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Antibiogram and Molecular Characterization of Extended-Spectrum Beta-Lactamase-Producing *Klebsiella pneumoniae* in a Nigerian Teaching Hospital

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Abstract

Background: *Klebsiella pneumoniae* is a gram-negative bacterium responsible for infections like urinary tract infections (UTIs) and is a leading cause of nosocomial infections in immunocompromised patients. Data on resistance profiles in Nigeria is limited. This study investigated the antibiogram and molecular characteristics of ESBL-producing *Klebsiella pneumoniae* at the University of Medical Sciences Teaching Hospital, Ondo State. **Methods**: A cross-sectional study analyzed 300 samples from urine, sputum, and wound infections. Isolates were cultured on MacConkey agar, followed by biochemical tests. The Kirby-Bauer method assessed resistance, and the double-disk synergy test confirmed ESBL production. PCR identified *bla*TEM, *bla*SHV, and *bla*CTX-M genes. SPSS was used for statistical analysis ($P \leq 0.05$). **Results**: Of 300 samples, 185 showed bacterial growth, with 54 confirmed as *Klebsiella pneumoniae*. Among these, 22 were multidrug-resistant (MDR), and 8 (14.8%) were ESBL producers. The *bla*TEM gene was present in all ESBL isolates, and the *bla*SHV gene in 7 of 8 (87.5%). Resistance to β-lactam antibiotics and aminoglycosides was high. Older patients and prolonged hospital stays correlated with higher MDR rates. A slight female predominance was noted. **Conclusions**: The high prevalence of MDR *Klebsiella pneumoniae* highlights the need for enhanced surveillance and deeper understanding of resistance patterns in the region.

Key word: *Klebsiella pneumoniae*, Multidrug Resistance, Extended-spectrum-Beta-Lactamase, Molecular Profiling, Healthcare facilities, Nigeria.

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Introduction

Klebsiella pneumoniae is an important gram-negative organism known to cause many infections, including UTIs, bloodstream infections, and pneumonia, especially in immunocompromised patients (1). It is also recognized as one of the leading causes of nosocomial infections worldwide, posing a significant challenge to public health (2). *Klebsiella pneumoniae* accounts for 20-30% of nosocomial pneumonia cases in the Americas and is among the top three pathogens isolated in hospital gram-negative bacteremias (3).

Recent studies in Nigeria and West Africa have shown an alarming spread of multidrug resistance among *Klebsiella pneumoniae* isolates. A systematic review and meta-analysis reported a pooled prevalence of ESBL-producing *Klebsiella pneumoniae* in Nigeria at 47.3%, reflecting the increasing trend of resistant strains and the need for effective infection control measures (4). The emergence of ESBL genes such as *bla*TEM, *bla*SHV, and *bla*CTX-M has further complicated treatment options for clinicians and scientists (5), leading to a global push to understand the genetic determinants of these resistance mechanisms (6). Additionally, a study in Lagos hospitals found that 69.8% of *Klebsiella pneumoniae* isolates were ESBL producers, with 7% exhibiting resistance to carbapenems, highlighting the need for prudent use of carbapenem antibiotics (7).

Studies indicate a high prevalence of ESBL-producing *Klebsiella pneumoniae* in Nigerian hospitals, where poor infection control practices and inadequate antimicrobial stewardship contribute to the spread of resistant isolates (4). Molecular characterization of these resistant isolates is critical for developing effective treatment protocols and infection control strategies (8). Despite these pressing concerns, there is still a shortage of data on the specific resistance profiles and genetic determinants in many regions of Nigeria (9). Therefore, this study aimed to investigate the prevalence of extended-spectrum beta-lactamase (ESBL)-producing *Klebsiella pneumoniae* at the University of Medical Sciences Teaching Hospital, Ondo, Nigeria. We assessed the associated resistance genes and examined the relationship between age and gender with the presence of these resistant strains. Understanding local resistance patterns will provide valuable insights for improving antibiotic therapy and infection control measures.

Methods

Study Design and Setting

This hospital-based, cross-sectional, and experimental design was carried out at the University of Medical Sciences Teaching Hospital, Ondo, Nigeria. Data were collected and analyzed to determine the prevalence of *Klebsiella pneumoniae* at the facility and identify the ESBL genes present in *Klebsiella pneumoniae* isolates from this region.

Source Materials

A total of 300 samples were collected from patients in various units of the University of Medical Sciences Teaching Hospital Complex in Ondo, Nigeria. Urine, sputum, and wound samples were collected from patients with urinary tract infections (UTIs) or a history of lung infection. Inclusion criteria for UTI patients involved symptoms such as dysuria, frequency, urgency, and flank pain. For patients with lung infections, inclusion criteria were based on clinical presentations including cough, purulent sputum production, chest pain, and fever. Medical history documentation, patient interviews, and reviews of electronic medical records confirmed patients' history of UTI or lung infection.

Microbiological Characterization

All suspected colony growths from clinical samples were characterized as *Klebsiella pneumoniae* through standard microbiological and biochemical tests, including Gram staining to evaluate the Gram reaction of the isolates. Biochemical tests such as oxidase production, citrate utilization, urease production, and the indole test were also conducted. These biochemical reactions were confirmed using the standard commercial identification system API 20E for accurate differentiation of *Klebsiella pneumoniae* from other closely related species (10).

Phenotypic Assessment of Antibiotic Resistance

The phenotypic pattern of susceptibility and resistance against *Klebsiella pneumoniae* was investigated using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar plates, following the Clinical Laboratory Standards Institute (CLSI) guidelines in the CLSI M100, 30th edition (2020) (11). The antibiotics tested included:

- Aminoglycosides: Amikacin (30 µg) and Gentamicin (10 µg)
- Fluoroquinolones: Ciprofloxacin (5 µg) and Levofloxacin (5 µg)
- β-lactam derivatives: Amoxicillin/Clavulanate (20/10 µg), Ampicillin (10 µg), Cefotaxime (30 µg), Ceftazidime (30 µg), Cefepime (30 µg), Ceftriaxone (30 µg), Aztreonam (30 µg), and Imipenem (10 µg)
- Trimethoprim-sulfamethoxazole: Trimethoprim (1.25 µg) and Sulfamethoxazole (23.75 µg)

Results were interpreted according to CLSI standards (CLSI, 2020) (12).

Phenotypic Detection of Extended-Spectrum Beta-Lactamase

The phenotypic confirmatory test for ESBL producers was performed using a double disc synergy test. A Mueller-Hinton agar plate was inoculated with the pathogen, and antibiotic discs containing Ceftriaxone (30 µg), Cefotaxime (30 µg), Ceftazidime (30 µg), and Amoxicillin/Clavulanic acid (20/10 µg) were placed on the agar. The distance between the discs was 20 mm. After 24 hours of incubation, an enhanced zone of inhibition between any cephalosporin antibiotic and the Amoxicillin/Clavulanic acid disc indicated the presence of an ESBL (13).

DNA Extraction

DNA was extracted from cultured isolates by alkaline lysis (14). A bacterial colony was suspended in 20 µl of lysis buffer (0.25% sodium dodecyl sulfate, 0.05M NaOH) and heated at 95 °C for 15 minutes. The cell lysate was diluted with 180 µl of distilled water, and cell debris was pelleted by centrifugation at 16,000 g for 5 minutes. The resulting supernatant was frozen at -20 °C.

Polymerase Chain Reaction Amplification of Beta-Lactamase Genes

Klebsiella pneumoniae isolates were screened for *bla*-TEM, *bla*-SHV, and *bla*-CTX-M genes using multiplex polymerase chain reaction (PCR) (Table 1). The PCR reaction was conducted using Solis Biodyne 5X HOT FIREPol Blend Master mix. Thermal cycling conditions included an initial denaturation at 95 °C for 15 minutes, followed by 34 amplification cycles (1 minute at 95 °C, 1 minute at 55 °C, and 1 minute at 72 °C), with a final extension at 72 °C for 10 minutes. Amplicons were separated on a 1.5% agarose gel and visualized using ethidium bromide staining (15).

Data Analysis

A power analysis determined the sample size needed for adequate statistical power. Data were analyzed using SPSS version 23 (IBM, Armonk, NY, USA). The chi-square and t-test compared categorical variables, with p-values 0.05 considered statistically significant at a 95% confidence interval.

Ethical Aspects

Ethical approval was obtained from the ethical review committee at the University of Medical Sciences Teaching Hospital, Ondo, Nigeria, through their letterhead. We ensured compliance with the ethical standards for research involving human subjects. For vulnerable patients, including minors, the elderly, and patients with terminal diseases, consent was sought from legal guardians or caregivers when necessary. Confidentiality was maintained by anonymizing and securely storing all samples to prevent unauthorized access. Only aggregated data were used for analysis and reporting to minimize the risk of identifying individual participants. Investigators were trained on confidentiality protocols and the ethical handling of sensitive information to protect participants' rights and ensure ethical integrity.

Table 1

Sequences of the primer used in this study

Gene	Primer Sequence	Product Size (bp)
bla_{TEMf}	TTT CGT GTC GCC CTT ATT CC	403
bla_{TEMr}	ATC GTT GTC AGA AGT AAG TTG G	403
bla_{SHVf}	CGC CTG TGT ATT ATC TCC CT	294
bla_{SHVr}	CGA GTA CTC CAC GAG ATC CT	294
bla_{CTX-Mf}	CGC TGT TGT TAG GAA GTG TG	500
bla_{CTX-Mr}	GGC TGG GTG AAG TAA GTG AC	500

Results

Sample Distribution of Isolates

A total of 185 bacterial isolates were obtained from the 300 samples collected (Table 2). Seven pathogenic organisms were identified: *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Streptococcus* spp., *Pseudomonas aeruginosa, Proteus* spp., and *Moraxella catarrhalis*.

Table 2

Frequency of bacterial isolates in clinical samples

Age (Years)	SA	EC	KP	SS	PSD	PRO	MC	NG	TOTAL
16-25	13	7	10	3	5	1	0	31	70
26-35	6	8	5	2	2	0	0	22	45
36-45	2	8	9	2	9	4	0	16	50
46-55	9	4	8	6	2	0	1	19	49
56-55	7	5	7	0	2	1	0	15	37
>65	1	7	15	3	2	5	4	12	49
TOTAL	38	39	54	16	22	11	5	115	300

Among the 185 isolates, *Klebsiella pneumoniae* was the most frequently recovered pathogen, accounting for 54 isolates (29.2%). Specifically, 50% of these isolates were derived from urine samples, 37% from sputum samples, and 13% from wound swabs (Table 3). The age of the patients ranged from 16 to over 65 years, with the highest number of *Klebsiella pneumoniae* isolates (15) observed in patients aged above 65. The prevalence of multidrug-resistant (MDR) *Klebsiella pneumoniae* strains was highest in the 16-25 and 36-45 age groups (Figure 1).

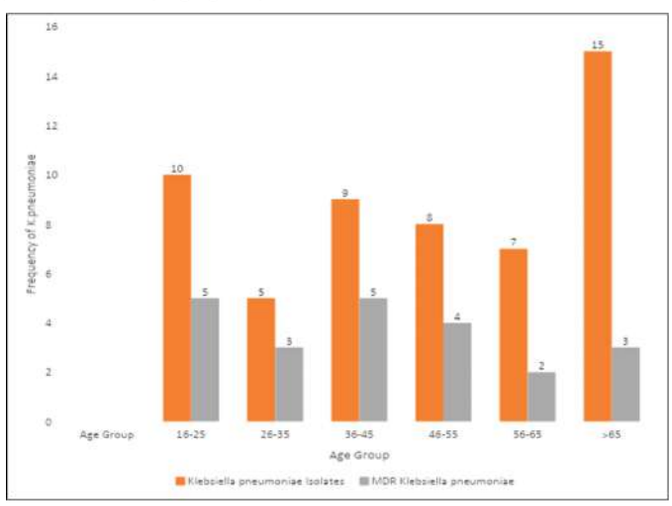
Table 3

Distribution of Klebsiella pneumoniae in clinical samples

S/N	Clinical Sample	Percentage (%)
1	Urine	50
2	Stupum	37
3	Wound swab	13

Figure 1

Distribution of Multidrug resistance (MDR) strains of Klebsiella pneumoniae by age group



Female patients exhibited a slightly higher prevalence of *Klebsiella pneumoniae* isolates (51.9%) compared to males (48.1%) (Table 4). However, no statistically significant difference was observed in the distribution of MDR *Klebsiella pneumoniae* between genders (T-test = 0.081; P > 0.05).

Table 4

Prevalence of Klebsiella pneumoniae based on gender

Gender	Frequency	Percentage (%)
Male	26	48.1
Female	28	51.9
Total	64	100

(t(df) = 0.081; p = 0.05)

Extended Spectrum Beta Lactamase Producing Klebsiella pneumoniae

Among the 54 Klebsiella pneumoniae isolates, 22 were identified as multi-drug resistant (MDR) (Table 5). Of

these, 8 (14.81%) were confirmed as Extended-Spectrum Beta-Lactamase (ESBL) producers. Statistical analysis using ANOVA revealed a significant variation in ESBL prevalence across different age groups (ANOVA = 0.049, P < 0.05).

Table 5

Frequency of ESBL Klebsiella pneumoniae in clinical isolates

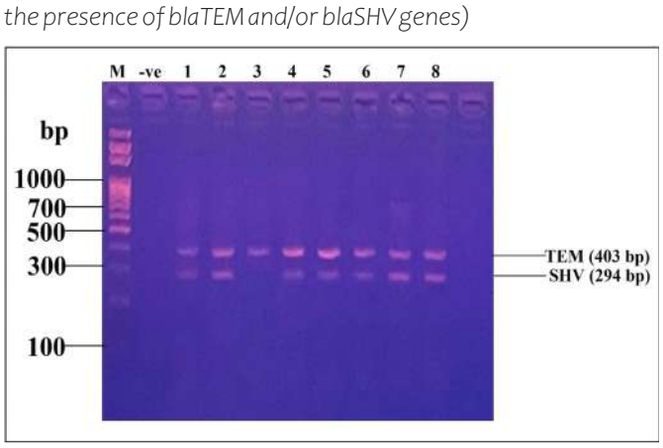
Age Group (Years)	Samples Collected	Klebsiella pneumoniae isolates	MDR Klebsiella pneumoniae	ESBL Klebsiella pneumoniae (%)
16-25	70	10	5	2 (25%)
26-35	45	5	3	2 (25%)
36-45	50	9	5	3 (37.5%)
46-55	49	8	4	1 (12.5%)
56-65	37	7	2	0 (0%)
>65	47	15	3	0 (0%)
TOTAL	300	54	22	8

Molecular Characterization of ESBL Producing Klebsiella pneumoniae

The molecular characterization of the 8 ESBL-producing Klebsiella pneumoniae isolates identified the presence of blaTEM and blaSHV genes (Figure 2). The co-existence of multiple bla genes in the same isolate was observed. 7 samples showed the presence of both blaTEM and blaSHV genes, and one sample showed the blaTEM gene without the blaSHV gene. However, blaCTX-M was not detected during the molecular characterization of the eight ESBL Klebsiella pneumoniae samples. blaTEM gene accounted for 100% of the ESBL isolates, while blaSHV was detected in 87.5% of the ESBL isolates.

Figure 2

Gel Electrophoresis showing amplification products of blaTEM (403bp product) and blaSHV (294bp product) genes in ESBL producing Klebsiella pneumoniae isolates. Lane M: 100bp DNA Ladder; Lane -ve: Negative control; Lanes 1-X: Isolates showing the presence of blaTEM and/or blaSHV genes)



Discussion

Klebsiella pneumoniae is a prominent cause of nosocomial infections, including urinary tract infections, pneumonia, septicemia, and soft tissue infections. The primary transmission routes are the gastrointestinal tract and the hands of healthcare professionals. Due to their rapid spread in hospital environments, these bacteria frequently trigger nosocomial outbreaks. The emergence of multidrug-resistant (MDR) strains, particularly Extended-Spectrum β-Lactamase (ESBL) producers, complicates treatment strategies (16, 17).

This study revealed that 29.2% of *Klebsiella pneumoniae* isolates were multidrug-resistant, indicating a high prevalence of ESBL-producing strains. This finding poses a significant threat to public health, especially for healthcare professionals at the University of Medical Sciences, Ondo. Similar results were reported by Charrouf and Husna, who documented the increasing proliferation of antibiotic-resistant *Klebsiella pneumoniae* strains driven by genetic resistance acquisition (18, 19).

As shown in Table 3, the prevalence of *Klebsiella pneumoniae* in clinical samples, notably in urine (50%) and sputum (37%), mirrors the findings of Gholipour et al. (20), who also identified elevated levels of ESBL-producing strains in urinary tract infections. Contributing factors include older age, pregnancy, and recurrent antibiotic use, which limit the efficacy of first-line treatments like third-generation cephalosporins (21, 22).

Our results indicate a higher prevalence of MDR *Klebsiella pneumoniae* isolates among patients over 65 years, consistent with Abdel-Rhman's 2020 study, which associated older age with increased susceptibility to MDR infections due to prolonged hospital stays and comorbidities (23). Additionally, a study in Saudi Arabia identified risk factors such as male gender, advanced age, ICU admission, diabetes mellitus, and chronic obstructive pulmonary disease as contributors to MDR infections (24).

The significant prevalence observed in patients aged 16-25 and 36-45 suggests that middle-aged adults are also at risk, potentially due to self-medication and frequent hospital visits. This aligns with findings from a multicenter observational study in Lebanese hospitals, which reported that prior antibiotic use within the last three months was significantly associated with MDR *Klebsiella pneumoniae* infections (25).

Gender distribution data revealed a higher prevalence in females (55.5%) compared to males (44.4%). However, this finding contrasts with Khuntayaporn's 2018 study, which found no statistically significant difference in *Klebsiella pneumoniae* infections between genders, though males exhibited higher odds of ESBL carriage (21). The increased prevalence among females in this study may be attributed to anatomical susceptibility to urinary tract infections, as supported by a Nigerian study (26).

Klebsiella pneumoniae employs various resistance mechanisms, including beta-lactamase production, efflux pumps, and alterations in penicillin-binding proteins (6). This study screened for ESBL genes *bla*CTX-M, *bla*TEM, and *bla*SHV, detecting *bla*TEM in 100% of isolates and blaSHV in

87.5%. These findings are consistent with Kadher and Jarallah's 2019 study (22). The prevalence of *bla*TEM and *bla*SHV genes may reflect local antibiotic usage practices and selective pressure from extensive use of penicillins and cephalosporins.

Global variations in ESBL gene distribution exist. While *blaCTX-M* predominates in Europe and parts of Asia, *blaTEM* and *blaSHV* are more common in Africa (27). For instance, a study in Kano, Nigeria, found that 73.3% of ESBL-positive Escherichia coli isolates harbored *blaTEM* genes (28). Understanding the localized prevalence of these genes is crucial for targeted interventions and antibiotic stewardship.

The high prevalence of ESBL-producing *Klebsiella pneumoniae* necessitates urgent global health interventions. As illustrated in Figure 1, the distribution of MDR strains across age groups highlights the need for institutional sensitization of healthcare professionals and patients. Implementing antimicrobial stewardship programs is essential to mitigate antibiotic abuse and curb resistance (29).

Additional measures include frequent training for healthcare providers on infection control policies, strict adherence to hand hygiene protocols, and monitoring contact exposure. Government regulation of antibiotic sales and the promotion of rapid diagnostic tests for appropriate therapy are also critical. Continuous surveillance and monitoring of resistance patterns are necessary to identify emerging threats and inform timely interventions.

Molecular epidemiology studies on ESBL-producing *Klebsiella pneumoniae* can elucidate the dissemination of resistant genes within healthcare facilities and the emergence of new strains. Continuous monitoring is vital to assess the efficacy of interventions and develop innovative solutions. A one-health approach that investigates environmental reservoirs of these pathogens can provide deeper insights into the factors driving MDR strains.

This study relied on phenotypic methods for initial resistance screening, which may underestimate or misclassify certain resistance mechanisms. The Kirby-Bauer disk diffusion method, while valuable for assessing antibiotic susceptibility, may fail to detect some ESBL variants. Additionally, the sample representativeness is limited, as the findings derive from specific clinical settings and may not reflect broader community resistance patterns.

Regional variations in ESBL gene distribution further emphasize the need for larger and more diverse sample sizes and the integration of molecular techniques to comprehensively understand resistance patterns. For instance, while *blaCTX-M* predominates in Europe and Asia, *blaTEM* and *blaSHV* are more prevalent in Africa (22). Future studies should focus on these discrepancies to inform effective, region-specific strategies for combating antibiotic resistance.

Conclusion

The widespread presence of *MDR Klebsiella* pneumoniae necessitates the immediate implementation of robust antibiotic stewardship programs and stringent infection control measures to mitigate the spread of resistant strains. Additionally, ongoing research into resistance mechanisms and the epidemiology of *ESBL*-producing strains is essential to guide evidence-based policies and treatment strategies.

A multidisciplinary approach that integrates molecular epidemiology, public health initiatives, and hospital-based interventions is crucial in addressing this escalating threat. Strengthening antimicrobial stewardship, promoting rapid diagnostic tools, and enforcing regulatory measures on antibiotic use will be pivotal in containing the spread of *MDR Klebsiella pneumoniae* and preserving the efficacy of existing treatment options.

Author's contribution Statement

Olabisi Promise Lawal, Joshua Odunayo Babatunde, and Sandra Owusu-Ansah: Conceptualization, Methodology Formal analysis, Data curation, Writing- Original draft preparation, Writing-Reviewing and Editing. **Hajara Hashim, Jennifer Chiagozie Okeke, Aimuanmwosa Andrew Igunma,** nyerovwo charity and Adegbesan Abiodun: Visualization, Investigation. **Olabisi Promise Lawal**: Supervision. **Chinaemerem Precious Ani , Daniel Osezuwa Ubebe, Kinglsey Ugonna Ugoagwu, Okabeonye Sunday Agbo, Charissa Favour Ani**: Software, Validation.

Ethics statement

The authors declare that the present study was conducted under the strictest ethical conditions.

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None.

Conflict of interest

The authors declare no conflict of interest.

Availability of data

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

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