3 (37.50%) were non-ESBL producers (Table 6). The difference between ESBL-positive and non-ESBL isolates was not statistically significant ($\chi^2 = 0.500$, df = 1, p = 0.480).

Table 6
Phenotypic Detection of ESBL-Producing *Escherichia coli* Isolated from Wound Samples

ESBL Status	Frequency n(%)	χ^2	df	P value
ESBL Positive E.coli	5(62.50)	0.500	1	0.480
Non ESBL E.coli	3(37.50)			
Total	8(100)			

Genotypic ESBL Detection

PCR analysis of the five phenotypically ESBL-positive isolates detected the blaCTX-M gene in 3 (60.00%), while 2 (40.00%) were negative. The difference was not statistically significant ($\chi^2 = 0.200$, df = 1, p = 0.655) (Table 7). Agarose gel electrophoresis showing amplification of the blaCTX-M gene is presented in Plate 1.

Table 7
Genotypic Detection of blaCTX-M Gene by PCR

blaCTX-M Gene Status	Frequency n(%)	χ^2	df	P value
Positive	3(60)	0.200	1	0.655
Negative	2(40)			
Total	5(100)			

Plate 1. Agarose gel electrophoresis showing amplification of the blaCTX-M gene (~ 585bp)

Lane 1 = Molecular Marker (585bp); lane 2 = E.coli 8, lane 3 = E. coli 3, lane 4 = E.coli 2, lane 5 = E.coli 1, lane 6 = E.coli 5. Gel image reproduced from the project work.

DISCUSSION

This study evaluated phenotypic and genotypic characteristics of ESBL-producing *Escherichia coli* isolated from wound infections at the Federal Teaching Hospital, Ido-Ekiti (FETHI), Ekiti State, Nigeria. Of the 95 wound samples examined, 66 (69.47%) yielded bacterial growth. *Staphylococcus aureus* was the predominant isolate (31.58%), while *Escherichia coli* accounted for 8.42% of the samples. The culture positivity rate was comparable with reports from Nigerian tertiary hospitals by [2] (68.50%) and [13] (71.20%).

Males constituted 58.95% of participants, although the sex distribution was not statistically significant ($p = 0.0811$). Participants aged 20–39 years formed the largest age group (30.53%), with no significant difference in the overall age distribution ($p = 0.1153$). Similar demographic patterns have been reported in previous Nigerian studies [14, 15]. Among the *Escherichia coli* isolates, however, age was significantly associated with isolation ($p = 0.0301$), with the highest rate observed among participants aged ≥ 60 years (20.83%). No significant association was found with sex ($p = 0.8310$).

Gram-negative organisms accounted for 37.89% of the samples, with *Pseudomonas aeruginosa* being the most frequently isolated Gram-negative pathogen (14.74%), followed by *Klebsiella aerogenes* (9.47%) and *Escherichia coli* (8.42%). The prevalence of *Escherichia coli* was comparable with the 10.20% reported by [15], although higher rates have been reported in some surgical wound studies [2, 16].

The *Escherichia coli* isolates demonstrated considerable antimicrobial resistance. Resistance was highest to levofloxacin (75.00%), followed by cefotaxime and ceftazidime (62.50% each), while imipenem, meropenem and ciprofloxacin each showed 50.00% resistance. In contrast, amikacin showed 100.00% susceptibility and gentamicin 87.50%, consistent with previous Nigerian reports [2, 17].

Phenotypic ESBL production was detected in 5 of the 8 *Escherichia coli* isolates (62.50%), comparable with rates reported by [18]. PCR detected blaCTX-M in 3 of these 5 isolates (60.00%). This is consistent

with reported Nigerian detection rates of 55–75% [15]. The two ESBL-positive isolates negative for blaCTX-M may possess other resistance determinants not investigated in this study.

Overall, the findings demonstrate the presence of ESBL-producing *Escherichia coli* among wound isolates at FETHI, alongside substantial resistance to several commonly used antibiotics, highlighting the need for continued antimicrobial susceptibility testing and surveillance.

CONCLUSION

This study demonstrates that *Escherichia coli* is a significant Gram-negative etiologic agent in clinical wound infections at FETHI. Infection risk is strongly age-dependent, disproportionately affecting elderly patients, whereas patient sex does not influence colonization rates.

The isolates exhibit alarming multidrug resistance, characterized by widespread failure of first-line beta-lactams and fluoroquinolones, as well as emerging resistance to reserve carbapenems. Conversely, aminoglycosides, particularly Amikacin, retain high in vitro efficacy, serving as vital bactericidal options for inpatient management.

Furthermore, extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* is highly prevalent among these wound isolates and is predominantly mediated by the blaCTX-M gene, confirming a significant burden of blaCTX-M-harboring ESBL-producing *Escherichia coli* in clinical wound infections at FETHI.

RECOMMENDATION

Avoid third-generation cephalosporins and fluoroquinolones as empirical monotherapy for wound infections at FETHI. Prioritize Amikacin for guided, targeted therapy in laboratory-confirmed MDR cases.

Mandate routine phenotypic ESBL screening (Double Disk Synergy or Combination Disk Test) for clinical Enterobacterales, and establish periodic PCR surveillance for blaCTX-M and carbapenemase genes.

Form a multidisciplinary Antimicrobial Stewardship Committee to regulate broad-spectrum antibiotic use, and enforce strict hygiene during wound dressing changes to prevent cross-transmission.

Expand molecular profiling using Whole Genome Sequencing (WGS) to detect non-blaCTX-M ESBLs/carbapenemases, and conduct multicenter studies to track high-risk clones (e.g., E. coli ST131) across Southwestern Nigeria.

Declarations

Ethical Approval

The source project reports approval from the Human Research and Ethics Committee, Federal Teaching Hospital, Ido-Ekiti (ERC/2026/04/08/1525B) and the FUOYE College of Medicine Research Ethics Committee (FUO/COMREC/MLS/2026/064).

Consent to Participate

Questionnaire were given to the participants to obtain their demographics characteristics and other relevant information to the study.

Consent form was given to the participants to obtain their consent to participate in the study. Informed consent was obtained from all participants (or their legal guardians where applicable), before their enrolment in the study.

Consent for publication

Not Applicable

Competing Interests

The authors declare that they have no competing interests

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Author Contribution

I. O.: Conceptualization, methodology, investigation, data collection, laboratory analysis, formal analysis, data interpretation, and writing of the original manuscript.B. O.: Supervision, conceptualization, methodology, validation, critical review of the manuscript, and overall guidance throughout the study.I. R.: Data validation, and review of the manuscript.F. A.: Laboratory investigation, and review of the manuscript.N. A.: Laboratory investigation, review and editing of the manuscript.I. S.: Data analysis support, and review of the manuscript.P. A.: Literature review, and review of the manuscript.K. S.: Review of the manuscript.All authors: Read and approved the final manuscript.

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Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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Plates

Plate 1 is available in the Supplementary Files section.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ESBLE.coliGelImage.png