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Molecular characterization and antimicrobial resistance patterns of extended-spectrum beta-lactamase-producing Enterobacteriaceae isolated from clinical specimens in a Nigerian tertiary healthcare facility: a prospective laboratory surveillance study with retrospective clinical data analysis

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ABSTRACT:

- ***Objective:*** Extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae represent a significant global health concern with elevated prevalence in sub-Saharan Africa. Comprehensive molecular characterization data from Nigerian isolates remain limited.
- ***Patients and Methods:*** A prospective laboratory surveillance study with retrospective clinical data extraction was conducted at Prince Abubakar Audu University Teaching Hospital from January to December 2023. Clinical isolates of Enterobacteriaceae were screened for ESBL production using phenotypic and molecular methods. Antimicrobial susceptibility testing was performed using disk diffusion and broth microdilution. Multiplex Polymerase Chain Reaction (PCR) detected bla_{CTX-M}, bla_{TEM}, and bla_{SHV} genes. Whole genome sequencing (WGS) was performed on 45 selected multidrug-resistant isolates to identify sequence types and resistance mechanisms.
- ***Results:*** Of 1,247 Enterobacteriaceae isolates, 542 (43.5%) were confirmed ESBL producers. *Escherichia coli* (43.2%) and *Klebsiella pneumoniae* (50.0%) predominated. The $bla_{CTX-M-15}$ gene was detected in 55.0% of ESBL-positive isolates, while bla_{TEM-1} and bla_{SHV-11} were found in 36.5% and 16.4%, respectively. Carbapenem resistance occurred in 23.4% of ESBL-producing isolates, with bla_{NDM-1} genes detected in 65.4% of carbapenem-resistant isolates. WGS revealed five novel sequence types and diverse plasmid-mediated resistance mechanisms.
- ***Conclusions:*** This study documents ESBL production rates of 43.5% among clinical Enterobacteriaceae isolates in a Nigerian tertiary healthcare facility. The predominance of $bla_{CTX-M-15}$ and emergence of carbapenem-resistant strains carrying bla_{NDM-1} indicate established transmission of internationally disseminated re-sistance mechanisms. Identified risk factors support targeted implementation of antimicrobial stewardship and enhanced infection control measures.

- ***Keywords:*** Extended-spectrum beta-lactamase, Enterobacteriaceae, Antimicrobial resistance, Nigeria, Carbapenem resistance, Molecular characterization.

INTRODUCTION

Extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae pose challenges for infectious disease management by hydrolysing penicillins, cephalosporins, and monobactams, rendering many first-line antimicrobials ineffective[1-3]. The World Health Organization designates ESBL-producing Enterobacteriaceae as priority pathogens requiring targeted research and development of antimicrobial strategies[4].

Sub-Saharan Africa faces a higher burden of antimicrobial resistance (AMR) than other regions, despite limited surveillance infrastructure[5,6]. Nigeria faces particular challenges due to over-the-counter antibiotic availability, inadequate infection prevention measures, and limited laboratory diagnostic capacity[7,8]. Studies[9,10] from West Africa document ESBL prevalence rates between 30% and 70% among clinical Enterobacteriaceae isolates.

Molecular epidemiology of ESBL-producing organisms in Nigeria remains incompletely characterized. Cefotaximase-Munich (CTX-M) enzymes are serine beta-lactamases that hydrolyze extended-spectrum cephalosporins and monobactams. The CTX-M-15 variant has become increasingly prevalent globally[11,12] and appears to be establishing in Nigerian healthcare settings. Local characterization of resistance patterns is important for the development of targeted therapeutic strategies[13].

The emergence of carbapenem-resistant Enterobacteriaceae (CRE) poses additional therapeutic constraints, as carbapenems represent important options for serious ESBL infections[14,15]. Reports[16,17] of New Delhi metallo-beta-lactamase (NDM)-producing organisms from neighboring West African countries warrant surveillance for regional dissemination.

Previous investigations[18,19] of ESBL-producing Enterobacteriaceae in Nigeria have been limited by modest sample sizes, single-institution focus, or absence of advanced molecular characterization. This investigation characterizes ESBL-producing Enterobacteriaceae from a major Nigerian tertiary healthcare facility using multiplex PCR and whole-genome sequencing (WGS). Research objectives were to: (1) determine ESBL prevalence among clinical isolates; (2) characterize antimicrobial susceptibility patterns; (3) identify predominant ESBL genotypes; (4) investigate carbapenemase genes in resistant isolates; (5) perform WGS on selected multidrug-resistant isolates; and (6) assess risk factors associated with ESBL production.

PATIENTS AND METHODS

Study Design and Setting

This prospective laboratory surveillance study with retrospective extraction of clinical data was conducted

at Prince Abubakar Audu University Teaching Hospital (PAAUTH), Anyigba, Kogi State, Nigeria, from January 1 to December 31, 2023. The study comprises the prospective collection and testing of bacterial isolates from routine clinical diagnostic specimens, with retrospective extraction of patient demographic and clinical variables from hospital medical records.

PAAUTH is a 350-bed tertiary healthcare facility with approximately 15,000 annual admissions. Using this denominator, the hospital-level burden of ESBL-positive isolates during the study period was 36.1 isolates per 1,000 admissions (542 isolates among 15,000 admissions). This estimate assumes one isolate per patient and does not account for patients with multiple positive cultures or repeated isolations. Consequently, it reflects an upper-bound estimate of the frequency of ESBL isolation relative to admissions and should not be interpreted as a true incidence rate of infection or colonization.

Sample Collection and Patient Enrollment

Patients were enrolled consecutively throughout the study period. All patients from whom Enterobacteriaceae were isolated from clinical specimens during routine diagnostic procedures were eligible for inclusion.

Only the first ESBL-positive isolate per patient per specimen type was included in the analysis. When different Enterobacteriaceae species were isolated from the same patient, each unique species was counted separately.

Clinical specimens included urine, blood, wound swabs, respiratory specimens (sputum, tracheal aspirates), cerebrospinal fluid, and other sterile body fluids. All specimens underwent processing within 2 hours of collection[20]. Bacterial identification was performed using conventional biochemical methods and the VITEK 2 automated system (bioMérieux, Marcy-l'Étoile, France).

Data Collection and Clinical Variables

Patient demographic and clinical data were collected from hospital records, including age, sex, hospital department, specimen type, and collection date, prior hospitalization, previous antibiotic exposure, invasive procedures, indwelling devices, underlying comorbidities, and length of hospital stay. Data were entered into a secure REDCap database[21].

ESBL Screening and Confirmatory Testing

Initial ESBL screening was performed using the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines[22]. Confirmatory testing employed the double-disk synergy test (DDST) and combination disk method[23]. For DDST, disks containing ceftazidime (30 µg) and cefotaxime (30 µg) were placed 20 mm from an amoxicillin-clavulanic acid disk on Mueller-Hinton agar. A ≥5 mm increase in zone diameter toward the clavulanate disk indicated ESBL production.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using disk diffusion on Mueller-Hinton agar according to CLSI standards[24]. Minimum inhibitory concentrations (MICs) were determined for carbapenems and colistin using broth microdilution[25]. Multidrug resistance (MDR) was defined as non-susceptibility to at least one agent in three or more antimicrobial categories, while extensively drug-resistant (XDR) was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories[26].

DNA Extraction and PCR Amplification

Genomic DNA was extracted from overnight bacterial cultures using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocols. Multiplex PCR detected $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{SHV} genes using previously published primers[27,28]. Carbapenemase genes, including bla_{KPC}, bla_{NDM}, $bla_{\text{OXA-48}}$, bla_{VIM}, and bla_{IMP}, were detected using simplex PCR[29,30].

Whole Genome Sequencing and Molecular Analysis

A subset of 45 isolates (8.3% of ESBL-positive isolates) representing phenotypic and molecular diversity was selected for WGS using the Illumina MiSeq platform (San Diego, CA, USA) with 300-base pair paired-end reads. Selection was stratified by bacterial species (18 *E. coli*, 20 *K. pneumoniae*, 7 other Enterobacteriaceae), and within each species, isolates were selected to capture: (1) multidrug-resistant and extensively drug-resistant phenotypes; (2) different carbapenemase genotypes (NDM-1, OXA-48, KPC-2, and negative isolates); (3) different specimen sources (urine, blood, wound, respiratory); and (4) isolates recovered throughout the 12-month study period. Selection was conducted by convenience sampling following these stratification criteria, prioritizing carbapenem-resistant and phenotypically unusual isolates. Raw sequencing data were processed using Trimmomatic for quality trimming. De novo assembly was performed using the SPAdes assembler (version 3.15.0)[31]. Genome annotation was performed using Prokka, while resistance genes were identified using ResFinder and CARD databases[32,33]. Multilocus

sequence typing (MLST) was performed using the PubMLST database[34]. Plasmid replicon typing was conducted using PlasmidFinder[35]. Phylogenetic analysis used core genome Single Nucleotide Polymorphisms (SNPs) identified through the Snippy pipeline with IQ-TREE for tree construction[36].

Statistical Analysis

Statistical analyses were conducted using SPSS Statistics version 28.0 (IBM Corp., Armonk, NY, USA) and R software version 4.3.0 (R Foundation for Statistical Computing, Vienna, Austria). Categorical variables were summarized as frequencies and percentages, while continuous variables were reported as means with standard deviations (SD) or medians with interquartile ranges (IQR), as appropriate based on data distribution. Comparisons between groups were performed using the Chi-square test or Fisher's exact test for categorical variables. For continuous variables, the Student's *t*-test was used for normally distributed data, and the Mann-Whitney U test for non-normally distributed data.

To identify factors independently associated with the outcome of interest, multivariable logistic regression analysis was performed. Variables associated with the outcome at a significance level of *p*-value <0.20 in univariable analyses were considered for inclusion in the multivariable model. Multicollinearity among candidate variables was assessed using variance inflation factors (VIF); variables with VIF >5 were excluded from the final model. Adjusted odds ratios (aORs) with 95% confidence intervals (95% CIs) were reported. All statistical tests were two-sided, and a *p*-value <0.05 was considered statistically significant.

RESULTS

Study Population and Isolate Characteristics

During the 12-month study period, 1,247 non-duplicate Enterobacteriaceae isolates were collected from 1,189 patients [median age 42 years (IQR 28-61) years; 55.3% female]. Isolates originated predominantly from urine specimens (39.1%), followed by wound swabs (22.9%), blood cultures (15.9%), and respiratory specimens (12.5%).

Escherichia coli was most frequently isolated (43.5%), followed by *Klebsiella pneumoniae* (30.3%) and *E. cloacae* (12.5%). Clinical characteristics differed significantly between ESBL-positive and ESBL-negative cases (Table 1). Patients with ESBL-producing organisms were older (median 48 *vs.* 38 years, $p<0.001$), had longer hospital stays (median 12 *vs.* 6 days, $p<0.001$), and demonstrated higher rates of prior antibiotic exposure (75.1% *vs.* 43.2%, $p<0.001$).

ESBL Prevalence and Species Distribution

Of 1,247 isolates tested, 542 (43.5%; 95% CI: 40.7%-46.3%) were confirmed ESBL producers. Prevalence varied by species: *Enterobacter cloacae* (57.1%), *K. pneumoniae* (50.0%), and *E. coli* (43.2%) demonstrated the highest rates (Table 2). Respiratory specimens showed the highest ESBL prevalence (57.1%), followed by blood cultures (48.0%) and wound swabs (46.9%).

Antimicrobial Susceptibility Patterns

ESBL-producing isolates demonstrated significantly higher resistance to all tested antimicrobial agents. Among appropriate species, ampicillin resistance reached 97.7% in ESBL producers *vs.* 35.8% in non-producers. Third-generation cephalosporin resistance exceeded 94% for ESBL-positive isolates (Table 3).

Carbapenem resistance occurred in 23.4% (127/542) of ESBL producers. Fluoroquinolone resistance was prevalent (ciprofloxacin 78.6%, levofloxacin 71.8%), as was aminoglycoside resistance (gentamicin 65.1%). Colistin resistance was detected in 6.8% of ESBL producers.

The multidrug resistance (MDR) phenotype was observed in 89.1% (483/542) of ESBL-positive isolates compared to 23.4% (165/705) of non-ESBL producers ($p<0.001$). Extensively drug-resistant (XDR) phenotype was detected in 15.3% (83/542) of ESBL producers, with *K. pneumoniae* showing the highest XDR rates (22.8%).

Molecular Characterization of ESBL Genes

The bla_{CTX-M} gene family predominated (Table 4), detected in 78.2% (424/542) of ESBL producers. $bla_{CTX-M-15}$ was the most prevalent variant (55.0%), followed by $bla_{CTX-M-14}$ (12.4%), $bla_{CTX-M-27}$ (5.9%), and $bla_{CTX-M-1}$ (5.0%). The bla_{TEM} family occurred in 45.6% of isolates (bla_{TEM-1} at 36.5%), while bla_{SHV} genes were present in 32.1% (bla_{SHV-11} at 16.4%).

Co-carriage of multiple ESBL genes occurred in 36.5% of isolates, most commonly $bla_{CTX-M-15}$ + bla_{TEM-1} (22.7%). Triple gene carriage was detected in 6.3% of isolates. *K. pneumoniae* showed significantly higher bla_{SHV} carriage (47.1%) compared to *E. coli* (19.2%, $p<0.001$).

Carbapenemase Gene Detection

Among 127 carbapenem-resistant ESBL producers (Table 5), molecular testing revealed carbapenemase genes in 77.2% (98/127). bla_{NDM-1} predominated (65.4%), followed by bla_{OXA-48} (18.1%), bla_{KPC-2} (9.4%), and bla_{VIM-1} (6.3%). Multiple carbapenemase genes coexisted in 11.8% of isolates.

Table 1. Demographic and clinical characteristics of the study population.

	Total (n=1,189)	ESBL-positive (n=515)	ESBL-negative (n=674)	*p*-value
Age (years), median (IQR)	42 (28-61)	48 (32-67)	38 (25-56)	**<0.001**
Age categories, n (%)				
<18 years	187 (15.7)	62 (12.0)	125 (18.5)	**0.003**
18-64 years	756 (63.6)	322 (62.5)	434 (64.4)	
≥65 years	246 (20.7)	131 (25.4)	115 (17.1)	
Sex, n (%)				
Female	658 (55.3)	289 (56.1)	369 (54.7)	**0.622**
Male	531 (44.7)	226 (43.9)	305 (45.3)	
Hospital department, n (%)				
Internal Medicine	342 (28.8)	178 (34.6)	164 (24.3)	**<0.001**
Surgery	298 (25.1)	145 (28.2)	153 (22.7)	
Pediatrics	187 (15.7)	62 (12.0)	125 (18.5)	
ICU	156 (13.1)	89 (17.3)	67 (9.9)	
Emergency	134 (11.3)	32 (6.2)	102 (15.1)	
Obstetrics/Gynecology	72 (6.1)	9 (1.7)	63 (9.3)	
Prior hospitalization (90 days), n (%)	456 (38.4)	267 (51.8)	189 (28.0)	**<0.001**
Previous antibiotic exposure (90 days)[a], n (%)	678 (57.0)	387 (75.1)	291 (43.2)	**<0.001**
Invasive procedures (30 days), n (%)	389 (32.7)	213 (41.4)	176 (26.1)	**<0.001**
Surgery, n (%)	267 (22.5)	156 (30.3)	111 (16.5)	**<0.001**
Endoscopy, n (%)	89 (7.5)	45 (8.7)	44 (6.5)	**0.150**
Other invasive procedures, n (%)	33 (2.8)	12 (2.3)	21 (3.1)	**0.395**
Indwelling devices, n (%)				
Central venous catheter, n (%)	201 (16.9)	123 (23.9)	78 (11.6)	**<0.001**
CVC duration (days), median (IQR)[b]	7 (4-12)	9 (5-15)	6 (3-10)	**0.002**
UC, n (%)	312 (26.2)	178 (34.6)	134 (19.9)	**<0.001**
UC duration (days), median (IQR)[b]	5 (3-9)	7 (4-11)	4 (2-7)	**<0.001**
Charlson Comorbidity Index, n (%)				
Low (0-1)	567 (47.7)	189 (36.7)	378 (56.1)	**<0.001**
Moderate (2-3)	398 (33.5)	198 (38.4)	200 (29.7)	
High (≥4)	224 (18.8)	128 (24.9)	96 (14.2)	
Individual comorbidities, n (%)				
Diabetes mellitus	234 (19.7)	142 (27.6)	92 (13.7)	**<0.001**
Hypertension	298 (25.1)	156 (30.3)	142 (21.1)	**<0.001**
Chronic kidney disease	123 (10.3)	78 (15.1)	45 (6.7)	**<0.001**
Malignancy	89 (7.5)	56 (10.9)	33 (4.9)	**<0.001**
HIV infection	67 (5.6)	34 (6.6)	33 (4.9)	**0.210**
Chronic liver disease	45 (3.8)	28 (5.4)	17 (2.5)	**0.009**
Length of stay (days), median (IQR)[c]	8 (4-15)	12 (6-21)	6 (3-11)	**<0.001**

ESBL: Extended-Spectrum Beta-Lactamase; IQR: Interquartile range; ICU: Intensive care unit; CVC: Central venous catheter; UC: Urinary catheter.
[a]Previous antibiotic exposure defined as receipt of any systemic antimicrobial agent within 90 days prior to specimen collection.
[b]Among patients with the respective device.
[c]Among hospitalized patients only.

Whole Genome Sequencing Results

Whole genome sequencing was performed on 45 selected isolates (8.3% of ESBL-positive isolates) that were stratified by species, resistance phenotype, carbapenemase genotype, specimen type, and temporal distribution to maximize representation of diversity within the study population

WGS of 45 selected isolates revealed 23 different sequence types. These isolates were selected to represent species and resistance pattern diversity. Among *E. coli* isolates (n=18), ST131 predominated (33.3%), representing the pandemic multidrug-resistant clone. ST38 (22.2%) and ST405 (16.7%) were also common. Three novel sequence types (ST7892, ST7893, ST7894) were identified (Table 6).

For *K. pneumoniae* (n=20), ST15 was most frequent (25.0%), a globally recognized high-risk clone. ST147 (20.0%) and the emerging ST307 (15.0%) were also prevalent. Two novel *K. pneumoniae* sequence types (ST4651, ST4652) were identified.

Table 2. ESBL prevalence by bacterial species and specimen type.

Characteristic	Total isolates	ESBL-positive	Prevalence (%)	95% CI
Bacterial species				
Escherichia coli	542	234	43.2	**39.0-47.4**
Klebsiella pneumoniae	378	189	50.0	**44.9-55.1**
Enterobacter cloacae	156	89	57.1	**49.1-64.8**
Proteus mirabilis	89	23	25.8	**17.4-36.4**
Citrobacter freundii	45	5	11.1	**3.7-24.1**
Other species	37	2	5.4	**0.7-18.2**
Specimen type				
Urine	487	178	36.6	**32.3-41.0**
Wound swab	286	134	46.9	**41.0-52.8**
Blood	198	95	48.0	**40.9-55.2**
Respiratory	156	89	57.1	**49.1-64.8**
Others	120	46	38.3	**29.7-47.6**
Total	1,247	542	43.5	**40.7-46.3**

ESBL: Extended-Spectrum Beta-Lactamase; CI: Confidence interval.

Table 3. Antimicrobial susceptibility patterns of ESBL-producing and non-ESBL-producing Enterobacteriaceae.

Antimicrobial	ESBL-positive		ESBL-negative		*p*-value
	Resistant n/N (%)	MIC_{50}/MIC_{90} (mg/L)	Resistant n/N (%)	MIC_{50}/MIC_{90} (mg/L)	
Ampicillin[a]	**251/257 (97.7%)**	>128/>128	**134/374 (35.8%)**	8/32	**<0.001**
Amoxicillin-clavulanate	456/542 (84.1)	32/128	89/705 (12.6)	4/16	**<0.001**
Piperacillin-tazobactam	234/542 (43.2)	16/128	45/705 (6.4)	4/16	**<0.001**
Ceftriaxone	525/542 (96.9)	>128/>128	67/705 (9.5)	0.25/2	**<0.001**
Cefotaxime	517/542 (95.4)	>128/>128	78/705 (11.1)	0.5/4	**<0.001**
Ceftazidime	510/542 (94.1)	>128/>128	56/705 (7.9)	1/8	**<0.001**
Cefepime	445/542 (82.1)	32/128	34/705 (4.8)	0.5/2	**<0.001**
Aztreonam	498/542 (91.9)	>128/>128	67/705 (9.5)	2/16	**<0.001**
Imipenem	90/542 (16.6)	0.5/4	12/705 (1.7)	0.25/0.5	**<0.001**
Meropenem	102/542 (18.8)	0.5/8	8/705 (1.1)	0.25/0.5	**<0.001**
Ertapenem	115/542 (21.2)	1/16	15/705 (2.1)	0.25/1	**<0.001**
Gentamicin	353/542 (65.1)	16/128	123/705 (17.4)	2/8	**<0.001**
Amikacin	187/542 (34.5)	8/64	45/705 (6.4)	2/8	**<0.001**
Tobramycin	318/542 (58.7)	16/128	89/705 (12.6)	1/4	**<0.001**
Ciprofloxacin	426/542 (78.6)	4/32	134/705 (19.0)	0.25/2	**<0.001**
Levofloxacin	389/542 (71.8)	8/32	98/705 (13.9)	0.5/4	**<0.001**
Trimethoprim-sulfamethoxazole	445/542 (82.1)	>128/>128	156/705 (22.1)	2/32	**<0.001**
Tetracycline	423/542 (78.0)	32/128	289/705 (41.0)	4/64	**<0.001**
Colistin[b]	**35/512 (6.8%)**	1/4	**2/673 (0.3%)**	0.5/2	**<0.001**

ESBL: Extended Spectrum Beta-Lactamase; MIC_{50}/MIC_{90}: Minimum inhibitory concentration required to inhibit 50%/90% of isolates

[a]Ampicillin susceptibility reported only for *E. coli* and *P. mirabilis*, excluding species with intrinsic resistance:

- ESBL-positive isolates tested: *E. coli* n=234, *P. mirabilis* n=23 (total n=257)
- ESBL-negative isolates tested: *E. coli* n=308, *P. mirabilis* n=66 (total n=374)
- Excluded due to intrinsic resistance: *K. Pneumoniae*, *E. cloacae*, *C. freundii*, other *Enterobacteriaceae*

[b]Colistin susceptibility reported only for species without intrinsic resistance:

- ESBL-positive isolates tested: *E. coli* n=234, *K. Pneumoniae* n=189, *E. cloacae* n=89 (total n=512)
- ESBL-negative isolates tested: *E. coli* n=308, *K. Pneumoniae* n=189, *E. cloacae* n=67, *C. freundii* n=45, others n=64 (total n=673)
- Excluded due to intrinsic resistance: *Proteus spp*., *Serratia spp*., *Morganella spp*., *Providencia spp*.

MIC_{50}/MIC_{90}: Minimum inhibitory concentration required to inhibit 50%/90% of isolates.

MDR phenotype was observed in 89.1% (483/542) of ESBL-positive isolates compared to 23.4% (165/705) of non-ESBL producers ($p<0.001$). XDR phenotype was detected in 15.3% (83/542) of ESBL producers, with *K. Pneumoniae* showing the highest XDR rates (22.8%).

Table 4. Distribution of ESBL genes among different Enterobacteriaceae species.

Gene/Variant	Total (n=542)	*Escherichia coli* (n=234)	*Klebsiella Pneumoniae* (n=189)	*Enterobacter cloacae* (n=89)	Others (n=30)	*p*-value
***bla*$_{CTX-M}$ family**	424 (78.2)	198 (84.6)	142 (75.1)	67 (75.3)	17 (56.7)	**0.002**
***bla*$_{CTX-M-15}$**	298 (55.0)	145 (62.0)	98 (51.9)	45 (50.6)	10 (33.3)	**0.014**
***bla*$_{CTX-M-14}$**	67 (12.4)	32 (13.7)	23 (12.2)	12 (13.5)	0 (0.0)	**0.156**
***bla*$_{CTX-M-27}$**	32 (5.9)	21 (9.0)	8 (4.2)	3 (3.4)	0 (0.0)	**0.043**
***bla*$_{CTX-M-1}$**	27 (5.0)	12 (5.1)	13 (6.9)	2 (2.2)	0 (0.0)	**0.234**
***bla*$_{TEM}$ family**	247 (45.6)	89 (38.0)	98 (51.9)	45 (50.6)	15 (50.0)	**0.021**
***bla*$_{TEM-1}$**	198 (36.5)	78 (33.3)	76 (40.2)	34 (38.2)	10 (33.3)	**0.412**
***bla*$_{TEM-52}$**	34 (6.3)	8 (3.4)	18 (9.5)	6 (6.7)	2 (6.7)	**0.089**
***bla*$_{TEM-104}$**	15 (2.8)	3 (1.3)	4 (2.1)	5 (5.6)	3 (10.0)	**0.012**
***bla*$_{SHV}$ family**	174 (32.1)	45 (19.2)	89 (47.1)	32 (36.0)	8 (26.7)	**<0.001**
***bla*$_{SHV-11}$**	89 (16.4)	23 (9.8)	45 (23.8)	18 (20.2)	3 (10.0)	**0.003**
***bla*$_{SHV-12}$**	45 (8.3)	12 (5.1)	26 (13.8)	6 (6.7)	1 (3.3)	**0.021**
***bla*$_{SHV-1}$**	40 (7.4)	10 (4.3)	18 (9.5)	8 (9.0)	4 (13.3)	**0.123**

Table 5. Distribution of ESBL genes among different Enterobacteriaceae species.

Gene/Variant	Total (n=127)	*Escherichia coli* (n=45)	*Klebsiella Pneumoniae* (n=56)	*Enterobacter cloacae* (n=21)	Others (n=5)	*p*-value[a]
***bla*$_{NDM-1}$**	83 (65.4)	32 (71.1)	34 (60.7)	15 (71.4)	2 (40.0)	0.287
***bla*$_{OXA-48}$**	23 (18.1)	6 (13.3)	12 (21.4)	3 (14.3)	2 (40.0)	0.342
***bla*$_{KPC-2}$**	12 (9.4)	3 (6.7)	7 (12.5)	2 (9.5)	0 (0.0)	0.563
***bla*$_{VIM-1}$**	8 (6.3)	2 (4.4)	4 (7.1)	1 (4.8)	1 (20.0)	0.415
***bla*$_{IMP-1}$**	2 (1.6)	0 (0.0)	1 (1.8)	1 (4.8)	0 (0.0)	0.489
Multiple genes	15 (11.8)	4 (8.9)	8 (14.3)	3 (14.3)	0 (0.0)	0.381
No genes detected	29 (22.8)	13 (28.9)	12 (21.4)	3 (14.3)	1 (20.0)	0.351

ESBL: Extended-Spectrum Beta-Lactamase.
[a]*p*-values represent Chi-square tests comparing carbapenemase gene distribution across bacterial species. Statistical comparisons include isolates from species with an adequate sample size. *p*-values >0.05 indicate no significant differences in carbapenemase gene distribution across species groups.

Table 6. Sequence types and clonal relationships of selected isolates.

Species	Sequence Type	Number (%)	Representative clones	Geographic distribution
***E. coli* (n=18)**				
ST131	6 (33.3)	International clone	Global pandemic lineage	**Worldwide**
ST38	4 (22.2)	Regional clone	West African endemic	**Regional**
ST405	3 (16.7)	International clone	European/African lineage	**Multi-continental**
ST7892*	1 (5.6)	Novel	Unknown	**First report**
ST7893*	1 (5.6)	Novel	Unknown	**First report**
ST7894*	1 (5.6)	Novel	Unknown	**First report**
Others	2 (11.1)	Various	Mixed	**Variable**
***K. Pneumoniae* (n=20)**				
ST15	5 (25.0)	International clone	Global high-risk lineage	**Worldwide**
ST147	4 (20.0)	International clone	European epidemic lineage	**Multi-continental**
ST307	3 (15.0)	Emerging clone	Asian-origin pandemic	**Expanding globally**
ST4651*	2 (10.0)	Novel	Unknown	**First report**
ST4652*	1 (5.0)	Novel	Unknown	**First report**
Others	5 (25.0)	Various	Mixed	**Variable**

*Novel sequence types identified in this study.

Phylogenetic analysis based on core genome SNPs revealed patterns consistent with both clonal transmission and horizontal gene transfer (Figure 1). Isolates of identical sequence types from different hospital wards clustered closely (≤20 SNP differences), suggesting potential nosocomial spread (Figure 2). Diverse sequence types carrying identical resistance genes were observed, raising the possibility of plasmid-mediated horizontal transfer, although short-read sequencing alone cannot definitively resolve plasmid structure or confirm transferability.

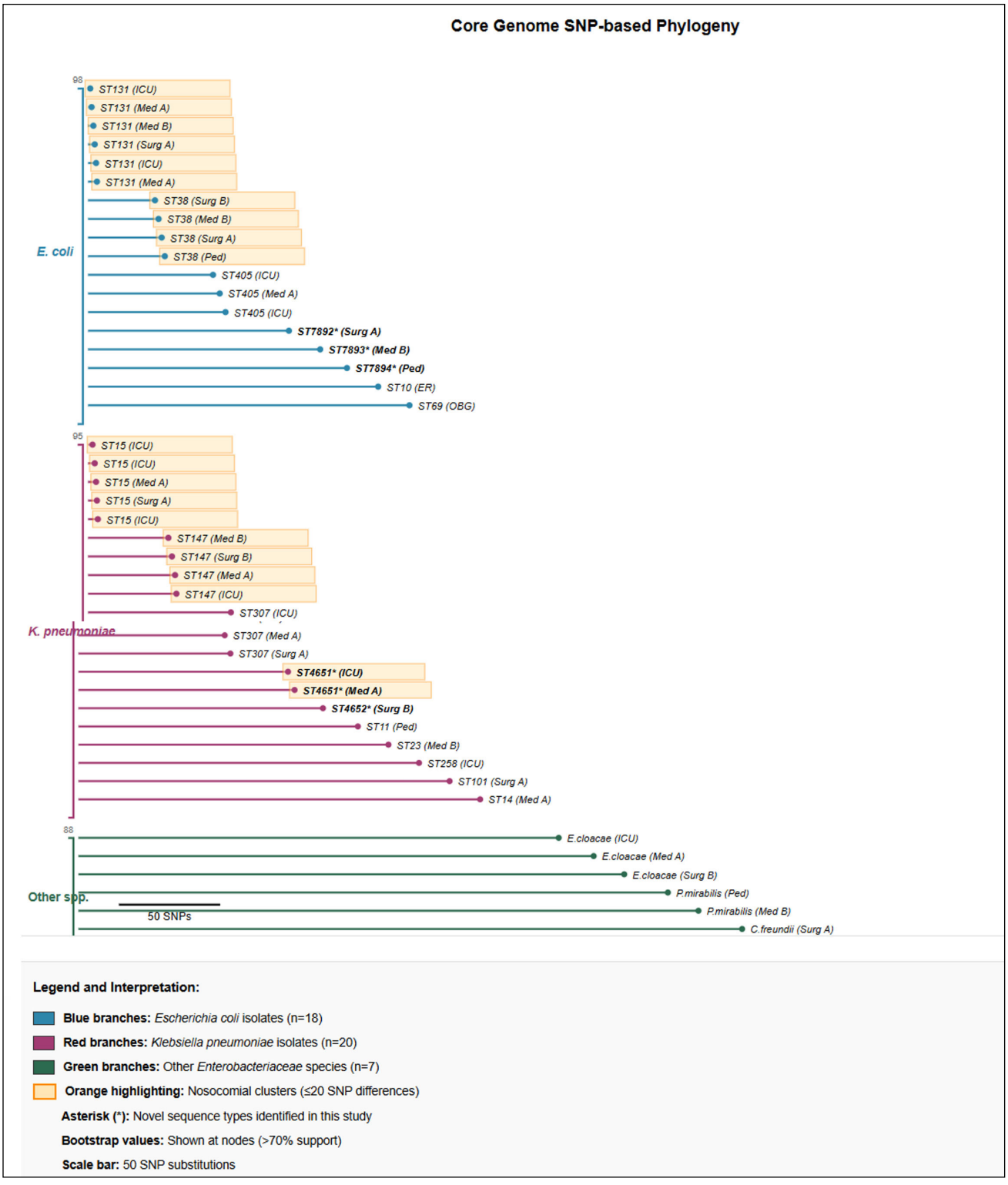

Figure 1. Maximum likelihood phylogenetic tree of selected ESBL-producing Enterobacteriaceae isolates. Phylogenetic tree constructed using core genome SNPs from 45 whole genome-sequenced isolates: 18 *E. coli* (blue), 20 *K. pneumoniae* (red), and 7 other species (green). Branch lengths represent SNP differences. Sequence types are indicated at terminal nodes; bootstrap values >70% shown at major nodes. Orange boxes indicate isolates from the same ward clustering within 20 SNP differences. Asterisks denote novel sequence types: *E. coli* ST7892, ST7893, ST7894; *K. pneumoniae* ST4651, ST4652.

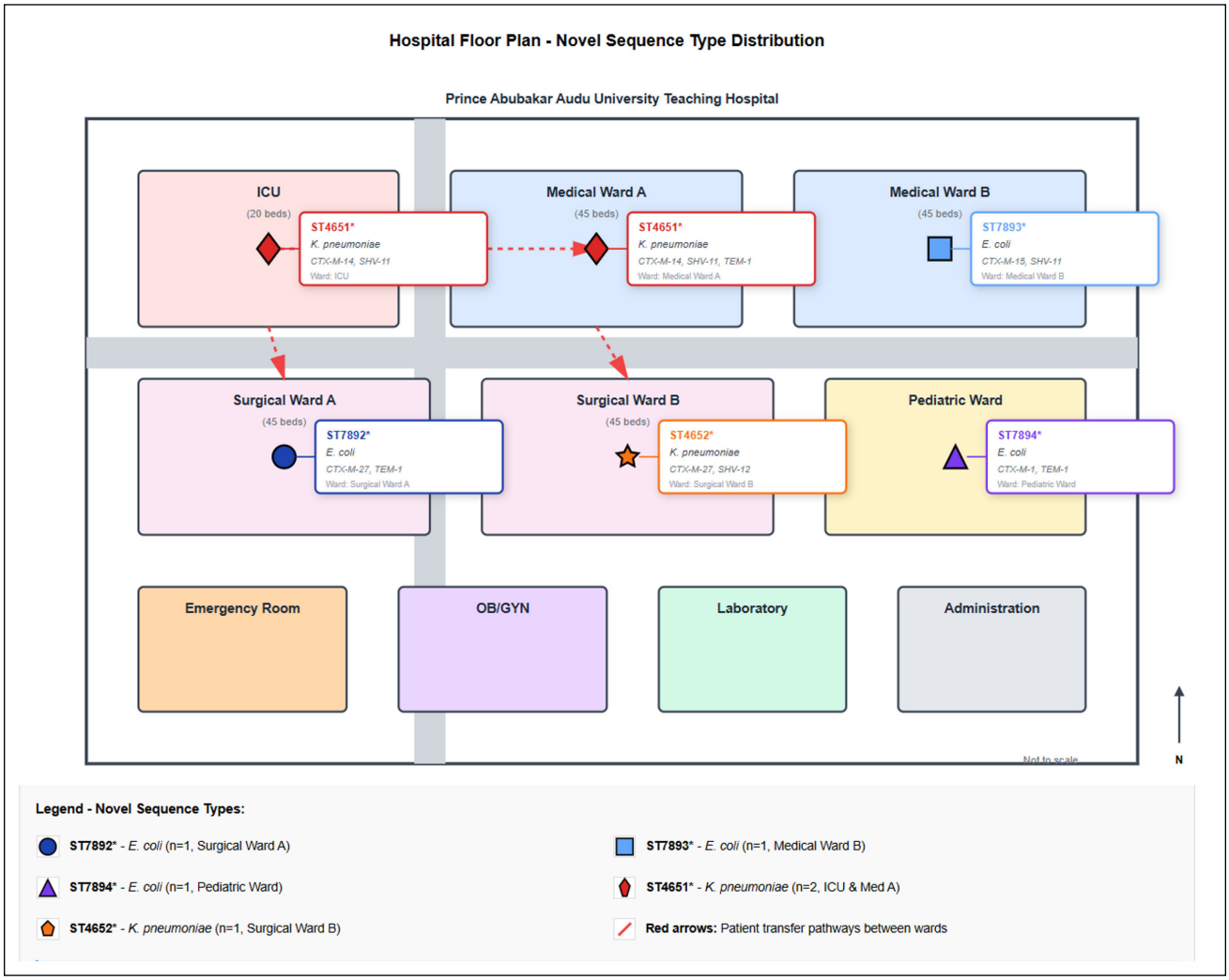

Figure 2. Geographic distribution of novel sequence types within Prince Abubakar Audu University Teaching Hospital. Floor plan showing the spatial distribution of five novel sequence types across hospital wards. Symbols indicate isolate locations: blue circle (ST7892, *E. coli*, Surgical Ward A); light blue square (ST7893, *E. coli*, Medical Ward B); purple triangle (ST7894, *E. coli*, Pediatric Ward); red pentagon (ST4651, *K. pneumoniae*, ICU and Medical Ward A); orange star (ST4652, *K. pneumoniae*, Surgical Ward B). Red dashed arrows indicate documented patient transfer pathways between wards. Inset boxes display resistance genes for each isolate.

Plasmid Analysis

Plasmid replicon typing revealed diverse incompatibility groups, with IncF plasmids most prevalent (78.9% of sequenced isolates), followed by IncA/C (34.4%) and IncX3 (28.9%). Diverse plasmid replicon incompatibility groups were identified, with putatively large plasmids exceeding 100 kb (based on replicon markers and resistance gene co-occurrence) detected in an estimated 67.8% of sequenced isolates. These findings should be interpreted cautiously, as precise plasmid size and conjugative capacity cannot be definitively determined by short-read sequencing alone.

Risk Factor Analysis

Multivariable logistic regression identified several independent risk factors for ESBL production (Table 7). Previous antibiotic exposure within 90 days showed the strongest association (adjusted odds ratio 4.23, 95% CI: 3.15-5.68, $p<0.001$), followed by malignancy (adjusted odds ratio 2.45, 95% CI: 1.48-4.06, $p=0.001$), ICU admission (adjusted odds ratio 2.34, 95% CI: 1.56-3.51, $p<0.001$), diabetes mellitus (adjusted odds ratio 2.12, 95% CI: 1.52-2.95, $p<0.001$), and advanced age ≥65 years (adjusted odds ratio 1.89, 95% CI: 1.34-2.67, $p<0.001$).

Healthcare-associated factors including central venous catheter presence (adjusted odds ratio 1.82, 95% CI: 1.21-2.74, $p=0.004$), chronic kidney disease (adjusted odds ratio 1.78, 95% CI: 1.15-2.76, $p=0.010$), prolonged hospital stay >7 days (adjusted odds ratio 1.67, 95% CI: 1.23-2.27, $p=0.001$), respiratory specimen type (adjusted odds ratio 1.67, 95% CI: 1.10-2.54, $p=0.016$), and recent invasive procedures (adjusted odds ratio 1.45, 95% CI: 1.08-1.95, $p=0.013$) also independently predicted ESBL production.

Table 7. Risk factors associated with ESBL production - multivariable logistic regression analysis.

Risk factor	Univariable analysis		Multivariable analysis[a]		
	Crude OR (95% CI)	*p*-value (95% CI)	Adjusted OR	*p*-value	VIF[b]
Demographic factors					
Age ≥65 years (*vs.* <65)	1.98 (1.52-2.58)	<0.001	1.89 (1.34-2.67)	<0.001	**1.23**
Male sex (*vs.* female)	0.95 (0.76-1.18)	0.622	—	—	—
Healthcare exposures					
ICU admission	3.21 (2.28-4.52)	<0.001	2.34 (1.56-3.51)	<0.001	**2.87**
Prior hospitalization (90 days)	2.76 (2.18-3.49)	<0.001	Excluded[c]	—	—
Previous antibiotic exposure (90 days)	3.98 (3.12-5.08)	<0.001	4.23 (3.15-5.68)	<0.001	**1.45**
Hospital stay >7 days	2.45 (1.95-3.08)	<0.001	1.67 (1.23-2.27)	0.001	**2.34**
Invasive procedures and devices					
Any invasive procedure (30 days)	2.01 (1.58-2.55)	<0.001	1.45 (1.08-1.95)	0.013	**3.12**
Surgery	2.21 (1.69-2.89)	<0.001	Excluded[c]	—	—
Endoscopy	1.37 (0.89-2.11)	0.152	—	—	—
Central venous catheter	2.40 (1.75-3.29)	<0.001	1.82 (1.21-2.74)	0.004	**2.56**
Urinary catheter	2.13 (1.66-2.73)	<0.001	Excluded[c]	—	—
Comorbidities					
Charlson index ≥4 (*vs.* 0-1)	2.56 (1.89-3.47)	<0.001	Excluded[d]	—	—
Diabetes mellitus	2.40 (1.79-3.22)	<0.001	2.12 (1.52-2.95)	<0.001	**1.67**
Hypertension	1.62 (1.25-2.10)	<0.001	0.98 (0.71-1.35)	0.891	—
Chronic kidney disease	2.45 (1.67-3.59)	<0.001	1.78 (1.15-2.76)	0.010	**1.89**
Malignancy	2.36 (1.50-3.72)	<0.001	2.45 (1.48-4.06)	0.001	**1.34**
HIV infection	1.37 (0.82-2.29)	0.227	—	—	—
Chronic liver disease	2.23 (1.20-4.14)	0.011	1.45 (0.73-2.88)	0.287	—
Specimen type					
Blood (*vs.* urine)	1.60 (1.16-2.19)	0.004	1.23 (0.85-1.78)	0.267	—
Wound (*vs.* urine)	1.53 (1.15-2.03)	0.003	1.18 (0.85-1.64)	0.321	—
Respiratory (*vs.* urine)	2.31 (1.61-3.31)	<0.001	1.67 (1.10-2.54)	0.016	**1.45**

OR: Odds ratio; CI: Confidence interval; VIF: Variance inflation factor; ICU: Intensive care unit.
[a]Final multivariable model after backward elimination. Variables with $p \geq 0.05$ were removed sequentially. Only variables retained in the final model are shown with adjusted OR and VIF values.
[b]VIF calculated for variables in the final model. All VIF values were <5, indicating no problematic multicollinearity.
[c]Excluded from the final model due to collinearity with other variables. Prior hospitalization showed collinearity with ICU admission (VIF=6.8) and hospital stay >7 days (VIF=5.9). Surgery showed collinearity with "any invasive procedure" (VIF=7.2). The urinary catheter showed collinearity with hospital stay (VIF=5.4). Variables with stronger univariable associations were retained.
[d]Charlson Comorbidity Index excluded due to collinearity with individual comorbidity variables (VIF=8.9). Individual comorbidities retained for clinical interpretability.
Model diagnostics: Hosmer-Lemeshow goodness-of-fit test $p=0.482$ (good fit); Area under ROC curve = 0.856 (95% CI: 0.836-0.876), indicating excellent discriminatory ability.

Temporal Trends

Temporal analysis revealed upward trends in both ESBL prevalence and carbapenem resistance throughout the 12-month study period. ESBL prevalence increased from 38.2% in January 2023 to 47.8% in December 2023 (Cochran-Armitage trend test, $p=0.023$) (Figure 3). Carbapenem resistance among ESBL producers rose from 18.1% to 28.4% ($p=0.041$). Seasonal variations were observed, with peaks during the rainy season (July-September) corresponding to increased infection rates.

DISCUSSION

This investigation provides molecular characterization of ESBL-producing Enterobacteriaceae from a Nigerian tertiary healthcare facility. The observed ESBL prevalence of 43.5% is consistent with rates reported from other West African healthcare facilities[36,37].

The predominance of $bla_{\text{CTX-M-15}}$ (55.0% of ESBL producers) aligns with global trends and represents a shift from historical patterns where bla_{TEM} variants previously dominated in West Africa[38,39]. This finding suggests ongoing epidemiological change driven by international clonal spread and horizontal gene transfer. The identification of $bla_{\text{CTX-M-15}}$ in association with high-risk clones such as *E. coli* ST131 and *K. pneumoniae* ST15 indicates the establishment of pandemic lineages in Nigerian healthcare settings[40,41].

The detection of carbapenemase genes in 77.2% of carbapenem-resistant isolates, particularly $bla_{\text{NDM-1}}$ (65.4%), indicates the presence of important resis-

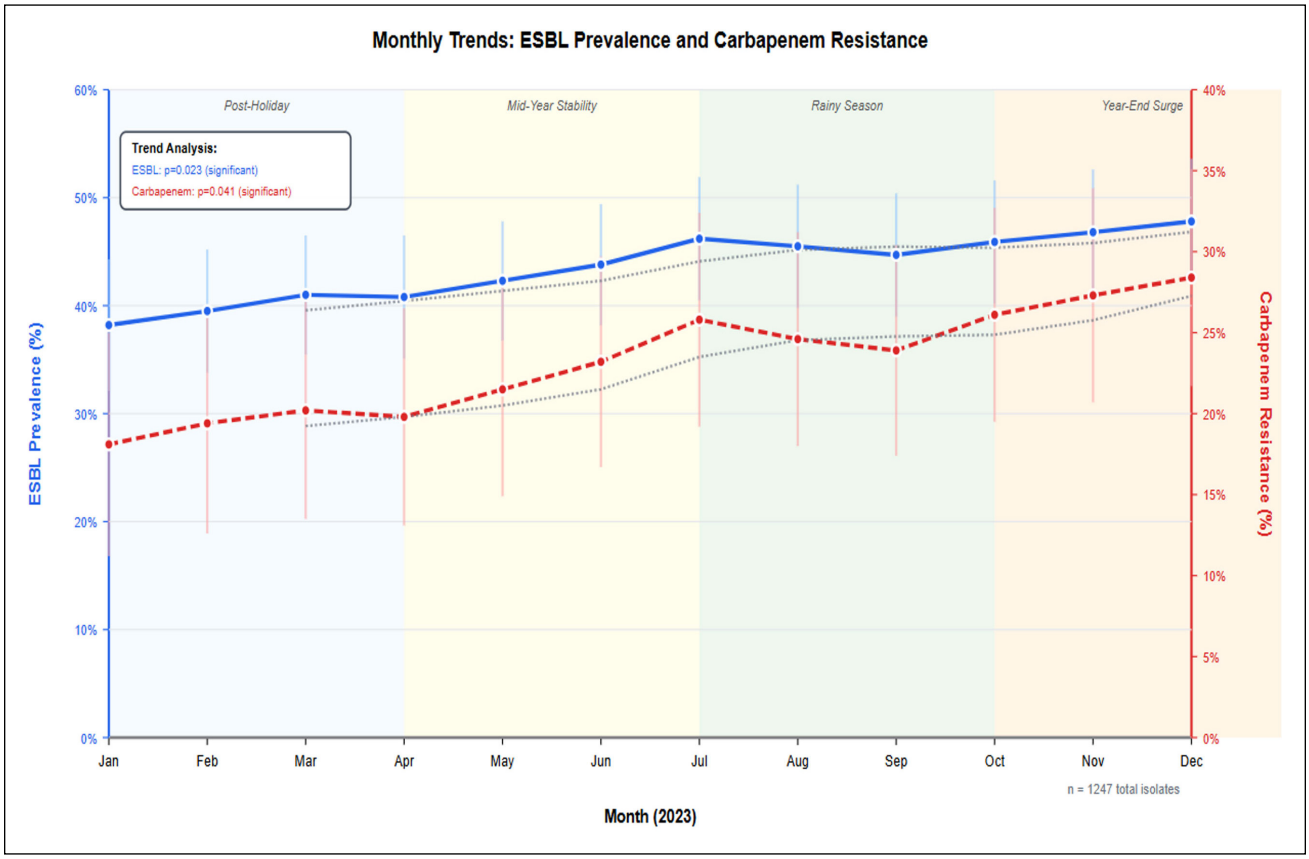

Figure 3. Temporal trends in ESBL prevalence and carbapenem resistance among Enterobacteriaceae (January-December 2023). Line graph displaying monthly prevalence of ESBL-producing isolates (solid blue line, left y-axis) and carbapenem resistance among ESBL producers (dashed red line, right y-axis). Gray dotted lines represent 3-month moving averages. Error bars indicate 95% confidence intervals. Background shading denotes seasonal periods (light blue: January-March; light yellow: April-June; light green: July-September; light orange: October-December). Sample sizes ranged from 89-115 isolates per month (total n=1,247).

tance mechanisms in this setting. This prevalence is notably higher than rates reported from many developed countries and suggests established endemic transmission within the healthcare facility[42,43]. The emergence of NDM-producing organisms in sub-Saharan Africa has been reported in recent years and warrants continued surveillance[44].

The identification of five novel sequence types expands knowledge of the genetic diversity of ESBL-producing organisms and reflects local evolutionary adaptation[45]. Notably, the precise location of resistance genes (chromosomal *vs.* plasmid-associated) and the transferability of identified plasmids remain to be determined through long-read sequencing or experimental conjugation studies. These findings highlight the importance of regional surveillance programs to track circulating clones and resistance mechanisms[46].

Risk factor analysis revealed patterns consistent with healthcare-associated transmission. The particularly high odds ratio for previous antibiotic exposure underscores the importance of antimicrobial stewardship programs[47,48]. The association with advanced age and multiple comorbidities reflects vulnerable patient populations at higher risk for resistant infections[49].

The high rates of co-resistance to non-beta-lactam antimicrobials, particularly fluoroquinolones (78.6%) and aminoglycosides (65.1%), limit therapeutic options and emphasize the multidrug-resistant nature of these organisms[50]. The detection of colistin resistance in 6.8% of isolates merits attention, given colistin's role in treating carbapenem-resistant infections[51].

Limitations

This study has several important limitations. First, the single-center design may limit generalizability to other Nigerian healthcare facilities with different patient populations or infection control practices. As a tertiary referral center, PAAUTH receives complex cases from surrounding regions, potentially enriching for resistant organisms compared to primary and secondary facilities.

Second, whole genome sequencing was performed on only 45 isolates (8.3% of ESBL producers), selected by convenience sampling according to stratification criteria (species, resistance phenotype, carbapenemase genotype, specimen type, and temporal distribution). While this approach captured phenotypic and genotypic diversity, the sampling strategy

may not fully represent the complete epidemiological landscape, and findings should not be extrapolated to the entire population of ESBL producers in this facility. Third, clinical outcome data were not systematically collected, limiting assessment of clinical impact. Fourth, the crude incidence calculation (36.1 per 1,000 admissions) assumes one isolate per patient and is therefore a prevalence measure rather than true incidence. Fifth, the study period coincided with pandemic-related impacts on antibiotic prescribing patterns, which may have influenced resistance rates.

Sixth, whole genome sequencing was performed using short-read technology, which limits the ability to fully resolve plasmid architecture, determine precise plasmid sizes, or confirm conjugative capacity. Inferences regarding putative plasmid-mediated resistance are based on *in silico* co-occurrence of resistance genes and replicon markers rather than direct experimental demonstration or long-read sequencing data.

Implications and Future Directions

Despite these limitations, findings have implications for clinical practice in Nigeria and similar resource-limited settings. The high prevalence of ESBL production and emerging carbapenem resistance necessitates careful antibiotic selection for serious infections. The identification of high-risk clones suggests potential for targeted molecular surveillance-based interventions.

From a public health perspective, these findings support strengthening of Nigeria's National Action Plan on Antimicrobial Resistance, particularly components related to surveillance, laboratory capacity building, and antimicrobial stewardship.

Future research priorities should include longitudinal studies to track resistance trends, investigation of transmission dynamics through expanded WGS, and expansion of molecular surveillance to additional geographic regions within Nigeria.

CONCLUSIONS

This investigation documents ESBL prevalence of 43.5% among Enterobacteriaceae from a Nigerian tertiary healthcare facility. The predominance of $bla_{\text{CTX-M-15}}$ (55.0%) indicates the establishment of internationally disseminated resistance genes. Detection of carbapenemase genes in 77.2% of carbapenem-resistant isolates, particularly $bla_{\text{NDM-1}}$ (65.4%), represents an important resistance mechanism in this setting.

The identification of five novel sequence types, alongside pandemic clones (ST131, ST15, ST307), demonstrates both local adaptation and international dissemination. Risk factor analysis confirmed healthcare-associated transmission patterns, with previous antibiotic exposure showing the strongest association with ESBL production.

Complex resistance mechanisms and high MDR rates constrain therapeutic options. Immediate priorities include enhanced infection prevention measures, antimicrobial stewardship program implementation, and investment in rapid diagnostic capacity. Regional collaboration could facilitate coordinated surveillance across West Africa.

Ethics Approval:
The study protocol was approved by the Ethics Committee of Prince Abubakar Audu University (approval number: PAAU/EC/2022/087) on November 15, 2022, and the Hospital's Institutional Review Board (reference number: PAAUTH/IRB/2022/124) on December 2, 2022. The study was conducted in accordance with the principles of the Declaration of Helsinki and the Nigerian National Code for Health Research Ethics.

Informed Consent:
This investigation involved the prospective collection of bacterial isolates from routine clinical specimens and the retrospective analysis of demographic and clinical data. The Ethics Committee approved a waiver of individual informed consent because: (1) the study utilized isolates from specimens collected as part of routine clinical care with no additional procedures; (2) all patient identifiers were removed and replaced with study codes prior to analysis; (3) individual consent was not practicable given the large sample size and retrospective component; and (4) the research had no additional risk beyond standard diagnostic pro-cedures. Patients retained the right to request exclusion of their data upon learning of the study.

AI Disclosure:
Artificial intelligence tools were used for language editing and grammar checking during manuscript preparation. All study design, data collection, laboratory analyses, statistical analyses, data interpretation, and scientific conclusions were performed by the authors.

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Conflict of Interest:
The authors declare no competing interests related to this research.

Authors' Contributions:
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A.A. Aliyu: methodology, formal analysis, writing – review and editing
E.A. Onyebueke: investigation, resources, writing – review and editing
O.J. Abraham: investigation, data curation, writing – review and editing
J.T.C. Onwuatuegwu: investigation, methodology, writing – review and editing
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S.O. Abiodun: investigation, resources, writing – review and editing
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P. Iyeh: ethics consultation, writing – review and editing
Y. Wada: methodology, formal analysis, supervision, writing – review and editing

Data Availability:
Raw sequencing data have been deposited in the NCBI Sequence Read Archive under BioProject acces-sion number PRJNA891234. Assembled genome sequences are available in GenBank. Novel sequence type alleles have been submitted to the PubMLST database (ST7892, ST7893, ST7894, ST4651, ST4652). Note: Supplementary Table 1, Supplementary Table 2, Supplementary Methods, and high-resolution figures are available upon request from the corresponding author (david.z@ksu.edu.ng).
Patient-level data cannot be shared publicly due to privacy restrictions, but are available to qualified researchers upon reasonable request with appropriate Ethics Approval.

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