Research Article

Bovine Mastitis in Nigeria: A High Burden of Phenotypically Characterized Methicillin- Resistant *Staphylococcus aureus*

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Abstract | The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) poses a serious public health threat and has been increasingly reported as an emerging problem in veterinary medicine. This study aimed to isolate and phenotypically characterize MRSA from mastitic cows on two institutional farms of Ahmadu Bello University, Zaria, Kaduna State, Nigeria. A total of 32 milk samples were collected; 12 (37.5%) from the Division of Agricultural Colleges farm and 20 (62.5%) from the National Animal Production Research Institute farm. Isolation and phenotypic identification of *Staphylococcus aureus* (*S. aureus*) was performed according to standard culture and characterization methods. The Kirby-Bauer disc diffusion method was used for antibiotic susceptibility testing (AST), using Ciprofloxacin (CP, 5µg), Gentamicin (GN, 10µg), Erythromycin (EM, 15µg), Amoxicillin-Clavulanic Acid (AMC, 30µg), and Cefoxitin (CFX, 30µg). Resistance to methicillin antibiotics was assessed using cefoxitin, and the AST results were interpreted according to the Clinical and Laboratory Standard Institute (CLSI) 2025 guideline. Bacterial isolates from mastitic milk were predominantly *S. aureus*, which represented 75% (24/32) of all isolates. Other identified bacteria include *Klebsiella* species (9.4%), *Streptococcus* species (6.3%), *Bacillus* species (6.3%), and *Corynebacterium* species (3.1%). Therefore, *S. aureus* was found to be the principal pathogen responsible for bovine mastitis in the study area. Importantly, five (20.8%) of the *S. aureus* were MRSA, and 2 (8.3%) were multidrug resistant. Consequently, we recommend the enforcement of strict hygiene, improved milking techniques, and prudent use of antibiotics.

Keywords | Cow, Institutional farms, Mastitis, MRSA, *Staphylococcus aureus,* Health

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INTRODUCTION

Milk is an animal-derived food that provides essential nutrients including protein, energy, vitamins and minerals which support tissue growth and maintenance (Rochmah *et al*., 2023). Biologically active compounds such as casein and whey proteins in milk have been found to be important for physiological and biochemical functions that have crucial impacts on human metabolism and health (Aliyo *et al*., 2022). However, the safety of milk and dairy products regarding food-borne diseases is a major global concern, particularly in developing countries where the production often occurs under poor hygienic, sanitary, and agricultural conditions (Umaru *et al*., 2019; Nisa *et al*., 2020; Ahmad *et al*., 2022). Bacterial contamination of milk is the major cause of food-borne diseases, which pose a serious threat to the health of millions of people worldwide. The magnitude of food-borne diseases, death or other complications associated with milk contamination increases day-to-day and imposes a substantial burden on health care systems (Aliyo *et al*., 2022). Due to its complex biochemical composition, good nutritional value, and high-water content, milk serves as an excellent growth medium for both pathogenic and spoilage microorganism associated with several disease conditions notably staphylococcal infections, salmonellosis, brucellosis, tuberculosis, shigellosis, cholera and numerous others (Unakal and Kaliwal, 2010; Esonu *et al*., 2021; Wijaya *et al*., 2021; de Klerk and Robinson, 2022).

Mastitis, the inflammation of mammary gland parenchyma of animals is endemic worldwide, and can affect all lactating livestock (Puspitasari *et al*., 2021; Girma and Tamir, 2022). The majority of animals especially dairy cattle are at risk of mastitis owing to poor milking procedures and unhygienic practices. This causes significant economic losses in terms of reduced milk production and quality, increased veterinary costs, deaths and impacting animal welfare. Accurate diagnosis and effective treatment are crucial, but current methods have limitations (Duarte *et al*., 2015). The emergence of antimicrobial resistance (AMR) is a critical global health concern, as it limits treatment options and leads to prolonged illness and higher mortality in both humans and animals (Thirafi *et al*., 2022). Assessing methicillin-resistant *Staphylococcus aureus* is crucial for developing effective treatment strategies and controlling the spread of resistant strains.

MATERIALS AND METHODS

Study area

This study was conducted on two Institutional Farms of Ahmadu Bello University, Zaria: The Division of Agricultural Colleges (DAC) and National Animal Production Research Institute (NAPRI) situated in Samaru and Shika of Sabon-Gari and Giwa Local Government Areas (LGAs), respectively in Kaduna State. These are located in the Northern Guinea Savannah ecological zone of Nigeria (Figure 1). Sabon-Gari is a major city located in the Northern part of Kaduna State. Sabon-Gari has six districts and eleven political wards, and boundaries with Zaria, Kudan, Soba and Giwa LGAs, and lies between latitude 11.06^{0}N to 11.12^{0}N and longitude 7.4^{0}E to 7.7^{0}E (Figure 1) with a total land area of 191.0km^2, a human population of 291,358 according to 2006 census (KDSG, 2008) and a density of 2,254/km^2. Giwa is a major city located in the Northern part of Kaduna State. Giwa has eleven districts and twelve political wards, and boundaries with Zaria, Sabon-Gari, Birnin Gwari and Igabi LGAs, and lies between latitude 12.20^{0}N to 12.52^{0}E and longitude 7.0^{0}E to 7.5^{0}E (Figure 1) with a total land area of 2,302 km^2, a human population of 292,384 according to 2006 census (KDSG, 2008) and a density of 187.6/km. They both have a tropical savanna climate, with a wet season lasting from April to September and a dry season from October to March. Their economy is primarily based on agriculture. Staples are guinea-corn and millet while cash crops include cotton, and groundnut. A major occupation of the people is animal rearing, namely; cattle, sheep, goats, and pigs (KDSG, 2019).

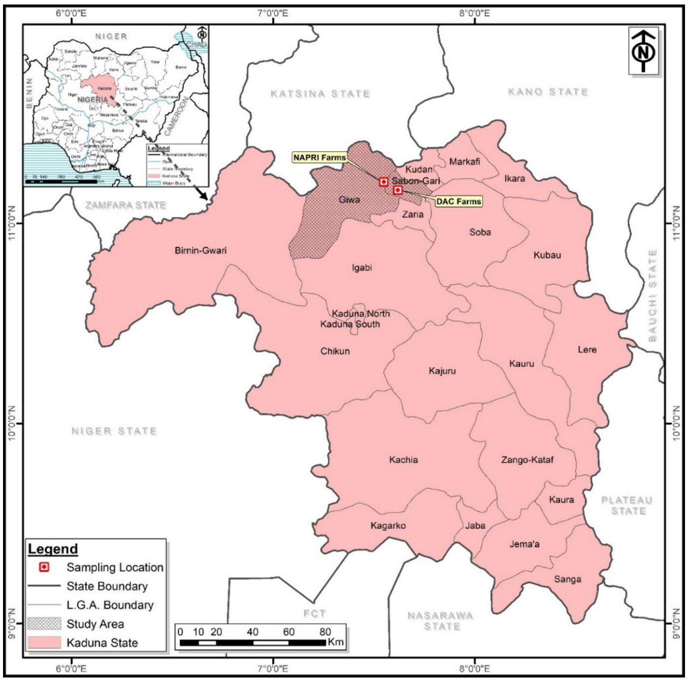

Figure 1: Map of Kaduna State showing Study Area (sampling points).
Source: Modified from https://www.openstreetmap.org/

Study design

This cross-sectional study employed purposive sampling. Animals displaying typical clinical sign of mastitis were sampled over a six-months period (January to June, 2024).

Sample collection, transportation, and storage

A total of 32 milk samples were aseptically collected from cows showing clinical signs of mastitis. This included 12 samples (37.5%) from DAC (4 White Fulani, 8 Sokoto Gudali) and 20 samples (62.5%) from NAPRI (5 White Fulani, 3 Sokoto Gudali, 12 Friesian). Prior to milking, the teat and udder were wash with water, dried with disposable towels, and disinfected with a mild antiseptic (Savlon). Five millilitres of milk were collected into a sterile bijou bottle. Samples were properly label and transported in an ice-packed cold box to the AMR Sentinel Laboratory of the Department of Veterinary Microbiology, Ahmadu Bello University, Zaria, for processing. The samples were stored at 4 °C until needed for analysis.

Phenotypic characterization of *Staphylococcus* species

All the pure colonies that appear purple or bluish Gram-positive cocci were subjected to catalase test, coagulase test, and mannitol fermentation test. Briefly, for catalase test, a sterilized Pasteur loop was used to pick an isolated colony from a blood agar plate and place it onto a drop of 6% hydrogen peroxide on a clean, grease-free glass slide. A foamy reaction indicated a catalase-positive result, and the negative was further confirmed using tube method. For the coagulase slide test, about 10 µl of deionized water or physiological saline was added to a slide, a sterilized Pasteur loop was used to pick an isolated colony from blood agar plate and was emulsified into the water to obtain a smooth milk-colored suspension. A drop of a rabbit plasma was added to the slide, and clumping was observed for 20 seconds, coagulase positive give agglutination within 20 seconds indicated a positive result, while the absence of agglutination indicated a negative result. For the mannitol fermentation test, an inoculum from a pure culture was transferred aseptically into a sterile tube containing phenol red mannitol broth. The inoculated tube was incubated at 37 °C for 24 hours. A color change from red to yellow indicated a positive result (mannitol fermentation), while no color change indicated a negative result. Although the DNase test would have further strengthened species-level identification, it was not available during the period of this study. Thus, phenotypically, *S. aureus* was identified as catalase positive, coagulase positive and ferment mannitol sugar.

Antimicrobial susceptibility testing

The Kirby-Bauer disc diffusion method was employed in this study, briefly, Mueller-Hinton agar plate was prepared according to manufacturer's instruction. A phenotypically identified colony was picked using a sterile Pasteur loop. Bacterial suspensions were prepared in sterile distilled water, and was standardized according to McFarland standards. The inoculum was lawned onto Mueller-Hinton agar plates. Five (5) different antibiotics from different classes including, Gentamycin (GN) 10 µg, Cefoxitin (CFX) 30 µg Ciprofloxacin (CIP) 5 µg, Amoxicillin clavulanic acid (AMC) 30 µg, and Erythromycin (EM) 15 µg were used. Similarly, Resistance to methicillin was assessed using cefoxitin. The antimicrobial disk was placed 15mm away from the edge of the plates and 20mm away from each other. The plates were incubated at 37 °C for 24 hours and the diameter of the inhibition zone for each antibiotic was recorded and interpreted using the interpretive standards of the Clinical and Laboratory Standard Institute (CLSI, 2025) guidelines.

Data analysis

The results from this study were summarized using descriptive statistics, including tables and percentages. Chi-square test using SPSS version 17 for Windows® was used to analyse the varied bacterial isolation rates. At 95% confidence interval, P-value ≤ 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

This study identified several bacterial isolates associated with bovine mastitis in two institutional farms in Nigeria. This includes the predominant *Staphylococcus aureus* (75%), followed by *Klebsiella* spp. (9.4%), *Streptococcus* spp. (6.3%), *Bacillus* spp. (6.3%), and *Corynebacterium* spp. (3.1%) (Table 1). Our findings confirm the earlier parallel reports of Anayo *et al*. (2013) and Yulianto *et al*. (2023) which documented *S. aureus* as the principal cause of bovine mastitis in different parts of the world. However, the observed high *S. aureus* isolation rate (75%) was relatively higher than the 52.4% reported by Sori *et al*. (2011) in south west Ethiopia. This is likely due to various risk factors for bacterial mastitis, such as poor milking hygiene (including inadequate udder disinfection, lack of glove use), indiscriminate antibiotic use, and the absence of dry cow therapy as documented by FAO (1990), Ningrum *et al*. (2022), and Khairullah *et al*. (2024). In addition, inadequate mastitis surveillance programs could further contribute to this high prevalence (Dufour *et al*., 2012; Shittu *et al*., 2012).

Table 1: Isolation rate of *Staphylococcus aureus* and other aerobic bacteria from mastitic cow milk in institutional farm.

S. No	Bacteria	No. of samples	% of isolate
1	*Staphylococcus aureus*	24	75.0
2	*Streptococcus* spp.	2	6.3
3	*Bacillus* spp.	2	6.3
4	*Klebsiella* spp.	3	9.4
5	*Corynebacterium* spp.	1	3.1
Total		32	100.0

The isolation rate of *S. aureus* varied across the two institutional farms sampled. Out of the 32 milk samples, 7 of 12 samples from DAC (58.3%) were positive, while NAPRI recorded a higher isolation rate of 17 out of 20 samples (85.0%). However, no statistically significant association was found between the farm of origin and *S. aureus* isolation (χ^2= 1.60, p= 0.206) (Table 2). Biochemically, all 24 staphylococcal isolates grown on Mannitol Salt Agar were Gram-positive cocci, occurring in clusters, non-motile, non-spore-forming, catalase positive, and fermentative, producing golden-yellow colonies characteristic of *S. aureus*, consistent with standard identification methods (Quinn *et al*., 2004; Kataria *et al*., 2013). Thus, the entire 24 staphylococci identified from both farms were exclusively *S. aureus* highlighting its significance as the major mastitis pathogen in cows.

Table 2: Farm-Specific Isolation rate of *Staphylococcus aureus*.

S. No	Farm	No. of samples	No. of positive	% of samples positive
1	DAC	12	7	58.3
2	NAPRI	20	17	85.0
Total		32	24	75.0

χ^2 = 1.60, p = 0.206

Table 3: Breed specific Isolation rate of *Staphylococcus aureus* from mastitic cow milk in institutional farm.

S. No	Breed	No. of samples	No. of positive	% of isolate
1	White Fulani	9	7	77.8
2	Sokoto Gudali	11	9	81.8
3	Friesian	12	8	66.7
Total		32	24	75.0

χ^2 = 0.75, p = 0.686

Similarly, among the three cattle breeds sampled, *S. aureus* isolation rates were comparable. Sokoto Gudali had the highest positivity (81.8%, 9/11), followed by White Fulani (77.8%, 7/9) and Friesians (66.7%, 8/12), though differences were not statistically significant (χ^2 = 0.754, p = 0.686) (Table 3). This indicates no strong breed-based predisposition for *S. aureus* infection in this study.

The antimicrobial susceptibility profile of the *S. aureus* isolates revealed high susceptibility to ciprofloxacin, gentamicin, and erythromycin. In contrast, widespread resistance was observed against amoxiclav, likely reflecting its easy availability in local markets. Variable resistance to cefoxitin was also detected, indicating the presence of MRSA strains. Notably, isolate M9 exhibited resistance to all tested antibiotics, while isolates M14, M17, M19, and M20 were fully susceptible, highlighting the heterogeneous resistance patterns present (Table 4). Phenotypic analysis identified five MRSA isolates (M9, M10, M11, M13, M28), representing 20.8% of the total *S. aureus* population. Among these, only two isolates (M9 and M10, 8.3%) met the criteria for multidrug resistance (MDR), displaying resistance to three or more antimicrobial classes (Figure 2).

Table 4: Antimicrobial susceptibility profile of *Staphylococcus aureus* isolated from mastitic cow milk in institutional farms, Nigeria.

S. No	Sample ID	Antibiotics (µg)				
		Ciprofloxacin (CP, 5)	Gentamicin (GN, 10)	Erythromycin (EM, 15)	Amoxiclav (AMC, 30)	Cefoxitin (CFX, 30)
1	M6	29 (S)	29 (S)	29 (S)	29 (S)	29 (S)
2	M7	16 (S)	16 (S)	17 (S)	14 (I)	13 (I)
3	M8	24 (S)	24 (S)	25 (S)	10 (R)	21 (S)
4	M12	29 (S)	15 (S)	25 (S)	6 (R)	26 (S)
5	M14	29 (S)	16 (S)	23 (S)	26 (S)	25 (S)
6	M16	28 (S)	13 (I)	24 (S)	26 (S)	25 (S)
7	M17	29 (S)	15 (S)	24 (S)	25 (S)	26 (S)
8	M18	29 (S)	13 (I)	21 (S)	6 (R)	25 (S)
9	M19	29 (S)	15 (S)	25 (S)	26 (S)	26 (S)
10	M20	28 (S)	17 (S)	25 (S)	26 (S)	26 (S)
11	M22	27 (S)	14 (I)	24 (S)	26 (S)	26 (S)
12	M24	29 (S)	16 (S)	21 (S)	26 (S)	25 (S)
13	M25	29 (S)	16 (S)	25 (S)	24 (S)	26 (S)
14	M26	29 (S)	16 (S)	25 (S)	25 (S)	26 (S)
15	M27	29 (S)	17 (S)	24 (S)	25 (S)	26 (S)
16	M29	29 (S)	13 (I)	25 (S)	26 (S)	26 (S)
17	M30	29 (S)	17 (S)	24 (S)	26 (S)	26 (S)
18	M31	29 (S)	15 (S)	23 (S)	26 (S)	25 (S)
19	M32	29 (S)	15 (S)	25 (S)	25 (S)	26 (S)
20	M9	6 (R)	6 (R)	6 (R)	6 (R)	6 (R)
21	M10	13 (R)	6 (R)	26 (S)	6 (R)	12 (R)
22	M11	29 (S)	15 (S)	15 (S)	6 (R)	6 (R)
23	M13	29 (S)	14 (S)	24 (S)	6 (R)	6 (R)
24	M28	29 (S)	17 (S)	25 (S)	25 (S)	6 (R)

Key: S= Susceptible; I= Intermediate; R= Resistance.

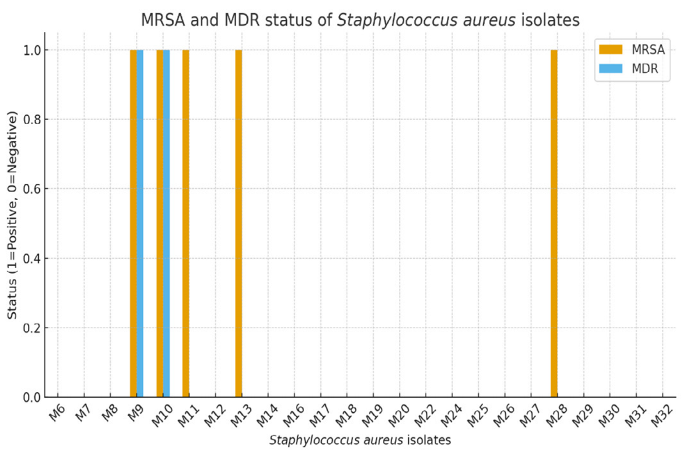

Figure 2: Occurrence of multidrug-resistant and methicillin-resistant *Staphylococcus aureus* among mastitis-affected cows in institutional farms, Nigeria.

Links Researchers

The co-occurrence of MRSA and MDR phenotypes in isolates M9 and M10 highlights potential treatment challenges and the risk of therapeutic failure, underscoring the importance of antimicrobial stewardship in dairy farms. Therefore, when MDR-MRSA is detected, the affected cow should be immediately isolated and its milk discarded to prevent spread. Similarly, staff must follow strict PPE, hygiene, and milking-order procedures, and in-contact cows should be screened, with cases reported to the appropriate authorities for monitoring.

The MRSA prevalence observed in this study is slightly higher than several previous studies in Nigeria. This includes the work of Umaru *et al.* (2019), who reported 7.8% MRSA among Fulani cattle herds in Kaduna State, and the 5.0% MRSA reported from fresh milk and milk products in Nasarawa State, Nigeria by Aliyu *et al.* (2020). This elevated prevalence may be driven by factors such as indiscriminate antibiotic use, suboptimal hygiene practices, close human-animal contact, and the widespread availability of amoxiclav, which likely drives resistance. Overall, the present study demonstrated that *S. aureus*, including MRSA and MDR strains, remains a major pathogen in bovine mastitis in institutional farms, with important implications for public health, animal health, and antimicrobial stewardship. The combination of farm management practices, antibiotic usage, and environmental factors likely drives the observed resistance patterns, emphasizing the need for monitoring, targeted interventions, and education to mitigate the risk of dairy-borne infections.

CONCLUSIONS

Using laboratory-based techniques, this study isolated and phenotypically characterized *S. aureus* from mastitic milk obtained from cattle on the Institutional Farms of Ahmadu Bello University, Zaria, Kaduna State, Nigeria. The findings revealed that *S. aureus* was the most commonly encountered pathogenic bacterium associated with bovine mastitis in the study area, accounting for 75% of the total isolates. Furthermore, the study demonstrated that the isolates exhibited resistance to beta-lactam antibiotics, particularly Amoxicillin-Clavulanic Acid (AMC) and Cefoxitin (CFX). The detection of MRSA in these university-affiliated farms, which ordinarily serve as national models for good dairy practice highlights a broader concern for the wider dairy sector and reinforces the need for stronger extension services and capacity-building efforts on hygiene, mastitis surveillance, and prudent antimicrobial use. Based on these findings, we recommend the exclusive use of veterinary-prescribed antimicrobials and strict adherence to proper hygienic practices on dairy farms to mitigate the emergence and spread of antimicrobial resistance.

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AUTHOR'S CONTRIBUTION

Ahmad, K.H. conceptualized and participated in the study; Musa, M. collected the samples, conducted the laboratory work, and prepared the initial draft of the manuscript; Umar, B.N., Zubair, F.Z., Ma'aruf, S.M., Fasogbon, I.V., Widodo, A., Wahyuni, R.M. and Dharianta, R provided assistance with laboratory analyses, proofreading, and manuscript preparation. All authors contributed to the final revision and approval of the manuscript.

NOVELTY STATEMENT

This study reports the first phenotypic characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) linked to bovine mastitis in institutional dairy farms in north-western Nigeria. It found a higher occurrence of MRSA (20.8%) compared to earlier reports from Nigerian dairy systems (5.0-7.8%). The study also identified multidrug-resistant MRSA strains (8.3%) in university-affiliated farms, which are typically considered models of good dairy management. These findings suggest that even such well-managed herds may act as underrecognized reservoirs of antimicrobial resistance, with potential implications for both animal and public health.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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