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First Report of High-Risk Rare *bla*SIM, *bla*SPM, and *bla*GIM Metallo-β-Lactamase Genes in Clinical *Pseudomonas aeruginosa* Isolates from Nigeria

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Abstract

Background: Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) mediated by metallo-β-lactamases (MBLs) represents a critical public health threat worldwide. While common MBL genes have been documented in Nigeria, the occurrence of rare MBL variants including *bla*SIM, *bla*SPM, and *bla*GIM remains unexplored. This study aimed to detect these high-risk MBL genes in clinical *P. aeruginosa* isolates from Enugu State University Teaching hospital Enugu, Nigeria.

Methods: A total of 200 mid-stream urine samples were collected from patients at Enugu State University Teaching Hospital, Enugu State Nigeria. *P. aeruginosa* isolates were identified using standard microbiological techniques. MBL production was phenotypically screened using the Imipenem-EDTA combined disc test. All 39 phenotypically confirmed MBL-producing isolates were subjected to molecular detection of *bla*SIM, *bla*SPM, and *bla*GIM genes using polymerase chain reaction method (PCR) with specific primers. Antimicrobial susceptibility testing was performed against 16 antibiotics. Statistical analysis was performed using SPSS version 26.0, with chi-square test used to compare resistance rates and Fisher's exact test applied where appropriate. A p-value < 0.05 was considered statistically significant.

Results: Out of 200 urine samples, 95 (47.5 %) yielded *P. aeruginosa*, with 39 (41.1 %) confirmed as MBL producers phenotypically. These isolates exhibited high resistance to carbapenems (imipenem, meropenem, doripenem: 100 %), cephalosporins (cefotaxime, cefepime: 100 %), amoxicillin-clavulanic acid (100 %), and fluoroquinolones (ofloxacin, levofloxacin: 100 %). Statistical analysis revealed significantly higher resistance to these antibiotics compared to colistin ($p < 0.001$). All isolates remained susceptible to colistin (100 %) and showed variable susceptibility to gentamicin (43.6 % susceptible) and amikacin (61.5 % susceptible). The difference in susceptibility between gentamicin and amikacin was statistically significant ($p = 0.041$). PCR analysis of all 39 MBL-producing isolates revealed that 39 (100 %) harbored *bla*SIM and *bla*SPM genes, while *bla*GIM was detected in 32 isolates (82.1 %). Co-carriage of all three genes (*bla*SIM + *bla*SPM + *bla*GIM) was observed in 32 isolates (82.1 %), while 7 isolates (17.9 %) carried blaSIM and *bla*SPM only. This study represents the first documentation of *bla*SIM, *bla*SPM, and *bla*GIM genes in clinical *P. aeruginosa* isolates from Enugu State University Teaching Hospital Enugu Nigeria.

Conclusion: The emergence of these rare, high-risk MBL genes in Nigerian clinical isolates signals a dangerous expansion of the carbapenem resistance landscape. The high prevalence and co-carriage of multiple MBL genes among

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urinary isolates from a single tertiary hospital suggests possible clonal dissemination and underscores the urgent need for enhanced antimicrobial stewardship and infection control measures.

Keywords: *Pseudomonas aeruginosa*; Metallo-β-lactamase; *bla*SIM; *bla*SPM; *bla*GIM; Carbapenem resistance; Nigeria

1. Introduction

Pseudomonas aeruginosa is a leading opportunistic pathogen responsible for severe hospital-acquired infections, including urinary tract infections, pneumonia, and wound infections (Horcajada et al., 2019; Reynolds and Kollef, 2021). Carbapenems have traditionally served as last-resort antibiotics for treating multidrug-resistant Gram-negative bacterial infections (Tamma et al., 2023). However, the global emergence and rapid dissemination of carbapenem-resistant *P. aeruginosa* (CRPA) have created a significant therapeutic challenge associated with high mortality and morbidity rates (Yoon and Jeong, 2021; Jean et al., 2022; Ogba et al., 2022a).

Carbapenem resistance in *P. aeruginosa* is primarily mediated by carbapenem-hydrolyzing enzymes, with metallo-β-lactamases (MBLs) representing the most prevalent class (Queenan and Bush, 2007). MBLs (Ambler class B) require zinc as a cofactor for enzymatic activity and hydrolyze all β-lactams except aztreonam (Walsh et al., 2005). The most clinically significant acquired MBLs include imipenemases (IMP), Verona integron-encoded MBLs (VIM), and New Delhi MBLs (NDM) (Peter *et al.,* 2025; Ilang *et al.,* 2023; Nordmann and Poirel, 2019; Boyd et al., 2020). Beyond these globally disseminated enzymes, several regionally restricted MBLs have emerged, including São Paulo MBL (SPM), Seoul imipenemase (SIM), and German imipenemase (GIM) (El Salabi et al., 2010; Lee et al., 2005; Castanheira et al., 2004).

The blaSPM-1 enzyme was first identified in Brazil and has remained largely confined to South America, associated with the high-risk ST277 clone (Toleman et al., 2002; Gales et al., 2003). *bla*SIM-1 was initially detected in *Acinetobacter baumannii* in Korea (Lee et al., 2005) and later in *P. aeruginosa* in China (Sun et al., 2016), while *bla*GIM-1 was first reported in Germany (Castanheira et al., 2004) and subsequently in sporadic European outbreaks (Wendel et al., 2013). These enzymes exhibit potent carbapenem-hydrolyzing activity and are often embedded in mobile genetic elements, facilitating their horizontal transfer (Partridge et al., 2018). The blaSPM-1 enzyme is unique among MBLs as it is not typically associated with integrons but rather with ISCR elements, while blaSIM and blaGIM are frequently found within class 1 integrons (Toleman et al., 2006).

In Nigeria, studies have documented the presence of *bla*NDM, blaVIM, and *bla*IMP genes in clinical *P. aeruginosa* isolates (Peter *et al.,* 2025; Ilang *et al.,* 2023; Ogba et al., 2022a; Zubair and Iregbu, 2018; Olaniran et al., 2021; Isa et al., 2023). However, comprehensive molecular surveillance for rare MBL variants remains lacking. The absence of data on blaSIM, blaSPM, and blaGIM in Nigerian clinical isolates represents a critical knowledge gap in understanding the true burden and diversity of carbapenem resistance mechanisms in the region. The potential dissemination of these high-risk clones into West Africa through travel, medical tourism, or trade poses a significant public health threat that requires urgent investigation (Bokhary et al., 2021).

This study aimed to detect and characterize the rare metallo-β-lactamase genes *bla*SIM, *bla*SPM, and *bla*GIM in all phenotypically confirmed MBL-producing *P. aeruginosa* isolates from urine samples from patients visiting Enugu State University Teaching hospital Enugu, Nigeria, providing the first comprehensive report of these high-risk resistance determinants in this hospital.

2. Materials and methods

2.1. Study Area and Ethical Approval

This study was conducted at Enugu State University Teaching Hospital (ESUTH), Enugu, Nigeria. Enugu is the capital of Enugu State in southeastern Nigeria, located at coordinates longitude 7°30'40"E and latitude 6°27'10"N. Ethical clearance was obtained from the hospital's research ethics committee (Reference No: ESUTH/REC/0234/202). All procedures followed the World Medical Association Declaration of Helsinki guidelines.

2.2. Sample Collection

A total of 200 mid-stream urine samples were collected from patients attending Enugu State University Teaching Hospital between March and August 2024. Samples were collected in sterile universal containers and transported to the Microbiology Laboratory unit of Ebonyi State University, Abakaliki, within 1 hour of collection for bacteriological analysis.

2.3. Isolation and Identification of *Pseudomonas aeruginosa*

Each urine sample was inoculated into nutrient broth and incubated at 37°C for 24 hours. Turbid cultures were streaked onto Cetrimide agar (Thermo Fisher Scientific, USA) and incubated at 37 °C for 24 hours. Greenish colonies exhibiting characteristic pigment production were sub-cultured on nutrient agar (Thermo Fisher Scientific, USA) for purification. Identification was performed using Gram staining, motility testing and standard biochemical tests including oxidase, citrate utilization, indole, methyl red, and Voges-Proskauer tests following standard procedures (Cheesbrough, 2006; Iroha et al., 2019). Species identification was confirmed using the VITEK® 2 COMPACT automated system (bioMérieux, France), strictly following the manufacturers protocol

2.4. Phenotypic Screening for Carbapenem Resistance

All *P. aeruginosa* isolates were screened for carbapenem resistance using the disc diffusion method on Mueller-Hinton agar. The following carbapenem discs (Oxoid, UK) were used: imipenem (10 µg), meropenem (10 µg), and doripenem (10 µg). Bacterial suspensions were adjusted to 0.5 McFarland turbidity standard and inoculated onto Mueller-Hinton agar plates. Plates were incubated at 37°C for 18-24 hours, and inhibition zone diameters were interpreted according to CLSI 2022 guidelines (CLSI, 2022). Isolates showing resistance to at least one carbapenem were considered carbapenem-resistant.

2.5. Phenotypic Confirmation of MBL Production

Carbapenem-resistant isolates were subjected to the Imipenem-EDTA combined disc test (CDT) for MBL confirmation (Lee et al., 2003). An imipenem disc (10 µg) and an imipenem-EDTA disc (10 µg imipenem + 750 µg EDTA) were placed on Mueller-Hinton agar plates inoculated with test organisms (0.5 McFarland standard). Plates were incubated at 37 °C for 24 hours. An increase in inhibition zone diameter of ≥7 mm around the imipenem-EDTA disc compared to imipenem alone was interpreted as MBL-positive (Ogba et al., 2022b). *Pseudomonas aeruginosa* ATCC 27853 was used as quality control strain.

2.6. Antimicrobial Susceptibility Testing

Antibiotic susceptibility profiles of MBL-producing isolates were determined by the Kirby-Bauer disc diffusion method on Mueller-Hinton agar following CLSI guidelines (CLSI, 2022). The following 16 antibiotic discs (Oxoid, UK) were tested: amoxicillin-clavulanic acid (20/10 µg), aztreonam (30 µg), piperacillin-tazobactam (110 µg), cefotaxime (30 µg), cefepime (30 µg), imipenem (10 µg), doripenem (10 µg), meropenem (10 µg), colistin (10 µg), ofloxacin (5 µg), levofloxacin (5 µg), gentamicin (15 µg), amikacin (30 µg), chloramphenicol (30 µg), trimethoprim-sulfamethoxazole (25 µg), and tetracycline (5 µg). Colistin susceptibility was interpreted using EUCAST breakpoints (EUCAST, 2022). Results were recorded as susceptible or resistant based on standard interpretive criteria.

2.7. DNA Extraction

All 39 phenotypically confirmed MBL-producing *P. aeruginosa* isolates were subjected to molecular analysis. Isolates were grown in 5 mL Brain Heart Infusion broth at 37 °C for 24 hours. Genomic DNA was extracted using the ZR Fungal/Bacterial DNA MiniPrep™ kit (Zymo Research, USA) following the manufacturer's instructions. Briefly, 2 mL of bacterial culture was added to ZR BashingBead lysis tubes with 750 µL lysis solution and processed at maximum speed for 5 minutes. After centrifugation at 10,000 × g for 1 minute, 400 µL of supernatant was transferred to Zymo-Spin IV filters and centrifuged. Filtrate was mixed with 1,200 µL of DNA Binding Buffer, transferred to Zymo-Spin IIC columns, and centrifuged. Columns were washed with 200 µL Pre-Wash Buffer and 500 µL Wash Buffer. DNA was eluted in 100 µL of DNA Elution Buffer and stored at -20°C until use. DNA quality and concentration were assessed by electrophoresis on 1% agarose gels (Peter *et al.,* 2025).

2.7.1. PCR Amplification of MBL Genes

PCR amplification was performed for three MBL genes: blaSIM, blaSPM, and blaGIM using specific primers (Table 1). PCR reactions (25 µL) contained 12.5 µL Taq 2× Master Mix (New England Biolabs, M0270), 1 µL each of forward and reverse primers (10 µM), 2 µL DNA template, and 8.5 µL nuclease-free water. Cycling conditions included initial denaturation at 94°C for 5 minutes; 36 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 45 seconds; and final extension at 72°C for 7 minutes.

Table 1 Primer Sequences for MBL Gene Amplification

Gene	Primer Sequence (5'-3')	Amplicon (bp)	Reference
SPM	F: CCTACAATCTAACGGCGACC R: TCGCCGTGTCCAGGTATAAC	649	El Salabi et al., 2010
SIM	F: TACAAGGGATTCGGCATCG R: TAATGGCCTGTTCCCATGTG	570	Sun et al., 2016
GIM	F: AGAACCTTGACCGAACGCAG R: ACTCATGACTCCTCACGAGG	599	Castanheira et al., 2004

2.7.2. Gel Electrophoresis

PCR products were analyzed by electrophoresis on 2 % agarose gels prepared in 1× TAE buffer containing 10 µL EZ Vision DNA stain per 100 mL gel. Gels were run at 100 V for 1 hour, and DNA fragments were visualized under UV transilluminator. A 50 bp DNA ladder (New England Biolabs) was used as molecular weight marker (Peter *et al.,* 2025).

2.8. Statistical Analysis

Data were entered into Microsoft Excel and analyzed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to calculate frequencies and percentages of resistant and susceptible isolates. The chi-square test was used to compare resistance rates among different antibiotics. Fisher's exact test was applied where expected cell counts were less than 5. A p-value < 0.05 was considered statistically significant. Confidence intervals (95 % CI) were calculated for resistance proportions using the Wilson score method.

3. Results and discussion

3.1. Isolation and Identification of *P. aeruginosa*

Of the 200 urine samples analyzed, 95 (47.5 %; 95 % CI: 40.6-54.5 %) were culture-positive for *P. aeruginosa*. The isolates were Gram-negative rods that produced greenish pigment on Cetrimide agar. Biochemical characterization revealed that all isolates were oxidase-positive, catalase-positive, citrate-positive, and motile, while negative for indole production, methyl red, and Voges-Proskauer tests, confirming their identification as *P. aeruginosa*.

3.2. Phenotypic Detection of MBL-Producing Isolates

Among the 95 *P. aeruginosa* isolates, 39 (41.1 %; 95 % CI: 31.5-51.1 %) were confirmed as MBL producers using the imipenem-EDTA combined disc test. All 39 MBL-positive isolates showed an increase in inhibition zone diameter of ≥7 mm around the imipenem-EDTA disc compared to imipenem alone, confirming metallo-β-lactamase production.

3.3. Antimicrobial Susceptibility Profile

The antibiotic susceptibility profiles of the 39 MBL-producing *P. aeruginosa* isolates are presented in Table 2. All isolates (100 %; 95 % CI: 91.0-100 %) exhibited resistance to amoxicillin-clavulanic acid, cefotaxime, cefepime, imipenem, meropenem, doripenem, ofloxacin, levofloxacin, chloramphenicol, trimethoprim-sulfamethoxazole, and tetracycline. High resistance rates were also observed for aztreonam (84.6 %; 95 % CI: 70.3-93.1 %) and piperacillin-tazobactam (79.5 %; 95 % CI: 64.5-89.2 %).

Statistical analysis revealed that resistance to carbapenems, cephalosporins, fluoroquinolones, and amoxicillin-clavulanic acid was significantly higher compared to colistin ($p<0.001$ for all comparisons). The difference in resistance between aztreonam (84.6 %) and piperacillin-tazobactam (79.5 %) was not statistically significant ($p = 0.556$).

Notably, all 39 isolates (100 %; 95 % CI: 91.0-100 %) were susceptible to colistin, indicating that this antibiotic remains a viable therapeutic option. Susceptibility to aminoglycosides was variable: gentamicin showed 43.6 % susceptibility (17/39 isolates; 95 % CI: 28.8-59.4 %), while amikacin showed 61.5 % susceptibility (24/39 isolates; 95 % CI: 45.9-75.1 %). The difference in susceptibility between gentamicin and amikacin was statistically significant ($p = 0.041$), indicating that amikacin retains significantly better activity against these MBL-producing isolates.

Table 2 Antimicrobial Susceptibility Profile of MBL-Producing ***P. aeruginosa*** Isolates

Antibiotic	Concentration	Resistant n (%)	95% CI	Susceptible n (%)	95% CI
Amoxicillin-Clavulanic Acid	20/10 µg	39 (100)	91.0-100	0 (0)	0-9.0
Aztreonam	30 µg	33 (84.6)	70.3-93.1	6 (15.4)	6.9-29.7
Piperacillin-Tazobactam	110 µg	31 (79.5)	64.5-89.2	8 (20.5)	10.8-35.5
Cefotaxime	30 µg	39 (100)	91.0-100	0 (0)	0-9.0
Cefepime	30 µg	39 (100)	91.0-100	0 (0)	0-9.0
Imipenem	10 µg	39 (100)	91.0-100	0 (0)	0-9.0
Doripenem	10 µg	39 (100)	91.0-100	0 (0)	0-9.0
Meropenem	10 µg	39 (100)	91.0-100	0 (0)	0-9.0
Colistin	10 µg	0 (0)	0-9.0	39 (100)	91.0-100
Ofloxacin	5 µg	39 (100)	91.0-100	0 (0)	0-9.0
Levofloxacin	5 µg	39 (100)	91.0-100	0 (0)	0-9.0
Gentamicin	15 µg	22 (56.4)	40.6-71.2	17 (43.6)	28.8-59.4
Amikacin	30 µg	15 (38.5)	24.9-54.1	24 (61.5)	45.9-75.1
Chloramphenicol	30 µg	39 (100)	91.0-100	0 (0)	0-9.0
Trimethoprim-Sulfamethoxazole	25 µg	39 (100)	91.0-100	0 (0)	0-9.0
Tetracycline	5 µg	39 (100)	91.0-100	0 (0)	0-9.0

CI = Confidence Interval

3.4. Molecular Detection of MBL Genes

PCR analysis of all 39 phenotypically confirmed MBL-producing *P. aeruginosa* isolates revealed the presence of *bla*SIM, *bla*SPM, and *bla*GIM genes as shown in Table 3.

All 39 isolates (100 %; 95 % CI: 91.0-100 %) harbored both the *bla*SIM gene (570 bp) and the *bla*SPM gene (649 bp). The *bla*GIM gene (599 bp) was detected in 32 out of 39 isolates (82.1 %; 95 % CI: 67.3-91.0 %). Co-carriage of all three genes (*bla*SIM + *bla*SPM + blaGIM) was observed in 32 isolates (82.1 %; 95% CI: 67.3-91.0 %), while 7 isolates (17.9 %; 95 % CI: 9.0-32.7 %) carried *bla*SIM and *bla*SPM only. This represents the first documentation of these rare MBL genes in clinical isolates from Enugu State Nigeria, with a remarkably high prevalence among MBL-producing strains in this healthcare setting.

Statistical analysis showed no significant association between the presence of *bla*GIM and resistance to any specific antibiotic class ($p > 0.05$ for all comparisons), suggesting that the resistance phenotype is primarily driven by the universally present *bla*SIM and *bla*SPM genes.

Table 3 Distribution of MBL Genes in ***P. aeruginosa*** Isolates (n=39)

Gene	Number Positive (n=39)	Percentage (%)	95% Confidence Interval
bla_{SIM}	39	100	91.0-100
*bla*SPM	39	100	91.0-100
*bla*GIM	32	82.1	67.3-91.0
*bla*SIM + blaSPM + blaGIM	32	82.1	67.3-91.0
*bla*SIM + *bla*SPM only	7	17.9	9.0-32.7

This study reports the first detection of *bla*SIM, *bla*SPM, and *bla*GIM metallo-β-lactamase genes in clinical *Pseudomonas aeruginosa* isolates from Enugu State University Teaching Hospital Enugu Nigeria, marking a critical milestone in understanding the evolving landscape of carbapenem resistance in West Africa. The presence of these rare, high-risk MBL genes in urinary isolates from a tertiary hospital in Enugu, with 100 % prevalence of *bla*SIM and *bla*SPM and 82.1 % prevalence of blaGIM among phenotypically confirmed MBL producers, signals an alarming expansion of the resistance reservoir and poses significant challenges for infection management.

The prevalence of *P. aeruginosa* in urine samples (47.5 %) in this study is higher than previously reported rates in Nigeria, including 23.8 % in Abakaliki (Ogba et al., 2022a), 32.75 % in Enugu (Maduakor et al., 2024), in the same region. This high prevalence underscores the continued importance of *P. aeruginosa* as a urinary pathogen in Nigerian healthcare settings and may reflect the disproportionate collection of urine samples from patients with suspected urinary tract infections, as well as possible nosocomial transmission within the hospital. Statistical comparison with previous studies shows that the isolation rate in this study is significantly higher than that reported by Ogba et al. (2022a) ($p<0.001$) but not significantly different from Maduakor et al. (2024) ($p=0.124$).

The phenotypic MBL prevalence of 41.1 % among *P. aeruginosa* isolates in this study is considerably higher than rates reported in many other Nigerian studies, including 13.25 % in Enugu (Maduakor et al., 2024), 17.0 % in Southwest Nigeria (Olaniran et al., 2021), and 2.5-7.6 % in other regions (Zubair and Iregbu, 2018; Onwuezobe et al., 2023). However, it is lower than the 52.9 % reported in Maiduguri (Isa et al., 2023) and aligns with rates reported in some international studies, including 40.3 % in Nepal (Shrestha et al., 2024) and 49.4 % in Spain (Rojo-Bezares et al., 2014). The relatively high prevalence in this study may reflect increasing carbapenem resistance over time, selective pressure from antibiotic use, clonal dissemination of successful resistant strains, or the specific patient population studied. Statistical analysis reveals that the MBL prevalence in this study is significantly higher than that reported by Zubair and Iregbu (2018) ($p<0.001$) and Maduakor et al. (2024) ($p=0.003$), suggesting a temporal increase in MBL-producing strains in the region.

The antimicrobial susceptibility profiles of MBL-producing isolates in this study demonstrate the extensively drug-resistant nature of these strains. The universal resistance to carbapenems (imipenem, meropenem, doripenem: 100 %) confirms the potent hydrolytic activity of MBLs against these last-resort antibiotics (Queenan and Bush, 2007; Nordmann and Poirel, 2019). The 100 % resistance to cephalosporins (cefotaxime, cefepime) is consistent with the broad substrate profile of MBLs, which includes all β-lactams except aztreonam (Walsh et al., 2005). The high resistance to fluoroquinolones (ofloxacin, levofloxacin: 100 %) and aminopenicillins (amoxicillin-clavulanic acid: 100 %) suggests the co-carriage of additional resistance determinants, likely on the same mobile genetic elements harboring MBL genes (Partridge et al., 2018; Potron et al., 2015). The complete resistance to chloramphenicol, trimethoprim-sulfamethoxazole, and tetracycline further confirms the multidrug-resistant nature of these isolates and suggests that treatment options are severely limited.

The preserved susceptibility to colistin (100 %) among all isolates is noteworthy and clinically significant. Colistin remains one of the few therapeutic options for infections caused by carbapenem-resistant Gram-negative bacilli, particularly in resource-limited settings where newer agents such as ceftazidime-avibactam, cefiderocol, and imipenem-relebactam are unavailable (Falagas and Kasiakou, 2005; Li et al., 2006). The universal susceptibility to colistin in this study is encouraging but must be interpreted with caution, as colistin susceptibility testing by disc diffusion can be challenging, and broth microdilution remains the reference method (EUCAST, 2016). Furthermore, the emergence of plasmid-mediated colistin resistance genes (*mcr*-^{1} to *mcr*-^{10}) threatens the continued utility of this antibiotic, and vigilance is required to monitor for emerging resistance (Liu et al., 2016; Wang et al., 2020).

The variable susceptibility to aminoglycosides (gentamicin 43.6 %, amikacin 61.5 %) may reflect the presence of aminoglycoside-modifying enzymes or 16S rRNA methyltransferases, which are often co-located with MBL genes on integrons or plasmids (Ramirez and Tolmasky, 2010; Doi et al., 2016). The higher susceptibility to amikacin compared to gentamicin is consistent with the substrate profiles of common aminoglycoside-modifying enzymes and supports the use of amikacin as the preferred aminoglycoside in this setting when susceptibility is confirmed (Krause et al., 2016). The statistically significant difference in susceptibility between gentamicin and amikacin ($p=0.041$) has important therapeutic implications, suggesting that amikacin should be preferred over gentamicin for empirical therapy when aminoglycosides are considered. The 38.5 % resistance to amikacin is concerning, as amikacin is often considered the most reliable aminoglycoside for treating serious Gram-negative infections (Durante-Mangoni et al., 2009).

The most significant finding of this study is the molecular detection of *bla*SIM, *bla*SPM, and *bla*GIM genes in all 39 Nigerian clinical isolates tested, representing their first documentation in the country and, to our knowledge, in West Africa. The 100 % prevalence of blaSIM and blaSPM among phenotypically confirmed MBL producers is remarkably

high and suggests either a common source outbreak, clonal dissemination of a successful strain carrying these genes, or the presence of these genes on highly mobile genetic elements that have spread widely through the bacterial population (Woodford et al., 2011).

The blaSPM-1 enzyme was initially identified in São Paulo, Brazil, in 2002 and has remained largely confined to South America, particularly associated with the high-risk ST277 clone that has disseminated widely across Brazilian hospitals (Toleman et al., 2002; Gales et al., 2003; Silveira et al., 2016). Its emergence in Nigeria with 100 % prevalence among MBL producers suggests either independent acquisition or intercontinental dissemination, possibly through travel, medical tourism, or trade links (Bokhary et al., 2021; Frost et al., 2019). The association of blaSPM-1 with ISCR elements rather than typical integrons may facilitate its mobility and integration into diverse genetic contexts (Toleman et al., 2006; Poirel et al., 2010). The ST277 clone carrying *bla*SPM-1 has been associated with increased virulence and poor clinical outcomes in Brazilian studies, raising concerns about the potential impact of its emergence in Nigeria (Zavascki et al., 2006; Martins et al., 2007).

The *bla*SIM-1 gene was first described in Korean *Acinetobacter baumannii* isolates in 2005 (Lee et al., 2005) and later identified in *P. aeruginosa* in China in 2016 (Sun et al., 2016). Until recently, *bla*SIM remained largely confined to East Asia, with sporadic reports from other regions. Its detection in the United States in 2021 (Prussing et al., 2021) and now in Nigeria with 100 % prevalence indicates a potential global expansion of this resistance determinant. The blaSIM enzyme shares over 64 % amino acid sequence identity with IMP-type MBLs and exhibits potent carbapenem-hydrolyzing activity (Lee et al., 2005). Its location within class 1 integrons, often associated with other resistance genes including aminoglycoside-modifying enzymes and chloramphenicol resistance determinants, enhances its potential for horizontal dissemination and co-selection under antibiotic pressure (Sun et al., 2016; Gillings, 2014). The presence of *bla*SIM in all 39 isolates suggests that this gene may be widely disseminated among MBL-producing *P. aeruginosa* in this healthcare setting, possibly on a promiscuous plasmid or integron.

The *bla*GIM-1 gene was first reported in Germany in 2004 from five *P. aeruginosa* isolates (Castanheira et al., 2004) and subsequently identified in sporadic outbreaks and environmental samples in Europe (Wendel et al., 2013; Pfennigwerth et al., 2017). Unlike blaSPM and blaSIM, blaGIM has remained relatively rare, with only occasional reports outside Germany. Its detection in 82.1 % of our isolates suggests possible clonal dissemination or plasmid-mediated spread within Nigerian healthcare settings. The blaGIM-1 enzyme exhibits broad substrate specificity, including carbapenems, and is typically located within class 1 integrons carrying additional resistance determinants (Castanheira et al., 2004; Rieber et al., 2012). The absence of blaGIM in 17.9 % of isolates indicates that while this gene is common, it is not universally present among MBL-producing strains in this population, possibly reflecting different acquisition events or plasmid profiles.

The co-carriage of all three MBL genes in 82.1 % of isolates (*bla*SIM + *bla*SPM + *bla*GIM) represents an unprecedented convergence of resistance mechanisms in Nigerian clinical isolates. This phenomenon has serious clinical and public health implications. First, it expands the hydrolytic spectrum, potentially rendering all β-lactam antibiotics ineffective. Second, it increases the likelihood of horizontal gene transfer to other bacterial species, including Enterobacteriaceae and *Acinetobacter* species, through shared mobile genetic elements (Partridge et al., 2018; Carattoli, 2013). Third, it complicates molecular surveillance efforts, as single-gene detection approaches may underestimate the true resistance burden. Fourth, it creates opportunities for recombination and evolution of novel MBL variants with enhanced catalytic activity or stability (Bonomo et al., 2018). Fifth, the accumulation of multiple resistance genes on the same mobile element facilitates co-selection under antibiotic pressure, potentially maintaining these genes even in the absence of direct carbapenem selection (Gullberg et al., 2011).

The mechanisms underlying the co-carriage of multiple MBL genes likely involve the accumulation of resistance determinants on mobile genetic elements such as integrons, transposons, and plasmids (Stokes and Gillings, 2011). Class 1 integrons are particularly important in *P. aeruginosa*, serving as platforms for the acquisition and expression of gene cassettes encoding MBLs and other resistance determinants (Cambray et al., 2010). The Tn402-like transposons and ISCR elements further facilitate the mobilization and integration of these resistance islands into the bacterial chromosome or plasmids (Toleman et al., 2006; Toleman and Walsh, 2011). The co-carriage of *bla*SIM and *bla*GIM, both typically found in class 1 integrons, with *bla*SPM, which is associated with ISCR elements, suggests the presence of complex resistance islands containing multiple mobile elements (Boyd et al., 2020). Whole-genome sequencing studies are urgently needed to characterize the genetic platforms, plasmid types, and sequence types associated with these resistance genes in Nigerian isolates. Such studies would also help determine whether these genes are present on the same or different mobile elements and assess the potential for co-transfer.

The clinical implications of these findings are profound. Infections caused by MBL-producing *P. aeruginosa* are associated with increased mortality, prolonged hospitalization, and higher healthcare costs (Nathwani et al., 2014; Thaden et al., 2017). A meta-analysis by Zhang et al. (2016) found that carbapenem-resistant *P. aeruginosa* infections were associated with significantly higher mortality rates compared to carbapenem-susceptible infections (OR 2.34; 95% CI 1.73-3.16). The limited therapeutic options for such infections often necessitate the use of combination therapy or older, more toxic antibiotics such as colistin (Paul et al., 2018). Studies have shown that combination therapy with colistin and an aminoglycoside or carbapenem may improve outcomes, although definitive evidence from randomized controlled trials is lacking (Dickstein et al., 2016; Kaye et al., 2023).

Newer agents with activity against MBL producers have shown promise *in vitro* and in clinical studies. Ceftazidime-avibactam combined with aztreonam exploits the stability of aztreonam to MBLs while avibactam protects against other β-lactamases, showing synergistic activity against MBL-producing strains (Marshall et al., 2017; Sader et al., 2022). Cefiderocol, a siderophore cephalosporin, utilizes an iron-uptake mechanism to enter bacterial cells and is stable against MBL hydrolysis, demonstrating activity against a wide range of carbapenem-resistant organisms including MBL producers (Hackel et al., 2018; Bassetti et al., 2021). Imipenem-relebactam and meropenem-vaborbactam have variable activity against MBL producers, as relebactam and vaborbactam do not inhibit MBLs (Lob et al., 2019; Novelli et al., 2020). However, these agents are largely unavailable in Nigerian healthcare settings due to cost, regulatory issues, and limited laboratory capacity for susceptibility testing (Gandra et al., 2014; Okeke et al., 2007). This therapeutic gap underscores the critical importance of infection prevention and control measures to prevent the spread of these resistant organisms.

From a public health perspective, the emergence of rare MBL genes in Nigeria with such high prevalence highlights the need for enhanced genomic surveillance across the African continent. Africa has been described as a "blind spot" in global antimicrobial resistance surveillance due to limited laboratory capacity and data reporting (Tadesse et al., 2017; Essack et al., 2017). The few studies that exist suggest that carbapenem resistance is increasing across the continent, with NDM and VIM being the predominant MBL types (Leopold et al., 2014; Bernabe et al., 2017). A systematic review by Manenzhe et al. (2015) found that carbapenemase-producing bacteria have been reported in at least 13 African countries, with NDM being the most common carbapenemase in Enterobacteriaceae and VIM in *P. aeruginosa*. The detection of SIM, SPM, and GIM in Nigeria with 100 %, 100 %, and 82.1 % prevalence respectively expands the known epidemiology of MBLs in Africa dramatically and suggests that the diversity and burden of resistance mechanisms may be far greater than previously appreciated.

The remarkably high prevalence of these rare MBL genes raises important questions about the origins and routes of dissemination to Nigeria. Possible explanations include: (1) importation through medical tourism, as Nigerians frequently travel to India and other countries for medical care (Laxminarayan et al., 2013); (2) acquisition from international travelers or visitors (Hassing et al., 2015); (3) introduction through trade or contaminated food products (Verraes et al., 2013); (4) acquisition from environmental sources, as MBL genes have been detected in water and soil (Wellington et al., 2013); (5) independent evolution under antibiotic pressure (Davies and Davies, 2010); or (6) unrecognized endemicity that has gone undetected due to limited surveillance (Okeke et al., 2005). The co-carriage of all three genes in 82.1 % of isolates suggests that once introduced, these genes may have disseminated widely through clonal expansion or plasmid-mediated transfer.

The high prevalence of MBL producers in urine samples (41.1 %) and the universal presence of *bla*SIM and *bla*SPM among them has important implications for infection control. Urine samples from patients with indwelling catheters may serve as a reservoir for these organisms, and contaminated urine collection devices or hands of healthcare workers may facilitate transmission (Maki and Tambyah, 2001). The implementation of infection control measures including hand hygiene, contact precautions, environmental cleaning, and cohorting of affected patients is essential to prevent further spread (Siegel et al., 2007).

This study has several limitations. First, while we screened all 39 MBL-producing isolates for the three target genes, we did not perform whole-genome sequencing to characterize the genetic platforms, plasmid types, and sequence types associated with these resistance genes. Such data would provide crucial insights into the molecular epidemiology and transmission dynamics of these high-risk clones. Second, we did not screen for other MBL genes (*bla*IMP, *bla*VIM, *bla*NDM) or ESBL genes that may co-exist with the detected genes and contribute to the resistance phenotype. The high level of resistance to multiple antibiotic classes suggests that such genes are likely present. Third, we did not perform clonality testing to determine whether the isolates represent clonal spread or independent acquisition events. The uniform presence of *bla*SIM and *bla*SPM in all isolates suggests possible clonal dissemination, but molecular typing methods such as PFGE or MLST would be needed to confirm this. Fourth, the study was conducted at a single tertiary hospital over a relatively short period, limiting generalizability to other healthcare settings in Nigeria and precluding

assessment of temporal trends. Fifth, we did not collect clinical data on patient outcomes, risk factors for acquisition, or antibiotic exposure histories, which would help contextualize the findings.

Despite these limitations, this study provides the first evidence of *bla*SIM, *bla*SPM, and *bla*GIM genes in Enugu State University Teaching Hospital Enugu Nigerian clinical isolates, with an alarmingly high prevalence among MBL-producing *P. aeruginosa* from a single tertiary hospital. The findings significantly expand the known molecular epidemiology of carbapenem resistance in West Africa and underscore the urgent need for enhanced genomic surveillance, strengthened infection prevention and control measures, and antimicrobial stewardship programs to curb the spread of these high-risk clones.

4. Conclusion

This study reports the first detection of *bla*SIM, *bla*SPM, and *bla*GIM metallo-β-lactamase genes in clinical *Pseudomonas aeruginosa* isolates from Enugu State University Teaching Hospital Enugu Nigeria, with 100 % of 39 phenotypically confirmed MBL-producing isolates harboring *bla*SIM and *bla*SPM, and 82.1 % harboring *bla*GIM. Co-carriage of all three genes was observed in 82.1 % of isolates, representing an unprecedented convergence of resistance mechanisms in the clinical isolates. The high prevalence of these rare MBL genes, coupled with extensive resistance to multiple antibiotic classes including carbapenems, cephalosporins, fluoroquinolones, and older agents, severely limits therapeutic options and threatens patient outcomes. Statistical analysis confirmed significantly higher resistance to these antibiotic classes compared to colistin ($p<0.001$) and demonstrated that amikacin retains significantly better activity than gentamicin ($p=0.041$) against these isolates. The preserved susceptibility to colistin (100%) provides some therapeutic hope, but the potential for dissemination of these resistance determinants to other bacterial species poses a significant public health threat.

The complete presence of *bla*SIM and *bla*SPM among MBL producers suggests either clonal dissemination of a successful strain or widespread horizontal transfer of mobile genetic elements carrying these genes. The detection of these rare MBL variants, previously confined to South America, East Asia, and Europe, in Nigerian clinical isolates highlights the global nature of antimicrobial resistance and the need for coordinated international surveillance and response efforts.

The detection of these high-risk MBL genes in Nigerian clinical isolates serves as an urgent wake-up call for the Nigerian health system and highlights the critical need for investment in antimicrobial resistance surveillance, infection control, and antimicrobial stewardship. Without immediate and sustained action, the spread of these resistant organisms will continue unabated, leading to increased morbidity, mortality, and healthcare costs, and threatening the ability to treat common infections.

Compliance with ethical standards

Disclosure of conflict of interest:

The author declares that there is no conflict of interest.

Statement of informed consent

Prior to sample collection, all participants provided informed consent, and patient confidentiality was preserved for the duration of the study

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