

Genotypic and Phenotypic Divergence of MRSA in Northwestern Nigeria: Strengthening Actionable Surveillance and Clinical Diagnostics

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Abstract

Background

Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a High Priority Pathogen on the World Health Organization (WHO) Bacterial Priority Pathogens List. However, phenotypic disc diffusion screening frequently overestimates *mecA*-mediated resistance in sub-Saharan Africa, creating significant diagnostic and therapeutic challenges. This study evaluated the phenotypic resistance profiles, *mecA* gene carriage, structural *SCCmec* cassette architectures, *spa* clonal diversity, and phylogenetic relationships of clinical *S. aureus* isolates across Northwestern Nigeria.

Methods

An 18-month (October 2019-July to 2021) multi-center cross-sectional surveillance study was executed across nine tertiary and specialist hospitals in Kano, Kaduna, Katsina, and Jigawa states. A total of 800 paired wound and nasal swab specimens were collected from 400 patients presenting with suppurative septic wounds. Phenotypic methicillin resistance was screened using 30µg cefoxitin disc diffusion per CLSI guidelines. Molecular confirmation of *mecA* and *SCCmec* structural types (I–V) was performed via multiplex PCR. Polymorphic X-region mapping of the *spa* gene was resolved through bidirectional Sanger chain-termination sequencing. Evolutionary relationships and ancestral distances were reconstructed using the Neighbor-Joining method in MEGA11.

Results

Phenotypic screening provisionally classified 144 clinical isolates (34.9%) as MRSA. Polymerase Chain Reaction (PCR) amplification confirmed the canonical *mecA* determinant in only 9 isolates (6.25%), leaving 135 phenotypic MRSA isolates (93.75%) as *mecA*-negative. Confirmed *mecA*-positive strains were restricted to urban tertiary centers in Kano and Kaduna states. *SCCmec* structural typing revealed Type III in 3 isolates (33.3%), Type II in 1 isolate (11.1%), and non-typeable (NT) elements in 5 isolates (55.6%). High-resolution *spa* typing identified four distinct clonal lineages: t037, t425, t1123, and t1223. Neighbor-Joining phylogenetic analysis demonstrated complete evolutionary separation of local Nigerian isolates from global NCBI reference genomes, forming distinct regional sub-clusters at ancestral nodes 47, 64, and 74.

Conclusions

A profound diagnostic divergence (93.75%) exists between phenotypic cefoxitin screening and *mecA* gene carriage in Northwestern Nigeria. Relying solely on disc diffusion over-diagnoses classical MRSA, driving inappropriate glycopeptide escalation and selection pressure for vancomycin resistance. Regional surveillance networks must integrate secondary target screening (*blaZ* hyper-expression, *pbp* point mutations, *mecC*) alongside genomic surveillance to accurately characterize non-*mecA*-mediated resistance phenotypes.

1.0 INTRODUCTION

Antimicrobial resistance (AMR) represents a significant challenge to global health [1], with *Staphylococcus aureus* (*S. aureus*) serving as a prominent contributor to both morbidity and mortality rates. This bacterium is responsible for a variety of infections, ranging from minor dermatological conditions to severe illnesses such as bacteremia and endocarditis [2, 3]. The World Health Organization (WHO) has classified methicillin-resistant *Staphylococcus aureus* (MRSA) as a High Priority Pathogen in its 2024 Bacterial Priority Pathogens List, highlighting the urgent need for

improved surveillance and responsible treatment protocols [1, 4]. The emergence of methicillin resistance in *S. aureus* primarily results from the horizontal transfer of the staphylococcal cassette chromosome *mec* (*SCCmec*), which harbors the *mecA* gene [3, 5]. This gene encodes penicillin-binding protein 2a (*PBP2a*), which exhibits a diminished affinity for beta-lactam antibiotics, consequently leading to cross-resistance [2, 5]. Polymerase chain reaction (PCR) for the detection of *mecA* is deemed the gold standard for identifying MRSA in clinical practice [6]

In sub-Saharan Africa, the limited diagnostic infrastructure often hinders the routine implementation of PCR testing for the *mecA* gene [7]. As a result, clinical microbiology laboratories predominantly employ phenotypic tests, notably the disc diffusion method utilizing a 30 µg cefoxitin disc, in accordance with the guidelines established by the Clinical and Laboratory Standards Institute, to evaluate methicillin resistance and guide treatment decisions [7, 8]. Cefoxitin is preferred over oxacillin due to its superior ability to stimulate *PBP2a* expression [6]. However, regional surveillance data collected from various parts of Africa indicate a growing divergence between phenotypic assessments of cefoxitin susceptibility and the molecular identification of *mecA* [7, 8]. Phenotypic testing frequently overestimates methicillin resistance, resulting in false-positive classifications of methicillin-resistant *Staphylococcus aureus* (MRSA) in isolates that do not harbor the *mecA* gene [8]. This diagnostic inconsistency considerably undermines antimicrobial stewardship, adversely affecting clinical decision-making and distorting regional epidemiological reporting [7, 8].

Phenotypic tests frequently overestimate methicillin resistance, resulting in false-positive classifications of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in isolates that do not possess the *mecA* gene [8]. This inconsistency complicates antimicrobial stewardship and adversely affects clinical decision-making and the accuracy of epidemiological data [7, 8]. In Northwestern Nigeria, major urban areas encounter significant challenges, as patients frequently present with severe wounds and infections [9, 10]. The prevalent practice of prescribing antibiotics empirically, coupled with the over-the-counter availability of these medications and limited infection control resources, exacerbates the situation [9, 10]. When clinical laboratories identify *S. aureus* as "phenotypically MRSA" through cefoxitin testing, healthcare providers are often inclined to avoid beta-lactam treatments in favor of glycopeptides such as vancomycin [7, 9]. Consequently, this practice generates two primary concerns: the misclassification of *mecA*-negative isolates leads to unnecessary and costly treatments, while the overuse of vancomycin increases selective pressure on staphylococcal populations, thereby heightening the risk of Vancomycin-Intermediate (VISA) and Vancomycin-Resistant *S. aureus* (VRSA) strains [7, 9]. The unnecessary administration of vancomycin exerts significant selection pressure on staphylococcal populations, raising the likelihood of VISA and VRSA strains [1, 11]. Biological factors contributing to *mecA*-negative resistance involve complex evolutionary mechanisms [12]; Among these are Borderline Oxacillin-Resistant *S. aureus* (BORSA), characterized by elevated expression of inducible beta-lactamase enzymes (*blaZ*), which can lead to increased minimum inhibitory concentrations (MICs) surpassing standard resistance thresholds [12].

Additionally, Modified Penicillin-Binding Protein *S. aureus* (MOD-SA) is identified by amino acid alterations in the transpeptidase domains of penicillin-binding proteins that diminish binding affinity for beta-lactams, even in the absence of *mecA* or *mecC* variants [13]. Various genetic variants such as *mecC*, *mecB*, and *mecD* exhibit approximately 70% similarity to *mecA*, enabling them to evade detection by standard polymerase chain reaction (PCR) tests, which may subsequently result in false-negative MRSA classifications [14, 15]. Furthermore, the molecular epidemiology and clonal lineages of *SCCmec* cassettes and *spa* types within Northwestern Nigeria remain inadequately defined [3, 16]. Although globally recognized epidemic clones exist, the divergence of local strains in West Africa from established reference genomes requires further investigation [16, 17]. The integration of genomic surveillance is critical to addressing these challenges [17]. In response to the diagnostic gap and the scarcity of genomic data, an 18-month multicenter study was conducted across nine hospitals situated in Kano, Kaduna, Katsina, and Jigawa states. The study aimed to quantify diagnostic divergence, profile structural and clonal diversity, map evolutionary trajectories, and strengthen actionable policies. Ultimately, the goal was to provide empirical data to enhance diagnostic stewardship and antimicrobial resistance (AMR) surveillance, thereby reducing the misuse of reserve glycopeptides in West Africa.

2.0 MATERIALS AND METHODS

2.1 Study Design

An observational epidemiological surveillance study with a multi-center, cross-sectional approach was conducted to assess the prevalence, phenotypic resistance characteristics, and molecular clonal structure of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates. This study design facilitated the concurrent evaluation of phenotypic resistance features and genotypic markers across various healthcare settings in different geographical areas.

2.2 Setting and Timeline

The fieldwork and laboratory assessments lasted for 18 months (from October 2019 to July 2021). Specimen collection and gathering of clinical data took place across nine chosen tertiary and specialist medical facilities located in four states (Kano, Kaduna, Katsina, and Jigawa) within the Northwestern region of Nigeria. The network of institutions included:

- Teaching Hospitals: Aminu Kano Teaching Hospital (AKTH), Abdullahi Wase Teaching Hospital, Ahmadu Bello University Teaching Hospital (ABUTH), and Federal Teaching Hospital, Katsina.
- Federal Medical Centers: Federal Medical Centre, Birnin Kudu (Jigawa State).
- Specialized and Specialist Hospitals: National Orthopaedic Hospital, Dala (Kano), Murtala Muhammad Specialist Hospital (Kano), Rasheed Shekoni Specialist Hospital (Jigawa), and General Amadi Rimi Specialist Hospital (Katsina).

2.3 Study Size

The minimum sample size (n) necessary to guarantee sufficient epidemiological power was calculated using the standard prevalence formula [18]:

$$n = Z^2 pq / d^2$$

Where:

- Z = Value from the standard normal distribution at a 95% confidence interval (1.96).
- p = Local prevalence of MRSA based on previous peer-reviewed studies (48.5% or 0.485) [19].
- q = Complementary probability of the anticipated prevalence (1 - p = 0.515).
- d = Absolute precision margin, or the allowable error margin (5% or 0.05).

The initial computation indicated a minimum requirement of 376 participants. After considering a 5% dropout rate to accommodate possible specimen damage or incomplete clinical records, the target enrollment was revised to 400 individuals. Since duplicate specimens (one wound swab and one nasal swab) were collected from each participant, a total of 800 distinct clinical specimens were analyzed.

2.4 Specimen Collection and Clinical Data Harvesting

Clinical data such as age, biological sex, lesion onset, anatomical location, and prior antibiotic treatment were recorded using structured epidemiological questionnaires that align with established surveillance systems [20]. Sterile cotton swabs, pre-wetted with sterile peptone water, were rotated across the deep margins of wound beds (including soft tissue infections, burns, surgical sites, ulcers, and osteomyelitis) as well as the anterior nares of each participant. Specimens were promptly immersed in Amies transport medium, kept under cold chain conditions (4°C) in insulated containers, and processed within 6 hours of collection in compliance with biosafety protocols [21].

2.5 Eligibility Criteria and Participant Selection

A consecutive sampling method was utilized to enroll participants from the designated institutional wards.

- **Inclusion Criteria:** Both male and female pediatric and adult patients (ages ranging from < 1 to 80 years) admitted to the orthopedic, surgical, pediatric, or burns units, who displayed clinically evident, suppurative septic wounds and provided written or parental informed consent.
- **Exclusion Criteria:** Individuals with clean or non-infected surgical wounds, outpatients, and those who had been under active anti-staphylococcal treatment within 48 hours before sampling.

2.6 Primary Microbiological Isolation and Morphological Profiling

Specimens were inoculated onto Mannitol Salt Agar (MSA) and 5% Sheep Blood Agar plates, followed by aerobic incubation at temperatures of 35°C to 37°C for duration of 24 hours [22]. Colonies exhibiting characteristic golden-yellow pigmentation with yellow zones on MSA (indicating mannitol fermentation) and clear beta-hemolytic zones on blood agar were provisionally selected. Morphological validation was conducted via Gram staining, identifying Gram-positive cocci arranged in grape-like clusters under light microscopy [22].

2.7 Biochemical Differentiation

Definitive validation of *S. aureus* was confirmed by demonstrating catalase-positive reactivity using a 3% hydrogen peroxide (H₂O₂) solution, followed by bound coagulase testing through slide agglutination with undiluted rabbit plasma [22]. The production of free coagulase was definitively confirmed using the tube coagulase test, during which a 0.5 mL volume of rabbit plasma was inoculated with the bacterial suspension, incubated at 35°C, and monitored macroscopically for definitive clot formation at both 4 hours and 24 hours [22].

2.8 Phenotypic Identification of Methicillin Resistance

Susceptibility testing was conducted using the Kirby-Bauer disc diffusion technique on Mueller-Hinton Agar (MHA) plates [22]. Bacterial suspensions were adjusted to a 0.5 McFarland turbidity standard and lawn-inoculated onto MHA, after which a 30 µg cefoxitin disc was applied aseptically. The plates were incubated at 35°C for duration of 18 to 24 hours. Inhibition zone diameters were measured with digital calipers and interpreted strictly according to CLSI guidelines [22]: breakpoints dictated that zones ≤ 21 mm indicated methicillin resistance, whereas zones ≥ 22 mm indicated methicillin susceptibility.

2.9 Genomic DNA Extraction

Bacterial isolates were conveyed under strict cold chain conditions (4°C) to the Nigerian Institute of Medical Research (NIMR) in Yaba, Lagos, for molecular assessment. Total genomic DNA was extracted utilizing a modified thermal lysis protocol that was optimized with a silica-membrane spin-column purification method [23]. Overnight bacterial pellets were resuspended in 1 mL of sterile nuclease-free water, then centrifuged at 10,000 rpm for 1 minute, treated with 200 µL of lysis buffer, and incubated for 10 minutes at 55°C in a digital heating block. After adding 200 µL of absolute ethanol, the mixture was transferred to a silica spin column, centrifuged at 10,000 rpm for 1 minute, and sequentially washed with 500 µL of Wash Buffers 1 and 2. The DNA that was retained was eluted in 50 µL of elution buffer and stored at -20°C.

2.10 Multiplex PCR Amplification for *mecA* and *SCCmec* Genotyping

Genotypic confirmation of the *mecA* gene (target fragments at 147 bp and 532 bp) and *SCCmec* structural types (I–V) was carried out using multiplex PCR assays [23, 24]. The reactions were performed in total volumes of 20 µL containing 1X HOT FIREPol Blend Master Mix (Solis BioDyne, Estonia) with 1.5 mM MgCl₂, 200 µM of each dNTP, 25 pmol of target-

specific primers (biomers.net, Germany), 2 units of Hot FIREPol DNA Polymerase, and 2 µL of template DNA. The thermal cycling profile for *mecA* began with an initial denaturation at 95°C for 5 minutes; followed by 35 cycles of 95°C for 30 seconds, an annealing phase at 58°C for 30 seconds, and an extension phase at 72°C for 45 seconds; concluding with a final extension at 72°C for 10 minutes. The *SCCmec* cycling framework included an initial denaturation at 95°C for 5 minutes; followed by 30 cycles of 94°C for 10 minutes, 55°C for 30 seconds, and 72°C for 80 seconds; ending with a Terminal extension at 72°C for 10 minutes. The amplicons were separated using 1.5% agarose gel electrophoresis at 80 V for 90 minutes, stained with 0.5 µg/mL ethidium bromide, and visualized under a UV transilluminator (Gel Doc XR, Bio-Rad, USA).

2.11 *spa* Gene Polymorphism Mapping

Polymorphic X-region mapping of the staphylococcal protein A (*spa*) gene was executed utilizing specific primers: 1095F (5'-AGACGATCCTTCGGTGAGC-3') and 1517R (5'-GCTTTTGCAATGTCATTACTG-3') within a 30 µL reaction matrix [25].

Amplification parameters included an initial denaturation phase at 95°C for 5 minutes, followed by 35 cycles consisting of denaturation (95°C for 30 seconds), annealing (52°C for 1 minute), and extension (72°C for 1 minute), concluding with a final extension at 72°C for 10 minutes. Amplicons were separated using 2% agarose gel electrophoresis at 80 V for 90 minutes and were analyzed for bands within the 300–350 bp range.

The purified amplicons underwent sequencing through the bidirectional Sanger chain-termination technique [26]. Following PCR, enzymatic cleanup was performed using the ExoSAP protocol, involving successive incubations at 37°C for 15 minutes and then at 80°C for another 15 minutes.

Cycle sequencing was carried out using the NimaGen BrilliantDye Terminator Cycle Sequencing Kit (V3.1) on an Applied Biosystems ABI 3500xL Genetic Analyzer that features a 50-cm capillary array and POP-7 polymer. Unincorporated dye terminators and salts were eliminated utilizing the ZR-96 DNA Sequencing Clean-up Kit from Zymo Research [27]. High-resolution base calling accuracy was confirmed using Kb Basecaller software alongside BigDye version 3.1 calibrations.

To reduce selection and measurement biases, standard reference control strains—*Staphylococcus aureus* ATCC 25923 (methicillin-susceptible) and *Staphylococcus aureus* ATCC 43300 (methicillin-resistant)—were utilized in every analytical phenotypic and genotypic run. Laboratory specimen labels were meticulously coordinated using automated alphanumeric codes to prevent data duplication or misclassification.

Possible sample degradation and plasmid loss biases were mitigated by maintaining strict, continuous cold chain conditions (4°C) throughout weekly batched sample transport. All molecular procedures were executed in duplicate by independent investigators who were blinded to the clinical information.

2.12 Statistical Analysis and Evolutionary Modeling

Descriptive statistics, such as percentage frequencies and absolute counts, were computed to summarize the prevalence rates of phenotypic and genotypic traits across various clinical presentations and states. Chromatogram data files underwent editing, trimming, and alignment using BioEdit software (Version 7.7.1) [28]. Sequence identities were established by searching the NCBI database through the Basic Local Alignment Search Tool (BLASTn). The *spa* repeats were categorized into defined types using the Ridom *SpaServer* software.

Phylogenetic structures and evolutionary timelines were modeled using the Neighbor-Joining method [29] within the MEGA11 software environment [30]. The structural integrity of the resulting trees was assessed using bootstrap testing that involved 1,000 replicates [31]. Evolutionary distances were calculated employing the Maximum Composite Likelihood method [32]. Codon positions (1st +2nd +3rd + non-coding) were meticulously analyzed, and all ambiguous

positions were addressed using the pairwise deletion approach, resulting in a final operational dataset consisting of 19,242 reference nucleotide positions.

3.0 RESULTS

3.1 Molecular Validation of the *mecA* Resistance Determinant

Among the 144 clinical isolates initially classified as methicillin-resistant *Staphylococcus aureus* (MRSA) through phenotypic methods, PCR amplification confirmed the presence of the *mecA* gene in just 9 isolates (6.25%). The remaining 135 isolates (93.75%) were determined to be *mecA*-negative by genotyping. The specific gel electrophoresis validation pathways for these amplifications are outlined below:

Plate 1: Representative Agarose Gel Electrophoresis patterns showing PCR amplified products for the *mecA* gene in clinical isolates.

Lane M: 1000-bp molecular weight DNA ladder; Lane N: Negative Control; Lane P=Positive Control; Lanes 1, 3, and 4: Positive for the *mecA* gene, demonstrating characteristic diagnostic bands at 147 bp or 532 bp; Lanes 2, 8, and 9: Positive for the *mecA* gene, indicated by distinct bands at 147 bp. Lanes 6, 7, 10, 11, 12, and 13: Negative for the *mecA* gene.

Plate 2: Representative Agarose Gel Electrophoresis patterns showing PCR amplified products for the *mecA* gene (Continuation).

Lane M = 1000-bp molecular weight DNA ladder; Lane N= Negative Control; Lane 3 = Positive Control; Lanes 1, 4, and 13 = Show positive for the *mecA* gene as identified by diagnostic bands at 147 bp or 532 bp; Lanes 2, 5, 6, 7, 8, 9, 10, 11, 12, and 14 = Indicate negative for the *mecA* gene.

3.2 Distribution of *SCCmec* Structural Cassette Types

SCCmec typing was successfully performed on the 9 *mecA*-positive MRSA isolates confirmed through genotyping. Three isolates (33.3%) were identified as having *SCCmec* type III structures, while one isolate (11.1%) presented *SCCmec* type II. The other 5 isolates (55.6%) could not be categorized into standard structural cassettes, thus were classified as non-typeable (NT).

Plate 3: Representative patterns from Agarose Gel Electrophoresis illustrating PCR amplified products for *SCCmec* types derived from *mecA*-positive MRSA amplicons.

Lane M= Molecular weight size marker; Lane N= Negative Control; Lanes 1 and 2 = Negative for the assessed *SCCmec* types; Lane 3 = Indicates positivity for *SCCmec* type II, marked by a diagnostic band at 398 bp; Lanes 4 to 6 = Positive for *SCCmec* type III, indicated by diagnostic bands at 280 bp.

3.3 Analysis of *Spa* Gene Polymorphism and Efficiencies of Sanger Sequencing

The polymorphic X-region of the staphylococcal protein A (*spa*) gene was identified in 6 out of 9 (66.7%) confirmed MRSA isolates, producing clean amplicon fragments that ranged between 300 bp and 350 bp. The gene was not detected in 3 isolates (33.3%), which were categorized as non-typeable. Successful downstream bidirectional Sanger sequencing was accomplished for 7 out of the 9 confirmed MRSA isolates (77.8%), while 2 isolates (22.2%) did not yield adequate amplification because of issues with nucleic acid integrity during quality assessment. Specifically for the *spa*-locus, 5 out of the 6 *spa*-positive amplicons (83.3%) were sequenced successfully, whereas 1 sample (16.7%) resulted in

an unsuccessful sequencing reaction. The bioinformatics analysis of the 5 refined *spa* sequences using Ridom *SpaType* software (Version 5.0) identified 4 distinct clonal *spa* types: t037, t425, t1123, and t1223.

Plate 4: Typical patterns of Agarose Gel Electrophoresis illustrating PCR amplification products for various *spa* types derived from *mecA*-positive MRSA amplicons.

Lane M=Molecular weight size marker; Lane N=Negative Control; Lanes 1, 2, and 3 = *Spa*-positive, showing diagnostic bands at 300 bp; Lane 4 = *Spa*-negative; Lane 5 = *Spa*-positive, showing a diagnostic band at 350 bp; Lanes 6 and 7 = *Spa*-negative; Lanes 8 and 9 = *Spa*-positive, showing diagnostic bands at 300 bp.

3.4 Molecular and Phenotypic Profiles of Confirmed *mecA*-Positive MRSA Isolates

Table 1 summarizes the comprehensive molecular traits, clinical sources of isolation, and phenotypic antibiotic resistance profiles of nine genotypically validated *mecA*-positive *Staphylococcus aureus* strains. The confirmed isolates were sourced from wound swabs (n = 5) and nasal swabs (n = 4), collected solely from three major tertiary institutions: National Orthopaedic Hospital, Dala (NOHD; n = 5), Aminu Kano Teaching Hospital (AKTH; n = 2), and Ahmadu Bello University Teaching Hospital (ABUTH; n = 2). Structural typing of *SCCmec* cassettes revealed that three isolates (020/PW/AKTH, 021/MSW/ABUTH, and 022/MSW/ABUTH) contained *SCCmec* Type III, one isolate (018/MS/NOHD) had *SCCmec* Type II, while five isolates were classified as non-typeable (NT). All nine *mecA*-positive isolates displayed extensive multidrug resistance (MDR) profiles. A universal co-resistance to vancomycin (VA, 100%) was noted in all genotypically confirmed isolates. High levels of concurrent resistance were also recorded against levofloxacin (LV, 77.8%; n = 7), gentamicin (GN, 66.7%; n = 6), erythromycin (E, 55.6%; n = 5), clindamycin (DA, 55.6%; n = 5), and doxycycline (DO, 55.6%; n = 5).

Table 1
Molecular and Phenotypic Profiles of Confirmed *mecA*-Positive MRSA Isolates

Isolate ID	Specimen Type	Healthcare Facility	Clinical Unit/Ward	<i>mecA</i> Status	<i>SCCmec</i> Type	Antibiotic Resistance Profile
22/PS/AKTH (W)	WS	AKTH	Paediatric Surgical	+	NT	E, DA, VA
10/MO/NOHD (N)	NS	NOHD	Male Orthopaedic	+	NT	E, DA, VA, GN, LV
12/CW/NOHD (N)	NS	NOHD	Children Ward	+	NT	E, DA, VA, LV
26/BP/NOHD (N)	NS	NOHD	Burns and Plastics	+	NT	VA, DO
35/BP/NOHD (N)	NS	NOHD	Burns and Plastics	+	NT	E, DA, VA, DO, GN
018/MS/NOHD (W)	WS	NOHD	Male Surgical	+	II	GN, VA, LV, DO
020/PW/AKTH (W)	WS	AKTH	Paediatric Ward	+	III	GN, E, VA, DA, LV
021/MSW/ABUTH (W)	WS	ABUTH	Male Surgical Ward	+	III	GN, VA, DA, LV, DO
022/MSW/ABUTH (W)	WS	ABUTH	Male Medical Ward	+	III	GN, DO, LV, VA

> Footnote: WS = Wound Swab; NS = Nasal Swab; AKTH = Aminu Kano Teaching Hospital; NOHD = National Orthopedic Hospital, Dala; ABUTH = Ahmadu Bello University Teaching Hospital; NT = Non-Typeable. E = Erythromycin; DA = Clindamycin; VA = Vancomycin; GN = Gentamycin; LV = Levofloxacin; DO = Doxycycline.

3.5 Epidemiological Prevalence Rates of Phenotypic and Genotypic MRSA across Surveyed States

The geographical distribution and diagnostic comparison between phenotypic screening using cefoxitin disc diffusion and genotypic confirmation of *mecA* via PCR across the four surveyed Northwestern states are illustrated in Table 2. Initially, phenotypic screening identified 144 MRSA isolates (34.9% overall prevalence), with the highest occurrence observed in Kano State (n = 67, 16.0%), followed by Kaduna (n = 37, 8.8%), Jigawa (n = 24, 5.7%), and Katsina (n = 16, 3.8%). However, molecular PCR confirmation for the *mecA* gene revealed a significant diagnostic disparity, confirming *mecA* carriage in only 9 of the 144 phenotypic MRSA isolates (6.25% overall genotypic prevalence). Genotypically confirmed MRSA strains were found exclusively in urban tertiary facilities in Kano (n = 7, 4.9%) and Kaduna (n = 2, 1.4%), while no *mecA*-positive isolates were found in Katsina (0.0%) and Jigawa (0.0%) despite the initial phenotypic resistance findings. Among the nine genotypically verified isolates, structural *SCCmec* cassettes were successfully identified in four isolates (44.4% of *mecA*+ pool), with equal representation from Kano (n = 2, 22.2%) and Kaduna (n = 2, 22.2%). Likewise, polymorphic *spa* gene detection resulted in six positive amplicons (66.7% of *mecA*+ pool), comprising four isolates from Kano (44.4%) and two isolates from Kaduna (22.2%).

Table 2
Epidemiological Prevalence Rates of Phenotypic and Genotypic MRSA across Surveyed States

Geographic State Location	Phenotypic MRSA Prevalence (Cefoxitin) (%)	Genotypic MRSA Prevalence (<i>mecA</i> PCR) (%)	<i>SCCmec</i> Positive Relative to Total Genotypic (%)	<i>spa</i> Positive Relative to Total Genotypic (%)
Kano	67 (16.0%)	7 (4.9%)	2 (22.2%)	4 (44.4%)
Katsina	16 (3.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Jigawa	24 (5.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Kaduna	37 (8.8%)	2 (1.4%)	2 (22.2%)	2 (22.2%)
Grand Total	144 (34.9%)	9 (6.25%)	4 (44.4%)	6 (66.7%)

3.6 Distribution Matrix of Screened *SCCmec* Structural Types

The structural categorization and frequency distribution of *SCCmec* cassette types among the evaluated cohort are provided in Table 3. Out of the total 144 clinically resistant isolates examined, structural cassettes were resolved in only four isolates, leading to an overall detection rate of 2.78% across the entire phenotypic pool. Within the genotypically confirmed *mecA*-positive group (n = 9), *SCCmec* Type III appeared as the predominant structural variant, accounting for 33.3% (n = 3; overall frequency 2.08%). *SCCmec* Type II was identified in one isolate, making up 11.1% (n = 1; overall frequency 0.69%). Notably, more than half of the *mecA*-positive isolates (n = 5, 55.6%; overall frequency 3.47%) did not align with standard I–V structural primers and were classified as non-typeable (NT) architectures.

Table 3
Distribution Matrix of Screened SCCmec Structural Types

Cassette Profile	Structural Variants Resolved	Total Detected (n = 144)	Total Confirmation Frequency (%)	Relative Distribution Within <i>mecA</i> + Pool (n = 9) (%)
SCCmec Type II	Type II Cassette Architecture	1	0.69%	11.1%
SCCmec Type III	Type III Cassette Architecture	3	2.08%	33.3%
Non-Typeable (NT)	Unassigned Structural Matrix	5	3.47%	55.6%
Total		9	6.25%	100.0%

3.7 Oligonucleotide Primers used for the various target genes in this Study

The specific oligonucleotide primers used in the amplification process of the target genes are provided in Table 4 as shown below.

Table 4
The lists of oligonucleotide primers used for the various target genes with their reference sources in this study

Primer	Nucleotide Sequence (5' – 3')	Amplicon Size (bp)	Source
<i>mecA</i>	F: GTG ATA TAC CAA GTG AAT R: ATG CGC TAT AGA TTG AAA GAA	147	[51]
<i>mecA</i>	F: AAA ATC GAT GGT AAA GGT TGG C R: AGT TCT GCA GTA CCG GAT TTG C	532	[52]
<i>SCCmec Type 1</i>	F: GCC ACT CAT AAC ATA TGG AA R: CAT CCG AGT GAA ACC CAA A	415	[52]
<i>SCCmec Type II</i>	F: ATT GCC TTG ATA GCC TTC T R: TAA AGG CAT CAA TCG ACA A	937	[52]
<i>SCCmec type III</i>	F: CGT CTA TTA CAA GAT GTT AAG R: CCT TTA TAG ACT GGA TTA TTC AAA ATA	518	[52]
<i>SCCmec type Iva</i>	F: GCC TTA TTC GAA GAA ACC G R: CTA CTC TTC TGA AAA GCG TCG	776	[52]
<i>SCCmec type IVb</i>	F: TCT GGA ATT ACT TCA GCT GC R: AAA CAA TAT TGC TCT CCC TC	493	[52]
<i>SCCmec type IVc</i>	F: ACA ATA TTT GTA TTA TCG GAG AGC R: TTG GTA TGA GGT ATT GCT GG	200	[52]
<i>SCCmec type IVd</i>	F: CTC AAA ATA CGG ACC CCA ATA CA R: TGC TCC AGT AAT TGC TAA AG	881	[52]
<i>SCCmec type V</i>	F: TAT ACC AAA CCC GAC AAC TAC R: CGT CTA TTA CAA GAT GTT AAG GAT AAT	359	[52]

3.8 Sequence Homology and Phylogenetic Analysis

- The bioinformatics analysis of the seven successfully sequenced *mecA* nucleotide fragments showed distinct evolutionary patterns when compared against global reference datasets obtained from the National Center for Biotechnology Information (NCBI) database (Fig. 1). The sequences were analyzed based on baseline similarities referenced against the prototype *mecA* gene sequence from *Staphylococcus aureus* strain JSCC 3621 (WIS).
- Global Standard Clusters: The reference control strains exhibiting $\geq 95\%$ sequence similarity—including *Staphylococcus aureus* JSCC 3621 (WIS), *Staphylococcus argenteus* strain SG144, *Staphylococcus aureus* subsp. *aureus* N315, *Staphylococcus aureus* TN/CN/1/12, and *Staphylococcus aureus* NCTC 10442—were closely clustered, sharing a well-supported common ancestor (Bootstrap value: 96).
- Local Sequence Topologies: The evolutionary distances across the branches indicated that ancestral node 66 is situated near node 98 but is significantly distant from nodes 25, 47, 64, and 74.
- Clonal Sub-clusters: Within the examined cohort, node 47 acts as the direct common ancestor connecting isolates 22/FS/AKTH (W) and 12/CW/NOHD (N). Node 64 designates a separate shared lineage for isolates 10/MO/NOHD

(N) and 20/PW/AKTH (W), placing them adjacent to the node 47 sub-cluster while establishing a considerable evolutionary distance between them. Likewise, isolates 26/BP/NOHD (N) and 21/MSW/ABUTH (W) were identified to have a distinct common ancestor at node 74.

- Regional Divergence Indicators: Importantly, none of the locally isolated clinical strains found in this investigation—specifically 35/BP/NOHD (N), 22/FS/AKTH (W), 12/CW/NOHD (N), 10/MO/NOHD (N), 20/PW/AKTH (W), 26/BP/NOHD (N), and 21/MSW/ABUTH (W)—were included within the tightly conserved clusters of the international NCBI reference database, suggesting separate regional micro-evolutionary paths.

Note

The evolutionary history was inferred using the Neighbor-Joining method in MEGA11. The optimal tree with the total branch length is displayed. Bootstrap values (1,000 replicates) are noted at significant ancestral points. Evolutionary distances were computed using the Maximum Composite Likelihood approach. Local clinical isolates from Northwestern Nigeria are specifically labeled with institutional codes.

4.0 Discussion

This study presents a comprehensive molecular and epidemiological analysis of *Staphylococcus aureus* isolates collected from tertiary healthcare institutions in Northwestern Nigeria, specifically in Kano, Katsina, Jigawa, and Kaduna States. A significant finding is the notable discrepancy observed between phenotypic antibiotic susceptibility and molecular gene validation. Among the 400 paired clinical isolates examined, 144 (34.9%) were initially classified as methicillin-resistant *Staphylococcus aureus* (MRSA) based on cefoxitin disc diffusion testing. However, polymerase chain reaction (PCR) analysis confirmed the presence of the *mecA* resistance gene in only 9 isolates (6.25%), indicating that 135 phenotypic MRSA isolates were *mecA*-negative. The *mecA*-positive strains were predominantly located within urban centers in Kano, including the Aminu Kano Teaching Hospital and the National Orthopaedic Hospital, as well as in Kaduna at the Ahmadu Bello University Teaching Hospital. In contrast, the phenotypic resistance rates in Katsina and Jigawa were 3.8% and 5.7%, respectively, with none harboring the *mecA* gene. All *mecA*-positive isolates exhibited extensive multidrug resistance (MDR) to various antibiotics.

The low detection rate of *mecA* (6.25%) among cefoxitin-resistant isolates reflects a broader trend observed in sub-Saharan Africa, wherein phenotypic resistance frequently overestimates *mecA*-mediated resistance [33]. Previous surveillance conducted in Nigeria has revealed substantial variability in *mecA* carriage among MRSA isolates, emphasizing geographical differences in resistance mechanisms [34, 35]. The identification of *SCCmec* Types II and III indicates the emergence of multidrug-resistant Healthcare-Associated MRSA (HA-MRSA) lineages in regional healthcare facilities. Furthermore, a significant proportion (55.6%) of non-typeable cassettes suggests that genetic modifications are actively occurring within these elements [36]. High-resolution analysis of the *spa* gene has revealed four distinct clonal types: t037, t425, t1123, and t1223. Notably, the *spa* type t037 is associated with the ST239-MRSA-III lineage, which is recognized for its multidrug resistance and rapid transmission within hospital settings [37]. The presence of *spa* type's t425, t1123, and t1223, alongside a 33.3% non-typeable rate, highlights the diverse clonal backgrounds present in Northern Nigeria.

The discrepancy between phenotypic beta-lactam resistance and the presence of the *mecA* gene has garnered significant attention within the field of clinical microbiology [38]. The World Health Organization's 2024 Bacterial Priority Pathogens List categorizes Methicillin-Resistant *Staphylococcus aureus* (MRSA) as a High Priority Pathogen due to its associated high mortality rates and resistance capabilities [39, 40]. Our findings corroborate global reports indicating that non-*mecA* mediated resistance varies from 10% to over 50% in clinical isolates [41].

However, the discordance rate of 93.75% reported in this study is among the highest documented, highlighting substantial diagnostic challenges.

Key Takeaway: The elevated discordance rate raises serious concerns regarding the efficacy of standard phenotypic diagnostic methods. This situation may result in unnecessary antibiotic usage and obscure underlying resistance mechanisms.

The notable discrepancy between phenotypic resistance to cefoxitin and the high prevalence of *mecA* negativity (93.75%) can be elucidated through three biological mechanisms within local staphylococcal lineages:

1. 1. Borderline Oxacillin-Resistant *Staphylococcus aureus* (BORSA): The overproduction of *blaZ*-encoded beta-lactamase enzymes can enhance the hydrolysis of beta-lactam rings, resulting in minimum inhibitory concentrations (MICs) that exceed phenotypic resistance thresholds without necessitating the expression of *PBP2a* [42].
2. 2. Modified PBP *Staphylococcus aureus* (MODSA) / *mec*-independent Oxacillin Non-Susceptible *Staphylococcus aureus* (MIONSA): Strains that lack *mecA* or *mecC* may still exhibit low-to-moderate beta-lactam non-susceptibility due to amino acid alterations in native penicillin-binding proteins [43, 44]. This complicates detection, as standard assays for *mecA* are incapable of identifying these variants [44].
3. 3. Undetected *mec* Homologues: Conventional *mecA* PCR primers may not sufficiently amplify variants such as *mecC* or novel *mec* structures, leading to false negatives in molecular diagnostics [45, 46]. Isolates that demonstrate phenotypic resistance with these divergent elements may not be adequately detected without advanced molecular assays [45, 46].

Phylogenetic analysis employing the Neighbor-Joining method underscores the biological uniqueness of local strains. International reference strains from NCBI form a closely clustered ancestral group (Bootstrap value: 96), whereas seven Nigerian isolates demonstrate notable divergence into independent sub-clusters at nodes 47, 64, and 74:

- - Node 47: Wound isolate 22/FS/AKTH correlates with nasal isolate 12/CW/NOHD.
- - Node 64: Nasal isolate 10/MO/NOHD links with wound isolate 20/PW/AKTH.
- - Node 74: Nasal isolates 26/BP/NOHD are associated with wound isolate 21/MSW/ABUTH. These local strains do not conform to international reference clusters, thereby suggesting a distinct micro-evolution influenced by regional antimicrobial pressures, including the prevalent use of sub-therapeutic antibiotics and localized infection control practices [47].

Phenotypic cefoxitin screening presents substantial challenges for healthcare in Northwestern Nigeria:

- - Diagnostic Over-estimation: The dependence on disc diffusion methods results in the misclassification of 94% of non-*mecA* resistant *Staphylococcus aureus* as methicillin-resistant *Staphylococcus aureus* (MRSA), thereby leading to unnecessary escalation in treatment.
- - Advanced Resistance: The emergence of vancomycin-resistant *Staphylococcus aureus* (VRSA) isolates underscores the imperative for glycopeptide susceptibility screening and the implementation of antimicrobial stewardship.
- - Infection Control Gaps: The detection of *SCCmec* Types II and III, combined with a high non-typeable rate, suggests the existence of unmonitored reservoirs of mobile genetic elements within healthcare environments.

These findings carry significant ramifications for global antimicrobial resistance (AMR) surveillance and health governance:

1. 1. Genomic Bias: Existing databases, such as NCBI GenBank, exhibit bias towards North American and European strains, inadequately representing the diversity found in sub-Saharan regions [49, 50].
2. 2. Stealth Resistance: *mecA*-negative strains may evade detection in rapid MRSA screening, increasing the risk of undetected transmission.
3. 3. Strengthening Global AMR Frameworks: In alignment with the World Health Organization's Global Antimicrobial Resistance and Infection Prevention and Control (WHO GLASS) initiative, it is essential for diagnostic networks to incorporate additional molecular targets for the accurate tracking of staphylococcal evolution [49].

Limitations of the Study

1. 1. Target Specificity and Uncharacterized Resistance Pathways: The molecular screening conducted in this study concentrated exclusively on the *mecA* gene, which left the genetic mechanisms underlying phenotypic resistance to cefoxitin in 135 *mecA*-negative isolates (93.75%) unresolved. While alternative mechanisms, such as hyper-expression of the *blaZ* gene or mutations in penicillin-binding protein (*pbp*) genes, were proposed, the examination of these factors could not be performed due to financial and technical restrictions.
2. 2. Structural Cassette Unassignability: The application of standard multiplex PCR for *SCCmec* Types I–V resulted in 55.6% ($n = 5$) of *mecA*-positive isolates being identified as non-typeable. Conventional PCR often encounters difficulties in identifying novel or altered cassettes, and comprehensive structural resolution necessitates the use of advanced Whole Genome Sequencing (WGS).
3. 3. Sample Transport and Sequencing Efficiency Constraints: Logistical challenges associated with specimen collection from geographically disparate sites adversely affected the quality of the samples. These challenges led to a decrease in sequence yield, resulting in unsuccessful Sanger sequencing of 2 *mecA* amplicons (22.2%) and 1 *spa* amplicon (16.7%), potentially attributable to low template concentration or nucleic acid degradation.
4. 4. Hospital-Centric Sampling Bias: The study cohort was exclusively derived from major tertiary centers, namely AKTH, NOHD, and ABUTH. This focus may not adequately represent the prevalence of community-acquired MRSA (CA-MRSA) or asymptomatic carriers within the broader population of Northern Nigeria

Declarations

Ethics Approval and Consent to Participate

This research was carried out in full compliance with the Declaration of Helsinki. The study protocol received ethical approval from the Health Research Ethics Committees (HREC) of the involved tertiary and specialist healthcare institutions across Kano, Kaduna, Katsina, and Jigawa states. All adult participants (or legal guardians in the case of minors) provided verbal informed consent before the collection of specimens and clinical data.

Consent for Publication

Not applicable. This manuscript does not contain any individual patient details, images, or personal identifying clinical records.

Availability of Data and Materials

The nucleotide sequence datasets for the *mecA* gene isolates created during this study have been submitted to the National Center for Biotechnology Information (NCBI) GenBank database with submission ID SUB16339088 (<https://submit.ncbi.nlm.nih.gov/subs/genbank/SUB16339088>). Formal accession numbers from NCBI GenBank are pending assignment and will be included in the final manuscript before publication. All other important phenotypic, molecular, and phylogenetic datasets generated or analyzed throughout this research are available in the published

article (and its supplementary materials) or can be requested from the corresponding author (H. B. Ali) upon reasonable inquiry.

Competing Interests

The authors declare that they have no financial or non-financial competing interests.

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Authors' Contributions

- JMA: Developed the study concept, conducted clinical specimen collection, laboratory processing, phenotypic assays, and wrote the initial manuscript.
- HBA: Oversaw the project, designed the molecular surveillance framework, managed data curation, critically reviewed the bioinformatics and phylogenetic analyses, and acted as the corresponding author and thorough reviewed of the manuscript.
- ASK: Supervised, co-developed the epidemiological protocol, oversaw molecular assay validation, and contributed to the editing of the manuscript.
- EB: Supported sample collection, regional lab logistics, and clinical data extraction in healthcare facilities in Kaduna State.
- NMB: Assisted with molecular typing methods, quality assurance, and provided a critical review of the manuscript.
- BE: Coordinated clinical specimen collection at the teaching hospital and contributed to study supervision, as well as proofreading the manuscript.

All authors reviewed and approved the final manuscript before submission.

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Plates

Plates 1 to 4 are available in the Supplementary Files section

Figures

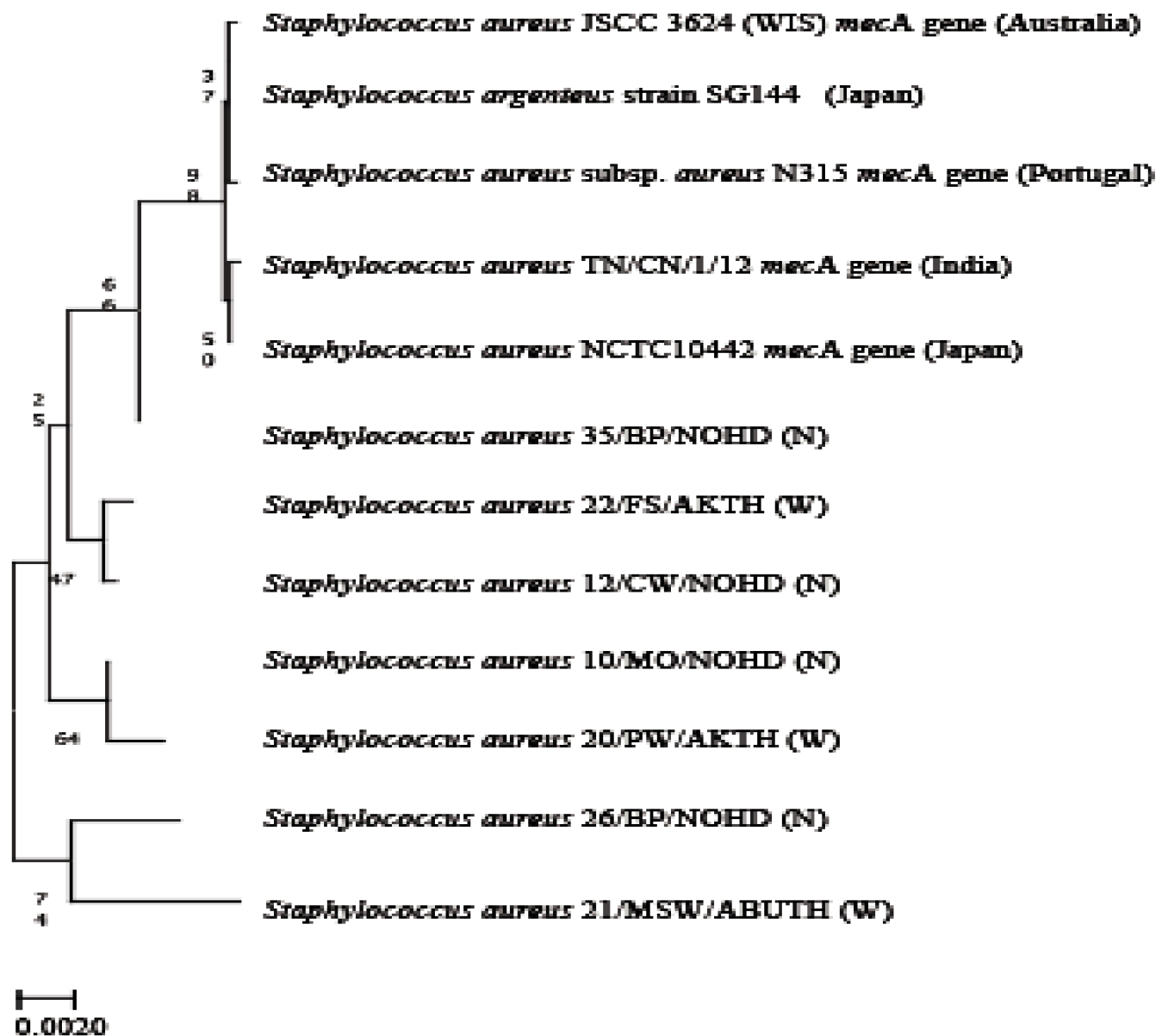


Figure 1

Phylogenetic Relationships of Reference Taxa and Confirmed Local MRSA Sequences Determined by the Neighbor-Joining Technique.

Note: The evolutionary history was inferred using the Neighbor-Joining method in MEGA11. The optimal tree with the total branch length is displayed. Bootstrap values (1,000 replicates) are noted at significant ancestral points. Evolutionary distances were computed using the Maximum Composite Likelihood approach. Local clinical isolates from Northwestern Nigeria are specifically labeled with institutional codes.

Supplementary Files

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- [Ethicalclearances.pdf](#)
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- [Plate3RepresentativepatternsfromAgaroseGelElectrophoresisillustratingPCRamplifiedproductsforSCCmectypes.png](#)
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