High prevalence of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* and *Escherichia coli* in clinical infections in a tertiary care hospital in southwestern Nigeria

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Summary

Background: Extended-Spectrum Beta-Lactamase (ESBL)-producing *Klebsiella pneumoniae* and *Escherichia coli* infections significantly impact healthcare delivery and contribute to global antibiotic resistance. Thus, knowledge of their prevalence and risk factors is essential for quick and effective treatment, prevention, and management. In this study, we investigated the prevalence of ESBL production among clinical *K. pneumoniae* and *E. coli* isolates, determined their susceptibility profile, and identified risk factors associated with their infections in a tertiary care hospital.

Materials and Methods: two hundred ninety-one non-duplicate isolates from diverse clinical samples were collected and matched with patients' biographical information. The isolates were identified by standard microbiological methods and their antimicrobial susceptibility was determined by the disk diffusion method. All isolates were screened for ESBL production. Risk factors such as age, sex, hospitalization, and source of infection were assessed for their association with ESBL infections. Multivariate logistic regression analysis was used to identify associated risk factors.

Results: out of the 291 isolates collected, 152 (52.2%) were *K. pneumoniae,* while 139 (47.8%) were *E. coli*. Approximately 43.3% (n=126) of the isolates were ESBL producers, with 54% as *K. pneumoniae* and 46% as *E. coli*. The ESBL producers were predominantly isolated from blood samples (100%) and exhibited higher resistance rates to ampicillin (96.1%), streptomycin (95.4%), trimethoprim (93.8%), tetracycline (92.3%) and other antibiotics (>70%), except for cefoxitin (34.4%) and imipenem (12.4%), compared with the non-ESBL producers ($p<0.05$). Multivariate analysis indicated that patients with sepsis or hospitalized were more likely to acquire ESBL infections ($p<0.05$).

Conclusions: this study reports a higher prevalence of ESBL-producing *E. coli* and *K. pneumoniae* than previously reported in our hospital. Antimicrobial stewardship programs and effective infection control practices could help manage this growing concern.

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Introduction

Escherichia coli and *Klebsiella pneumoniae*, are the main opportunistic pathogens that have a considerable impact on hospitalized patients, leading to bacteremia as well as urinary and respiratory tract infections. The primary treatment for these infections involves the use of various antibiotics, particularly β-lactam agents. However, both species often produce enzymes such as Extended-Spectrum Beta-Lactamases (ESBLs), which lead to resistance against beta-lactam antibiotics [25,29,33].

ESBLs are reducing enzymes that act on cephalosporins (different generations) and monobactams, with reduced activity against carbapenems and cefoxitin; however, clavulanic acid and

tazobactam inhibit their action [16,32]. These enzymes bind to specific regions of the drugs before they can acquire their target sites and inactivate them. Genes encoding for ESBL can be found on both internal genetic structures and external genetic vehicles like plasmids and transposons, which are highly transferable intra and inter-bacterial species [16,17]. ESBLs are frequently transmitted by plasmids, which can result in their distribution among hospitalized patients, thereby fueling their spread across regions [15,30].

E. coli and *K. pneumoniae* are currently considered the two most common ESBL-producing pathogens in the hospital setting [21,29]. These pathogens pose significant concerns in healthcare settings due to their high adaptability and effective transmission. Infections attributable to these pathogens have escalated, correlating with heightened healthcare expenditures, extended hospitalizations, and increasing mortality rates. According to the reports of the World Health Organization (WHO), emerging ESBL-Producing Enterobacterales (ESBL-PE) are considered the most serious and life-threatening challenges of the 21st century and top the list of priority pathogens for research and development of new antibiotics [7,18,37].

The growing occurrence of ESBL-producing pathogens has been reported globally and varies by country [23,25]. A recent and detailed systematic review and meta-analysis of studies on ESBL-producing *E. coli* and *K. pneumoniae* worldwide from 1990 to 2022 was extremely informative. Africa had the greatest frequency of *E. coli* and *K. pneumoniae*, with 44.3% and 32.8%, respectively. Asia had 35.7%, Europe had 23.1% and 25.4%, while North America had the lowest, with 28.1% and 25.0% [29]. Previous studies in Nigeria have shown prevalence rates ranging from 7.5% to 82.5% [23,34]. The variable reported rates may be primarily related to local epidemiology, current antibiotic prescribing policies and practices, underreporting of rates from some places, and the methodology of ESBL detection [23].

The increasing occurrence of clinical infections due to ESBL-producing bacteria limits patient treatment options. This issue is exacerbated by inherent challenges in developing countries, such as insufficient drug supply chains, ineffective health insurance systems, the financial burden of out-of-pocket drug procurement by patients, rudimentary laboratory diagnostics, and inadequate hospital infection prevention and control measures. Infections caused by these resistant bacteria have escalated the utilization of limited 'last resort' antibiotics, such as carbapenems, resulting in heightened resistance to these drugs and significant mortality rates [11].

The factors that predispose patients to ESBL infections are many and diverse. Prolonged exposure to antibiotics and recent hospitalization were among the most frequently documented risks for the acquisition of ESBL infections. Additionally, it has been reported that the presence of invasive devices, such as an intravenous line, endotracheal tube, or urinary catheter, as well as underlying conditions like diabetes mellitus and benign prostatic hyperplasia, are independent risk factors for infections by hospital-acquired ESBL-producing enterobacteria in hospitalized patients [3,14].

The current study was initiated due to the significant increase in antimicrobial selection pressure (a growing local challenge caused by too frequent antibiotic prescription and use), the growing challenge in treating severe infections caused by ESBL-producing multidrug-resistant bacteria, the worldwide spread of ESBLs, and the necessity for enhanced information and continuous monitoring and assessment of antimicrobial resistance. This study aimed to investigate the prevalence of ESBL production among clinical *K. pneumoniae and E. coli* isolates, determine their susceptibility profile, and identify risk factors associated with their infections in a tertiary care hospital.

Materials and Methods

Study setting, sample size, and isolate identification

This laboratory-based study was done at the Medical Microbiology and Parasitology laboratory of the Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. A total of 291 non-repetitive Enterobacterales isolates from different clinical samples, including urine, faecal, blood, sputum, wound samples, eye and ear swabs, and aspirates of other anatomical sites of in and outpatients of ages ranged from less than five years old to over 60 years old were collected at the Microbiology laboratory of Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife, Nigeria.

All isolates were re-identified by culturing on Eosin Methylene Blue (EMB) agar (MAST Group Ltd., Bootle, United Kingdom), Gram staining, the use of conventional biochemical tests, and the analytical profile index (API) 20E identification kit (bioMérieux, Marcy-l'Étoile, France). The identified Enterobacterales were stored in glycerol broth (20%, v/v) and kept at 4°C in a refrigerator for further examination.

Antimicrobial susceptibility testing

The antimicrobial susceptibility of the isolates was determined by the Kirby-Bauer disc diffusion method on Mueller Hinton Agar (MHA) plates following the Clinical and Laboratory Standard Institute (CLSI) guidelines [12]. Three to four colonies of each isolate of interest were transferred into tubes containing sterile saline, and the samples were then adjusted to obtain the 0.5 McFarland turbidity standard. The bacterial suspensions were homogeneously spread on MHA plates with a sterile cotton swab. Antimicrobial discs were then placed on the plates. The following antibiotics were tested: Nalidixic Acid (NA) (30µg), Tetracycline (T) (30µg), Streptomycin (S) (10µg), Cefoxitin (FOX) (30µg), Chloramphenicol (C) (30µg), Imipenem (IMI) (10µg), Ampicillin (AP) (10µg), Cefepime (CPM) (30µg), Amoxicillin+Clavulanic Acid (AUG) (30µg), Ciprofloxacin (CIP) (5µg), Gentamicin (GM) (30µg), and Trimethoprim (TM) (5ug) (MAST Group Ltd.). The plates were incubated overnight at 37°C for 18-20 hours, and zones of inhibition were measured to the nearest millimetre. *Escherichia coli* ATCC 25922 was used as a control strain [6].

Extended-Spectrum Beta-Lactamase screening

Presumptive Extended-Spectrum Beta-Lactamase screening

Three-to-five-hour tryptic soy broth suspensions of the test organisms were inoculated onto Mueller-Hinton agar plates using sterile swab sticks. Presumptive ESBL test was carried out by placing cefotaxime (30ug) and ceftazidime (30ug) antibiotic discs (MAST Group Ltd.) on the culture using a multidisc applicator (MAST Group Ltd.) or a sterile pair of forceps. The plates were then incubated overnight at 37°C for 24 hours. *E. coli* ATCC 25922 was used as a control strain. Using the interpretative chart provided by the Clinical and Laboratory Standards Institute [8], the diameters of the zones of inhibition were interpreted, and isolates resistant at specified breakpoints to at least one of the antimicrobial screening agents (≤27mm for cefotaxime and ≤22 mm for ceftazidime) were reported as presumptively ESBL-positive and selected for confirmatory testing.

Confirmatory Extended-Spectrum Beta-Lactamase screening

A combined disc diffusion method that involved the use of ceftazidime (30µg) with clavulanic acid (10ug) and cefotaxime (30µg)

with clavulanic acid (10µg) disks was employed for the ESBL confirmatory screening. The discs were placed on Mueller-Hinton agar inoculated with the test organisms. The plates were then incubated overnight. Zones of inhibition were measured using a ruler. Once obtained in the case of any test isolate, an increase by ≥5mm in zone diameter for ceftazidime or cefotaxime, when used in combination with clavulanic acid, compared with that for the same discs without clavulanic acid (obtained during the presumptive screening) was used to confirm the isolate to be an ESBL producer [9]. *Escherichia coli* ATCC 25922 (ESBL-negative) and *Klebsiella pneumoniae ATCC* 700603 (ESBL-positive) served as negative and positive control strains, respectively.

Assessment of risk factors

Possible risk factors for ESBL acquisition, such as age, sex, and source of infection (based on the nature of the clinical specimen from which the isolates were obtained) and hospitalization, were considered. Information concerning these risk factors was obtained using a proforma based on the patients' laboratory request forms.

Data analysis

The data obtained were analyzed by descriptive and inferential statistics using Microsoft Excel 2021 and WINPEPI (Version 11.65) [2]. Univariate analysis was conducted to identify the variables linked to ESBL infections. Categorical variables were analyzed utilizing Fisher's exact tests, and statistical evaluations were conducted employing two-tailed tests. Multivariate logistic regression assessed the relationship between ESBL infection and previously identified risk factors while controlling for confounding variables. All variables linked to the acquisition of ESBLs in the bivariate analysis were incorporated into the initial (full) model. The final multivariate regression model was utilized to calculate the adjusted Odds Ratio (OR) and the corresponding 95% Confidence Intervals (CI). The threshold for statistical significance was established at a p-value of less than 0.05.

Results

Distribution of Enterobacterial isolates among clinical specimens

Two hundred and ninety-one non-duplicate *Klebsiella pneumoniae* and *Escherichia coli* isolates of in- and outpatients were obtained from the clinical samples sent to the Medical Microbiology Laboratory of OAUTHC, Ile-Ife. Strict inclusion criteria were observed. The included samples must have adequate demographic data, among others. One hundred and twenty-six (43.3%) of the 291 patients from whom the samples were obtained were male, while 165 (56.7%) were female. The ages of the patients ranged from less than five years to over 60 years. Patients between the ages of 18 and 40 were the most common (n=113; 38.8%), followed by those over 60 (n=75; 25.8%) and 41 to 60 (n=66; 22.7%). The most common form of infection among the patients was urinary tract infection (n=53; 18.2%), followed by wound infections (n=50; 17.2%) and benign prostatic hyperplasia-urethral stricture (n=30; 10.3%) (Table 1).

Table 1. Socio-demographic and clinical characteristics of patients.

Variable		Frequency	Percentage
Sex	F	165	56.7
	M	126	43.3
Age (years)	<6	18	6.2
	6-17	19	6.5
	18-40	113	38.8
	41-60	66	22.7
	>60	75	25.8
Type of specimen	Aspirate	6	2.1
	Blood	7	2.4
	Cerebrospinal fluid	1	0.3
	Ear and eye	4	1.4
	Endo cervical swab	11	3.8
	High vaginal swab	12	4.1
	Sputum	24	8.2
	Stool	9	3.1
	Urine	165	56.7
	Wound	46	15.8
	Catheter tip	6	2.1
Ward/clinic	Inpatient	225	77.3
	Outpatient	66	22.7
Clinical diagnosis	Benign prostatic hyperplasia-urethral stricture	30	10.3
	Chronic liver disease	3	1.0
	Fever	2	0.7
	Gastrointestinal tract infection	2	0.7
	Inflammation	6	2.1
	Otitis media	2	0.7
	Reproductive tract Infection	13	4.5
	Respiratory tract infection	17	5.8
	Sepsis	17	5.8
	Urinary tract infection	53	18.2
	Wound infection	50	17.2

The isolates were obtained from various clinical specimens: aspirates from various anatomical sites (n=6), blood (n=7), Cerebrospinal Fluid (CSF) (n=1), eye and ear swabs (n=4), Endocervical Swabs (ECS) (n=11), High Vaginal Swabs (HVS) (n=12), catheter tip (n=6), sputum (n=24), stool (n=9), urine (n=165) and wound (n=46), as shown in Table 2.

A total of 152 (52.2%) *K. pneumoniae* and 139 (47.8%) *E. coli* were isolated from the samples. *K. pneumoniae* was more commonly isolated from the urogenital tract (n=90), than *E. coli* (n=75). However, the reverse was the case with the gastrointestinal tract (*E. coli* n=5; *K. pneumoniae* n=4).

Susceptibility patterns of isolates

The majority of the isolates were resistant to trimethoprim (n=256, 88%), ampicillin (n=247; 84.9%), tetracycline (n=242; 83.2%), streptomycin (n=227; 78%) and nalidixic acid (n=211; 72.5%). Moderate resistant rates were seen against cefotaxime (n=190; 65.3%), ciprofloxacin (n=187; 64.3%), chloramphenicol (n=164; 56.4%), cefepime (n=177; 60.8%), augmentin (n=170; 58.4%), gentamicin (n=161; 55.3%), ceftazidime (n=160; 55%) while the least rate was against imipenem (n=42; 14.4%) (Table 3).

E. coli demonstrated the least susceptibility to ampicillin (90.6%), trimethoprim (89.2%), tetracycline (87.1%), and streptomycin (84.9%). However, *K. pneumoniae* showed the least susceptibility to trimethoprim (86.8%), ampicillin (79.6%), and tetracycline (79.6%) (Table 3). There were no significant differences in the percentages of isolates of *E. coli* and *K. pneumoniae* that were resistant to most of the antimicrobial agents (p>0.05). However, significantly higher percentages of *E. coli* isolates were resistant to streptomycin (p=0.007) and ampicillin (p=0.009) compared to *K. pneumoniae* isolates. In addition, significantly higher percentages of isolates of *K. pneumoniae* were resistant to imipenem (p=0.001) and cefepime (p=0.006) compared to *E. coli* isolates.

Prevalence of Extended-Spectrum Beta-Lactamase-producing isolates among the isolates

One hundred and twenty-six (43.3%) of the 291 isolates were identified as ESBL producers. *K. pneumoniae* accounted for 54% (n=68) of the 126 ESBL producers, while *E. coli* accounted for 46% (n=58). Within species, the order remained the same, although the gap in the prevalence of expression of ESBL phenotype between *K. pneumoniae* and *E. coli* was narrowed to 44.7% and 41.7%, respectively. There was no significant difference between the prevalence of ESBL in *Escherichia coli* and *Klebsiella pneumoniae* (OR=0.88; CI=0.54-1.45; p=0.637).

Sixty-five of the 165 isolates (39.4%) from urine, 7 of 7 isolates from blood, 1 of 1 isolate from the CSF, and 5 of 9 isolates from stool samples were ESBL producers. The frequency of isolating an ESBL producer from the urinary tract was relatively less than that of isolating an ESBL producer from the cerebrospinal fluid, catheters, and blood (Table 4).

Table 2. Distribution of Isolates of *Escherichia coli* and *Klebsiella pneumoniae* among clinical specimens.

Organisms	Clinical specimens											
	ASP	Blood	CSF	E&E	ECS	HVS	CT	Sputum	Stool	Urine	Wound	Total
Escherichia coli	5	3	1	0	8	9	4	12	5	75	17	139
Klebsiella pneumoniae	1	4	0	4	3	3	2	12	4	90	29	152
Total (%)	6 (2.1)	7 (2.4)	1 (0.3)	4 (1.4)	11 (3.8)	12 (4.1)	6 (2.1)	24 (8.2)	9 (3.1)	165 (56.7)	46 (15.8)	291 (100)

ASP, aspirates from various anatomical sites; CSF, Cerebrospinal Fluid; E&E, ear and eye swabs; ECS, Endocervical Swab; HVS, High Vaginal Swab; CT, Catheter Tip.

Table 3. Resistance rates of the isolates.

Antimicrobial agents	Total n=291 (%)	*Escherichia coli* n=139 (%)	*Klebsiella pneumoniae* n=152 (%)	p-value
Nalidixic acid	211 (72.5)	106 (76.3)	105 (69.1)	0.190
Tetracycline	242 (83.2)	121 (87.1)	121 (79.6)	0.116
Streptomycin	227 (78)	118 (84.9)	109 (71.7)	0.007*
Cefoxitin	104 (35.7)	45 (32.4)	59 (38.8)	0.272
Chloramphenicol	164 (56.4)	73 (52.5)	91 (59.9)	0.237
Imipenem	42 (14.4)	8 (5.8)	34 (22.4)	0.001*
Cefotaxime	190 (65.3)	85 (61.2)	105 (69.1)	0.176
Ampicillin	247 (84.9)	126 (90.6)	121 (79.6)	0.009*
Cefepime	177 (60.8)	73 (52.5)	104 (68.4)	0.006*
Augmentin	170 (58.4)	79 (56.8)	91 (59.9)	0.635
Ciprofloxacin	187 (64.3)	93 (66.9)	94 (61.8)	0.393
Gentamicin	161 (55.3)	72 (51.8)	89 (58.6)	0.288
Trimethoprim	256 (88)	124 (89.2)	132 (86.8)	0.591
Ceftazidime	160 (55)	72 (51.8)	88 (57.9)	0.345

*statistically significant.

Prevalence of antimicrobial resistance among Extended-Spectrum Beta-Lactamase and non-Extended-Spectrum Beta-Lactamase-producing isolates

The susceptibility pattern of ESBL-producing isolates relative to that of non-ESBL-producing isolates is represented in Table 5. The ESBL producers significantly ($p<0.05$) demonstrated higher rates of resistance than those of the non-ESBL producers to nalidixic acid (86.1%, 62.6%), tetracycline (92.3%, 76.6%), streptomycin (95.4%, 65.5%), chloramphenicol (71.3%, 45%), ampicillin (96.1%, 67.3%), cefepime (93%, 36.3%), augmentin (75%, 41.59%), ciprofloxacin (84.5%, 49.1%), gentamicin (86.1%, 32.1%), trimethoprim (91.18%, 81.31%), and to the two extended-spectrum cephalosporins, cefotaxime (94.6%, 39.2%) and ceftazidime (92.3%, 26.9%). Nevertheless, relative to the non-ESBL producers, the ESBL producers did not show increased resistance to imipenem (12.4%, 15.7%) and cefoxitin (34.4%, 35.1%).

Risk factors associated with Extended-Spectrum Beta-Lactamase infections

As shown in Table 6, there was no significant association between ESBL infections, the sex of patients ($p=0.094$), and the ages of patients ($p>0.05$). However, there was a significant association between ESBL infections and the type of clinical specimens collected, as these infections were common in patients with sepsis ($p=0.005$). Also, these infections were significantly associated with the ward of the patients ($p= 0.035$), with the prevalence of ESBL isolates among inpatients standing at 83.3% and among patients diagnosed with sepsis (10.3%).

The associated factors were further subjected to multivariate analysis using the binomial logistic regression model to determine independent predictors of ESBL infections. Out of the three variables that were tested, sepsis (OR=3.8333; 95%CI=1.2010-12.2348; $p=0.0232$) and inpatients (OR=1.8750; 95%CI=1.0494-3.3502; $p=0.0338$) were found to be independent predictors of ESBL infections in this study (Table 7).

Discussion

Infections caused by ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* in recent years have been recognized globally as a public health problem [33]. ESBL-producing Enterobacterales are now on the WHO priority pathogen list of organisms that urgently require the development of new drugs [37]. The occurrence of ESBL-producing isolates in clinical specimens is dynamic and exhibits global and regional variations. Systematic ESBL identification in hospitals will function as a crucial tool for determining initial medication and perhaps decreasing the expenses

Table 4. Prevalence of Extended Spectrum Beta-Lactamase (ESBL) producers among the isolates.

Organisms	Clinical specimens												
	Within species	Total	ASP	Blood	CSF	E&E	ECS	HVS	CT	Sputum	Stool	Urine	Wound
Escherichia coli	58/139 (41.7)*	58/126 (46)	1/5 (20)	3/3 (100)	1/1 (100)	0 (0)	3/8 (37.5)	5/9 (55.6)	4/4 (100)	6/12 (50)	2/5 (40)	24/75 (32)	9/17 (52.9)
Klebsiella pneumoniae	68/152 (44.7)	68/126 (54)	1/1 (100)	4/4 (100)	0 (0)	1/4 (25)	1/3 (33.3)	1/3 (33.3)	1/2 (50)	2/12 (16.7)	3/4 (75)	41/90 (45.6)	13/29 (44.8)
Total (%)	126 (43.3)	126 (100)	2 (33.3)	7 (100)	1 (100)	1 (25)	4 (36.4)	6 (50)	5 (83.3)	8 (33.3)	5 (55.6)	65 (39.4)	22 (47.8)

ASP, aspirates from various anatomical sites; CSF, Cerebrospinal Fluid; E&E, ear and eye swabs; ECS, Endocervical Swab; HVS, High Vaginal Swab: CT, Catheter Tip; *p>0.05.

Table 5. Antimicrobial resistance rates of Extended Spectrum Beta-Lactamase (ESBL)-and non-ESBL-producing isolates.

Antibiotics	ESBL-producing isolates (n=126) (%)	Non-ESBL-producing isolates (n=165) (%)	p-value†
Nalidixic acid	108.5 (86.1)	103 (62.6)	0.001*
Tetracycline	116 (92.3)	126 (76.6)	0.005*
Streptomycin	120 (95.4)	108 (65.5)	0.001*
Cefoxitin	43 (34.4)	58 (35.1)	0.810
Chloramphenicol	90 (71.3)	74 (45)	0.001*
Imipenem	16 (12.4)	26 (15.7)	0.407
Cefotaxime	119 (94.6)	65 (39.2)	0.001*
Ampicillin	121 (96.1)	111 (67.3)	0.001*
Cefepime	117 (93.0)	60 (36.3)	0.001*
Augmentin	100 (79.1)	70 (42.7)	0.001*
Ciprofloxacin	106 (84.5)	81 (49.1)	0.001*
Gentamicin	108 (86.1)	53 (32.1)	0.001*
Trimethoprim	118 (93.8)	138 (83.6)	0.007*
Ceftazidime	116 (92.3)	44 (26.9)	0.001*

†Fisher exact test; *statistically significant.

of treatment and the duration of hospitalization for patients. This study presents data on the frequency of ESBL occurrence in *Klebsiella pneumoniae* and *Escherichia coli* at a tertiary care hospital in Nigeria.

In this study, the prevalence of ESBL-producing Enterobacterales (*E. coli and K. pneumoniae*) was 43.3%, higher than 37.9% previously documented in the study environment [26]. In Nigeria, the reported incidence of ESBL-producing organisms varies between 7.5% and 82.5% from one region to another. Our prevalence of ESBL-PE was consistent with previous studies from the region [34]. The global distribution of ESBL producers in *K. pneumoniae* and *E. coli* was found to be 32.7% and 33.0%, respectively, lower than that in this study. It is interesting to note that the African rates of 32.8% and 44.3%, respectively, largely agree with that of our study, showing the magnitude of the problem. It is important to note that healthcare-based studies often report elevated incidences due to the concentration of cases and their status as 'hotbeds' of microbial distribution [29,34]. The high incidence of ESBLs found in this study could be attributed to the frequent use and misuse of third-generation cephalosporins [31,35]. In several parts of the world, ESBLs are becoming an increasingly prominent resistance mechanism among Enterobacterales [10,24,27]. Clinical infections caused by ESBL-producing bacteria are associated with high costs, extended hospital stays, and a high mortality rate [16]. Consequently, properly monitoring the prevalence and type of ESBL in clinical isolates may aid in defining appropriate therapeutic options.

The ESBL-producers were predominantly *K. pneumoniae* (54%) compared to *E. coli* strains (46%). This is consistent with the findings of [4,21], who reported that more *Klebsiella pneumoniae* than *Escherichia coli* produced ESBL. The prevalence of ESBL-PE was significantly higher in isolates obtained from inpatients compared to those from outpatients, consistent with previous findings [1,4,20]. The observed pattern may be attributed to the widespread use of ceftriaxone and cefotaxime as standard empirical antibiotic therapy in Nigerian medical facilities. In addition, hospitalization has been recognized as a significant risk factor for ESBL-PE infection. This is because ESBL-encoding genes are carried by plasmids,

Table 6. Factors associated with infections caused by Extended Spectrum Beta-Lactamase (ESBL)-producing enterobacterial isolates.

Variable (n)		ESBL (n=126) n (%)	Non-ESBL (n=165) n (%)	Crude OR	95% CI	p-value†
Sex	F (165)	64 (50.4)	101 (61.3)	0.65	0.40-1.07	0.094
	M (126)	62 (49.6)	64 (38.9)			
Age	<6 (18)	8 (6.4)	10 (6.1)	1.05	0.35-3.06	1.000
	6-17 (19)	7 (5.6)	12 (7.3)	0.75	0.24-2.14	0.637
	18-40 (113)	42 (33.3)	71 (43.3)	0.66	0.40-1.10	0.114
	41-60 (66)	32 (25.4)	34 (20.6)	1.31	0.73-2.36	0.397
	>60 (75)	36 (28.6)	39 (23.6)	1.34	0.77-2.35	0.284
Type of specimen	Aspirate (6)	2 (1.6)	4 (2.4)	0.65	0.06-4.62	0.701
	Blood (7)	7 (5.6)	0 (0)	∞	1.9474-∞	0.003*
	Cerebrospinal fluid (1)	1 (0.8)	0 (0)	∞	0.0336-∞	0.433
	Ear and eye (4)	1 (0.8)	3 (1.8)	0.43	0.01-5.47	0.636
	Endo cervical swab (11)	4 (3.2)	7 (4.2)	0.74	0.16-2.99	0.762
	High vaginal swab (12)	6 (4.7)	6 (3.6)	1.32	0.34-5.09	0.768
	Sputum (24)	8 (6.4)	16 (9.7)	0.63	0.23-1.63	0.391
	Stool (9)	5 (3.9)	4 (2.4)	1.66	0.35-8.55	0.508
	Urine (165)	65 (51.6)	100 (60.6)	0.69	0.42-1.14	0.152
	Wound (46)	22 (17.5)	24 (14.6)	1.24	0.63-2.45	0.520
	Catheter tip (6)	5 (3.9)	1 (0.6)	6.78	0.74-322.43	0.088
Ward/clinic	Inpatient (225)	105 (83.3)	120 (72.7)	1.88	1.02-3.53	0.035*
	Outpatient (66)	21 (16.7)	45 (27.3)			
Clinical diagnosis	Benign prostatic hyperplasia-urethral stricture (30)	13 (10.3)	17 (10.3)	1.00	0.43-2.29	1.000
	Chronic liver disease (3)	3 (2.4)	0 (0)	∞	0.5444-∞	0.080
	Fever (2)	2 (1.6)	0 (0)	∞	0.2464-∞	0.187
	Gastrointestinal tract infection (2)	0 (0)	2 (1.2)	0.00	0.000-6.9714	0.507
	Inflammation (6)	0 (0)	6 (3.6)	0.00	0.000-1.0961	0.038
	Otitis media (2)	0 (0)	2 (1.2)	0.00	0.0000-6.9714	0.507
	Reproductive tract Infection (21)	9 (7.1)	13 (7.9)	0.90	0.33-2.36	1.000
	Respiratory tract infection (17)	7 (5.6)	10 (6.1)	0.91	0.29-2.74	1.000
	Sepsis (17)	13 (10.3)	4 (2.4)	4.63	1.38-19.90	0.005*
	Urinary tract infection (53)	23 (18.3)	30 (18.2)	1.00	0.52-1.91	1.000
	Wound infection (50)	21 (16.7)	29 (17.6)	0.94	0.48-1.81	0.876

†Fisher exact test for dichotomous predictors, *statistically significant. OR, Odds Ratio; CI, Confidence Interval.

Table 7. Independent predictors of Extended Spectrum Beta-Lactamase (ESBL) infection in multivariate logistic regression model.

Predictor	Adjusted OR	95% CI	p-value
Blood	0.0000	0.0000	0.9974
Inpatient	1.8750	1.0494-3.3502	0.0338*
Sepsis	3.8333	1.2010-12.2348	0.0232*

*statistically significant. OR, Odds Ratio; CI, Confidence Interval.

which may be readily spread among other bacteria present in hospitalized patients [20].

A total of 65 ESBL-producing isolates, accounting for 51.6% of the 126 isolates, were recovered from urine samples. These 65 isolates constituted 39.4% of all the isolates acquired from urine samples. In contrast, only 7 of the ESBL-producing isolates (5.6% of 126) were acquired from blood samples, yet they accounted for 100% of the blood sample isolates collected. It is equally interesting to observe that in cases of sepsis, 13 of the 17 isolates identified were positive for ESBL production, and there was a significant association between sepsis and ESBL infections. These observations are worrisome, as previous research indicates that ESBL-producing bacterial infection caused more severe sepsis compared to non-ESBL bacterial infection [28,30], and infections caused by ESBL-producing bacteria are more fatal compared to those caused by non-ESBL-producing bacteria. Moreover, earlier investigations have indicated that the production of ESBL serves as a significant predictor of hospital mortality in patients with sepsis [19]. This serves as a caution regarding invasive enterobacterial infections, which could potentially exhibit greater virulence compared to other enterobacterial infections, as well as increased drug resistance, making treatment more challenging. To enhance the clinical management of patients exhibiting these potential risk factors for ESBL production, such as sepsis and hospitalization, it is crucial for the therapeutic regimen to include treatment options that specifically target these issues.

In our study, both organisms were obtained from various age groups and in similar proportions from both males and females. No significant differences were seen which is similar to observations in a study that found no relationship between ESBL-producing *E. coli* or *K. pneumoniae* infection with age or sex [5,36]. However, in other studies that assessed risk factors, most patients infected with ESBL producers were older [3,30]. Besides, the observation of higher ESBL-PE prevalence in patients in the age group of 18 to 40 years may be due to self-medication practice [22].

Our study shows a significant association between ESBL-PE and resistance to quinolones, aminoglycosides, tetracycline, chloramphenicol, trimethoprim, and augmentin as previously reported [3,24]. The high level of resistance exhibited by ESBL-PE isolates to other antibiotic classes is concerning, as it may limit the availability of effective empirical therapy options for treating infections caused by these bacteria. Also, the high resistance to trimethoprim, ampicillin, and tetracycline by both organisms may be attributed to their huge over-the-counter use and abuse in the wider study setting area. Antibiotics can easily be gotten in the open market, and people use them for virtually every ailment. The majority of the isolates (86%) showed susceptibility to imipenem. This is similar to the findings of a study [24], which found that imipenem had the highest level of antimicrobial activity against both ESBL-producing and non-ESBL-producing Gram-negative bacilli. Even though imipenem was only recently introduced, 12.4% and 15.7% of ESBL-producers and non-ESBL-producers, respectively, were resistant to it. As a result, the treatment choices for infections caused by these organisms are severely limited. The isolates that were resistant to imipenem may have plasmids encoding carbapenemase, an enzyme that hydrolyzes carbapenems, the class to which imipenem belongs [27]. A prior study connected imipenem's therapeutic failure to the formation of multiple beta-lactamases [13]. In this resource-constrained scenario, the evolution of carbapenem resistance portends a gloomy future for antimicrobial options and availability.

Some limitations, including the non-availability of data regarding prior antibiotic usage and past colonization with drug-resistant bacteria, constrained this investigation. These two risk variables were not examined due to the retrospective nature of the study. Another constraint was the absence of molecular investigations on the ESBL genes. Further analyses would have provided a more comprehensive understanding of the specific types of ESBL genes present in this area. We recommend advanced molecular analysis for species classification and ESBL typing and a more in-depth look at other risk variables in future studies. This would provide a more complete picture of current realities.

Despite these limitations, our study has demonstrated that antibiotic resistance remains a significant local burden that must be addressed as a public health issue. It has highlighted the necessity of ESBL testing in cases of sepsis due to the high probability of it being caused by ESBL-producing enterobacteria. Both ESBL-positive and ESBL-negative isolates were resistant to a wide range of antibacterial drugs. Although imipenem showed potential against ESBL producers as many isolates were sensitive to it, observation of 28.1% resistance to it limits the treatment choices for infections caused by these organisms. Therefore, there is a need for continuous surveillance of bacterial resistance, improved antibiotic stewardship and infection control strategies, and regular screening of ESBLs in clinical isolates to prevent treatment failure.

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