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PREVALENCE AND PHENOTYPIC DETECTION OF BETA-LACTAMASES IN CLINICAL ISOLATES OF GRAM-NEGATIVE BACTERIA FROM SOUTHWESTERN NIGERIA

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ABSTRACT

Antimicrobial resistance among Gram-negative bacteria represents a major public health challenge globally, with extended-spectrum β-lactamase (ESBL) and metallo-β-lactamase (MBL) production significantly limiting therapeutic options. In Nigeria, increasing resistance to β-lactam antibiotics has been reported, yet comprehensive phenotypic data across multiple tertiary healthcare facilities remain limited. This study determined the prevalence and phenotypic detection of ESBL- and MBL-producing Gram-negative bacteria isolated from clinical specimens in southwestern Nigeria. A total of 285 non-duplicate Gram-negative bacterial isolates were collected between August 2021 and April 2023 from urine, wound swabs, stool, sputum, and blood samples across three tertiary hospitals. Isolates were identified using standard microbiological and biochemical methods. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method. Isolates exhibiting resistance or reduced susceptibility to third-generation cephalosporins were screened phenotypically for ESBL and MBL production using the double-disk synergy and combination disk tests. The isolates demonstrated extremely high resistance to β-lactam antibiotics, particularly third-generation cephalosporins. Resistance rates exceeded 85% for cefotaxime, cefixime, cefuroxime, and ceftriaxone-sulbactam among *Klebsiella* and *Escherichia coli* isolates. High resistance was also observed against β-lactam/β-lactamase inhibitor combinations and carbapenems. Phenotypic assays confirmed a high prevalence of ESBL producers, with the additional detection of MBL activity in a subset of isolates, indicating the emergence of carbapenem resistance. The high prevalence of ESBL- and MBL-producing Gram-negative bacteria in southwestern Nigeria poses a serious threat to effective antimicrobial therapy. Routine resistance surveillance, strengthened antimicrobial stewardship, and improved diagnostic capacity are urgently needed to curb the spread of these multidrug-resistant pathogens.

Keywords Antimicrobial Resistance, Extended-Spectrum β-lactamase, Metallo-β-lactamase, Gram-Negative Bacteria, Nigeria

INTRODUCTION

Antimicrobial resistance (AMR) poses a profound and growing threat to global health, eroding many of the therapeutic advances achieved over the past century (Walsh *et al.*, 2023). Gram-negative members of the Enterobacteriaceae family including *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., and related organisms are major causes of both community-acquired and healthcare-associated infections (WHO, 2024). These bacteria are implicated in a broad spectrum of clinical manifestations, such as urinary tract infections, bloodstream infections, pneumonia, and intra-abdominal sepsis, and they account for a substantial proportion of morbidity, mortality, and healthcare resource use worldwide (Bittencourt *et al.*, 2025).

In Nigeria, the pooled prevalence of extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-PE) has been estimated at approximately 34.6%, indicating a substantial burden in both hospital and community settings. Similarly, in a study in Edo State, clinical Enterobacteriaceae isolates showed high rates of multidrug resistance and a predominance of the CTX-M-15 gene, further confirming the public health threat at a national level (Jesumirhewe *et al.*, 2022).

β-Lactam antibiotics (penicillins, cephalosporins, monobactams, and carbapenems) remain central to the empirical and targeted management of many infections caused by Enterobacteriaceae because of their broad activity, predictable pharmacology, and generally favourable safety profile (Freed & Hanson, 2024). The clinical utility of this class, however, has been seriously undermined by the global emergence and diversification of β-lactamase enzymes (Rawson *et al.*, 2025). β-Lactamases hydrolyse the β-lactam ring, the structural moiety essential for antibiotic activity, thereby inactivating agents that would otherwise prevent bacterial cell wall synthesis. Over recent decades, these enzymes have evolved and proliferated into multiple families with distinct substrate spectra and inhibitor susceptibilities (Göpel *et al.*, 2025). Across sub-Saharan Africa, the prevalence of carbapenemase-producing bacteria in hospital settings has been reported in various studies, ranging from low to moderately high levels depending on country and diagnostic capability (Manenzhe *et al.*, 2015).

Extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases, and carbapenemases represent clinically significant β-lactamase groups because of their capacity to eliminate common therapeutic options (Gatti *et al.*, 2025).

ESBLs typically confer resistance to penicillins and third-generation cephalosporins and are usually susceptible to inhibition by classical β-lactamase inhibitors; AmpC enzymes can hydrolyse a wider range of cephalosporins and are less reliably inhibited. Carbapenemases, a heterogeneous group that includes metallo-β-lactamases (MBLs), KPC-type class A enzymes, and OXA-type oxacillinases, can inactivate carbapenems, which are often reserved as last-line agents for severe infections (Rodríguez-Baño *et al.,* 2018; Hickson *et al.,* 2025). Importantly, MBLs are not inhibited by conventional β-lactamase inhibitors, and their presence markedly reduces available therapeutic choices.

The clinical and public-health impact of infections caused by ESBL-, AmpC- or carbapenemase-producing Enterobacteriaceae is substantial. These infections are associated with higher rates of treatment failure, longer hospital stays, and increased mortality, particularly among critically ill or immunocompromised patients (Yang *et al.,* 2025). Isolates that produce β-lactamases frequently carry additional resistance determinants to non-β-lactam classes such as aminoglycosides, fluoroquinolones, and trimethoprim-sulfamethoxazole; such multidrug resistance further restricts treatment options and often necessitates the use of older, more toxic or less well-studied agents (Bittencourt *et al.,* 2025). In Nigeria, environmental and animal sources have been shown to harbor ESBL-producing Enterobacteriaceae, including *E. coli* and *K. pneumoniae*, with significant multidrug resistance, posing risks of zoonotic or environmental transmission (Adebiyi *et al.,* 2023).

Accurate, timely detection of β-lactamase producers is therefore essential for effective clinical management, antimicrobial stewardship, and infection-control interventions (Rawson *et al.,* 2025). Molecular diagnostics, such as polymerase chain reaction and sequencing, provide definitive gene-level identification but remain resource-intensive and are not universally accessible in routine diagnostic settings. Phenotypic screening approaches and confirmatory assays, when implemented according to standardized laboratory procedures, offer practical alternatives for many laboratories and are critical for guiding empiric therapy and containment measures Gatti *et al.,* 2025).

Despite widespread acknowledgement of the problem, the prevalence and distribution of β-lactamase enzymes vary substantially by geography and healthcare setting, and many regions lack comprehensive surveillance data. Tertiary care centers are particularly important sentinel sites for the emergence and amplification of multidrug-resistant Enterobacteriaceae due to high antimicrobial pressure and the concentration of vulnerable patients (WHO, 2024). In this context, the present study aims to characterize the prevalence and co-occurrence of ESBL and MBL enzymes among Gram-negative bacterial clinical isolates collected at a tertiary care center.

MATERIALS AND METHODS

This study was carried out between August 2021 and April 2023 at three tertiary healthcare facilities in southwestern Nigeria. A total of 285 non-duplicated Gram-negative (bacillus) clinical isolates were collected from various samples, including urine, wound swabs, stool, sputum, and blood. Isolates were identified and characterized using standard microbiological protocols. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method. Isolates that were resistant or showed reduced susceptibility to two or three third-generation cephalosporins were further phenotypically confirmed for ESBL and MBL production using the double-disk synergy test and the combination disk test.

Collection of Isolate

Gram-negative isolates were collected consecutively from the three hospitals until the desired number was reached. The isolates were collected on an already prepared peptone water and transported on ice to the Department of Microbiology Laboratory, Babcock University, where they were plated on several selective media.

Identification of Isolates

The isolates were sub-cultured onto nutrient agar and incubated at 37°C for 18 to 24 hours. Preliminary identification was done by reconfirming their Gram reaction, and a series of biochemical tests was also carried out. The identity of the isolates was confirmed using conventional biochemical methods such as API 20E.

Antimicrobial susceptibility testing

The pattern of antibiotic susceptibility of the isolates was determined using the standard Kirby-Bauer disc diffusion method, as outlined in the Clinical and Laboratory Standards Institute (CLSI, 2016) guidelines. The antibiotic discs that were used includes ceftriaxone sulbactam (45μg), chloramphenicol (30 μg), nalidixic acid (30 μg), cefixime (5μg), ofloxacin (5μg), cefotaxime (30 μg), imipenem/cilastain (10μg), ampiclox (10 μg), cefuroxime (25μg), nitrofurantoin (300μg), levofloxacin (5μg), ceftazidime (30 μg), amoxicillin/clavulanate (30μg), and imipenem (10 μg). Resistance to three or more different antibiotic families was regarded as multidrug resistance (MDR).

Phenotypic Detection of MBL and ESBL

Combination Disc Test (CDT)

According to Sachdeva *et al.* (2017), a 0.5 McFarland bacterial suspension was prepared and inoculated onto Mueller–Hinton agar plates to produce a lawn culture. Two discs were placed on the agar surface: imipenem (10 µg) and imipenem + EDTA (10 µg/750 µg) positioned at least 25 mm apart.

Following incubation at 37 °C for 18 hours, inhibition zone diameters were measured. An increase of ≥7 mm in zone diameter with the carbapenem disc combined with EDTA, compared with the carbapenem disc alone, was interpreted as positive for MBL production. Quality control was ensured using appropriate positive and negative control strains.

For the detection of extended-spectrum β-lactamase (ESBL) activity, a 0.5 McFarland suspension was prepared from fresh overnight colonies and uniformly inoculated onto Mueller–Hinton agar plates to obtain a lawn culture. Two sets of antibiotic discs were placed on the agar surface: ceftazidime (30 µg) and ceftazidime–clavulanic acid (30/10 µg), and cefotaxime (30 µg) and cefotaxime–clavulanic acid (30/10 µg). The discs were positioned at least 25 mm apart (centre-to-centre).

The plates were incubated aerobically at 37 °C for 18 hours. After incubation, the inhibition zone diameters around the cephalosporin discs alone and in combination with clavulanic acid were measured and compared. An increase of ≥5 mm in the inhibition zone diameter for either antimicrobial agent tested in combination with clavulanic acid compared to the zone diameter of the agent tested alone was interpreted as positive for ESBL production. Quality control was performed using known ESBL-positive and ESBL-negative control strains (Peter-Getzlaff *et al.,* 2011).

Double Disc Synergy Test (DDST)

The Double Disc Synergy Test (DDST) was also used for the phenotypic detection of metallo-β-lactamase (MBL) production in carbapenem-resistant Gram-negative isolates. The test is based on the ability of EDTA to chelate zinc ions required for MBL enzyme activity.

A 0.5 McFarland suspension was prepared from fresh overnight bacterial colonies and uniformly inoculated onto Mueller–Hinton agar plates to obtain a lawn culture. An EDTA disc was prepared by impregnating a sterile blank filter paper disc with 10 µL of 0.5 M EDTA (pH 8.0) and allowing it to dry. A carbapenem disc (imipenem 10 µg) was placed on the inoculated agar surface, and the EDTA disc was positioned at a 20 mm (centre-to-centre) distance from the carbapenem disc. The plates were incubated aerobically at 37 °C for 18 hours.

Following incubation, the plates were examined for enhancement of the inhibition zone between the carbapenem and EDTA discs. The presence of a clear expansion of the inhibitory zone (keyhole effect) in the direction of the EDTA disc was considered as positive for MBL production, while the absence of zone enhancement was considered negative. Quality control was ensured using known MBL-producing and non-MBL control strains.

The Double Disk Synergy Test (DDST) was performed for the phenotypic detection of extended-spectrum β-lactamase (ESBL) production in the Gram-negative bacterial isolates as well.

A 0.5 McFarland suspension was prepared from fresh overnight colonies and uniformly inoculated onto Mueller–Hinton agar plates to produce a lawn culture. A disc of amoxicillin–clavulanic acid (20/10 µg) was placed at the center of the plate. Discs of ceftazidime (30 µg) and cefotaxime (30 µg) were then placed at a distance of 20–30 mm (centre-to-centre) from the amoxicillin–clavulanic acid disc.

The plates were incubated aerobically at 37 °C for 18 hours. After incubation, the plates were examined for enhancement of the inhibition zones of the cephalosporin discs toward the amoxicillin–clavulanic acid disc. The presence of a clear expansion of the inhibition zone (keyhole effect) indicated ESBL production, whereas the absence of zone enhancement indicated a non-ESBL-producing isolate. Quality control was ensured using known ESBL-positive and ESBL-negative control strains.

RESULTS AND DISCUSSION

A total of 267 isolates (as the remaining 18 were contaminated) were classified into eight genera (fig. 2), with a predominance of *Klebsiella* spp. (n=112, 42%) and *E. coli* (n=68, 26%). Together, these two taxa accounted for more than two-thirds (68%) of all isolates, indicating their central role as leading etiological agents among the Enterobacteriaceae in the sampled clinical settings. *Pseudomonas* spp. represented the third most frequently encountered group, contributing 19% (n=51) of all isolates, followed by *Proteus* spp. (n=26, 10%). The recovery of these two genera reflects their established role as opportunistic pathogens in nosocomial and community-acquired infections. Other species were encountered less frequently, including *Acinetobacter* spp. (n=2. 1%), *Enterobacter* spp. (n=6, 2%), *Morganella* spp (n=1, <1%), and *Serratia* spp. (n=1, <1%)

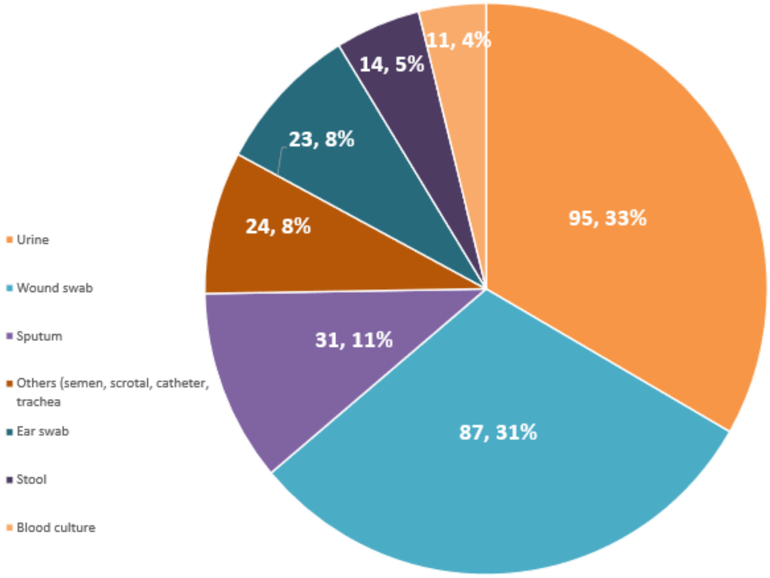

Figure 1: Pathological Distribution of the Isolates

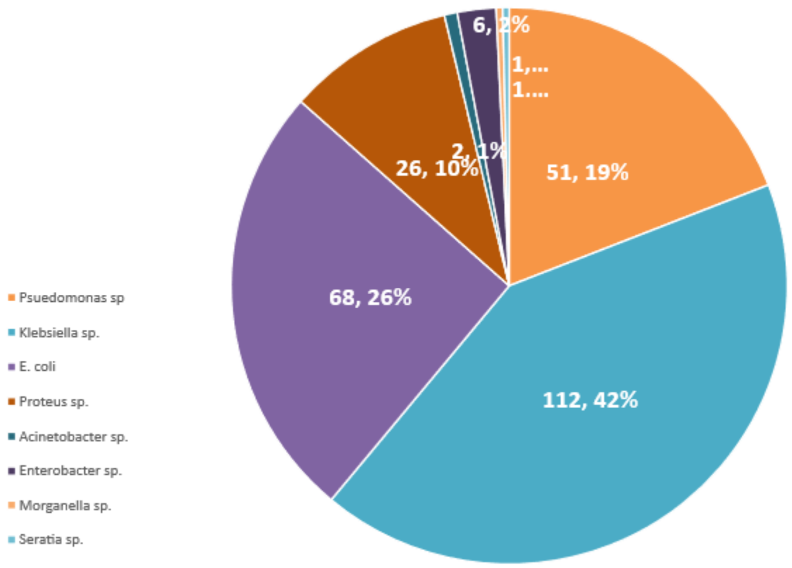

Figure 2: Identified GNB from the Three Study Sites Using the Biochemical Method

High antimicrobial resistance was recorded among the isolates tested from all three study sites. Twelve antibiotics belonging to six different antibiotic classes were tested against all 267 isolates. The CLSI 2016 breakpoint for the antibiotic disc diffusion test was used in analyzing the isolates for sensitivity and resistance (CLSI, 2016).

Most antibiotics tested showed a very high resistance rate (>85%) among *Klebsiella* isolates. Resistance was highest for cefotaxime (93%; 105), and then nalidixic acid and imipenem/cilastin (88.4%; 99), followed by amoxicillin/clavulanate and ampicillin/cloxacillin (85.7%; 96), and then cefuroxime, cefixime and ceftriaxone sulbactam at 83% (93), 82% (92), and 80.3% (90) respectively. No antibiotics show overwhelming sensitivity across all isolates; however, ofloxacin, levofloxacin, and gentamicin maintain relatively higher sensitivity rates compared to the others. From the data distribution, resistance is statistically predominant across nearly all tested antibiotics (p<0.05), indicating that the difference between the proportions of resistant and sensitive strains is not due to random chance. The large margin between resistance and sensitivity rates for most antibiotics suggests a significant shift toward multidrug resistance in *Klebsiella* isolates from this study (Fig. 3).

The majority of *E. coli* isolates exhibit resistance to most antibiotics. Notably, cefotaxime (100%), nalidixic acid, and amoxicillin/clavulanate (98.4%), cefixime (87.3%), and ampicillin/cloxacillin (92%) show extremely high resistance rates. Fluoroquinolones and aminoglycosides retained partial activity as they show relatively better sensitivity: ofloxacin (46%), levofloxacin (31.7%), and gentamicin (30%). The pattern suggests widespread multidrug resistance (MDR) among *E. coli* isolates, with nearly all β-lactam antibiotics showing very high resistance levels. Even last-resort drugs like carbapenems (imipenem/cilastin at 95%) have alarmingly high resistance (fig. 4). The partial sensitivity to fluoroquinolones (ofloxacin, levofloxacin) and aminoglycosides (gentamicin) could guide therapy. Still, caution is needed given the high resistance rates. Clinically, this means treatment options are severely limited, and empirical therapy should be guided by susceptibility testing.

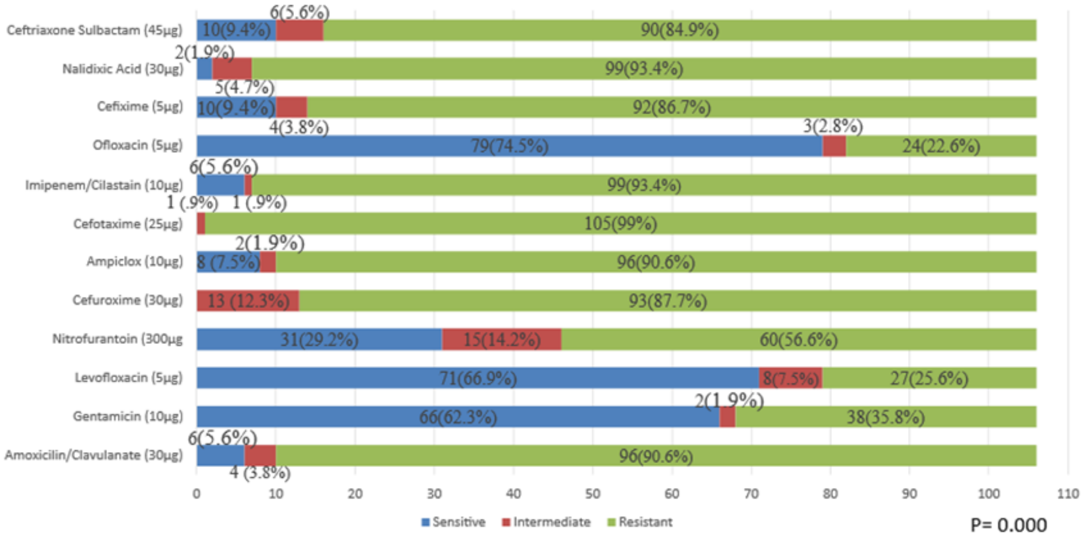

Figure 3: Relationship Between *Klebsiella* Strains and the Antibiotics Tested

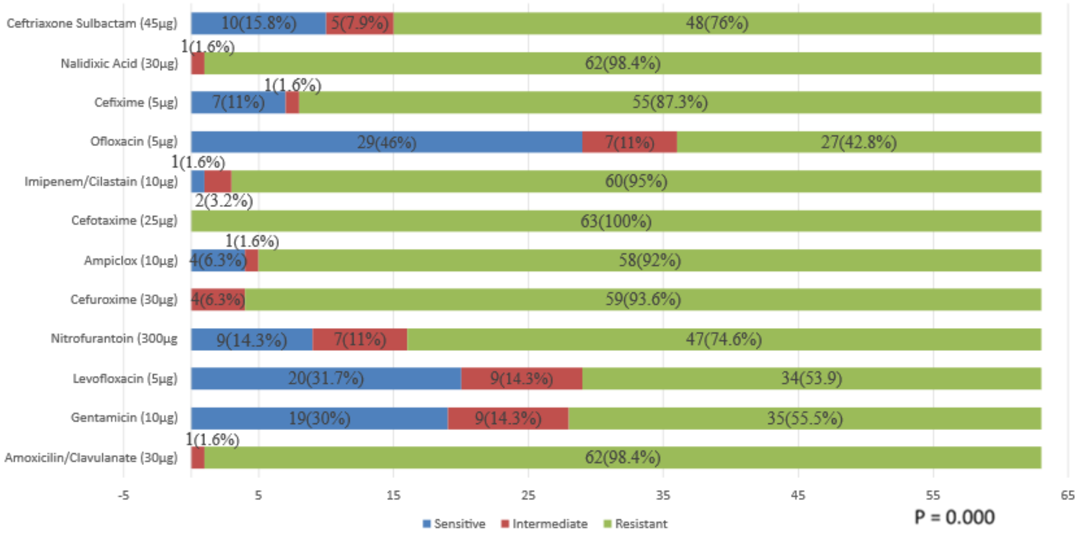

Figure 4: Relationship Between *E. coli* Strains and the Antibiotics Tested

The distribution of *E. coli* and *Klebsiella* isolates across antibiotic resistance classes is presented in Figure 5. A clear gradient of multidrug resistance was observed in both genera, with the majority of isolates clustering within higher resistance categories. Only a small proportion of isolates demonstrated resistance to one or two classes of antibiotics, with *E. coli* represented by 2 (3.1%) isolates in each category, and *Klebsiella* represented by 5 (4.7%) isolates resistant to two classes. This suggests that single or dual-class resistance is relatively uncommon in this study.

At the intermediate level (resistance to 3 and 4 classes of antibiotics), there was a little increase in the number of *Klebsiella* isolates compared to *E. coli*. Specifically, *Klebsiella* accounted for 18 (16.9%) isolates resistant to three classes and 37 (34.9%) resistant to four classes of antibiotics, while *E. coli* recorded 6 (9.5%) and 20 (31.7%) isolates in the same categories, respectively. This indicates that *Klebsiella* was disproportionately represented among isolates with moderate multidrug resistance. The highest frequencies were recorded in isolates resistant to 4 antibiotic classes. *Klebsiella* peaked at 37 (34.9%) isolates resistant to four classes, which was almost twice the number recorded for *E. coli* (20 isolates). However, in the category of isolates resistant to six antibiotic classes, *E. coli* slightly outnumbered *Klebsiella*, with 23 (36.5%) isolates compared to 19 (17.9%).

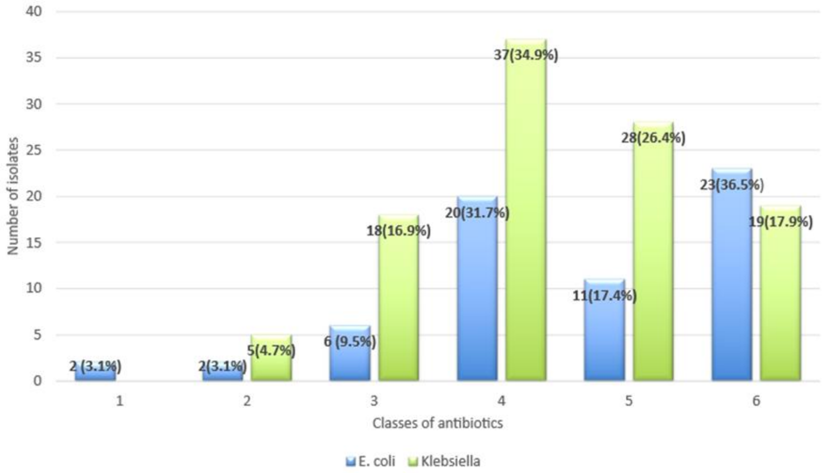

Figure 5: Comparison of Multidrug Resistance Between *E. coli* and *Klebsiella* Strains

Figure 6 shows the distribution of ESBL-, MBL-, and co-producing (ESBL & MBL) isolates across bacterial species. Among the isolates, *Klebsiella* spp. demonstrated the highest proportion of ESBL production (40%), followed closely by *E. coli* (39.6%) and *Pseudomonas* spp. (39%). *Proteus* showed a comparatively lower ESBL prevalence (30.6%).

For MBL production, *Klebsiella* spp. still had the highest proportion (38%), followed by *Pseudomonas* spp. and *E. coli* (35% each). *Proteus* exhibited a lower MBL rate (19%).

Co-production of ESBL and MBL enzymes was also most frequently observed in *Klebsiella* spp. (29.4%), followed by *E. coli* (25%) and *Pseudomonas* spp. (21.5%). *Proteus* had the lowest co-production rate (15%). No ESBL, MBL, or co-producing isolates were observed among *Acinetobacter*, *Enterobacter*, *Morganella*, or *Serratia* species in the dataset. Additionally, 20% of isolates were classified as unidentified for both ESBL and MBL categories.

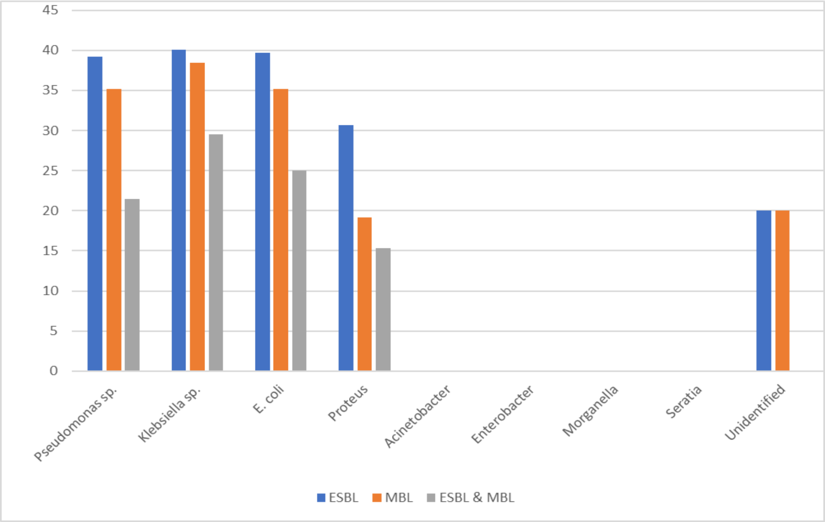

Figure 6: ESBL and MBL Producers Among Various Isolates

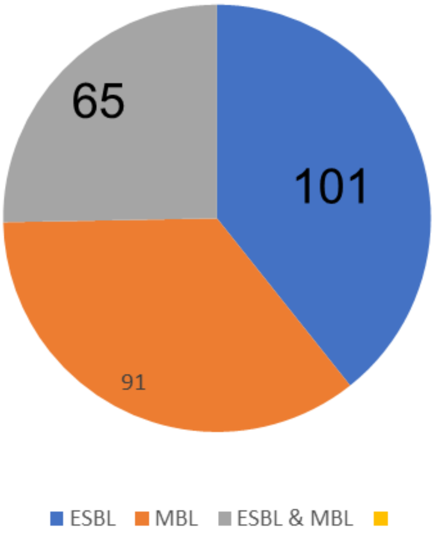

Figure 7: Overall Beta-Lactamase Production Across Sites

Discussion

The distribution of Gram-negative bacteria (GNB) identified across the study sites demonstrates patterns that are consistent with both national and global trends in clinical microbiology. The predominance of *Klebsiella* spp. and *E. coli* underscores their central role as major etiological agents of infections in hospital and community settings. This aligns with earlier Nigerian studies where *K. pneumoniae* and *E. coli* were the most frequently isolated Enterobacteriaceae from bloodstream and urinary tract infections (Aibinu *et al.*, 2017; Olalekan *et al.*, 2020) Similarly, Iregbu (2025), reported high recovery rates of these two genera in Abuja, further highlighting their significance in local antimicrobial resistance epidemiology.

The detection of *Pseudomonas* spp. (19%) as the third most abundant isolate is noteworthy. *P. aeruginosa* is a notorious opportunistic pathogen frequently associated with multidrug resistance and hospital-acquired infections. Comparable rates have been reported in Lagos and Ibadan, where *Pseudomonas* accounted for approximately 15-20% of GBN isolates (Ogbolu *et al.*, 2011; Adeyankinnu *et al.*, 2014). The presence of *Proteus* spp. (10%) also corresponds with findings from Northern Nigeria, where *P. mirabilis* was identified in both urinary and wound infections, albeit at lower prevalence than *E. coli* and *Klebsiella* (Yusuf *et al.*, 2021).

The occurrence of less frequently isolated genera, including *Acinetobacter*, *Enterobacter*, *Morganella*, and *Serratia*, though limited, is clinically significant as members of these genera are increasingly being recognized as reservoirs of ESBL and carbapenemases (Peirano & Pitout 2020). Their recovery in this study echoes reports from South Africa and Ghana, where *A. baumannii* and *E. cloacae* have emerged as critical healthcare-associated pathogens despite their lower prevalence (Musicha *et al.*, 2017; Mbelle *et al.*, 2020).

The susceptibility pattern of the *Klebsiella* isolates shows very high resistance to multiple β-lactams and notable variation across other classes. Resistance to third-generation cephalosporins was extreme: cefotaxime, cefixime, cefuroxime, and ceftriaxone-sulbactam. Similarly, high resistance was recorded to amoxicillin/clavulanate and ampicillin/cloxacillin. These results are highly consistent with the established role of ESBLs in *Klebsiella*, which confer resistance to penicillins and first to third-generation cephalosporins and are frequently plasmid-encoded, enabling rapid spread (Paterson & Bonomo, 2005; Bush & Bradford, 2020).

By contrast, fluoroquinolone resistance was lower, though still clinically relevant. These rates are somewhat lower than several regional reports that have documented ≥30-40% non-susceptibility. (Elsayed *et al.,* 2017; Jomehzadeh *et al.,* 2022). Gentamicin resistance fell in the mid-range of African estimates and is mechanistically compatible with the spread of aminoglycoside-modifying enzymes (AMEs) frequently co-located with ESBL-associated β-lactam resistance and co-resistance to aminoglycosides, while diverging markedly in the very high carbapenem resistance signal – suggesting local CRKP transmission.

The antimicrobial susceptibility profile of *E. coli* isolates presented in this study demonstrates widespread resistance to multiple agents. Nearly universal resistance was observed against third-generation cephalosporins and β-lactam/β-lactamase inhibitor combinations, including cefotaxime, cefixime, cefuroxime, ceftriaxone-sulbactam, and amoxicillin/clavulanate. High resistance was also documented against ampicillin/cloxacillin and imipenem/cilastatin. These results are consistent with the well-established dominance of ESBL-producing *E. coli* in clinical settings, particularly bla_{CTX-M}, and bla_{SHV} producers that confer broad resistance to β-lactams (Pitout & Laupland 2008; Bevan *et al.,* 2017). Similar high-level cephalosporin resistance has been reported across Nigeria (Oduyebo *et al.,* 2016; Olalekan *et al.,* 2020) and other sub-Saharan African (Tansarli *et al.,* 2014), underscoring the regional burden of ESBL-producing Enterobacteriaceae.

By contrast, resistance to fluoroquinolones and aminoglycosides showed greater heterogeneity. Ofloxacin resistance was recorded in almost half of the isolates, while levofloxacin and gentamicin resistance stood at over average. These findings align with global reports of high fluoroquinolone resistance in *E. coli* (Rodríguez-Baño *et al.,* 2018; Baba *et al.,* 2020). However, resistance rates in this study appear higher than those reported in Europe and North America, where fluoroquinolone resistance in *E. coli* bloodstream isolates remains below 30% (ECDC, 2022). This disparity may reflect regional differences in antimicrobial prescribing practices and limited regulation of fluoroquinolone use in Nigeria, factors that accelerate selection.

The prevalence of ESBL producers detected by phenotypic methods varied by hospital, with UCH having the highest rates of production across all enzymes. This is somewhat similar to the study of Kaur *et al.* (2017), which reported moderately high ESBL and MBL producers among Gram-negative isolates. In the current study, the highest prevalence of both ESBL and MBL was found in *Klebsiella* spp, followed by *E. coli,* then *Pseudomonas* spp and the lowest in *Proteus* spp, which is contrary to Kaur *et al.* (2017), who reported the highest frequency in *E. coli* followed by *Klebsiella pneumonia*. The highest MBL production was seen in *Klebsiella* spp, followed by *E. coli*, which is comparable to Kaur *et al.* (2017), who also reported the highest MBL production in *Klebsiella,* and to Ugwu *et al.* (2020), who reported the highest prevalence of MBL producers in *K. pneumoniae* followed by *E. coli*.

The high prevalence of ESBL and MBL producers observed in this study, particularly among *Klebsiella* spp., *E. coli*, and *Pseudomonas* spp., is consistent with trends reported in Nigeria and globally. Similar investigations in tertiary hospitals across Nigeria have documented significant rates of ESBL production among clinical Gram-negative isolates, although the exact prevalence varies depending on region, sample type, and methodology. A systematic review of ESBL-producing Gram-negative bacteria in Nigeria reported prevalence values as high as 82.3% in some zones and as low as 7.5% in others, indicating considerable heterogeneity but overall high occurrence in clinical settings (Tanko *et al.,* 2020). Additionally, a meta-analysis focusing on *K. pneumoniae* in Nigeria estimated a pooled ESBL prevalence of approximately 47%, which supports our finding that *Klebsiella* remains a major contributor to ESBL dissemination (Ezeh *et al.,* 2024).

Several Nigerian hospital-based studies have similarly identified ESBL producers predominantly in *E. coli* and *Klebsiella* spp. isolates. A Southwest Nigerian study reported 69% ESBL production among *E. coli* isolates and 31% among *K. pneumoniae* from urine specimens, indicating levels that are comparable or even higher than those observed in the present dataset (Mofolorunsho *et al.,* 2021). Contrastingly, other phenotypic surveys have noted lower ESBL and MBL proportions, such as 32.4% ESBL and only 7.6% MBL among isolates in another hospital study, highlighting the influence of clinical setting, detection methods, and local antimicrobial use on prevalence estimates (Alabi *et al.,* 2020).

Compared with international data, our findings also align with reports from other African countries demonstrating *Klebsiella* and *E. coli* as dominant ESBL producers. In Ethiopia, pooled ESBL prevalence among Gram-negative isolates reached over 50%, with *Klebsiella* spp. frequently identified as the most common ESBL producer, similar to the pattern seen here (Tufa *et al.,* 2020). A meta-analysis in Egypt reported even higher pooled ESBL prevalence (around 60%), exceeding the estimates from Nigeria and other countries such as Nepal, Pakistan, and East Africa, demonstrating that ESBL production is a widespread and escalating problem across diverse (Azzam *et al.,* 2024).

Unlike ESBLs, phenotypic and molecular data on MBL producers in Nigeria are relatively scarce, but available studies confirm the presence of carbapenemase and MBL activity among *E. coli, K. pneumoniae,* and *Pseudomonas aeruginosa* isolates in clinical and environmental settings. For example, animal and abattoir-based research in Nigeria identified MBL genes among Gram-negative isolates, further indicating that MBL production is increasingly detected beyond hospital environments (Ejikeugwu *et al.,* 2021).

Globally, studies in Asia and Europe also report substantial ESBL and MBL production among Gram-negative pathogens, with *Pseudomonas* spp. frequently showing lower ESBL rates compared to Enterobacteriaceae, reflecting intrinsic resistance mechanisms and varying epidemiology across regions (Fazlul *et al.,* 2019). This international context emphasizes that β-lactamase–mediated resistance is a worldwide challenge, though the burden and dominant species may differ by geography, antibiotic use patterns, and infection control practices.

Resistance caused by ESBLs is often associated with resistance to other classes of antibiotics, and this makes it difficult to choose effective therapy. ESBL-producing *E. coli* associated mortality has been reported to be three-times higher than non-ESBL producing *E. coli* (Ismail & Haydar, 2016; Ugwu *et al.,* 2020). The high levels of ESBL producers are a major threat to infection management as this may have contributed to the antibiotic resistance reported in this study. ESBL-producing organisms are known to contain plasmids with genes that encode resistance to quinolones, aminoglycosides, and cotrimoxazole.

MBLs have been recognized as one of the most notable resistance determinants in Enterobacteriaceae. MBL production is a significant problem in hospital isolates of Gram-negative bacteria. MBL production remains the most clinically important mechanism responsible for carbapenem resistance.

CONCLUSION

The Gram-negative bacteria studied were found to be resistant to various classes of antimicrobials. The study showed a high prevalence of drug-resistant genes among the clinical isolates. The majority of the isolates were multidrug resistant. This study also showed that the prevalence of ESBL and MBL producers is high, posing a serious challenge for therapy as well as infection control.

Author Contributions

Conceptualization, M.G.O. and A.O.E.; methodology, M.G.O., A.A.A. and N.B.L; validation, M.G.O., and A.O.E.; formal analysis, M.G.O.; investigation, M.G.O., N.B.L; O.O.C. and O.O.G.; resources, M.G.O., A.A.A., O.O.C. and O.O.G.; data curation, M.G.O. and N.B.L; writing—original draft preparation, M.G.O.; writing—review and editing, M.G.O.; supervision, A.O.E.; project administration, M.G.O.; funding acquisition, M.G.O.; All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

Patient consent was waived because the researcher did not collect or interact with the human subjects directly. The researcher only obtained Gram-negative isolates from the various study sites.

Data Availability Statement

The raw data supporting the conclusions of this article will be made available by the corresponding author on request.

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ABBREVIATIONS

The following abbreviations are used in this manuscript:
UI - University of Ibadan
UCH - University College Hospital
BUTH - Babcock University Teaching Hospital
LUTH - Lagos University Teaching Hospital
AST - Antimicrobial Susceptibility Testing
MRD - Multidrug Resistance
GNB - Gram-negative Bacteria
AMR - Antimicrobial Resistance
MBL - Metallo-beta-lactamase
ESBL - Extended-Spectrum Beta-lactamase

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