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A Study on the Molecular Genes of ESBL-Producing Enterobacteriaceae from Patients Hospitalized in the Internal Medicine Department of a Tertiary Hospital in Lagos, Nigeria

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Abstract

Background: Multidrug-resistant Enterobacteriaceae pose a significant global health burden. These bacteria produce extended-spectrum beta-lactamase (ESBL) enzymes, which render them resistant to many beta-lactam antibiotics. This study aimed to identify both the phenotypic traits and molecular genes associated with ESBL production in Enterobacteriaceae strains isolated from hospitalized patients in the Internal Medicine Department of Lagos University Teaching Hospital (LUTH), Idi-Araba. **Materials and Methods:** This cross-sectional study was carried out in the Internal Medicine Department of Lagos University Teaching Hospital, Idi-Araba. All consenting patients admitted

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with clinical signs of infection were enrolled. Relevant clinical samples were collected and analyzed using standard microbiological identification methods, while multiplex polymerase chain reaction (PCR) was employed to genotypic markers. **Result:** 300 bacterial pathogens were isolated, of which 176 belonged to the Enterobacteriaceae family. *Escherichia coli* was the most frequently identified pathogen, accounting for 32% of the isolates, followed by Klebsiella pneumoniae, 23.9% and Klebsiella oxytoca, 17.6%, among others. More than 60% of the Enterobacteriaceae isolates were found to produce ESBL. Among the ESBL genes detected, TEM was the most prevalent, followed by SHV and CTX-M. **Conclusion:** The study revealed a high prevalence of extended-spectrum beta-lactamase (ESBL) production. Plasmid-mediated resistance genes, including TEM, SHV, and CTX-M, were identified.

Keywords

Enterobacteriaceae, ESBL, Beta-Lactamase, TEM, SHV, CTX-M

1. Introduction

Background: Enterobacteriaceae is a heterogeneous group of gram-negative straight rods and non-sporulated bacteria. Members of Enterobacteriaceae are widely distributed in nature, and many of their species live in the gut of humans and animals, including insects, where they can cause enteric diseases or remain as commensal organisms. The members of this family play a role as plant pathogens and biotechnological microorganisms for the heterologous production of proteins [1]. However, only a small group of species are considered strict pathogens in human [2].

To survive the effects of antibiotics, microorganisms are constantly finding new defense strategies. Some Enterobacteriaceae produce enzymes called Extended-Spectrum Beta-Lactamases (ESBLs), which are enzymes that cause resistance to some of the most commonly used antibiotics, including all penicillin, cephalosporins and monobactams [3]. Antibiotic resistance of bacteria is commonly seen in daily medical practice, with multidrug-resistant gram-negative bacteria posing the greatest threat to human health [4].

Extended spectrum beta lactamases (ESBLs) are bacterial enzymes that hydrolyze oxyimino-cephalosporins and confer resistance to broad spectrum cephalosporin and aztreonam [5], they give the bacteria ability to resist penicillins and cephalosporins of the first, second and third generations as well as aztreonam through hydrolysis of these antibiotics [6] and are encoded by mobile genetic elements [7]. Alarmingly, these genes code resistance to not only cephalosporins and penicillin but also other antibiotics such as aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol, and sulfamethoxazole (trimethoprim) [7].

ESBL-producing Enterobacteriaceae infections became a significant therapeutic challenge worldwide in daily clinical practice since their resistances to additional classes of antibiotics reduce effective therapeutic options [8]. In many cases, even common infections such as urinary tract infections caused by ESBL-produc-

ing organisms require more complex antibiotics and are of major concern since infections caused by these resistant strains are associated with prolonged hospital stay and increased case-fatality rate [9]. Nosocomial risk factors such as the presence of intravascular catheters, undergoing surgery, staying at an intensive care unit, and international travel have been shown to increase the risk of being colonized with ESBL-producing Enterobacteriaceae [10]-[12].

The prevalence of beta-lactamase-producing organisms has been rising globally, including in European countries. [13] In North America, the estimated prevalence of ESBL-producing *E. coli* is 9.8%.[14] Numerous studies have also documented the growing emergence of ESBL-producing *Enterobacteriaceae* across Africa [15] [16]. However, prevalence rates vary significantly across the continent, ranging from 16.4% to 77.8% in North Africa, and from 8.8% to 13.1% in South Africa. In East Africa, reported rates fall between 37.4% and 62.8% [17]. A pooled analysis by Toy *et al*. reported a prevalence of 9.3% in sub-Saharan Africa and as high as 58.0% in North West Nigeria [16] [18].

The wide regional differences underscore the need to take into account at the level of the country, the region, the hospital, and at times the individual hospital unit when making decisions about empirical therapy for serious infections. Moreover, infections caused by ESBL producers range from uncomplicated urinary tract infections to life-threatening sepsis. ESBL—producing organism exhibit co-resistance to many other classes of antibiotics, resulting in limitation of therapeutic options. The data generated in this study will enable physicians handling infections caused by ESBL—producing Enterobacteriaceae to consider possible antibiotic susceptibility patterns when formulating decisions pertaining to developing management options. Therefore, this study aimed to identify both the phenotypic features and molecular genes associated with ESBL production in Enterobacteriaceae pathogens isolated from hospitalized patients in the Internal Medicine Department of Lagos University Teaching Hospital (LUTH), Idi-Araba.

2. Methods

2.1. Study Area

This study was undertaken in Lagos University Teaching Hospital (LUTH) Idi-Araba, Lagos State of Nigeria. This is the largest tertiary hospital in Lagos state and one of the foremost hospitals in Nigeria. It serves people in South west geopolitical zone and epicenter for referrals in Nigeria.

2.2. Study Design

The study was a cross-sectional hospital-based survey of all the patients with a clinical diagnosis of infection admitted to the adult medical wards of Lagos University Teaching Hospital. The study started from May 2019 to April 2020. Clinical samples and relevant data were collected from consenting participants who met the eligibility criteria and were processed and analyzed for relevant genes responsible for extended-spectrum beta-lactamase in Enterobacteriaceae.

2.3. Sample Size

The sample size was determined by using the formula below:

$$N_o = \frac{Z^2 pq}{d^2}$$

N = The desired sample size.

Z = The standard normal deviation, usually set at 1.96 corresponding to 95% confidence interval.

p = The prevalence.

$q = 1 - p$.

d = The standard error (margin of error) set at 0.05.

where Extended Spectrum Beta-lactamase (ESBL) prevalence rate among Enterobacteriaceae of 9.25% was used [19].

The sample size was calculated using the formula above where: $Z = 1.96$; $p = 0.226$; $q = 1 - p = 0.774$; $d = 0.05$.

$N = (1.96)^2 \times 0.0925 \times 0.91/(0.05)^2 = 3.8416 \times 0.0925 \times 0.91/0.0025 = 129.3 = 129$.

The number of Enterobacteriaceae isolates used for this study 176 in order to cover for attrition and possible loss due storage.

2.4. Sampling Method

Systematic consecutive sampling method was used to recruit participants into this study.

2.5. Inclusion Criteria

Patients with clinical diagnosis of infection on admission for more than 24 hours at the Lagos State University Teaching Hospital were included in the study.

2.6. Exclusion Criteria

Patients with infections who were hospitalized for one day were excluded from the study.

2.7. Sample Collection and Analysis

Screening specimen included clinically relevant samples collected from patients presenting with infections before administration of antibiotics. The samples analyzed were blood, urine, wound swabs, ascitic fluid, aspirate, wound biopsy, sputum and others. There were aseptically collected into the appropriate specimen bottles and transported to the medical microbiology laboratory through cold chain. The various specimens were carefully registered and processed through macroscopy, microscopy and culture in appropriate media according to the standard laboratory practice. Enterobacteriaceae isolates were further identified with the use of Microbact Identification System (**MICROBACT™ 24E)** according to the manufacturer's guideline (Oxoid UK).

2.8. Antibiotics Susceptibility Testing

Antimicrobial susceptibility testing of isolates was done using Clinical and Laboratory Standards Institute (CLSI) guidelines [20]. This was carried out by modified Kirby-Bauer Disk diffusion method on Mueller-Hinton agar plates incubated aerobically at 37˚C for 18 - 24 hours. The zones of inhibition around the disc were measured in millimeters and classified as Sensitive (S), Intermediate (I), or Resistant (R). The antibiotics tested were Piperaciin-Tazobactam (TZP), Cefuroxime (CXM), Amoxicilin/clavulanate (AMC), Ciprofloxacin (CIP), Cefotaxime (CTX), Meropenem (MEM), Amikacin (AK), Ceftriaxone (CRO), Cefipime (FEP), Gentamicin (CN), Levofloxacin (LEV) [20].

2.9. Phenotypic Detection and Confirmation of Extended Spectrum *β*-Lactamase (ESBL) Producing Enterobacteriaceae

The Enterobacteriaceae isolates were screened for ESBL production by using disc diffusion of ceftazidime (30 µg) and cefotaxime (30 µg) placed on inoculated plate containing Muller Hinton agar according to CLSI recommendation. The zone diameters of ≤22 mm and ≤27 mm for ceftazidime and cefotaxime respectively, were indicated as suspected ESBL production [20]. Positive isolates were subjected to a confirmatory test using the double disk synergy test.

Confirmatory test: Double disc synergy test (DDST) was used to test for presence of ESBL in Enterobacteriaceae. Discs containing cephalosporin (cefotaxime and ceftazidime) was placed next to a disc with clavulanic acid (amoxicillin-clavulanic acid). The distance between the discs was 20 mm centre to centre. The agar was incubated at 35˚C to 37˚C for 18 hours in ambient air.

3. Result

Positive result was indicated by augmenting of zone of inhibition towards the direction of the amoxicillin-clavulanic acid with dumb-bell or keyhole appearance.

3.1. Quality Control

Klebsiella pneumoniae ATCC 700603 was used as positive control organism *Escherichia coli* ATCC 25922 was used as negative control organism [20].

3.2. Molecular Detection of the Resistance Genes

The molecular analysis was done in Nigeria Institute of Medical Research (NIMR) Yaba Lagos, Nigeria. Genotypic determination of the resistance genes responsible for ESBL producing enterobacteriaceae phenotype was done. Polymerase chain reactions (PCR) were used to detect the resistant genes from the isolates following the result of the phenotypic resistance testing.

3.3. DNA Extractions

Bacterial DNA extractions were done by extraction column method using Zymo Bacterial/Fungal DNA Mini Prep™ Kit [21] [22].

Table 1. Primer sequence.

Phenotypic resistance	Target gene	Primer name	PRIMER SEQUENCE $5^1 \rightarrow 3^1$	Amplicon size (base pair)	References
ESBL	i. CTX-M	Forward (F)	GACAAAGAGAGTGCAACGGATG	501	[23]
	CTX-M	Reverse (R)	TCAGTGCGATCCAGACGAAA		
	ii. TEM	Forward (F)	AGTGCTGCCATAACCATGAGTG	431	[23]
		Reverse ®	CTGACTCCCCGTCGTGTAGATA		
	iii. SHV	Forward (F)	GATGAACGCTTTCCCATGATG	214	[23]
		Reverse (R)	CGCTGTTATCGCTCATGGTAA		

3.4. Choice of Primer

Table 1 shows primers used for detection of antibiotic resistance genes were chosen from database sequences with consideration to the common target of resistance genes (TEM, SHV, and CTX-M). The primers were synthesized by STAB VIDA, Lda, FCT/UNL (Lab. 0072829-516) Caparica in Portugal.

3.5. Amplification

The PCR was performed in a final volume of 20 µL reaction master mixture using Solis Biodyne (from Estonia) 5X firepol ready to load master mix. 4 µL of extracted DNA was added to 16 µL of PCR master mixture. The amplification reactions were performed in Bio-Rad T100 Thermal Cycler under the described amplification conditions. The runs were done as multiplex PCR and the condition was optimized to ensure that each amplicon was the correct base pair. The annealing temperature of each of the primer set was optimized with due consideration to the melting temperature (Tm) of the various primers in the set to ensure optimum reaction. 35 cycles were performed to ensure enough PCR products were generated to enable easy detection after agarose gel electrophoresis. The runs were done according to the groups of genes responsible for a particular phenotypic resistance.

ESBL-producing Enterobacteriaceae genes (CTX-M, TEM and SHV): the previously described protocol was used and the multiplex assay detected TEM, SHV, and CTX-M genes [23] The amplification condition involved denaturation at 94˚C for 5 minutes followed by 35 cycles of 94 C for 1 minute, 61˚C for 1 minute and 72˚C for 1 minute, and a final extension of 72˚C for 5 minutes [23].

3.6. Agarose Gel Electrophoresis

Agarose gel electrophoresis technique was used to separate amplified gene products according to their base pairs. The assay products were electrophoresed for 30 minutes at 100 V in 0.5X TBE buffer. The DNA was stained with ethidium bromide (1 µ/mL). The gels were imaged under ultraviolet (UV) light. The PCR amplicon size was calculated by comparing the molecular weight with the molecular ladders [23].

3.7. Data Analysis

The data collected were entered in Microsoft Excel version 2010 and subsequently analyzed using the International Business Machine Statistical Package for Social Sciences (IBM SPSS) statistics for Windows, version 25 (IBM Corp., Armonk, New York, USA). The data were presented in frequency tables and summary statistics.

4. Results

4.1. Demographic Characteristics of the Patients Enrolled in the Cross-Sectional Study

A total of 717 patients were investigated, 300 were positive for different bacterial infections and 176 were positive for infections caused by bacteria of the family Enterobacteriaceae. Seventy-four (42%) males and one hundred and two (58%) females were participants with infections caused by Enterobacteriaceae. The age range of the patients was (18 to 80) years, with a mean age of 45.9 ± 12.1 years. The highest number of patients was in the 30 to 39 age group followed by the 40 to 49 and 50 to 59 years age groups, with 42% and 38% respectively (Table 2).

4.2. Types of Clinical Infections Involved in the Study

Urinary tract infections 72 (41%) were the most predominant infection, followed by lower respiratory tract infections 37 (21%), bloodstream infections 30 (17%), skin and soft tissue infections 30 (17%) and 7 (4%) others (Figure 1). The most common organism isolated was *Escherichia coli* 56 (32%), followed by *Klebsiella pneumoniae* 42 (23.9%), *Klebsiella oxytoca* 31 (17.6%), and others (Table 3).

4.3. Antibiotic Susceptibility Profile of Pathogens

The susceptibility profiles of the isolates showed that over 60% Enterobacteriaceae were susceptible to amikacin, levofloxacin, piperacillin tazobactam and meropenem while more than 50% were resistant to second and third generation cephalosporins such as cefuroxime, cefotaxime and ceftriaxone. Most of the Enterobacteriaceae were susceptible to piperacillin tazobactam (81%) and meropenem (89%) (Table 4).

4.4. Extended Spectrum Beta-Lactamase Producing Enterobacteriaceae

Of 176 Enterobacteriaceae isolates screened for extended spectrum beta-lactamase (ESBL) production using ceftazidime and cefotaxime disc diffusion, 95 (54%) isolates were positive (Table 5). Those positive with screening test were subjected to confirmatory test using double disc synergy test (DDST). The test revealed that 52 (29.5%) of Enterobacteriaceae were extended spectrum beta-lactamase (ESBL) producers (Table 5). The distribution of the ESBL producing Enterobacteriaceae according to their various species is shown in Table 5. *Klebsiella pneumoniae* 19 (36.5%) was the most common ESBL producers followed by *Escherichia coli* 15 (28.8%), *klebsiella oxytoca* 9 (17.3%), *Enterobacter cloacae* 2 (3.8%), and one

(1.9%) each of *Citrobacter koseri*, *Enterobacter aerogenes*, *Enterobacter agglomerans*, *Enterobacter gergoviae* and *proteus vulguris*.

4.5. Molecular Analysis of ESBL Producing Enterobacteriaceae

All the 52 extended spectrum beta-lactamase (ESBL) producing Enterobacteriaceae were subjected to molecular analysis by polymerase chain reaction (PCR). The result showed that TEM genes 43 (56.6%) were the highest followed by SHV genes 31 (40.8%), with the lowest of 2 (2.6%) for CTX-M genes. Extended spectrum beta-lactamase genes were most detected in *Klebsiella pneumoniae* 19 (41.3%), followed by *Klebsiella oxytoca* 10 (21.7%), *Escherichia coli* 10 (21.7%), *Enterobacter cloacae* 3 (6.5%), and 1 (2.2%) each for *Enterobacter aerogenes*, *Proteus mirabilis, Proteus vulgaris*, and *Citrobacter koseri*. There was no resistance gene detected in 6 isolates (Table 6). There were multiple occurrences of genes in some of the isolates. The co-existence of CTX-M, TEM and SHV was seen in an isolate of *Escherichia coli*, while TEM and SHV genes were seen in 27 isolates (*Klebsiella pneumoniae* (16), *Klebsiella oxytoca* (6), *Escherichia coli* (3), *Enterobacter aerogenes* (1) and *Citrobacter koseri* (1)). The co-existence of TEM and CTX-M genes was seen in only one isolate of *Klesbsiella oxytoca.*

Table 2. Baseline characteristics.

Variable	Frequency (n = 176)	Percentage (%)
Age group (Years)		
18 - 29	25	14.0
30 - 39	42	24.0
40 - 49	38	22
50 - 59	38	22
≥60	33	19
Mean ± SD Age	45.9 ± 12.1	
Gender		
Male	74	42
Female	102	58

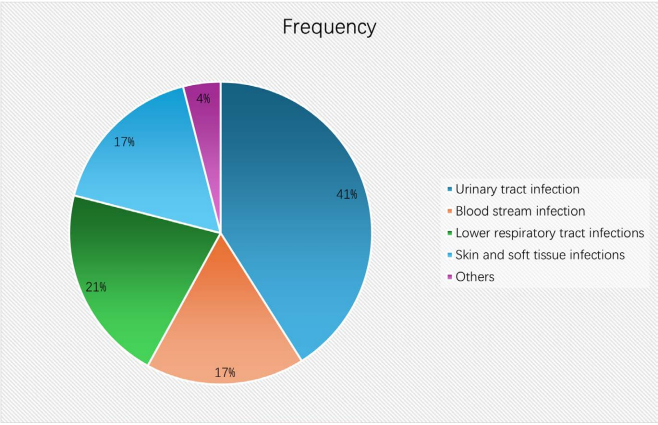

Figure 1. Various types of infections.

Table 3. Enterobacteriaceae isolated from the clinical specimens.

Enterobacteriaceae		Blood (%)	Sputum (%)	Urine (%)	Wound specimen (%)	Others (%)
E. coli	56 (32%)	6 (20.0)	14 (37.8)	26 (36.1)	8 (26.6)	2 (28.6)
Klebsiella pneumoniae	42 (23.9%)	10 (33.3)	16 (43.2)	8 (11.1)	6 (20)	2 (28.6)
Klebsiella oxytoca	31 (17.6%)	2 (6.6)	5 (13.5)	19 (26.4)	4 (13.3)	1 (14.3)
Citrobacter freundii	2 (1.1%)	2 (6.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Citrobacter koseri	5 (2.8%)	1 (3.3)	0 (0.0)	2 (2.8)	1 (3.3)	1 (14.3)
Enterobacter aerogenes	5 (2.8%)	1 (3.3)	1 (2.7)	2 (2.8)	1 (3.3)	0 (0.0)
Enterobacter agglomerans	5 (2.8%)	2 (6.6)	0 (0.0)	3 (4.1)	0 (0.0)	0 (0.0)
Enterobacter cloacae	3 (1.7%)	1 (3.3)	0 (0.0)	0 (0.0)	2 (6.6)	0 (0.0)
Enterobacter gergoviae	2 (1.1%)	0 (0.0)	0 (0.0)	1 (1.4)	1 (3.3)	0 (0.0)
Klebsiella ozaenae	1 (0.5%)	0 (0.0)	1 (2.7)	0 (0.0)	0 (0.0)	0 (0.0)
Morganella morganii	1 (0.5%)	0 (0.0)	0 (0.0)	1 (1.4)	0 (0.0)	0 (0.0)
Proteus mirabilis	10 (5.7%)	3 (10.0)	(0.0)	2 (2.8)	4 (13.3)	1 (14.3)
Proteus vulgaris	2 (1.1%)	(0.0)	(0.0)	1 (1.4)	1 (3.3)	0 (0.0)
Providencia rettgeri	4 (2.3%)	0 (0.0)	0 (0.0)	2 (2.8)	2 (6.6)	0 (0.0)
Serratia marcescens	4 (2.3%)	1 (3.3)	0 (0.0)	3 (4.1)	0 (0.0)	0 (0.0)
Serratia liquefaciens	3 (1.7%)	1 (3.3)	0 (0.0)	2 (2.8)	0 (0.0)	0 (0.0)
Total	**176 (100)**	**30 (100)**	**37 (100)**	**72 (100)**	**30 (100)**	**7 (100)**

Table 4. Antibiotics susceptibility profile.

S/N	ISOLATE	TOTAL NUMBER OF ISOLATES	GENTAMICIN (%S)	AMIKACIN (%S)	CIPROFLOXACIN (%S)	LEVOFLOXACIN (%S)	CEFUROXIME (%S)	CEFOTAXIME (%S)	CEFTRIAXONE (%S)	CEFEPIME (%S)	PIPERACILLIN TAZOBACTAM (%S)	AMOXICILLIN CLAVULANATE (%S)	MEROPENEM (%S)
1.	*Escherichia coli*	56	36 (64)	36 (64)	25 (45)	41 (73)	16 (29)	29 (52)	29 (52)	35 (63)	48 (86)	31 (55)	51 (91)
2.	*Klebsiella pneumoniae*	42	23 (55)	30 (72)	19 (45)	25 (60)	12 (29)	15 (36)	14 (33)	23 (55)	32 (76)	25 (60)	33 (79)
3.	*Klebsiella oxytoca*	31	17 (55)	24 (77)	16 (52)	21 (68)	8 (26)	11 (35)	13 (42)	17 (55)	22 (71)	17 (55)	28 (90)
4.	*Klebsiella ozaenae*	1	0	1 (100)	0	1 (100)	0	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
5.	*Proteus mirabilis*	10	6 (60)	5 (50)	6 (60)	8 (80)	3 (30)	7 (70)	6 (60)	7 (70)	9 (90)	6 (60)	9 (90)
6.	*Proteus vulgaris*	2	1 (50)	1 (50)	1 (50)	1 (50)	0	1 (50)	1 (50)	1 (50)	2 (100)	1 (50)	2 (100)
7.	*Enterobacter aerogenes*	5	4 (80)	5 (100)	4 (80)	5 (100)	2 (40)	3 (60)	3 (60)	3 (60)	4 (80)	2 (40)	4 (80)
8.	*Enterobacter agglomerans*	5	1 (20)	2 (40)	3 (60)	4 (80)	1 (20)	2 (40)	3 (60)	4 (80)	4 (80)	4 (80)	5 (100)
9.	*Enterobacter cloacae*	3	0	1 (33)	3 (100)	3 (100)	0	0	0	1 (33)	2 (67)	2 (67)	3 (100)
10.	*Enterobacter gergoviae*	2	2 (100)	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)	2 (100)	1 (50)	2 (100)

Continued

11. *Citrobacter koseri*	5	5 (100)	3 (60)	4 (80)	4 (80)	4 (80)	3 (60)	4 (80)	4 (80)	4 (80)	4 (80)	5 (100)
12. *Citrobacter freundii*	2	1 (50)	2 (100)	0	0	0	1 (50)	1 (50)	1 (50)	2 (100)	1 (50)	1 (50)
13. *Serratia marcescens*	4	4 (100)	4 (100)	1 (25)	3 (75)	3 (75)	4 (100)	4 (100)	4 (100)	4 (100)	2 (50)	4 (100)
14. *Serratia liquefaciens*	3	2 (67)	3 (100)	1 (33)	2 (67)	1 (33)	3 (100)	3 (100)	3 (100)	3 (100)	2 (67)	3 (100)
15. *Providencia rettgeri*	4	4 (100)	4 (100)	3 (75)	4 (100)	2 (50)	3 (75)	1 (25)	2 (50)	3 (75)	3 (75)	4 (100)
16. *Morganella morganni*	1	1 (100)	1 (100)	1 (100)	1 (100)	0	0	0	0	1 (100)	1 (100)	1 (100)
17. Total	176	107 (61)	123 (70)	88 (50)	124 (70)	53 (30)	84 (48)	84 (48)	107 (61)	143 (81)	103 (59)	156 (89)

Table 5. Extended Spectrum Beta-lactamase resistance phenotype screening and confirmatory test.

Bacterial Isolate	ESBL Screening	ESBL Confirmation with DDST
Klebsiella pnuemoniae (n = 42)		
a. ESBL Positive	27 (64.3%)	19 (45.2%)
b. ESBL Negative	15 (35.7%)	-
Escherichia coli (n = 56)		
a. ESBL Positive	26 (46.4%)	15 (26.8%)
b. ESBL Negative	30 (53.6%)	-
Klebsiella oxytoca (n = 31)		
a. ESBL Positive	18 (58.1%)	10 (32.3%)
b. ESBL Negative	13 (41.9%)	-
Enterobacter cloacae (n = 3)		
a. ESBL Positive	3 (100%)	3 (100%)
b. ESBL Negative	0 (0.0%)	-
Proteus mirabilis (n = 10)		
a. ESBL Positive	7 (70%)	2 (20%)
b. ESBL Negative	3 (30%)	-
Proteus vulgaris (n = 2)		
a. ESBL Positive	1 (50%)	1 (50%)
b. ESBL Negative	1 (50%)	-
Enterobacter aerogenes (n = 5)		
a. ESBL Positive	3 (60%)	1 (20%)
b. ESBL Negative	2 (405)	-
Citrobacter koseri (n = 5)		
a. ESBL Positive	3 (60%)	1 (20%)
b. ESBL Negative	2 (40%)	-
Klebsiella ozaenae (n = 1)		
a. ESBL Positive	1 (100%)	0 (0.0)
b. ESBL Negative	0 (0.0)	0 (0.0)
Enterobacter agglomerans (n = 5)		
a. ESBL Positive	2 (40%)	
b. ESBL Negative	3 (60)	0 (0.0)

Continued

Enterobacter gergoviae (n = 2)		-
a. ESBL Positive	1 (50%)	0 (0.0)
b. ESBL Negative	1 (50%	-
Citrobacter freundii (n = 2)		
a. ESBL Positive	1 (50%)	0 (0.0)
b. ESBL Negative	1 (50%)	-
Serratia marcescens (n = 4)		
a. ESBL Positive	0 (0.0)	0 (0.0)
b. ESBL Negative	4 (100%)	-
Serratia liquefacciens (n = 3)		
a. ESBL Positive	0 (0.0)	0 (0.0)
b. ESBL Negative	3 (100%)	-
Providencia rettgeri (n = 4)		
a. ESBL Positive	1 (25%)	0 (0.0)
b. ESBL Negative	3 (75%)	-
Morganella morganni (n = 1)		
a. ESBL Positive	1 (100%)	0 (0.0)
b. ESBL Negative	0 (0.0)	-

Table 6. Molecular Genes Identified from the isolates.

Bacteria isolate	No. (%) of ESBL positive isolates (n = 52)	No (%) of isolates harbouring ESBL gene (n = 46)	No of various ESBL genes detected (n = 76)		
			CTX-M (n = 2)	TEM (n = 43)	SHV (n = 31)
Klebsiella pneumoniae	19	19 (41.3%)	0 (0.0)	16 (37.2%)	19 (61.3%)
Klebsiella oxytoca	10	10 (21.7%)	1 (50.0%)	10 (23.3%)	6 (19.4%)
Escherichia coli	15	10 (21.7%)	1 (50.0%)	10 (23.3%)	4 (12.9%)
Enterobacter cloacae	3	3 (6.5%)	0 (0.0)	3 (7.0%)	0 (0.0%)
Enterobacter aerogenes	1	1 (2.2%)	0 (0.0)	1 (2.3%)	1 (3.2%)
Proteus mirabilis	2	1 (2.2%)	0 (0.0)	1 (2.3%)	0 (0.0)
Proteus vulgaris	1	1 (2.2%)	0 (0.0)	1 (2.3%)	0 (0.0)
Citrobacter koseri	1	1 (2.2%)	0 (0.0)	1 (2.3%)	0 (0.0)

5. Discussion

The emergence and rapid spread of multidrug-resistant strains of ESBL-producing *Enterobacteriaceae* pose a significant global public health concern [24]. Findings from this study reveal a high prevalence of ESBL-producing organisms based on phenotypic analysis. Specifically, the study recorded a 29.5% prevalence of ESBL-producing *Enterobacteriaceae* in various clinical samples, highlighting a serious health challenge in Nigeria. This aligns with previously reported prevalence rates in the country, which have ranged from 7.5% to 82.3% [25] [26]. Similarly,

high rates of ESBL producers have been documented in other African nations. For example, a systematic review from Nepal reported a pooled prevalence of 29% among *Enterobacteriaceae* isolates, with *Escherichia coli* being the most common ESBL producer [27]. In Egypt, a meta-analysis estimated an overall prevalence of 60%, primarily involving *E. coli* and *Klebsiella pneumoniae* [28]. Likewise, in Ghana, 49.1% of *Enterobacteriaceae* isolates were found to be ESBL producers, with *E. coli* being the most prevalent [29]. Even more concerning are reports from Nigeria and other countries that have recorded even higher prevalence rates [30]-[33]. These results indicate that beta-lactam antibiotics are becoming less effective in treating infections caused by *Enterobacteriaceae* across different regions. The elevated prevalence rates highlight the urgent need for ongoing monitoring and the implementation of antibiotic stewardship strategies.

Klebsiella pneumoniae and *Escherichia coli* were identified as the most common ESBL-producing *Enterobacteriaceae* in this study. This finding is consistent with earlier reports from healthcare institutions in South West Nigeria as well as several European countries [31] [34]. However, research conducted by Nwankwo and colleagues in Kano [35] revealed a slightly different pattern, with *E. coli* emerging as the predominant ESBL producer, followed by *Klebsiella pneumoniae*. These discrepancies in the prevalence and distribution of ESBL phenotypes may be due to variations in study methodologies, timing, geographic regions, patient populations, clinical conditions, infection prevention and control measures across different healthcare settings [25] [26].

Several evidence has shown that carbapenems are drugs of choice in the management of ESBL producing Enterobacteriaceae [25] and in this study, many ESBL producers were found susceptible to carbapenems.

The ESBL-producing *Enterobacteriaceae* isolates in this study harbored **CTX-M**, **TEM**, and **SHV** genes. Among these, the **TEM** gene was the most frequently detected, followed by **SHV**, while **CTX-M** was the least common. This pattern aligns with the findings of a systematic review by Tanko and colleagues on the prevalence of ESBL-producing Gram-negative bacteria in Nigeria [25]. The results also support earlier studies reporting a high prevalence of **TEM** and **SHV** genes in Nigeria [26] [30] [36]. However, some studies have reported a higher occurrence of the **CTX-M** gene [37]-[39] both within and outside the country. *Klebsiella* species were the most prevalent carriers of ESBL resistance genes, followed by *Escherichia coli*, a trend consistent with the findings from the systematic review of ESBL phenotypes in Nigeria [25]. In contrast, studies from North African countries such as Egypt reported **CTX-M** as the most dominant gene, identified in 73% of phenotypically confirmed ESBL-producing *E. coli* isolates, followed by **TEM** (60%) and **SHV** (22%) [28]. These differences suggest that the prevalence and distribution of ESBL genes can vary significantly across different geographic regions.

The identification of **TEM**, **SHV**, and **CTX-M** genes in *Enterobacteriaceae* (key pathogens in both community-acquired and hospital-associated infections) offers important insights into their epidemiology and the risk factors linked to their

transmission [28]. These findings are particularly significant for infection prevention and control efforts, as these genes are plasmid-mediated and often associated with transposons and insertion sequences. This genetic configuration facilitates their horizontal transfer between bacterial strains, even across different species. Such plasmid-mediated gene exchange plays a crucial role in the acquisition and dissemination of multidrug resistance among bacterial populations [28] [36]. The detection of ESBL genes, especially the **CTX-M** genotype, is a major public health concern, given its association with numerous outbreaks in healthcare settings and communities worldwide [28] [40].

Throughput technologies, including DNA sequencing and pulsed-field gel electrophoresis, have been utilized in numerous studies to detect ESBL genes and trace their transmission patterns [41]. However, the absence of these tools in certain regions limits the scope of effective surveillance and containment strategies.

6. Conclusions

This study identified a high prevalence of ESBL production among *Enterobacteriaceae* isolates from patients admitted to the medical wards of Lagos University Teaching Hospital (LUTH). While most of the isolates remained susceptible to meropenem, a considerable proportion exhibited strong resistance to third-generation cephalosporins. The detection of plasmid-mediated ESBL genes, namely **TEM**, **SHV**, and **CTX-M**, raises concerns about the potential for widespread outbreaks of multidrug-resistant superbugs.

Therefore, routine surveillance and screening for ESBL-producing *Enterobacteriaceae* are highly recommended. Additionally, the use of carbapenems is strongly advised for the effective management of infections caused by these resistant organisms.

Limitation

Although genetic sequencing of the identified ESBL genes would have provided deeper insights, it was not feasible due to financial constraints. Expanding the study to include a larger population would also enhance the validity and generalizability of the findings.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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