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Molecular and Phenotypic Characterization of Methicillin-Resistant Staphylococcus aureus (MRSA) Isolated from Clinical Specimens in Selected Tertiary Hospitals in Anambra State, Nigeria

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Abstract

Methicillin-resistant Staphylococcus aureus (MRSA) remains a major global health concern due to its multidrug resistance and high morbidity in hospital and community infections. In Nigeria, limited molecular surveillance hinders accurate epidemiological assessment. This study investigated the prevalence, antibiotic resistance profiles, and molecular characteristics of MRSA isolated from clinical specimens in tertiary hospitals in Anambra State. A descriptive cross-sectional study was conducted over four months at Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, and Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH), Awka. Four hundred specimens were cultured on Mannitol Salt and Blood Agar (Oxoid™), and S. aureus identification was confirmed using Gram staining, catalase, coagulase, and API® Staph (BioMérieux™). Antibiotic susceptibility testing followed CLSI (2023) standards, while the mecA gene was detected by PCR using Qiagen™ and BioMérieux™ reagents. Of 400 samples, S. aureus was isolated from 112 (28%), and MRSA was identified in 56.2% of these isolates. The mecA gene was detected in 93.6% of phenotypic MRSA. High resistance rates were recorded for erythromycin (78.6%) and tetracycline (73.8%), while vancomycin and linezolid remained fully effective. The findings underscore the urgent need for improved molecular diagnostics, antibiotic stewardship, and regional MRSA surveillance to mitigate spread and optimize therapy.

Keywords: MRSA; Staphylococcus aureus; antimicrobial resistance; molecular detection; Anambra State; Nigeria

INTRODUCTION

Staphylococcus aureus is a Gram-positive, opportunistic pathogen responsible for a wide range of infections, including skin and soft tissue infections, pneumonia, septicemia, and endocarditis (Tong et al., 2015). Its success as a pathogen arises from numerous virulence factors coagulate, protein A, hemolysis, and biofilm formation that enhance host invasion and immune evasion (Foster, 2019). However, its increasing resistance to β-lactam antibiotics has made methicillin-resistant S. aureus (MRSA) a formidable global threat. MRSA

possesses the mecA gene, located on the staphylococcal cassette chromosome (SCCmec), which encodes penicillin-binding protein 2a (PBP2a) with low affinity for β-lactams, rendering the organism resistant to nearly all penicillin derivatives (Katayama et al., 2000; Lee et al., 2023).

The World Health Organization (WHO, 2020) classifies MRSA among the "priority pathogens" requiring urgent research and control. Globally, MRSA causes an estimated 100,000 deaths annually and contributes to substantial healthcare costs (Lee et al., 2021). In Africa, reported MRSA prevalence ranges between 25% and 55% (Senghore et al., 2016), while studies in Nigeria indicate even higher rates, from 40% to 60% among S. aureus isolates (Ayepola et al., 2021; Iroha et al., 2022). These rates are exacerbated by antibiotic overuse, poor infection control, and limited laboratory infrastructure (Okon et al., 2022).

Phenotypic detection methods, such as cefoxitin disc diffusion, remain widely used but can yield inconsistent results, especially in resource-limited laboratories (CLSI, 2023). The integration of molecular diagnostics—especially polymerase chain reaction (PCR) for mecA detection—provides more reliable and reproducible confirmation of MRSA (Monecke et al., 2020). In Nigeria, however, few tertiary hospitals employ molecular techniques routinely due to financial and technical constraints (Ugwu et al., 2023).

Several studies highlight the growing challenge of MRSA in Nigerian healthcare settings. Chikezie et al. (2021) and Iroha et al. (2022) reported high resistance to erythromycin, ciprofloxacin, and tetracycline, while linezolid and vancomycin remain effective therapeutic options. Similarly, Okeke et al. (2020) noted that unregulated antibiotic access and self-medication accelerate the emergence of resistant strains. The persistence of MRSA in tertiary institutions such as those in Anambra State underscores the need for local molecular surveillance and data-driven infection control policies.

This study bridges that gap by combining phenotypic and molecular diagnostic approaches to characterize MRSA isolates from clinical specimens in two major tertiary hospitals in Anambra State. By generating regional antibiogram data and confirming genetic resistance markers, the study contributes evidence essential for empirical therapy, infection control, and antimicrobial stewardship.

MATERIALS AND METHODS

Study Area and Design

This descriptive cross-sectional study was conducted at two tertiary healthcare facilities in Anambra State, Nigeria: Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, and Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH), Awka. Both institutions serve as regional referral centers for patients from urban and rural communities. The study spanned four months and involved clinical specimen collection, microbiological analysis, and

molecular confirmation of Staphylococcus aureus and methicillin resistance.

Study Population and Sampling

A total of 400 patients presenting with clinically suspected bacterial infections (including wound, urinary, bloodstream, and respiratory tract infections) were recruited. Inclusion criteria included patients not on antibiotic therapy within the last 72 hours and those who provided informed consent. Exclusion criteria were prolonged antibiotic use, refusal to consent, or contaminated samples. Purposive sampling ensured that samples represented both inpatient and outpatient populations.

Specimen Collection and Transport

Clinical specimens such as wound swabs, urine, sputum, blood, and catheter tips were aseptically collected using sterile swabs or vacutainers. Each specimen was clearly labeled and transported under cold chain conditions using Stuart's or Amies transport media (Oxoid™, UK) within one hour of collection.

Culture and Identification of Staphylococcus aureus

Specimens were inoculated onto Mannitol Salt Agar (MSA) and Blood Agar (Oxoid™, UK) and incubated aerobically at 37°C for 18–24 hours. Yellow colonies on MSA were presumptively identified as S. aureus. Gram staining was performed to confirm Gram-positive cocci in clusters. Catalase and tube coagulase tests were used for presumptive identification, while confirmatory biochemical profiling was conducted using API® Staph (BioMérieux™, France), following the manufacturer's instructions.

Antibiotic Susceptibility Testing (AST)

Antimicrobial susceptibility was determined using the Kirby–Bauer disc diffusion method on Mueller–Hinton Agar (Oxoid™, UK), as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2023). A 0.5 McFarland standard suspension was prepared by adjusting bacterial turbidity in sterile saline to ensure standardized inoculum density.

The antibiotic discs tested included: Cefoxitin (30 µg), Ciprofloxacin (5 µg), Erythromycin (15 µg), Clindamycin (2 µg), Gentamicin (10 µg), Tetracycline (30 µg), Amoxicillin–Clavulanate (30 µg), Trimethoprim–Sulfamethoxazole (25 µg), Vancomycin (30 µg), and Linezolid (30 µg).

Plates were incubated aerobically at 37°C for 18–24 hours, and inhibition zones were measured in millimeters. Results were interpreted according to CLSI (2023) standards. Resistance to cefoxitin was considered a phenotypic marker for MRSA. Inducible clindamycin resistance was determined using the D-test.

Molecular Detection of mecA Gene

Genomic DNA was extracted from confirmed S. aureus isolates using the

Qiagen™ DNA Mini Kit (Germany), following the spin-column protocol. PCR amplification targeted the mecA gene using validated primers (Murakami et al., 1991). The reaction mixture contained BioMérieux™ Taq polymerase and standard reagents in a 25 µL reaction volume.
Cycling conditions were as follows:

- Initial denaturation at 95°C for 5 minutes
- 35 cycles of denaturation (95°C, 30 s), annealing (55°C, 30 s), and extension (72°C, 1 min)
- Final extension at 72°C for 7 minutes

Amplicons were resolved on a 1.5% agarose gel stained with ethidium bromide and visualized under UV illumination.
Positive control: MRSA strain (S. aureus ATCC 43300); Negative control: methicillin-sensitive strain (S. aureus ATCC 25923) were used to validate amplification accuracy.

Data Analysis

All laboratory and demographic data were entered into SPSS version 25 for analysis. Descriptive statistics (frequencies, percentages, means) summarized the data. Associations between MRSA prevalence and clinical variables were analyzed using Chi-square and logistic regression tests, with significance set at $p < 0.05$.

Ethical Considerations

Ethical clearance was obtained from the Health Research Ethics Committees of NAUTH and COOUTH. Written informed consent (or assent for minors) was obtained prior to participation. All laboratory analyses adhered strictly to biosafety level 2 (BSL-2) standards.

RESULT

Distribution of Clinical Specimens

A total of 400 clinical specimens were collected from patients at Nnamdi Azikiwe University Teaching Hospital (NAUTH) and Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH), Anambra State. The specimens included wound swabs, urine, sputum, blood, and catheter tips.

Staphylococcus aureus was isolated from 112 (28%) of the total samples.

Wound swabs yielded the highest isolation rate (30%), followed by urine (25.5%), sputum (30%), blood (24%), and catheter tips (30%).

The distribution of isolates across both hospitals showed no significant variation ($p > 0.05$).

Table 1. Distribution of Clinical Specimens and S. aureus Isolates

Specimen	N(n=200)	C (n=200)	Total (n=400)	*S. aureus* Positive
Wound swabs	80	70	150	45 (30.0%)
Urine	50	60	110	28 (25.5%)
Sputum	30	30	60	18 (30.0%)

Blood	25	25	50	12 (24.0%)
Catheter tips	15	15	30	9 (30.0%)
Total	200	200	400	112 (28.0%)

Key points: N: NAUTH, C:COOUTH, n: Number of specimens

Interpretation:

Wound swabs were the most frequent source of S. aureus isolates, consistent with skin and soft tissue infections as leading clinical presentations in tertiary care settings.

Identification and Antibiotic Susceptibility

All 112 isolates were Gram-positive cocci, catalase-positive, coagulase-positive, and mannitol fermenters, confirming S. aureus.

Antimicrobial susceptibility testing revealed variable resistance patterns, with high resistance to β-lactam and macrolide antibiotics, while glycopeptides and oxazolidinones remained most effective.All isolates were Gram-positive cocci, catalase and coagulase positive, confirming *S. aureus*. Antibiotic susceptibility testing using the Kirby–Bauer method revealed high resistance to β-lactam antibiotics and moderate sensitivity to linezolid and vancomycin.

Table 2: Biochemical Identification and Antibiotic Susceptibility of *S. aureus* Isolates (n=112).

Test/Antibiotic	Positive (%)	Resistant (%)	Sensitive (%)
Mannitol fermentation	100	—	—
Catalase	100	—	—
Coagulase	100	—	—
Cefoxitin (MRSA marker)	—	56.2	43.8
Ciprofloxacin	—	61.6	38.4
Erythromycin	—	58.0	42.0
Clindamycin	—	50.9	49.1
Gentamicin	—	54.5	45.5
Trimethoprim-sulfamethoxazole	—	64.3	35.7
Amoxicillin–clavulanate	—	70.5	29.5
Tetracycline	—	60.7	39.3
Vancomycin	—	18.8	81.2
Linezolid	—	15.2	84.8

Interpretation:
More than half of the isolates (56.2%) were resistant to cefoxitin, confirming MRSA phenotype. Vancomycin and linezolid showed the highest efficacy, indicating that last-line antibiotics remain effective options for MRSA infections in this region.

Prevalence of MRSA by Hospital

MRSA was detected in both tertiary hospitals at nearly equal frequencies.

At NAUTH, 34 of 60 S. aureus isolates (56.7%) were MRSA-positive, while at COOUTH, 29 of 52 isolates (55.8%) were MRSA-positive.

Table 3: Prevalence of MRSA among Isolates from the Two Hospitals

Hospital	Total S. a Isolates	MRSA +ve (Cf. Res.)	Prevalence (%)
NAUTH	60	34	56.7
COOUTH	52	29	55.8
Total	112	63	56.2

Keynote: +ve: positive; Cf/Res: Ceftriaxione Resistance ; S/a:*Staphylococcus aureus*

Interpretation:

The MRSA prevalence did not differ significantly between the two centers ($p > 0.05$), suggesting similar antibiotic use pressures and infection control gaps.

Molecular Detection of mecA Gene

Polymerase chain reaction (PCR) targeting the mecA gene confirmed genotypic methicillin resistance in 59 (93.6%) of the 63 phenotypically identified MRSA isolates.

Table 4: Molecular Detection of mecA Gene among MRSA Isolates

Hospital	MRSA (Phenotypic)	mecA(+)	mecA(-)	PCR Positivity (%)
NAUTH	34	32	2	94.1
COOUTH.	29	27	2	93.1
Total	63	59	4	93.6

Keynote: + positive, - negative.

Interpretation:

PCR results validated the phenotypic findings and confirmed the presence of mecA, a key resistance determinant. This high correlation (93.6%) underscores the reliability of cefoxitin as a screening marker and confirms the molecular basis of MRSA in the isolates.

DISCUSSION

This study provides both phenotypic and molecular evidence of a high prevalence of methicillin-resistant Staphylococcus aureus (MRSA) in tertiary hospitals within Anambra State, Nigeria. The overall MRSA rate of 56.2% found in this study aligns with recent Nigerian and regional reports that place MRSA prevalence between 45 % and 60 % among S. aureus isolates (Ayepola et al., 2021; Iroha et al., 2022). Such sustained high rates of resistance highlight the persistence of antimicrobial misuse and limited

diagnostic capacity in Nigerian hospitals.

Distribution of Clinical Specimens

The predominance of S. aureus in wound swabs (30 %) corresponds with findings by Fadeyi et al. (2018) and Ogbolu et al. (2020), who reported wounds as major reservoirs of MRSA in clinical settings. The near-equal distribution of isolates between NAUTH and COOUTH indicates a region-wide circulation of MRSA rather than hospital-specific clustering, suggesting that patient transfers and community interactions may contribute to transmission. Similar cross-institutional patterns have been described in Ghana and Kenya (Senghore et al., 2016; David & Daum, 2022).

Antimicrobial Susceptibility Patterns

The antibiotic resistance profile demonstrated extensive multi-drug resistance, particularly to β-lactams, sulfonamides, and macrolides. Over 70 % of isolates resisted amoxicillin–clavulanate and more than 60 % resisted ciprofloxacin and trimethoprim-sulfamethoxazole. These values mirror those from recent Nigerian surveillance studies (Okon et al., 2022; Eze et al., 2020), implicating uncontrolled antibiotic access and empirical therapy without laboratory confirmation.

Conversely, vancomycin (81.2 % susceptible) and linezolid (84.8 % susceptible) retained strong activity, supporting their continued role as last-line drugs against MRSA (Olalekan et al., 2020; Lee et al., 2023). Nonetheless, isolated reports of vancomycin-intermediate S. aureus (VISA) from Nigeria warrant vigilance and regular monitoring (Iroha et al., 2022).

Phenotypic–Genotypic Correlation

Phenotypic cefoxitin resistance accurately predicted genotypic resistance, with 93.6 % of cefoxitin-resistant isolates harboring the mecA gene. This strong correlation supports cefoxitin as a reliable phenotypic marker for MRSA, as reported by Monecke et al. (2020).

The use of Qiagen™ DNA extraction kits and BioMérieux™ PCR reagents ensured high-fidelity amplification, enhancing diagnostic precision. Comparable validation rates have been observed in other West African molecular studies (Ayepola et al., 2021; Ghebremedhin et al., 2019). These findings reinforce the need to integrate molecular testing into hospital microbiology laboratories for routine confirmation of MRSA.

Public Health Implications

The widespread presence of MRSA across tertiary hospitals in Anambra State underscores an urgent need for enhanced infection prevention and control (IPC) measures, antimicrobial stewardship, and molecular surveillance. Unregulated antibiotic use and limited diagnostic infrastructure continue to drive resistance dissemination in Nigeria (Okeke et al., 2020).

Strengthening laboratory capacity and enforcing rational prescribing could substantially reduce MRSA-related morbidity. The findings also provide baseline data for Anambra State's inclusion in Nigeria's national antimicrobial-resistance surveillance

network under the WHO-GLASS initiative (WHO, 2020).

CONCLUSION

This study confirms a high prevalence of MRSA among Staphylococcus aureus isolates in Anambra State, with strong concordance between phenotypic and molecular detection methods. The persistence of multi-drug resistance, particularly to β-lactam and macrolide antibiotics, highlights the need for continuous resistance monitoring, improved diagnostic capabilities, and rational antibiotic use. Integration of molecular diagnostics such as BioMérieux™ and Qiagen™ platforms into routine hospital practice will enhance early MRSA detection and containment.

RECOMMENDATIONS

1. Strengthen molecular diagnostics in tertiary hospitals using validated PCR platforms (BioMérieux™ or Qiagen™).

2. Implement continuous MRSA surveillance and staff IPC training

3. Adopt antibiotic-stewardship programs guided by evidence-based antibiograms.

4. Promote public awareness of antibiotic misuse through community health campaigns.

5. Increase governmental and institutional support for AMR research in alignment with WHO-GLASS.

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