PSM
Microbiology

Research Article **2026 | Volume 11 | Issue 1 | 37-47**

Open Access

Article Information

Received: January 14, 2026

Accepted: February 17, 2026

Published: April 16, 2026

Authors' Contribution
B.T.O and S.A designed the study; A.O and B.T.O performed experiments; V.A.O and SA analyzed, while A.O and B.T.O. wrote the first draft. All the authors read and approved the final manuscript.

Citation
Odumosu, B.T., Olofu, V.A., Okiki, A., Adesida, S., 2026. Characterization of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Dairy Products from Local Farms in Lagos. PSM Microbiol., 11(1): 37-47.

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Characterization of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Dairy Products from Local Farms in Lagos

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Abstract:

Staphylococcus aureus, a coagulase-positive Gram-positive bacterium, is a major foodborne pathogen commonly associated with raw milk and dairy products, causing food poisoning and mastitis in dairy cattle. Methicillin-resistant *Staphylococcus aureus* (MRSA) strains present significant public health risks due to their resistance to common antibiotics. This study aimed to investigate the prevalence and antimicrobial resistance profile of *S. aureus* in raw cow milk from Lagos State, Nigeria. A total of 132 raw milk samples were collected from cattle farms in Kara, an abattoir in Agege, Lagos. Out of 114 bacterial colonies, 64 were Gram-positive cocci, and 17 isolates (14.9%) were identified as *S. aureus* using cultural and biochemical methods. Antimicrobial susceptibility testing revealed that all *S. aureus* isolates were resistant to methicillin, 5 (29.4%) were resistant to cefoxitin, 1 (5.9%) was vancomycin-resistant, and all 17 (100%) were susceptible to imipenem. Broth microdilution testing showed cefoxitin MIC values of ≥4 µg/mL for the five resistant isolates, confirming MRSA status. PCR amplification further detected the mecA gene in these isolates, supporting phenotypic resistance findings. The prevalence of *S. aureus* was higher in Kara (58.9%) compared to Agege (41.1%). The findings of this study highlight the significant presence of antibiotic-resistant *S. aureus* in raw milk, which poses health risks to consumers and emphasizes the need for enhanced surveillance, antibiotic stewardship, and public education on milk safety.

Keywords: *Staphylococcus aureus*, MRSA, antimicrobial resistance, cow milk, Lagos State, dairy hygiene, public health.

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INTRODUCTION

Staphylococcus aureus is an opportunistic pathogen often implicated in human and animal diseases, especially foodborne infections. *S. aureus* is a Gram-positive, coagulase-positive bacterium that is commonly found in the nasal passages, skin, and on surfaces of both humans and animals. It is an important pathogen in foodborne infection due to its ability to produce heat-stable enterotoxins, which cause foodborne illness when ingested and have been ranked among the leading causes of food-related illness worldwide (Fang *et al.*, 2024).

Milk is a highly nutritious food that is widely consumed in different households; however, raw milk often favours the growth of many microorganisms. Milk and its derivatives, in particular, are considered a major source of *S. aureus* infection in man (González-Machado *et al.*, 2024; Jahan *et al.*, 2015). The bacterium is frequently found associated with subclinical mastitis in dairy cattle and may be present in milk and other dairy products, which can result in substantial economic losses for the dairy industry (Jahan *et al.*, 2015; Shah *et al.*, 2025). Raw milk is especially vulnerable to *S. aureus* contamination due to factors such as poor milking hygiene, inadequate sanitation, and suboptimal handling or storage conditions (Liu *et al.*, 2022). The pathogen is capable of multiplying rapidly in milk at ambient temperatures, making it a particular concern in settings where milk is not properly refrigerated (Lalani, 2021; Liu *et al.*, 2022). Methicillin-resistant *Staphylococcus aureus* (MRSA)-causing bovine mastitis was first reported in 1972, and its transmission appears to occur between animals, and from animals to humans (Hassen *et al.*, 2026; Khanal *et al.*, 2022; Matyi *et al.*, 2013).

Raw cow milk, while popular for its natural nutrients and beneficial bacteria, can also be a medium for the proliferation of harmful pathogens, including *S. aureus* and its methicillin-resistant form (MRSA) (Tegegne *et al.*, 2024). Investigating these pathogens and their susceptibility to antibiotics in raw cow milk is essential to address significant public health and economic concerns. The presence of *S. aureus* and MRSA in raw cow milk poses severe health risks to consumers. *S. aureus* infection can cause food poisoning, leading to gastrointestinal issues such as nausea, vomiting, and diarrhoea, while MRSA infections are particularly of interest due to their resistance to commonly used antibiotics, which can result in therapeutic failure, extended hospital stays, and death (Weleslassie *et al.*, 2026; Zhang *et al.*, 2023).

Several studies have demonstrated the occurrence of *Staphylococcus aureus*, including methicillin-resistant *S. aureus* (MRSA), in raw cow milk and bovine subclinical mastitis in Nigeria, emphasizing its relevance to food safety and public health. In Jos South Local Government Area of Plateau State, a study reported *S. aureus* in 20.4% of quarter milk samples from cows with subclinical mastitis, with 11.9% of the *S. aureus* isolates identified as MRSA, and these isolates showed resistance to antibiotics such as oxacillin and cotrimoxazole (Anueyiagu *et al.*, 2022). Similarly, *S. aureus* contamination of raw cow milk has been reported in Northern Oyo State with a prevalence of 2.7% as part of a broader assessment of milk quality, although MRSA was not specifically detected in that study (Ghali-Mohammed *et al.*, 2025). Beyond Plateau and Oyo States, evidence synthesized from a national systematic review demonstrates that the prevalence of *S. aureus* in raw cow milk and milk products varies markedly across Nigeria, reflecting differences in dairy practices, hygiene, and antimicrobial use. The prevalence rates of *S. aureus* in milk ranged from as low as 3.1–5.0% in some northern locations to values exceeding 70% in certain traditional dairy settings (Odetokun *et al.*, 2023).

In Ogun State (Abeokuta), a notably higher MRSA burden was documented, detecting methicillin resistance in 18.5% of *S. aureus* isolates recovered from raw cow milk (Omoshaba *et al.*, 2020). In Kaduna State, prevalence rates of *S. aureus* from raw and bulk milk varied from 8.7% to 12.4% (Okpo *et al.*, 2016; Umaru *et al.*, 2014), whereas a lower prevalence of 3.1% was observed in milk and milk products sampled in Zaria (Esonu *et al.*, 2021). These findings collectively corroborate earlier reports of multidrug-resistant *S. aureus* in dairy samples from Oyo, Ogun, Kaduna, and Nasarawa States, while also confirming that MRSA generally represents a smaller but clinically significant fraction of *S. aureus* isolates in raw cow milk across Nigeria (Odetokun *et al.*, 2023). The wide regional variation in prevalence and resistance patterns underscores the importance of localized surveillance and targeted antimicrobial resistance control strategies within Nigeria's dairy value chain.

Although MRSA has been reported in food products within Lagos (Mujeeb *et al.*, 2025) and in dairy systems in Southwest Nigeria (Omoshaba *et al.*, 2020), data specifically on dairy products in Lagos remain scarce. This present study represents the first comparative analysis of antimicrobial resistance patterns in Kara and Agege—two high-risk locations characterized by distinct socio-economic and sanitary conditions. The study underscores significant regional differences in resistance profiles, reinforcing the need for context-specific monitoring and control strategies. There are scanty reports on the state of milk consumed in Lagos State, which is the hub of business activities. This absence of local data leaves a margin in deciphering the gravity of contamination and the hazards posed by the pathogen in Lagos.

Hence, investigating the prevalence and antimicrobial susceptibility of *S. aureus* in raw milk is essential to mitigate potential health risks and develop effective control measures. This study aims to isolate *S. aureus* from raw cow milk in Lagos State and determine its susceptibility profile to commonly used antibiotics.

MATERIALS AND METHODS

Sample Size Justification

The sample size was determined using the sample size determination for the estimation of the single population proportion formula (Thrusfield, 2018).

$N = (Z^2pq)/D^2$

where $q = 1-p$, N = sample size, Z = Z-score, p = prevalence rate, D = absolute allowable error.

With a confidence interval of 95%

$p = 42.1\% = 0.421$ (Alembo *et al.,* 2020),

$Z = 1.96$, $q = 1-p = 0.58$, $D = 20\%$ of $p = 0.0842$

$N = (1.96^2 * 0.421 * 0.58)/0.0842^2$

$N = 132.3$

A total of 132 raw cow milk samples were obtained and used for this study.

Sample Collection

A total of 132 raw cow milk samples were collected at a major cattle market in Kara (a boundary between Lagos and Ogun States) and an abattoir at Agege, Lagos State. 66 milk samples were collected from each location. The milk samples were collected in a universal bottle on ice and transported to the Central Research Laboratory of the University of Lagos, Akoka, for microbiological analysis.

Identification of Staphylococcal Isolates

Samples were initially inoculated into sterile nutrient agar and incubated at 37°C for 24 h. The overnight cultures were then sub-cultured on freshly prepared Mannitol Salt agar (MSA) by streaking and incubation at 37°C for 18 – 24 h. Cultural characteristics of the resulting colonies were noted and further characterized using standard laboratory procedures (Aernan *et al.*, 2023; Bergey, 1994; Cheesbrough, 2005; Iqbal *et al.*, 2024).

Antibiotics Susceptibility Testing (AST)

The antibiotic susceptibility of *S. aureus* isolates was determined using the standardized disk diffusion method in accordance with CLSI guidelines (Clinical and Laboratory Standards Institute, 2020). Briefly, discrete colonies were emulsified in 5 mL sterile saline, adjusted to 0.5 McFarland turbidity (≈1.5×10^8 CFU/mL), and uniformly swabbed onto Mueller–Hinton agar. AST was performed for cefoxitin (FOX) 30 µg (the recommended MRSA screening agent due to superior mecA detection compared to oxacillin), methicillin (MET) 5µg, vancomycin (VA) 30µg, imipenem (IPM) 10µg, gentamicin (GEN) 10µg, erythromycin (ERY) 15µg, and tetracycline (TET) 30µg. Antibiotic disks were firmly placed at equidistance on Mueller-Hinton agar and then incubated at 37 °C for 24 hours. After incubation, the zone of inhibition was measured, recorded, and interpreted following previous studies (Aernan *et al.*, 2024; Clinical and Laboratory Standards Institute, 2020; Iqbal *et al.*, 2016).

The differing disk concentrations reflect CLSI/EUCAST standardization rather than arbitrary choice. Each antibiotic has a defined content (for example, cefoxitin 30 µg, methicillin 5 µg, gentamicin 10 µg) selected to provide reliable detection of resistance mechanisms and to ensure consistent interpretation of inhibition zones across laboratories. These values take into account differences in molecular weight, diffusion behaviour in agar, and established clinical breakpoints, thereby allowing isolates to be accurately categorized as susceptible, intermediate, or resistant.

Amplification of resistance genes by polymerase chain reaction (PCR)

A total of 17 isolates were selected for the amplification of the mecA gene by multiplex PCR. The strain's DNA was extracted by suspending fresh colonies in a 200 µL Tris-EDTA and boiling for 10 minutes at 100 °C, and immediately cooled on ice. The boiled suspension was centrifuged at 12,000 rpm, and the supernatant was collected and used as the template for the amplification of genes as previously described with slight modifications (Odumosu *et al.*, 2013). Multiplex Polymerase Chain Reaction (Multiplex-PCR) was carried out using a 25 µl reaction comprising both forward and reverse primers, Forward AAAATCGATGGTAAAGGTTGGC, reverse AGTTCTGCAGTACCGGATTTGC (533bp) primer specific for the mecA gene (Shopsin *et al.*, 1999).

Statistical Analysis

All data were entered into Microsoft Excel 2021 spreadsheets. Descriptive statistics were used to summarize resistance patterns. Fisher's exact test was applied to determine significant associations between MRSA in samples from Kara and Agege. Statistical significance was set at $p < 0.05$.

RESULTS

Identification and Purification of Isolates

Out of the 132 samples analyzed in this study, a total of 114 bacterial colonies were recognized, of which 64 were Gram-positive cocci, 42 showed a yellow colony on MSA, and 17 (14.9%) tested positive for catalase and tube coagulase test, and *Staphylococcus aureus* was confirmed presumptively. In all, 17 *S. aureus* were identified from the cow milk samples (Table 1).

Distribution of Isolates (*Staphylococcus aureus*) across the Locations

A total of 17 *Staphylococcus aureu***s** isolates were identified in both locations. 10 isolates were identified in Kara and 7 in Agege, as shown in Table (2).

Result of Antimicrobial Susceptibility Test

The antimicrobial susceptibility test shows that 5 (29.4%) were resistant to cefoxitin, 1 (5.9%) to vancomycin, and 1 (5.9%) to erythromycin, and none were resistant to imipenem, gentamicin, clindamycin, and tetracycline, respectively. The highest average zone of inhibition was shown in Imipenem (30.18 mm), as shown in Table (3).

Table 1. Biochemical Identification of *Staphylococcus aureus*

Samples/Isolates Tested	Test	Result	No. of positive isolates (%)	Inference
132 milk samples	Growth on Nutrient media	Visible growth/colony	114 (86.36%)	Bacterial growth
114 isolates	Gram staining	Gram-positive (purple) cocci	64 (56.1%)	Gram-positive cocci
64 isolates	Growth on Mannitol Salt Agar	Golden yellow	42 (65.6%)	*Staphylococcus* spp
64 isolates	Catalase test	Bubbles	40 (62.5%)	*Staphylococcus* spp
40 isolates	Agglutination test	Coagulase	17 (42.5%)	*Staphylococcus aureus*
17 isolates	Lecithinase test	Opaque zone	17 (100%)	*Staphylococcus aureus*

Table 2. Distribution of the S. aureus isolates in the two Locations.

Isolates	Kara	Agege	Total
No. *S. aureus* identified	10	7	17
Percentage of *S. aureus* identified	58.9	41.1	100

Table 3: Mean Zone of Inhibition of the *S. aureus* against different antibiotics.

Antibiotic	Average Zone (mm)
Cefoxitin	24.12
Methicillin	13.20
Vancomycin	21.59
Imipenem	30.18
Gentamicin	23.94
Erythromycin	27.06
Clindamycin	21.94
Tetracycline	23.76

The study shows that 5 of the isolates were cefoxitin resistant (MRSA), 1 vancomycin-resistant staphylococci, and 1 clindamycin-resistant, as shown in the table (4).

A total of 5 isolates were Methicillin Resistance *Staphylococcus aureus* (MRSA); resistance to cefoxitin, which is the surrogate for MRSA, as shown in the table below.

Table 4. Summary of the Antimicrobial Susceptibility Result

Antibiotic	Susceptible (S)	Intermediate (I)	Resistant (R)
Cefoxitin	12 (70.6%)	0 (0.0%)	5 (29.4%)
Methicillin	0 (0.0%)	0 (0.0%)	17 (100%)
Vancomycin	15 (88.2%)	1 (5.9%)	1 (5.9%)
Imipenem	13 (76.5%)	4 (23.5%)	0 (0.0%)
Gentamicin	17 (100.0%)	0 (0.0%)	0 (0.0%)
Erythromycin	9 (52.9%)	8 (47.1%)	0 (0.0%)
Clindamycin	12 (70.6%)	4 (23.5%)	1 (5.9%)
Tetracycline	17 (100.0%)	0 (0.0%)	0 (0.0%)

Table 5. Table of MRSA (cefoxitin-resistant) distribution in the two locations

Location	MRSA	Non-MRSA
Kara	3	7
Agege	2	5
Total	5	12

Statistical Correlation

Fisher's exact test was used to compare the prevalence of MRSA between Kara (3 out of 10) and Agege (2 out of 7). The difference was not statistically significant (p = 0.05), suggesting there is no clear variation in MRSA distribution between the two locations.

Detection of mecA gene by PCR

The result of the PCR amplification targeting the *mec*A gene identified 8 out of 17 isolates (47.06%) as mecA positive, as evidenced by a distinct expected amplicon size of 533bp (Figure 1). The remaining 9 isolates were negative. Despite partial *mec*A carriage, all isolates were phenotypically resistant to methicillin (17/17, 100%) while cefoxitin resistance was observed in 5/17 isolates (29.40%), all of which were positive for *mec*A amplification.

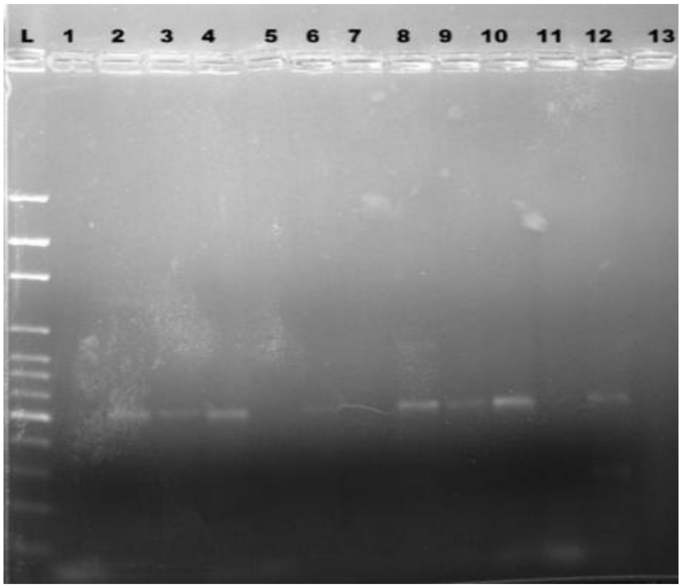

Fig. 1. Agarose gel electrophoresis of *mec*A PCR products. Lane L, DNA ladder; lanes 1–13, clinical isolates. Eight lanes display bands at the expected *mec*A amplicon size of 533bp; non-banding lanes indicate *me*cA-negative results.

DISCUSSION

Staphylococcus aureus is a significant foodborne pathogen that poses a substantial public health risk, especially in dairy products. In this study, a prevalence of 12.8% for *S. aureus* isolates was found across the two locations studied. This was lower than the previous investigation (Omoshaba *et al.*, 2020), which reported a higher prevalence of 18.5% of *S. aureus* in raw cow milk in Abeokuta. Similarly, a study reported an even higher prevalence of 31% (Esonu *et al.*, 2021), while another study reported a prevalence of 5% in Nassarawa State

(Yakubu *et al.*, 2020), which was lower compared to the present study. The differing prevalence rates observed across studies may be explained by variations in hygiene practices, farm management, and environmental conditions. For instance, although Lagos and Ogun are geographically close, differences in herd density, informal milk handling, and sanitary practices may contribute to the lower prevalence reported here compared with Abeokuta (Omoshaba *et al.*, 2020). Regional differences are also evident when comparing South-Western Nigeria with North-Central Nigeria, which demonstrated only 5% prevalence of *S. aureus* (Yakubu *et al.*, 2020). Climatic factors such as temperature and humidity, as well as socio-economic differences in dairy production systems, have been shown to influence bacterial survival and transmission (Hassen *et al.*, 2026). However, the presence of *S. aureus* in raw cow milk is worrisome due to the ability of the bacteria to produce coagulase, a virulence factor that facilitates the formation of blood clots, helping the bacterium to evade the host's immune system. This characteristic is commonly associated with pathogenic strains of staphylococci, implicating these isolates as potential public health hazards.

The antimicrobial susceptibility profile of *Staphylococcus aureus* isolates from cow milk in this study revealed significant resistance patterns, particularly to β-lactam antibiotics. The cefoxitin resistance rate (29.4%) observed in this study is indicative of the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) strains. This finding aligns with previous research that reported cefoxitin resistance in milk-derived isolates in Nasarawa State (Yakubu *et al.*, 2020). Similarly, a study found MRSA in 18.5% of milk samples from Ogun State (Omoshaba *et al.*, 2018), and a meta-analysis reported an average MRSA prevalence of approximately 46% in food and animal sources across Nigeria (Ezeh *et al.*, 2023).

The distribution of MRSA between Kara and Agege did not show a statistically significant difference ($p = 0.05$), indicating a potentially widespread presence of resistant strains across dairy locations in Lagos. This finding is consistent with the notion of endemic MRSA distribution in Nigerian livestock, as supported by findings in Plateau State and other regions.

Regarding susceptibility, all isolates were susceptible to gentamicin and tetracycline (100%). These results are consistent with findings from Plateau and Nasarawa States, where gentamicin and tetracycline susceptibility remained high among dairy isolates (Anueyiagu *et al.*, 2022; Yakubu *et al.*, 2020). However, contrasting reports, such as those of Ayeni et al. (2016), found high resistance to gentamicin and tetracycline in Ogun State, suggesting geographic variation in resistance patterns likely influenced by antimicrobial usage practices.

Vancomycin resistance was rare (5.9%), aligning with previous estimates that reported a pooled vancomycin resistance prevalence of approximately 13% in Nigeria. Imipenem resistance was not detected, consistent with global trends of low carbapenem resistance in animal-derived *S. aureus* (Ezeh *et al.*, 2023)

Moderate levels of intermediate susceptibility were observed for erythromycin (47.1%) and clindamycin (23.5%), with only one isolate (5.9%) showing resistance to clindamycin. These results reflect lower macrolide-lincosamide resistance levels compared to clinical human isolates but align with findings from dairy-associated *S. aureus* in Plateau and Nasarawa States (Yakubu *et al.*, 2020).

The cefoxitin and methicillin resistance isolates (*n*=17) in this study were subjected to genotypic confirmation of the presence of the *mec*A gene, which confirms the MRSA positive strains. The PCR amplification of the *mec*A gene was confirmed among five of the cefoxitin-resistant strains and three out of the 17 methicillin-resistant strains, with an amplicon size of 533bp each. According to the CLSI guidelines, resistance to methicillin alone does not guarantee the presence of MRSA because methicillin is unstable *in vitro,* whereas cefoxitin is a better inducer of the *mec*A gene because it can increase the expression of PBP2 (Koupahi *et al.*, 2016).

This study clearly shows that MRSA is present in milk from local dairy farms in Nigeria, suggesting that it could find its way into the food we consume. This aligns with nationwide surveillance reports (Federal Ministries of Agriculture and Health, 2017; Gaddafi *et al.*, 2025; Musa *et al.*, 2026), which point to the widespread occurrence of MRSA in animals raised for food. Of particular concern is the risk this poses to vulnerable individuals—young children, the elderly, and those with weakened immune systems—who are especially prone to serious illness from drug-resistant infections (El-Ghany, 2021; Gould *et al.*, 2012; Iqbal and Ashraf, 2021; Vestergaard *et al.*, 2019). To better safeguard these groups, we recommend practical, health-focused actions grounded in the One Health approach. Promoting the pasteurization of raw milk, for instance, can greatly reduce the risk of harmful bacteria (Oliver *et al.*, 2005) and should be emphasized through public awareness campaigns. Additionally, educating farmers on clean milking practices and responsible antibiotic use can help limit the spread of MRSA on farms (Organization *et al.*, 2016). When combined with regular monitoring of antimicrobial resistance in the food chain, these strategies can go a long way in reducing the threat of MRSA transmission from farm to table.

CONCLUSION

This study demonstrates the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) in cow milk samples from Lagos State, with a prevalence of 29.4%, aligning with trends reported in other regions of Nigeria. While resistance to β-lactam antibiotics remains a concern, the high susceptibility to gentamicin, tetracycline, and imipenem indicates that these antibiotics remain effective options. The presence of MRSA in dairy products poses a heightened risk to vulnerable populations such as immunocompromised individuals and young children, who may experience more severe or prolonged infections. Actionable interventions should include community-based pasteurization campaigns to ensure raw milk is heat-treated before consumption, and targeted farmer education programs emphasizing hygienic milking practices and judicious antibiotic use.

A call for routine surveillance among dairy farms is highly recommended to ensure a holistic One Health approach towards the mitigation of AMR, and antibiotic stewardship should be promoted among farmers and veterinarians.

ACKNOWLEDGMENTS

The author would like to acknowledge the authorities of the Department of Microbiology, Faculty of Science, University of Lagos, for their support in this research.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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