Antibiogram and Molecular Detection of β-Lactamase Genes (blaTEM and blaSHV) in Clinical Isolates from Nigeria

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ABSTRACT

Antimicrobial resistance (AMR) among Gram-negative bacteria represents a major public health challenge in Nigeria and across Sub-Saharan Africa, particularly resistance to β-lactam antibiotics mediated by plasmid-encoded β-lactamase genes. This study assessed phenotypic antibiotic resistance patterns and the presence of blaTEM and blaSHV genes among 40 archived Gram-negative clinical bacterial isolates obtained from urine and wound samples in Nigeria. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method following Clinical and Laboratory Standards Institute guidelines. Molecular detection of resistance genes was carried out using polymerase chain reaction (PCR). Very high resistance rates were observed against ceftazidime (97.7%), cefuroxime (≈99%), amoxicillin–clavulanic acid (≈92–100%), and ciprofloxacin (92%). Relatively higher susceptibility was recorded for nitrofurantoin, gentamicin, and ofloxacin. Molecular analysis showed that 75% (15/20) of isolates screened carried the blaTEM gene, while all isolates screened for blaSHV (24/24; 100%) were positive. The study demonstrates extensive phenotypic resistance and a high prevalence of β-lactamase–encoding genes among Gram-negative clinical isolates in Nigeria, underscoring the urgent need for integrated phenotypic and molecular AMR surveillance and strengthened antimicrobial stewardship.

Keywords: Antimicrobial resistance; Gram-negative bacteria; β-lactamase genes; blaTEM; blaSHV; Antibiogram

INTRODUCTION

Antimicrobial resistance (AMR) is a major global public health challenge that threatens the effective prevention and treatment of infectious diseases, undermines health system resilience, and compromises progress toward universal health coverage (Pamela Cipriano *et al*., 2024; Binagwaho and Ghebreyesus, 2019). The rapid emergence and dissemination of resistant microorganisms have significantly reduced the effectiveness of commonly used antimicrobial agents, resulting in increased morbidity, mortality, and healthcare costs worldwide. According to recent global estimates, AMR is responsible for millions of infections annually and poses a substantial burden, particularly in low- and middle-income countries (LMICs), where surveillance and antimicrobial stewardship programs remain limited (J. Lewnard *et al*., 2024; S. Gandra *et al*., 2020; G. Sulis *et al*., 2021).

The development and spread of AMR are driven by multiple interconnected factors, including inappropriate and excessive antimicrobial use, weak infection prevention and control practices, poor sanitation, population growth, urban overcrowding, globalization of trade and travel, and disruptions caused by conflicts and environmental changes. These factors facilitate the persistence and transmission of resistant pathogens across human, animal, food, and environmental reservoirs, highlighting the need for a coordinated One Health approach to combat antimicrobial resistance (Luong Nguyen-Thanh *et al*., 2025; Samreen *et al*., 2021).

In sub-Saharan Africa, including Nigeria, the burden of infections caused by β-lactam-resistant Gram-negative bacteria has increased substantially over the past decade. Factors such as unregulated access to antibiotics, widespread empirical prescribing, limited diagnostic capacity, and inadequate antimicrobial resistance surveillance systems have contributed to the dissemination of resistance to β-lactam antibiotics in clinical settings (D. Ogbolu *et al*., 2018; Iheanacho and Eze, 2022). Although extended-spectrum β-lactamase (ESBL)-producing organisms represent a major component of this burden, comprehensive molecular data on β-lactamase genes circulating in Nigerian healthcare facilities remain limited.

The blaTEM and blaSHV genes encode β-lactamase enzymes that are commonly associated with resistance to penicillins and cephalosporins, and certain variants within these gene families have been implicated in ESBL phenotypes (Guzmán *et al*., 2013). Molecular detection of these β-lactamase genes, in combination with conventional antibiogram analysis, provides valuable insight into resistance mechanisms and supports informed antimicrobial therapy and infection prevention strategies. Therefore, this study was conducted to determine antibiotic resistance patterns and to detect blaTEM and blaSHV β-lactamase genes among bacterial isolates obtained from clinical samples in Nigeria.

MATERIALS AND METHODS

Study Design and Sample Collection

An experimental laboratory-based study design was employed. A total of forty (40) archived clinical bacterial isolates, comprising 20 urine isolates and 20 wound isolates, were obtained for analysis. The isolates had been previously cultured and stored under appropriate laboratory conditions. For this study, the isolates were revived by subculturing in nutrient broth and subsequently processed for antimicrobial susceptibility testing and molecular analysis. All laboratory procedures were conducted at the Microbiology Laboratory, Babcock University, Nigeria.

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar, and results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (2017). Commercially prepared Gram-negative antibiotic discs (Abtek Biologicals Limited, United Kingdom) were used. The antibiotics tested included ceftazidime (30 µg), cefuroxime (30 µg), gentamicin (10 µg), ofloxacin (5 µg), amoxicillin–clavulanic acid (30 µg), nitrofurantoin (30 µg), cefixime (5 µg), and ciprofloxacin (5 µg). Zones of inhibition were measured and classified as susceptible, intermediate, or resistant in accordance with CLSI interpretive criteria. Multidrug resistance (MDR) was defined as resistance to three or more classes of antimicrobial agents, as previously described by Magiorakos et al. (2012).

Genomic DNA Extraction

Genomic DNA was extracted from overnight bacterial cultures using the Quick-DNA™ Miniprep Plus Kit (Zymo Research, USA), following the manufacturer's instructions. The purity and integrity of the extracted DNA were assessed by agarose gel electrophoresis, and DNA samples were stored at −20 °C until further molecular analysis.

PCR Detection of β-Lactamase Genes (blaTEM and blaSHV)

Polymerase chain reaction (PCR) was used to detect the presence of blaTEM and blaSHV β-lactamase genes. PCR amplification was performed in a final reaction volume of 25 µL, consisting of 12.5 µL of PCR master mix (New England Biolabs, USA), 9.5 µL of nuclease-free water, 0.5 µL each of forward and reverse primers, and 2.0 µL of template DNA. Amplification was carried out using a G-STORM PCR system (Gene Technologies Co., Ltd.).

The cycling conditions included an initial denaturation at 94 °C for 3 minutes, followed by 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 56 °C for 30 seconds, and extension at 68 °C for 30 seconds, with a final extension at 68 °C for 5 minutes. PCR products were visualized using agarose gel electrophoresis.

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Agarose Gel Electrophoresis

PCR amplicons were resolved on 1.2% agarose gels prepared by dissolving 0.3 g of agarose powder in 25 mL of 1× TBE buffer (89 mM Tris base, 89 mM boric acid, and 2 mM EDTA). The gel solution was heated until fully dissolved, allowed to cool to approximately 50–60 °C, and stained with ethidium bromide before casting. Following solidification, the gel was placed in an electrophoresis tank containing 1× TBE buffer. PCR products were loaded into the wells alongside a molecular weight marker and electrophoresed at 100 V for 15 minutes. DNA bands were visualized under ultraviolet illumination using a UV transilluminator.

Data Analysis

Data obtained from antimicrobial susceptibility testing and molecular detection assays were analyzed using descriptive statistics and presented in tables and graphical formats for interpretation.

RESULT

Distribution of Clinical Isolates and Antibiotic Susceptibility Patterns

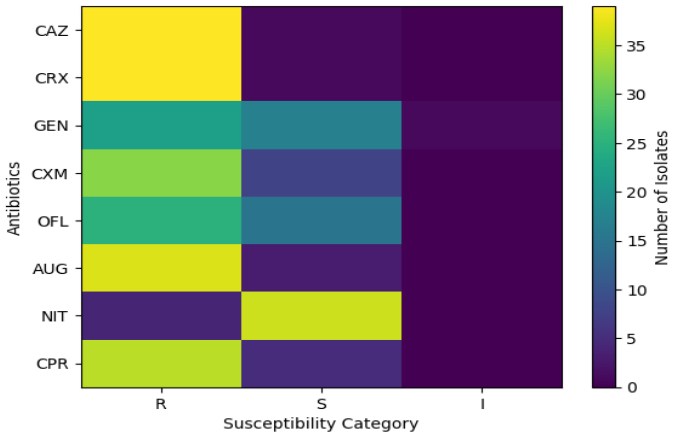
A total of 40 Gram-negative clinical bacterial isolates were analyzed in this study, comprising equal numbers obtained from urine and wound samples. All isolates were subjected to antimicrobial susceptibility testing using eight commonly prescribed antibiotics.

The antibiotics tested included ceftazidime (30 µg), cefuroxime (30 µg), gentamicin (10 µg), ofloxacin (5 µg), amoxicillin–clavulanic acid (30 µg), nitrofurantoin (30 µg), cefixime (5 µg), and ciprofloxacin (5 µg). The susceptibility profiles of the isolates are summarized in Figure 1.

The results showed a high level of resistance to several β-lactam and fluoroquinolone antibiotics. Resistance was most frequently observed against ceftazidime (39/40; 97.5%), cefuroxime (39/40; 97.5%), amoxicillin–clavulanic acid (37/40; 92.5%), and ciprofloxacin (35/40; 87.5%). In contrast, higher levels of susceptibility were observed for nitrofurantoin (36/40; 90.0%), gentamicin (17/40; 42.5%), and ofloxacin (15/40; 37.5%).

Overall, the antibiogram patterns revealed widespread resistance to commonly used antibiotics among the clinical isolates, with nitrofurantoin demonstrating the highest activity.

Figure 1. Antibiotic Susceptibility Profile Heatmap of Isolates



Key: Ceftazidime (CAZ), Cefuroxime (CRX), Gentamicin (GEN), Cefixime (CXM), Ofloxacin (OFL), Augmentin (AUG), Nitrofurantoin (NIT), Ciprofloxacin (CPR), Resistant(R), Susceptible (S), Intermediate (I)**.**

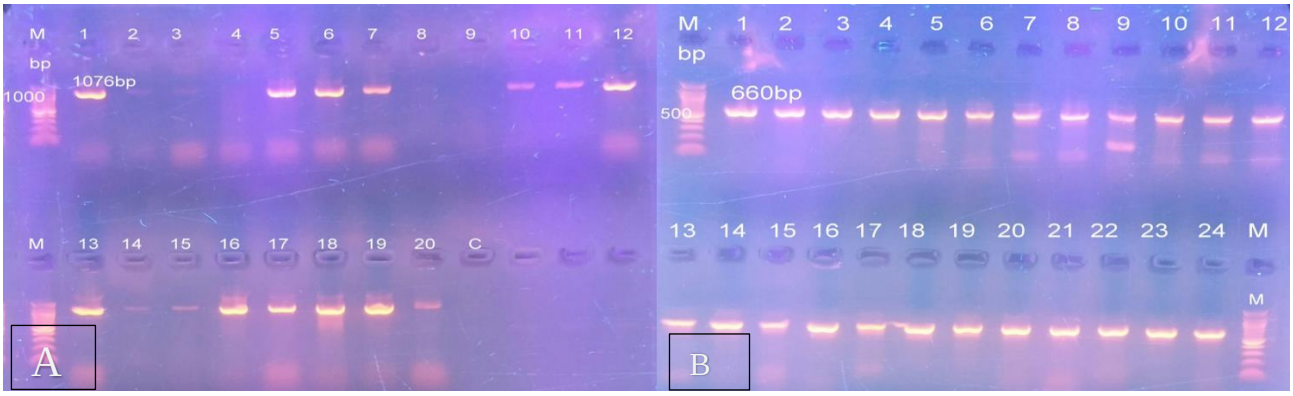
Molecular Detection of blaTEM and blaSHV Genes

Polymerase chain reaction (PCR) analysis was performed to detect the presence of blaTEM and blaSHV β-lactamase genes among the clinical bacterial isolates. Amplification of the blaTEM gene yielded a PCR product

of approximately 1076 bp, while amplification of the blaSHV gene produced an expected amplicon size of approximately 660 bp. of the 20 isolates screened for the blaTEM gene, 15 isolates (75.0%) showed positive amplification, corresponding to isolates 1, 5, 6, 7, and 10–20. In contrast, 24 isolates were screened for the blaSHV gene, and all 24 isolates (100%) demonstrated positive amplification. No amplification was observed in the negative control lanes for either gene.

The PCR findings indicate a high prevalence of blaSHV β-lactamase genes and a substantial presence of blaTEM genes among the tested clinical isolates. Representative agarose gel electrophoresis images showing PCR amplification products are presented in Figure 2.

Figure 2. Agarose gel electrophoresis of PCR amplification products for blaTEM and blaSHV genes.



(A) Detection of the blaTEM gene showing an expected amplicon size of approximately 1076 bp. (B) Detection of the blaSHV gene showing an expected amplicon size of approximately 660 bp. Lane M: molecular weight marker; lanes 1–20 (blaTEM) and 1–24 (blaSHV) represent clinical isolates; C: negative control.

DISCUSSION

This study provides combined phenotypic and molecular evidence of widespread β-lactam resistance among clinical bacterial isolates from Nigeria, underscoring the growing public health challenge posed by antimicrobial resistance in Sub-Saharan Africa. The integration of antibiogram data with molecular detection of β-lactamase–encoding genes strengthens the evidence base by demonstrating not only reduced susceptibility to commonly used antibiotics but also the genetic mechanisms underpinning this resistance. Such findings are particularly concerning in settings where β-lactam antibiotics remain first-line agents for the empirical treatment of infections, often in the absence of routine susceptibility testing.

Across Sub-Saharan Africa, limited diagnostic capacity, unregulated access to antimicrobials, and inconsistent antimicrobial stewardship have created conditions that favor the selection and persistence of resistant bacterial populations (Porter *et al*., 2022; Totaro *et al*., 2025). The detection of β-lactamase genes in this study reflects broader regional trends, where plasmid-mediated resistance mechanisms are increasingly reported in clinical isolates from both community and hospital settings. Importantly, the coexistence of phenotypic resistance with molecular confirmation highlights the potential for sustained transmission of resistant strains and resistance determinants within healthcare environments.

Collectively, these findings reinforce the urgent need for strengthened antimicrobial resistance surveillance that combines phenotypic testing with molecular characterization, particularly in low- and middle-income countries. Such integrated approaches are essential for informing treatment guidelines, supporting infection prevention and control strategies, and guiding policy-level interventions aimed at curbing the spread of β-lactam resistance in the region.

Phenotypic resistance patterns in a regional context

The extremely high levels of resistance observed in this study against ceftazidime (97.7%), cefuroxime (≈99%), amoxicillin–clavulanic acid (≈92–100%), and ciprofloxacin (92%) among Gram-negative clinical bacterial

isolates are consistent with, and in some cases exceed, resistance rates reported across Nigeria and the wider Sub-Saharan African region. In a large multicenter Nigerian study involving 307 Gram-negative isolates, Ogbolu *et al*. (2018) reported resistance rates of 92.2% to third-generation cephalosporins and 78.1% to fluoroquinolones, closely mirroring the resistance patterns observed in the present study. These convergent findings indicate that resistance to commonly prescribed first-line agents has become widespread and deeply entrenched among Gram-negative bacteria in Nigerian clinical settings.

Regional evidence further supports these observations. A comprehensive systematic review of antimicrobial resistance in West Africa demonstrated moderate to high resistance rates among Gram-negative pathogens across multiple clinical syndromes, including urinary tract, bloodstream, and wound-related infections. Notably, studies focusing on urinary tract infections, one of the primary specimen sources in the present study, reported increasing resistance to cephalosporins and other empirically prescribed antibiotics, raising concerns about the continued effectiveness of standard treatment regimens. The high resistance to β-lactam antibiotics observed here, therefore, reflects not an isolated phenomenon, but a broader regional trend driven by sustained antimicrobial pressure (K. Bernabé *et al*., 2017; Williams-Walker *et al*., 2025).

The persistence of such resistance patterns is likely attributable to the widespread empirical use of broad-spectrum antibiotics, unregulated access to antimicrobials, and limited routine susceptibility testing in many healthcare settings (Mariita *et al.*, 2018; Bebell and Muiru, 2014). These factors collectively promote the selection and maintenance of resistant Gram-negative populations, particularly those harboring plasmid-mediated resistance mechanisms. The close alignment between the phenotypic resistance patterns observed in this study and those reported in large-scale Nigerian and West African investigations reinforces the reliability of the findings and highlights the urgency of addressing antimicrobial misuse at both institutional and community levels.

In contrast, the relatively higher susceptibility observed to nitrofurantoin, gentamicin, and ofloxacin aligns with trends reported across Sub-Saharan Africa, where older or more narrowly used antibiotics often retain partial activity against Gram-negative bacteria. Nitrofurantoin, in particular, continues to demonstrate comparatively preserved efficacy in urinary tract infections, likely owing to its restricted clinical indications and limited availability outside regulated healthcare environments. These findings underscore the importance of antibiotic stewardship and the use of locally generated antibiogram data to inform targeted therapy and slow the further emergence of resistance (Teixeira *et al*., 2025; Ampaire et al., 2016). Although stratification of resistance patterns by bacterial species would provide additional epidemiological insight, this was not feasible in the present study due to incomplete species-level identification of archived isolates.

Molecular detection of β-lactamase genes in a regional context

The molecular findings of this study further highlight the widespread dissemination of β-lactamase–encoding genes among clinical bacterial isolates in Nigeria and are consistent with trends reported across Sub-Saharan Africa. The detection of blaTEM in 75% (15/20) of amplified isolates and blaSHV in 100% (24/24) of amplified isolates underscores the substantial burden of plasmid-mediated β-lactam resistance in this setting. Although the proportion of isolates subjected to PCR differed between genes, the high positivity rates suggest extensive circulation of these resistance determinants within clinical environments.

Comparable studies across the region report variable but consistently high prevalence of these genes. In Sudan, Altayb *et al*. (2021) reported blaTEM and blaSHV prevalences of 61% and 38%, respectively, while Dirar *et al*. (2020) documented an even higher blaTEM prevalence of 86%, alongside blaSHV at 28%. In contrast, a study from Burkina Faso by Kpoda *et al*. (2018) reported substantially lower rates (blaTEM 26.2% and blaSHV 5.9%) among ESBL-producing isolates. These variations likely reflect differences in antimicrobial usage practices, infection control policies, healthcare infrastructure, and the selective pressures exerted by commonly prescribed antibiotics across different settings.

The remarkably high detection of blaSHV in the present study, relative to many regional reports, is particularly noteworthy. This finding may indicate localized amplification and dissemination of SHV-associated resistance plasmids, possibly driven by sustained exposure to β-lactam antibiotics and limited antimicrobial stewardship.

Similar high prevalences have been reported in hospital-based studies outside West Africa, such as the South African study by Ehlers *et al*. (2009), which documented an overall ESBL gene prevalence of 87%, emphasizing that healthcare environments can act as focal points for the accumulation and spread of resistance determinants.

Importantly, the coexistence of phenotypic resistance and molecular confirmation observed in this study mirrors findings from multiple SSA investigations, where isolates frequently harbor one or more β-lactamase genes. This genetic redundancy enhances bacterial survival under antimicrobial pressure and contributes to treatment failure and persistent transmission. Collectively, these results reinforce the concept that β-lactam resistance in Sub-Saharan Africa is driven not by isolated events but by entrenched, region-wide dissemination of mobile resistance genes, underscoring the need for continuous molecular surveillance alongside routine susceptibility testing.

Study limitations

This study has numerous limitations that should be considered when the findings are interpreted. Firstly, the sample size and the use of archived isolates from a single laboratory setting are relatively small and may limit the generalizability of the results to other healthcare facilities or regions in Nigeria. Secondly, the molecular screening was limited to just two β-lactamase genes (blaTEM and blaSHV), and other clinically important resistance determinants, including blaCTX-M and carbapenemase genes, were not investigated in this study. Consequently, the full molecular landscape of β-lactam resistance among the isolates may not have been properly captured. Despite these study limitations, the phenotypic and molecular approach combined provides valuable baseline data on resistance patterns and highlights the urgent need for broader genomic surveillance using larger, multi-center datasets.

CONCLUSION

This study provides evidence of widespread antimicrobial resistance among Gram-negative clinical bacterial isolates from Nigeria, marked by extremely high resistance to commonly used β-lactam and fluoroquinolone antibiotics. The detection of blaTEM and the universal presence of blaSHV among screened isolates highlight the extensive dissemination of plasmid-mediated β-lactamase genes in clinical settings. The strong concordance between phenotypic resistance and molecular findings suggests sustained selective pressure driven by the continued empirical use and misuse of broad-spectrum antibiotics.

These findings emphasize the urgent need for strengthened antimicrobial resistance surveillance systems that integrate routine susceptibility testing with molecular characterization of resistance mechanisms. Improved antimicrobial stewardship, enhanced diagnostic capacity, and regulation of antibiotic use are critical to preserving the effectiveness of existing therapeutic options and limiting the further spread of β-lactam resistance in Nigeria and across Sub-Saharan Africa.

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Conflict Of Interest

The authors declare no conflict of interest.

Ethical Considerations

Ethical approval was waived as the study involved archived bacterial isolates with no associated patient identifiers. The study was conducted in accordance with institutional and national ethical guidelines for research involving biological materials.

Data Availability

The data generated and analyzed during this study are available from the corresponding author upon reasonable request.

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