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Extended-Spectrum β-Lactamases in *Klebsiella pneumoniae* Isolates and Carbapenem-Resistant Enterobacteriaceae in Two Public Hospitals: Detection of SHV, CTX-M, and TEM β-Lactamases

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Abstract

Multidrug-resistant Klebsiella pneumoniae *remains a serious challenge in clinical settings due to its ability to produce extended-spectrum beta-lactamases (ESBLs) and carbapenemases. This study investigated the phenotypic and molecular characteristics of resistant* Klebsiella pneumoniae *isolates from Lagos University Teaching Hospital (LUTH), Idi-Araba, and Ikorodu General Hospital, Lagos State. A total of 200 samples were examined, with 143 from LUTH and 57 from Ikorodu. Antibiotic susceptibility testing showed high resistance to β-lactam antibiotics, particularly amoxicillin-clavulanate (69.23% in LUTH and 61.4% in Ikorodu) and third-generation cephalosporins. Imipenem showed the highest susceptibility, especially among Ikorodu isolates (100%). Among 26 LUTH isolates tested, all were positive for carbapenemase production using the Modified Carbapenem Inactivation Method and were also confirmed as ESBL producers using the Double Disk Synergy Test. The prevalence of carbapenemase- and ESBL-producing isolates in LUTH was 18.2%. Molecular analysis revealed the presence of the SHV gene in one isolate, while CTX-M and TEM genes were not detected in the others, suggesting alternative resistance mechanisms. Continuous surveillance of antimicrobial resistance is essential for effective infection control and treatment.*

Keywords: Klebsiella pneumoniae; ESBL; Carbapenemase; Antibiotic resistance; SHV gene; CTX-M gene; TEM gene.

Introduction

Antimicrobial resistance (AMR) emergence and transmission have emerged as a major global public health issue, endangering the successful treatment of infectious diseases. It is a major threat because the efficacy of the common antibiotics against most bacterial infections is being eroded due to increased resistance to antibiotics globally (World Health Organization, 2021). The latest report on Global Antimicrobial Resistance and Use Surveillance System (GLASS) is characterized by alarming rates of resistance in common bacterial pathogens (World Health Organization, 2022). Increased treatment failure of infectious diseases caused by microbes globally is attributed to antimicrobial resistance (AMR), which occurs due to widespread or indiscriminate use of antibiotics. AMR significantly impacts mortality and morbidity, bringing substantial economic burdens to people and countries (Salam et al., 2023). A projection showed that there were five million deaths associated with AMR in 2019, which suggests that the ten million deaths and 24 million individuals under the poverty lines projected to occur in 2030 due to AMR could be achieved earlier than was expected (Murray et al.,2022)

According to the report by Centers of Disease Control and Prevention (CDC), AMR causes more than 2.8 million infections and 35,000 deaths every year in the United States alone (Centers for Disease Control and Prevention, 2022). Klebsiella spp, especially Klebsiella pneumoniae is a widely distributed Gram-negative, non-motile mostly encapsulated bacterium that belong to the Enterobacteriaceae family. The bacterium is usually linked with nosocomial and opportunistic medical significance infections. It is linked to the pneumoniae, urinary tract infections, septicemia, bacterial meningitis, biliary tract infection, wound infections, and Surgical infections. They have a special relation to ventilator-associated pneumoniae (VAP), and they are

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the major cause of bacteraemia in the Gram-negative group of ESKAPE pathogens, and they have a rather high mortality rate (Alabi et al., 2025). One of the greatest concerns of K. pneumoniae has been the rate at which they develop resistance to antibiotics since they are rich in AMR plasmids. Thus, K. pneumoniae is listed as a critical priority pathogen in need of urgent drug development by the World Health Organisation (WHO). The bacterium has become resistant to various antimicrobial agents, thus becoming difficult to treat (Srisrattakarn et al., 2025).

The number of mechanisms that this pathogen employs to avoid host immune defence and antibiotics was found to be enormous by several studies, including the synthesis of antibiotic-modifying enzymes like Extended Spectrum Beta-Lactamase (ESBL) (Silago et al., 2021, Sabença et al., 2023). The past two decades have witnessed a dramatic rise in the health risk to the population due to ESBL producing bacteria. Based on the information provided it is evident that the bacteria that develop and acquire ESBL-type resistance are predominantly members of the family Enterobacteriaceae with K. pneumoniae being the most important. Extended Spectrum Beta-Lactamases (ESBL) are enzymes that can hydrolyze and break down the beta-lactam ring of Penicillins and Cephalosporins. This implies that the activation of these specific antimicrobial drugs will result in bacteria resistance and uselessness of the host defense (Abreu et al., 2025) under the circumstances.

The significance of ESBLs lies in the fact that they pose an increasing risk to the public health, which is linked to the excessive and inappropriate use and abuse of antibiotics. To begin with, ESBL producing bacteria are also resistant to most of the commonly used antibiotics and as such, these infections are hard to cure. As a result, ESBL-infected patients can experience a delay in getting the most effective treatment as doctors attempt to figure out which antibiotics the infecting bacteria is sensitive to. This increases the mortality rates. Patients with ESBL-producing bacterial infections are found to be 57% likely to die in comparison to a patient with a non-ESBL-producing strain (Husna et al.,2023). Public and community health are also at risk due to the dissemination of ESBLs, because hospital-infected patients can also have extended stay in a healthcare setting that can become a focal point of transmission of ESBL-producing bacteria. It is challenging to control the spread of these bacteria in hospital environments as the patients are already highly susceptible to infection (Tufa et al., 2020).

Nevertheless, most K. pneumoniae strains are resistant to the Carbapenem antibiotics, the most effective medications at the moment that can be used to treat and cure serious infections, this kind of resistance is increasingly common in hospitals, as well as in non-hospitalized patients. Therefore, if the spread of ESBLs is not effectively managed, it is very likely that there will be a new wave of antibiotic resistance that will pose a significant worldwide health risk (Prapti et al., 2026). Moreover, with the probability of sepsis and other serious illnesses caused by bacterial infection, the pressure both on healthcare providers and systems may become overwhelming. This scenario perfectly illustrates the wider social and economic importance of combating ESBLs and other antibiotic resistance mechanisms (Muteeb et al., 2023). Thus, the necessity to examine the antimicrobial susceptibility patterns of *Klebsiella pneumoniae* predominant in the public hospitals in Lagos.

Problem Statement

The increasing rise of ESBL-producing *Klebsiella pneumoniae* is a major public health issue, particularly in healthcare environment (World Health Organization, 2022). Studies have revealed that TEM, SHV- and CTX-M-type β-lactamases are dominant and widespread among *Klebsiella spp*, especially *Klebsiella pneumoniae* isolates (Bush & Bradford, 2020: Pitou*t et al.*, 2022,). However, there is limited data on the molecular characteristics of these enzymes in our locality. The lack of understanding of the molecular epidemiology of ESBL-producing *Klebsiella pneumoniae* in our locality hinders the development of effective infection control strategies and treatment guidelines (Martin & Bachman, 2021). Therefore, this study aims to investigate the prevalence and molecular characteristics of ESBL-producers among the carbapenem resistant *Klebsiella pneumoniae* isolates in public hospitals in Lagos State. The specific objectives are to: isolate *Klebsiella pneumoniae* among patient in two public hospitals in Lagos; determine the antibiotics susceptibility pattern of the isolates; investigate the prevalence of carbapenem-resistant Enterobacteriaceae in the clinical isolates from the two hospitals; determine the prevalence of extended-spectrum beta-lactamase (ESBL)-producing *Klebsiella pneumoniae* isolates in two public hospitals; identify and characterize the

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specific beta-lactamase genes (SHV, CTX-M, TEM) responsible for the extended-spectrum beta-lactamase production in *Klebsiella pneumoniae*.

Literature Review

Antimicrobial resistance (AMR) is a major global health challenge responsible for increasing treatment failure, prolonged hospital stays, and higher mortality rates. It arises mainly from the misuse and overuse of antibiotics. In 2019, AMR was linked to about five million deaths worldwide, with projections suggesting that this figure may reach ten million deaths annually by 2050 if urgent action is not taken. It is also expected to push millions into poverty due to increased healthcare costs and loss of productivity. A major group of organisms driving this crisis are the ESKAPE pathogens, which include *Klebsiella pneumoniae*, among others (Prapti *et al.,* 2026).

Klebsiella pneumoniae is a Gram-negative, non-motile, facultative anaerobic bacterium that is part of the Enterobacteriaceae family. It is commonly found in the human gut but can become pathogenic, causing urinary tract infections, pneumonia, bloodstream infections, wound infections, and meningitis. Its virulence is linked to a polysaccharide capsule, lipopolysaccharide (LPS), fimbriae, biofilm formation, and siderophore production, all of which enhance immune evasion, adhesion, and survival in the host (Brown et al., 2022; Smith et al., 2022).

A major concern is its ability to acquire resistance genes through horizontal gene transfer, especially those encoding extended-spectrum β-lactamases (ESBLs) such as SHV, TEM, and CTX-M. These enzymes confer resistance to commonly used β-lactam antibiotics, including penicillins and cephalosporins (Kulaç et al., 2024). In addition, the emergence of carbapenem-resistant Enterobacteriaceae (CRE) has further limited treatment options and increased clinical complications (Olaitan et al., 2025). Resistance mechanisms include β-lactamase production, efflux pumps, and reduced membrane permeability. The rise of multidrug-resistant and carbapenem-resistant *K. pneumoniae* has made treatment increasingly difficult, highlighting the urgent need for antimicrobial stewardship and continuous surveillance (Eslami et al., 2024; IDSA, 2024).

Review of Related Studies

Eslami et al. (2025) examined innovative antibiotic therapies for carbapenem-resistant Gram-negative infections, focusing on treatment effectiveness and safety. The study highlighted drugs such as cefiderocol and polymyxin derivatives, showing their potential against resistant pathogens. It also compared monotherapy and combination therapy approaches and emphasized infection control practices. However, the study is largely clinical and treatment-focused, with little attention to the molecular characterization of resistance genes. It does not specifically explore *Klebsiella pneumoniae* isolates or the distribution of ESBL genes such as SHV, CTX-M, and TEM in hospital settings. The absence of localized epidemiological data limits its relevance to specific healthcare environments. This study addresses this gap by focusing on both phenotypic and molecular detection of resistance genes in clinical isolates from Nigerian hospitals.

Srisrattakarn et al. (2025) investigated antimicrobial-resistant bacteria in environments near animal farms in Thailand, identifying ESBL-producing *Escherichia coli* and *Klebsiella* species. The study demonstrated the presence of CTX-M and other resistance genes in environmental samples, highlighting the role of agricultural practices in resistance spread. Despite its contribution, the study is limited to environmental samples and does not address clinical isolates or hospital-based infections. It also lacks detailed analysis of carbapenem resistance. This study fills this gap by focusing on clinical *Klebsiella pneumoniae* isolates in hospital settings and examining both ESBL and carbapenemase production, thereby providing direct clinical relevance. Desai et al. (2022) assessed the prevalence of ESBL-producing *Klebsiella pneumoniae* in a tertiary hospital in India and identified the dominance of blaTEM, blaSHV, and blaCTX-M genes. The study provides valuable insight into the genetic basis of resistance and highlights the coexistence of multiple resistance genes. However, it does not explore carbapenemase production or resistance patterns beyond ESBLs.

Abreau et al. (2025) investigated ESBL-producing *Klebsiella pneumoniae* in dogs, emphasizing the role of animals in spreading antimicrobial resistance. The study identified high prevalence of CTX-M, SHV, and TEM genes and highlighted multidrug resistance patterns. While the findings are important for One Health perspectives, the study is limited to veterinary samples and does not

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address human clinical infections. It also lacks analysis of carbapenemase-producing strains. This study differs by focusing on human clinical isolates and incorporating both ESBL and carbapenemase detection, making it more relevant to hospital infection control. El-Zayat et al. (2026) adopted a One Health approach to examine seasonal variation in ESBL-producing bacteria across humans, animals, and the environment. The study demonstrated that ESBL gene distribution varies with season and source, providing a broad understanding of resistance dynamics. However, it does not focus specifically on carbapenem-resistant Enterobacteriaceae or provide in-depth analysis of hospital-based isolates.

Prapti et al. (2026) explored ESBL- and carbapenemase-producing *Klebsiella pneumoniae* in street food, revealing the presence of multiple resistance genes and highlighting food safety concerns. The study contributes to understanding community-level transmission of resistant bacteria. However, it is limited to food samples and does not examine clinical outcomes or hospital-based infections. It also does not provide detailed comparison between different healthcare facilities. This study fills this gap by focusing on clinical isolates from two hospitals and comparing resistance patterns across settings. Al-Sheboul et al. (2023) investigated ESBL-producing *Klebsiella pneumoniae* in hospital patients in Jordan, identifying a high prevalence of SHV, CTX-M, and TEM genes. The study also explored plasmid-mediated resistance. While informative, it does not examine carbapenemase production or compare multiple healthcare institutions. Its findings are also context-specific and may not apply to other regions. This study extends the scope by including carbapenem resistance and comparing isolates from two hospitals, providing a broader and more comparative perspective.

Elsafi (2020) examined ESBL-producing Enterobacteriaceae in a hospital setting, identifying CTX-M as the most dominant gene and highlighting multidrug resistance patterns. The study provides useful baseline data on resistance gene distribution. However, it includes multiple bacterial species and does not focus specifically on *Klebsiella pneumoniae*. It also lacks investigation into carbapenemase production. This study improves on these limitations by focusing specifically on *Klebsiella pneumoniae* and incorporating both ESBL and carbapenemase analysis. Alabi et al. (2025) reviewed antimicrobial resistance trends in Nigeria, emphasizing the role of poor regulation, misuse of antibiotics, and lack of surveillance. The study provides a broad overview of AMR challenges and advocates for a One Health approach. However, it is a narrative review and does not provide empirical data or molecular analysis of resistance genes. It also lacks focus on specific pathogens such as *Klebsiella pneumoniae*.

The reviewed studies collectively highlight the growing threat of antimicrobial resistance across clinical, environmental, veterinary, and food systems. Many studies focus on ESBL gene distribution, treatment strategies, or One Health perspectives, while others emphasize environmental and community transmission. However, key gaps remain, including limited focus on carbapenemase-producing organisms, lack of combined phenotypic and molecular analysis, and insufficient hospital-based comparative studies, particularly in Nigeria. Few studies specifically examine *Klebsiella pneumoniae* in clinical settings while simultaneously assessing ESBL and carbapenem resistance. This study is unique as it integrates phenotypic and molecular approaches, focuses on clinical isolates, compares two hospitals, and provides localized data, thereby offering a more comprehensive and context-specific understanding of antimicrobial resistance.

Materials and Methods

Study Area

This study was conducted in Lagos State, Nigeria, at two public healthcare facilities: Lagos University Teaching Hospital (LUTH), Idi-Araba, a tertiary institution, and Ikorodu General Hospital, a secondary healthcare facility.

Study Design

A laboratory-based cross-sectional design was adopted to investigate the prevalence and molecular characteristics of carbapenem-resistant Klebsiella pneumoniae, with emphasis on extended-spectrum beta-lactamase (ESBL) production and associated resistance genes (SHV, CTX-M, and TEM).

Study Population and Sampling

The study population consisted of hospitalized patients receiving care in the selected hospitals. A total of 200 clinical samples of Klebsiella pneumoniae were obtained, including 143 samples from LUTH and 57 from Ikorodu General Hospital.

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Only clinical samples confirmed as Klebsiella pneumoniae were included, while non-Klebsiella species and non-clinical samples were excluded. A convenience sampling method was used, whereby all available samples that met the inclusion criteria were selected. The sample size was guided by standard recommendations for molecular studies and calculated using Yamane's formula, yielding a minimum of 133 samples; however, a total of 200 samples were analyzed to improve reliability.

Collection, Transportation, and Storage of Isolates

Bacterial isolates were obtained from the microbiology laboratories of the selected hospitals, where they had been previously identified and stored. The isolates were preserved in cryovials on Mueller-Hinton agar slants and transported under appropriate conditions to the laboratory for further analysis.

Identification and Biochemical Characterization

All isolates were confirmed as Klebsiella pneumoniae using standard microbiological techniques. Sub-culturing was performed on MacConkey agar, followed by incubation at 35–37°C for 18–24 hours. Colonies showing typical mucoid and pink characteristics were presumptively identified. Confirmation was achieved through Gram staining and a series of biochemical tests, including oxidase, urease, indole, citrate utilization, Voges-Proskauer, catalase, and sugar fermentation tests. These procedures were carried out according to standard laboratory guidelines and reference manuals.

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. Standardized bacterial suspensions equivalent to 0.5 McFarland were inoculated onto Mueller-Hinton agar plates. Antibiotic discs used included imipenem, ceftazidime, cefotaxime, cefuroxime, cefepime, ciprofloxacin, and amoxicillin-clavulanate. After incubation, zones of inhibition were measured and interpreted based on CLSI standards. Carbapenem resistance was further assessed using the Modified Carbapenem Inactivation Method (mCIM). The mCIM procedure was performed according to CLSI guidelines. Briefly, a 10 µg meropenem disk was incubated in a suspension of test organisms prepared in tryptic soy broth for 4 hours at 35°C ± 2°C. After incubation, the disk was removed and placed on a Mueller-Hinton agar plate previously inoculated with a 0.5 McFarland suspension of *Escherichia coli* ATCC 25922 as the indicator strain. Plates were incubated at 35°C for 18–24 hours. Zones of inhibition were interpreted as follows: ≥19 mm (negative for carbapenemase production), 16–18 mm (indeterminate), and ≤15 mm (positive for carbapenemase production). *Klebsiella pneumoniae* ATCC 1705 was used as the positive control, while *E. coli* ATCC 25922 served as the negative control.

Detection of ESBL Production

Screening for ESBL production was carried out using ceftazidime, cefotaxime, and cefpodoxime discs. Isolates showing reduced susceptibility were subjected to confirmation using the double-disk synergy test (DDST) and combined disk method. The double-disk synergy test (DDST) was performed using the standard CLSI-recommended configuration, placing amoxicillin-clavulanate (20 µg/10 µg) at the center with ceftazidime and cefotaxime discs positioned at a distance of 20 mm (center to center). The standard DDST format was adopted rather than modified variants, ensuring consistency in ESBL detection. A significant increase in inhibition zone diameter in the presence of clavulanic acid confirmed ESBL production. Standard control strains were included to ensure accuracy of results.

Molecular Characterization

Molecular analysis was performed to detect ESBL-associated genes, including blaSHV, blaTEM, and blaCTX-M, using polymerase chain reaction (PCR). Genomic DNA was extracted from bacterial isolates using a commercial DNA extraction kit following the manufacturer's instructions. PCR amplification was carried out in a reaction mixture containing DNA template, primers, master mix, and nuclease-free water. The amplification process involved initial denaturation, followed by repeated cycles of denaturation, annealing, and extension, and a final extension step. Gene-specific primers targeting blaSHV, blaTEM, and blaCTX-M were used. Primer sequences were as follows: blaSHV (F: 5′-…-3′, R: 5′-…-3′), blaTEM (F: 5′-…-3′, R: 5′-…-3′), blaCTX-M (F: 5′-…-3′, R: 5′-…-3′). PCR amplification was performed in a thermocycler under the following conditions: initial denaturation at 95°C for 5 minutes; 35 cycles of denaturation at 95°C for 30 seconds, annealing at 52–58°C (gene-specific) for 30 seconds, and extension at 72°C for 1 minute; followed by a final extension at 72°C for 7 minutes. Positive control strains carrying known blaSHV, blaTEM, and

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blaCTX-M genes were included alongside nuclease-free water as a negative control.

The amplified products were separated using agarose gel electrophoresis and visualized under ultraviolet light. Band sizes were compared with a molecular weight marker to confirm the presence of target genes. Positive and negative controls were included in all runs to validate the results.

Data Analysis

Data obtained from laboratory analyses were entered and analyzed using statistical software. Descriptive statistics were used to summarize the prevalence of ESBL and carbapenem-resistant isolates, while chi-square tests were used to examine associations between resistance patterns and gene distribution. The chi-square test of independence was applied to assess associations between categorical variables, including resistance phenotypes and presence of ESBL genes. Statistical significance was determined at $p < 0.05$ with a 95% confidence level. Degrees of freedom and p-values were reported alongside chi-square statistics in the results section.Results were presented in tables and charts for clarity.

Ethical Considerations

Ethical approval for the study was obtained from the appropriate institutional review body. Since the study involved already collected and anonymized bacterial isolates, no direct patient contact occurred and informed consent was not required. All samples were coded to maintain confidentiality and ensure compliance with ethical standards.

Results

A total of 200 isolates were collected and investigated, 143 isolates from Lagos University Teaching Hospital, (LUTH). Idi-Araba. Lagos, and 57 isolates from Ikorodu General Hospital in Lagos State Nigeria.

The 200 clinical isolates were subjected to antibiotic susceptibility test and the results showed Imipenem having the highest susceptibility (79.72%) followed by Ciprofloxacin (60.84%), Cefepime (50.35%) Ceftazidime (46.85%), Cefotaxime (45.45%), and, Amoxicillin–Clavulanate had the least susceptibility (30.75%) for isolates from LUTH. For Isolates from Ikorodu General Hospital, the results showed Imipenem having the highest susceptibility (100%) followed by Ciprofloxacin (91.23%), Cefepime (64.91%) Ceftazidime and Cefotaxime (59.65%), and, Amoxicillin–Clavulanate had the least susceptibility (38.60%).

Among the clinical isolates from LUTH, 26 were resistant to both Ceftazidime, and Cefepime Ceftazidime (3rd and fourth Generation Cephalosporins) and Imipenem. These isolates were tested for ESBL production. The result showed the 26 isolates (*Klebsiella pneumoniae*) were positive for ESBL production (See figure 2).

Table 1: Showing the resistance pattern of *K. pneumoniae* isolated from clinical samples against different classes of antibiotics in LUTH.

S/N	Drugs	Total Isolate (Count)	Resistant (Count)	Susceptible (Count)	Resistant (Percentage %)	Susceptible (Percentage %)	Average Zone of Inhibition for Susceptible (mm)
1	AMC	143	99	44	69.23	30.77	1.37
2	CAZ	143	76	67	53.15	46.85	1.89
3	FEP	143	71	72	49.65	50.35	2.12
4	CTX	143	78	65	54.55	45.45	1.71
5	CIP	143	56	87	39.16	60.84	1.48
6	IPEM	143	29	114	20.28	79.72	1.78

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Table 2: Showing the resistance pattern of *K. pneumoniae* isolated from clinical samples against different classes of antibiotics in general hospital, Ikorodu.

S/N	Drugs	Total Isolate (Count)	Resistant (Count)	Susceptible (Count)	Resistant (Percentage %)	Susceptible (Percentage %)	Average Zone of Inhibition for Susceptible (mm)
1	AMC	57	35	22	61.40	38.60	0.85
2	CAZ	57	23	34	40.35	59.65	1.53
3	FEP	57	20	37	35.09	64.91	1.57
4	CTX	57	23	34	40.35	59.65	2.07
5	CIP	57	5	52	8.77	91.23	2.29
6	IPEM	57	0	57	0.00	100.00	2.64

Table 3: Showing antibiotic resistance pattern of *K. pneumoniae* isolates from LUTH and Ikorodu general hospital

ANTIBIOTICS	Ikorodu (N=57)%	Luth(N=143)%
AMC	61.40	69.23
CAZ	40.35	53.15
FEP	35.09	49.65
CTX	40.35	54.55
CIP	8.77	39.16
IPEM	0.00	20.28

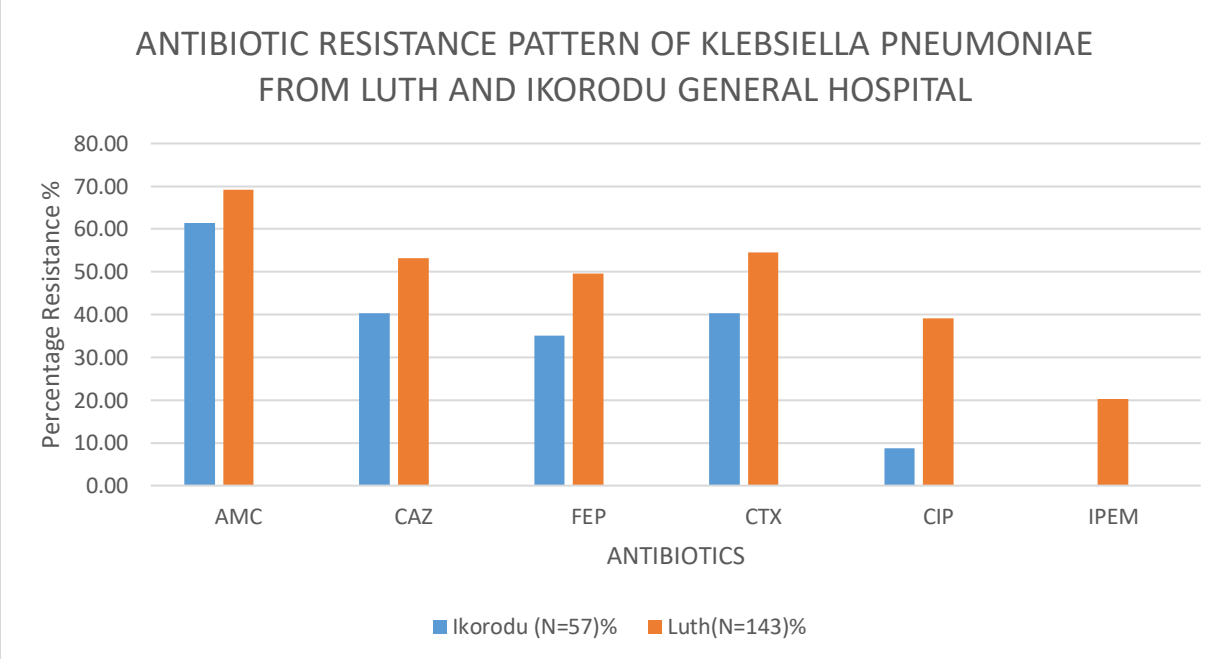


Figure 1: Chart Showing Antibiotic Resistance Pattern of *Klebsiella pneumoniae*

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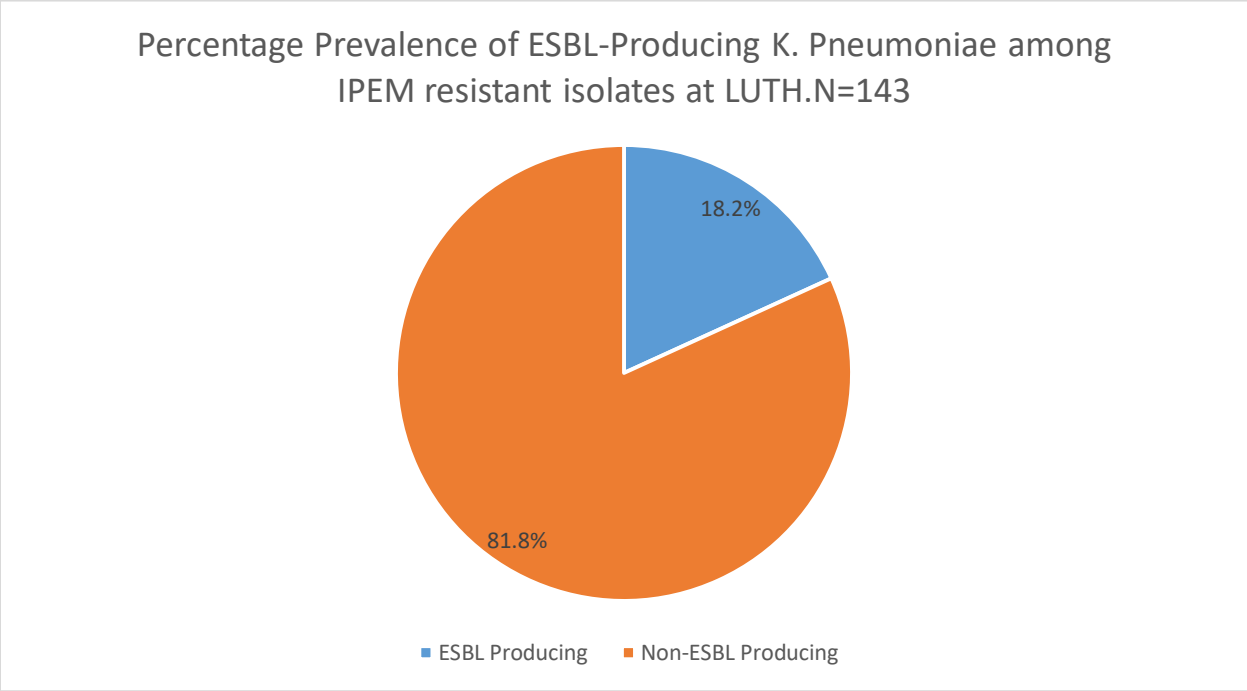


Figure 2: Percentage Prevalence of ESBL-producing *Klebsiella pneumoniae*

Table 4 below showed that 26 isolates were selected based on resistance to imipenem and all were confirmed as carbapenemase producers.

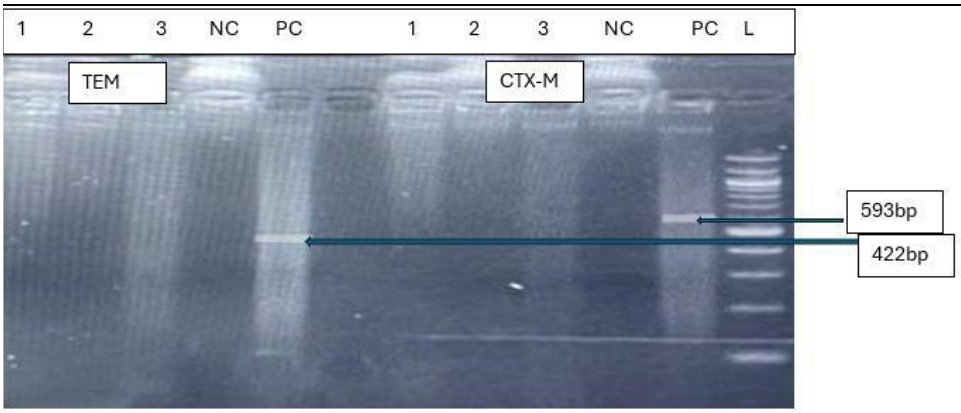
Result revealed that the antimicrobial susceptibility pattern of *Klebsiella pneumoniae* from LUTH revealed high resistance to β-lactam antibiotics, with 69.23% of isolates resistant. About 18 isolates showed no zone of inhibition (0 mm) against amoxicillin–clavulanate, ceftazidime, cefotaxime, cefepime, ciprofloxacin, and imipenem, indicating strong multidrug resistance and possible ESBL and carbapenemase production. Resistance to third-generation cephalosporins (ceftazidime and cefotaxime), with inhibition zones of 0–9 mm, strongly suggests ESBL activity due to enzymatic hydrolysis of oxyimino-cephalosporins. PCR analysis of three selected isolates (1B5, 8B5, and B19) showed that only isolate B19 carried the SHV gene (739 bp), while CTX-M (593 bp) and TEM (422 bp) genes were not detected in any isolate. Gel electrophoresis confirmed a single SHV band, and sequencing validated its identity through strong similarity with reference database sequences.

Hence, there was widespread β-lactam resistance driven mainly by ESBL and carbapenemase activity, with limited detection of common resistance genes, suggesting other mechanisms may also be involved.

Table 4: Gene responsible for ESBL production in the *Klebsiella pneumoniae* in LUTH

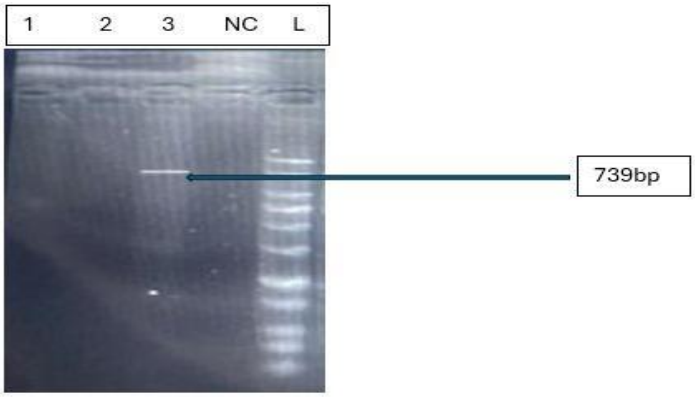
Gene	Expected Band Size (bp)	Observed Band Size (bp)	Amplification Success
SHV	739	Detected in 1 isolate	Success
CTX-M	593	Not detected	N/A
TEM	422	Not detected	N/A

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Lanes 1-5 TEM, lane 7 space, lanes 7-11 CTX, lane 12 ladder (100bp))

Figure 3: PCR Amplification Results



Gel Image for SHV amplification. Lanes 1-4 SNV, 5 ladder,(50bp)

Figure 4: Some of the confirmed phenotypically positive ESBL isolates

Discussion

In this study, inhibition zone diameters ranging from 0–11 mm suggest possible carbapenemase production (EUCAST, 2021). According to CLSI guidelines, isolates with imipenem inhibition zones ≤19 mm are considered potential carbapenemase producers and require confirmation using the Modified Carbapenem Inactivation Method (CLSI, 2024). The observed loss of activity against imipenem among several isolates supports the presence of carbapenemase enzymes, particularly Class A *Klebsiella pneumoniae* carbapenemase (KPC) and Class B metallo-β-lactamases such as NDM, VIM, and IMP (Logan et al., 2022; WHO, 2023). However, isolates with minimal inhibition (e.g., LUTH B8: 11 mm) may indicate reduced enzyme production or permeability defects rather than full carbapenemase activity (EUCAST, 2024).

The co-resistance observed to both third-generation cephalosporins and carbapenems suggests the co-existence of extended-spectrum β-lactamases (ESBLs) and carbapenemase genes, often located on transferable plasmids. This pattern indicates possible clonal spread of multidrug-resistant *K. pneumoniae* within the hospital environment. In addition, resistance may be enhanced by mechanisms such as porin loss or efflux pump overexpression, which act alongside ESBL enzymes to reduce antibiotic susceptibility even in the absence of carbapenemase production (Bush et al., 2020; WHO, 2023). The high level of β-lactam resistance observed in isolates from LUTH highlights the growing therapeutic challenges in managing infections in tertiary healthcare settings,

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with resistance to key drugs such as imipenem, cefepime, and ceftazidime suggesting the emergence of multidrug-resistant or even pan-drug-resistant strains (Olowo-Okere et al., 2022).

The prevalence of carbapenemase-producing *K. pneumoniae* in this study was 18.2% (26/143), indicating a significant burden of resistance to last-line antibiotics. Molecular analysis of three selected isolates (LUTH 1B5, LUTH 8B5, and LUTH B19) showed that only isolate B19 harbored the SHV gene, while CTX-M and TEM genes were not detected. This corresponds to a low detection rate (33.3%) of SHV among the tested isolates and suggests that other resistance mechanisms or genes may be involved. Despite this, all 26 isolates tested for carbapenemase production were positive, reinforcing the clinical importance of carbapenem resistance in the study setting.

The detection of the SHV gene only in isolate LUTH B19 suggests a limited presence of this ESBL type among the studied isolates, although SHV enzymes are widely associated with broad-spectrum β-lactam resistance (Abed et al., 2025; Kahil et al., 2025). The absence of CTX-M and TEM genes in other isolates indicates the possible presence of alternative ESBL genes such as OXA or PER (Abed et al., 2025; Hussein et al., 2022). Hence, the complexity of antibiotic resistance patterns and the need for larger, regionally representative studies to better understand resistance trends and guide effective treatment strategies (Salawudeen, 2023; Mohammed & Anwar, 2023).

Conclusion and Recommendations

This study demonstrated that *Klebsiella pneumoniae* isolates obtained from Lagos University Teaching Hospital and Ikorodu General Hospital showed varying patterns of antibiotic susceptibility and resistance. Most isolates were sensitive to imipenem, ciprofloxacin, and cefepime, indicating their continued effectiveness in treatment. However, a high level of resistance was observed against amoxicillin–clavulanate and third-generation cephalosporins such as ceftazidime and cefotaxime. This suggests increasing difficulty in treating infections caused by these organisms using commonly available antibiotics. The detection of carbapenem-resistant isolates further raises serious clinical concerns, as these drugs are often considered last-resort options. Molecular analysis revealed the presence of the SHV gene in one isolate, while CTX-M and TEM genes were not detected in others. This finding implies that resistance in some isolates may be due to other mechanisms or less commonly studied genes. It highlights the complexity of antimicrobial resistance and the need for broader genetic investigations.

Based on these findings, continuous monitoring of antimicrobial resistance in hospital settings is strongly recommended. Routine screening for ESBL-producing organisms should be encouraged to support accurate diagnosis and proper treatment decisions. The rational use of antibiotics must be promoted to reduce misuse and slow down the spread of resistance. In particular, carbapenems should be carefully controlled to preserve their effectiveness. Further studies should include a larger sample size and explore other resistance genes such as OXA and PER. Strengthening infection control practices, improving laboratory capacity, and promoting antibiotic stewardship programs are essential steps in limiting the spread of resistant *Klebsiella pneumoniae*.

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