Prevalence of *Mec*A and *Mec*C Genes from Cattle and Livestock Marketers at Shinge Livestock Market in Lafia, Nasarawa State, Nigeria

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ABSTRACT

Background: Methicillin Resistant *Staphylococcus aureus* (MRSA), one of the main causes of infections is associated with livestock, the community, and healthcare facilities. The *mecA* gene primarily mediates resistance, and the advent of *mecC*-positive MRSA presents difficulties for diagnosis and treatment. The purpose of this study was to identify the molecular features of the *mecA* and *mecC* genes from cattle and livestock marketers at Shinge livestock market in Lafia, Nasarawa State, Nigeria.

Materials and Methods: In September and October of 2023, a laboratory-based observational study was carried out at the Shinge livestock market. Standard microbiological methods were used to identify MRSA from one hundred (100) cattle skin swabs and one hundred (100) nasal secretions from livestock marketers. The swabs were cultured on Manitol Salt Agar (MSA) at 37℃ for 24 hours. Cefoxitin disk using Kirby-Bauer diffusion technique was used to confirm methicillin resistance. Multiplex Polymerase Chain Reaction (PCR) was used to detect the *mecA* and *mecC* genes molecularly.

Result: Twenty-six (13%) of the 200 samples examined, sixteen (16) from livestock traders and ten (10) from cattle skin swabs were found to be MRSA. Molecular analysis revealed that 19.2% of isolates had *mecA* and 26.9% had *mecC* genes.

Conclusion: In the study populations, the prevalence rates of the *mecA* and *mecC* genes were 19.2% and 26.9%, respectively. Therefore, increased surveillance is essential to addressing MRSA resistance trends.

Key words: Methicillin resistance, *mecA*, *mecC*, Cefoxitin, PCR

INTRODUCTION

Staphylococcus aureus is a Gram-positive bacterium that can cause infections ranging from benign infections of the skin and soft tissues to invasive diseases including osteomyelitis, pneumonia, bacteremia, and endocarditis. Resistant to methicillin, the emergence and global spread of *Staphylococcus aureus* has created serious therapeutic and epidemiological challenges, especially in hospitals where resistance to many antibiotics strains raise morbidity, death, and medical costs (Murugan *et al*., 2015). Among the most common reasons for infections, acquired in hospitals and the community, Methicillin-Resistant *Staphylococcus aureus* (MRSA) has a great morbidity and fatality rate. MRSA is a major issue in hospitals, and infection prevention and control depend heavily on its detection (Lakhundi and Zhang, 2018).

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The primary cause of methicillin resistance in *S. aureus* is the *mecA* gene, which is located on a moveable genetic element called staphylococcal cassette chromosome mec (SCCmec). PBP2a, an alternative penicillin-binding protein that decreases affinity for all β-lactam antibiotics, including carbapenems, cephalosporins, and penicillins, is produced by the *mecA* gene. This permits bacterial survival and peptidoglycan synthesis to persist in the face of β-lactam exposure (Lakhundi and Zhang, 2018; Arumugam *et al*., 2016). Horizontal gene transfer is made possible by the incorporation of SCCmec into the *S. aureus* chromosome, which aids in the quick clonal growth of MRSA in both community-acquired and hospital-acquired environments (Becker *et al*., 2016).

2011 saw the discovery of *MecC*, a novel *mecA* homolog. Located on the SCCmec type XI element, *mecC* shares roughly 70% nucleotide similarity with *mecA* and encodes PBP2c, another β-lactam-insensitive penicillin-binding protein. Like PBP2a, PBP2c confers resistance to β-lactams; however, its expression often eludes detection by conventional diagnostic modalities such as mecA-specific PCR assays and PBP2a latex agglutination tests, potentially misclassifying *mecC*-positive MRSA as methicillin-susceptible *S. aureus* (MSSA) (Cheng and Huang, 2014;Wangai *et al*., 2019). Furthermore, *mecC*-positive MRSA isolates are often linked to environmental and zoonotic reservoirs, especially in wastewater, companion animals, and livestock, highlighting the importance of a One Health strategy to antimicrobial resistance surveillance (Gurumsamy *et al*., 2013).

The prevalence of *mecA* and *mecC* genes in cattle and livestock marketers at the Shinge livestock market in Lafia, Nasarawa State, is not well documented. Therefore, the purpose of this study was to determine the prevalence of the *mecA* and *mecC* genes in cattle and livestock marketers at Shinge livestock market in Lafia, Nasarawa State, Nigeria.

MATERIALS AND METHODS

Ethical approval

The research was given ethical clearance by the Nasarawa State Ministry of Health Lafia Ethical Committee following a compelling presentation to their panel. The owners' permission was obtained before samples were collected from the cattle. On September 1, 2023, the ethical approval was given with reference number 18/06/2017.

Study site and population

The Shinge cattle market in Lafia, Nasarawa State, Nigeria, served as the site of the cross-sectional study. The Federal University of Lafia's Microbiology Laboratory in Nasarawa State, Nigeria, was the site of the laboratory work. The skins of one hundred (100) cattle were swabbed, and livestock marketers provided 100 nasal secretions. Samples were gathered during September and October of 2023.

Inclusion and exclusion criteria

The study included marketers older than ten (10) years, as well as livestock and marketers who had not taken antibiotics in the past two weeks. However, marketers under the age of ten (10) and cattle with a history of antibiotic use within the previous two (2) weeks were not allowed to participate in the study.

Sample collection

Sterile normal saline was used to moisten the sterile cotton swabs used to collect the samples The method earlier outlined by Collahan *et al*. (2021) was employed to collect nasal secretions. Swabs were placed into the nasal passage and rotated for ten seconds, same process was repeated on the other nasal passage, and the swab was then carefully withdrawn and labeled. One hundred cattle skin surfaces were swabbed and one hundred nasal secretions were gathered from livestock marketers. The samples were transported to the Federal University of Lafia Microbiology Laboratory in a sterile transport container so they could be processed.

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Cultural Procedure

Samples were cultured via a slightly amended kind of the method outlined by Garoy *et al*. (2019). After being inoculated on Mannitol Salt Agar (MSA), the swabs were incubated at 37°C for a whole day. The bacterial colonies were identified using biochemical tests and Gram staining.

Screening for Methicillin Resistant *Staphylococcus aureus*

In compliance with the standards established by the Clinical and Laboratory Standards Institute, all *Staphylococcus aureus* isolates were screened for MRSA using the Kirby Bauer disc diffusion method using a 30µg cefoxitin disc on Muller Hinton agar (CLSI, 2016). The bacterial suspension was standardized for each strain using 0.5 McFarland turbidity criteria. After flooding the Mueller Hinton Agar plate with the suspension and letting it absorb, using sterile forceps, cefoxitin discs were positioned on the agar. Zone of inhibitions were identified 24 hours after the sensitivity plates were incubated at 37°C. According to Clinical and Laboratory Standard Institute recommendations, a zone diameter of $\geq$ 21 mm was considered sensitive, and $\leq$ 21 mm was considered resistant (2016). The twenty six (26) MRSA screened were further analyze for the detection of *mecA* and *meC* genes.

Bacterial DNA Extraction

With a small adjustment, the bacterial DNA was extracted using a technique previously reported by Abimiku *et al*. (2016). Ten (10) milliliters of the bacterial isolate's overnight broth culture in one milliliter of Luria Bertani (LB) were spun for three minutes at 14000 rpm. After discarding the supernatant, the extracted cell pellet was resuspended in 1 mL of sterile distilled water, put into a 1.5 mL centrifuge tube, and centrifuged for 10 minutes at 14000 rpm. After carefully discarding the supernatant, the pellet was vortexed to resuspend it in 100 microliters of sterile distilled water. After centrifuging the tube once more for ten minutes at 14,000 rpm, the supernatant was properly disposed of. The cells were re-suspended in 500 µL of regular saline and heated for 20 minutes at 950℃ in a water bath. After cooling on ice for ten minutes, the heated bacterial suspension was spun at 14,000 rpm for three minutes. The DNA-containing supernatant was transferred to a 1.5 mL microcentrifuge tube and stored at -20°C for use in further processes.

DNA Quantification

Deoxyribonucleic acid (DNA) was quantified by means of the Nanodrop 1000 spectrophotometer. The equipment's application was started by double-clicking the Nanodrop icon. The device was initialized with two microliters of sterile distilled water and blanked with ordinary saline. The higher pedestal was lowered to make contact with the extracted DNA after two (2) µL of it had been loaded onto the lower pedestal. The DNA concentration was ascertained by clicking the "measure" button (Abimiku *et al.*, 2019).

DNA Amplification of *MecA* and *MecC* Genes by Multiplex Polymerase Chain reaction (PCR)

MecA-specific primers were used to amplify the DNA, resulting in a 533 bp fragment (Forward: 5′-AAAATCGATGGTAAAGGTTGGC-3′, *MecC*-specific primers that yield a 356 bp fragment (Forward: 5′-TCACCAGGTTCAAC[Y]CAAAA3-3′) and reverse: 5′-AGTTCTGGAGTACCGGATTTGC-3′ 5′-CCTGAATC[W]GCTAATAATATTTC5-3′ is the reverse. The final volume of 15 µL utilized for the amplification on an ABI 9700 thermal cycler from Applied Biosystems consisted of 2 µL of DNA (10 ng/µL), 7.5 µL of 2× Taq master mix (Zymo Research, United States), 1 µL of forward primer (10 µM), 1 µL of reverse primer (10 µM), and 3.5 µL of deionized water. The PCR of the *mecA* and *mecC* genes was conducted under the following conditions: first denaturation at 95 °C for 5 minutes, then 35 cycles of 95 °C for 1 minute, 55 °C for 30 seconds, 72 °C for 1 minute, and 72 °C for 5 minutes (Lee, 2003; Stegger *et al*., 2012).

Table 1: Primer for identifying *S. aureus* and MRSA genetic variants

Target gene	primer	Oligonucleotide sequence (5^I-3^I)	Amplicon size (bp)	Reference

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Nuc	Nuc F Nuc R	5^I –GCGATTGATGGTGATACGGTI-3^I 3^I –AGCCAAGCCTTGACGAACTAAAGC-5^I	279	Brakstad *et al*., 1992
MecA	MecA-F MecA-R	5^I –AAAATCGATGGTAAAGGTTGGC-3^I 3^I-AGTTCTGGAGTACCGGATTTGC-5^I	533	Lee, 2003
mecC	mecC-F mecC-R	5^I-TCACCAGGTTCAAC[Y]CAAAA-3^I 3^I-CCTGAATC[W]GCTAATAATATTTC-5^I	356	Stegger *et al*., 2012

RESULTS

Table 2 and 3 presents results obtained for zone of inhibition (mm) for MRSA isolates from cattle skin swab (n=10) and Livestock marketers (n=16) respectively. Based on CLSI guideline, a zone diameter ≥ 21mm is to considered sensitive and a zone diameter ≤ 21 mm to cefoxitin (30$\boldsymbol{\mu g}$) is considered resistant.

Table 2: Zone of Inhibition of Methicillin Resistant *Staphylococcus aureus* from Livestock Marketers

Isolates	Zones of Inhibitions (mm)
M3	15.0
M5	12.0
M19	21.0
M24	16.0
M38	13.0
M44	9.00
M73	20.0
M74	15.0
M75	15.0
M79	10.0
M84	15.0
M85	16.0
M86	14.0
M90	15.0
M94	10.0
M96	14.0

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Table 3: Zone of Inhibition of Methicillin Resistant *Staphylococcus aureus* from Cattle

Isolates	Zones of Inhibitions (mm)
A9	0.00
A18	20.0
A22	16.0
A33	19.0
A41	12.0
A55	20.0
A56	15.0
A61	20.0
A74	21.0
A81	20.0

Figure 1 show 16SrRNA *nuc* gene confirming isolates were *S. aureus*. Lane L represents 1,500 bp DNA molecular ladder. Presence of bands on the gel indicates the expression of 16SrRNA *nuc* gene for *Staphylococcus aureus.* Lanes M5, A55 and A81 did not show the appearance of the 16SrRNA-*nuc* (279 bp) gene for *Staphylococcus aureus.*

The amplified *mecC* gene of the isolated bacteria is electrophoresed on an agarose gel in Figure 2. Lane L represents 1,500 bp DNA molecular ladder. In the ladder, lane M3, M19, M24, M84, M96, A9 and A18 represents the expression of the *mecC* (356) gene.

Agarose gel electrophoresis of the amplified *mecA* gene for isolated bacteria is shown in Figure 3. Lane L represents 1,500 bp DNA molecular ladder and lane M24, M74, M90, M94 and A32 represents the expression of the *mecA* gene

Plate I: 16SrRNA *nuc* gene confirming isolates were *S. aureus.*

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Figure 2 Amplified MecC gene of isolated bacteria using agarose gel electrophoresis.

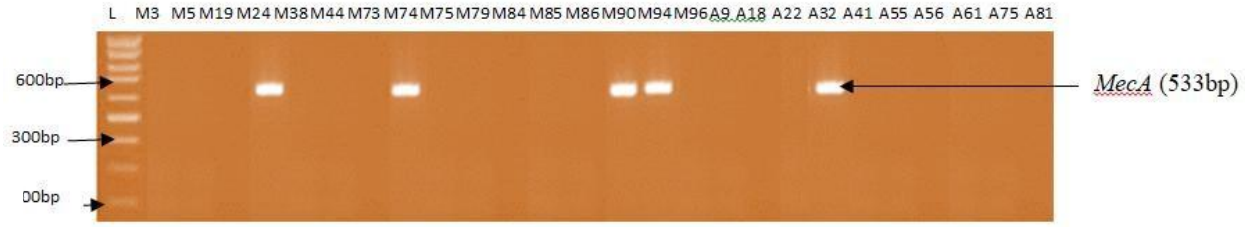

Figure 3 Amplified MecA gene of isolated bacteria using agarose gel electrophoresis.

Table 4 shows the occurrences of the resistant genes from cattle and their marketers. Five (19.2 %) were positive for *mecA*, 4 from marketers and 1 from cattle. Seven (26.9 %) were positive for *mecC*, 5 from marketers and 2 from cattle.

Table 4 Occurrence of the resistant genes among cattle marketers and cattle

Isolates	*MecA* No. (%)	*MecC* No. (%)
No. of positive	5.00 (19.2)	7.00 (26.9)
No. of negative	21.0 (80.8)	19.0 (73.0)
Total	26.0 (100)	26.0 (100)

DISCUSSION

The current study's molecular characterisation of MRSA strains from isolates obtained from cattle and livestock traders reveals that 26.9% of the isolates have the *mecC* gene, whereas 19.2% of the isolates have the *mecA* gene. *mecA* and *mecC* both produce a modified penicillin-binding protein (PBP2a or PBP2c) that is inherently resistant to methicillin-associated antibiotics. The prevalence of *mecA* and *mecC* in this study has the challenge of tackling staphylococcal agents by both human and animal health sectors.

The prevalence of *mecA* (19.2%) as reported in this study is in consistent with what is widely acknowledged that *mecA* is a determinant gene encoding for MRSA in the world, since it has frequently been detected in MRSA from both livestock and human being and a high prevalence in LA-MRSA is consistently demonstrated in available researches. The present study prevalence rate (19.2 %) of *mecA* gene is lower than the previous prevalence rate of *mecA* reported by Rao *et al*. (2022), who reported a prevalence rate of 21.4 % in their study. The prevalence rate of *mecA* gene in the current study also differs from the prevalence reported by Godwin. (2016) and Hamim & Salman. (2017), who reported a higher prevalence of 73.3 % and 68 % respectively in their studies. In another study previously reported by Shebl *et al*. (2020), a prevalence rate of 100 % was recorded for *mecA* genes which also differ from the current study finding on *mecA* prevalence. Also Rajdeep *et al*. (2025), and Idakari *et al.* (2025) reported a very higher prevalence rates (90% and 94% respectively) of *mecA* genes in their study which also differ from the current prevalence rate. However, prevalence rate lower than the current prevalence have been reported by Taban *et al*. (2021), they reported a prevalence rate of 7.4% in their study. The variation in *mecA* gene prevalence reported across studies may be attributed to differences in geographical location, antimicrobial usage patterns, livestock management practices, animal species, sample types, occupational exposure, and laboratory detection methods. Furthermore, differences in sample size, study design, circulating MRSA clones, and the implementation of antimicrobial stewardship programmes may contribute to the observed disparities.

The present study prevalence rate (26.9%) of *mecC* gene contrasts with most published studies. Shebl *et al*. (2020), and Rajdeep *et al*. (2025), reported the prevalence rates of 6% and 10% respectively. Similarly, a prevalence rate of 7.9% has been reported by Idrees *et al*. (2023), which also differ from the current prevalence. A higher proportion of *mecA* and *mecC*-positive MRSA isolates might result from regional differences in MRSA prevalence, variations in cattle farming system management, patterns of antimicrobial prescribing, contact with animals ivestock merchants is closely associated with cattle from many farms and regions of diverse settings and their movement may increase likelihood of dissemination and gene flow of resistance traits arises during livestock transactions. Therefore, the increased exposure of people interacting with colonized cattle might enhance transmission of *mecA* and mecC positive LA-MRSA through human-cattle contact, illustrating the One Health principles governing the spread of antimicrobial resistance in humans, animals, and the environment.

Co-detection of *mecA* and *mecC* genes in cattle and marketers of livestock animals carry significant implications for public health. Livestock market workers who handle cattle with MRSA infections (whether due to *mecA* or *mecC*) through skin-to-skin contact with the animals, with instruments contaminated with MRSA, or exposure in the live animal marketplace risk being colonized. Colonization can further contribute to the ongoing spread of MRSA in the home, in hospitals, the community and beyond by serving as another avenue for transmission of MRSA to human hosts. This finding underscores the importance of surveillance for livestock-associated MRSA strains in live animal marketing operations and their workers; of implementing of infection control measures in these settings; of promoting good personal hygiene by marketers in livestock animals and judicious antimicrobial use in animal agriculture.

Our study provides insight into the prevalence of *mecA* and *mecC* genes from cattle and livestock marketers in Lafia, Nasarawa State. However the research findings are limited by the fact that the study was conducted in a single livestock market and the gels photographs did not display the positive and negative controls.

CONCLUSION

In the study populations, the prevalence rates of the *mecA* and *mecC* genes were 19.2% and 26.9%, respectively. Therefore, increased surveillance is essential to addressing MRSA resistance trends

RECOMMENDATION

Surveillance for MRSA and resistance genes (*mecA* and *mecC*) amongst livestock and in livestock products should be regularly enhanced. Prudent use of antimicrobials should be practiced through well-established antibiotic stewardship programmes in animal farming. Hand hygiene and standard biosecurity protocols amongst livestock dealers ought to be advocated for reducing interspecies and human exposure. Further molecular-epidemiological investigations with larger sample numbers in variety of livestock animals could add extra insights into transmission patterns.

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Conflict of Interest

No conflict of interest

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