Original Article

Occurrence of Methicillin-resistant *Staphylococcus aureus* (MRSA) From Dairy Cows in Kebbi, Nigeria

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ABSTRACT

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important healthcare-associated pathogen that is also an emerging zoonosis.

Objectives: A cross-sectional study was conducted to investigate the prevalence of MRSA in dairy cattle farms in Kebbi, North-Western Nigeria.

Methods: A total of 200 milk samples, consisting of 50 samples each from lactating cows, were collected and used. The samples were analyzed using bacterial culture and isolation and polymerase chain reaction (PCR). Suspected MRSA isolates were identified via PCR detection of the mecA gene, and antimicrobial susceptibility profiles of the isolates were assessed using the Kirby-Bauer disk diffusion method.

Results: Of the two hundred milk samples examined, the prevalence of MRSA was recorded at 18% (36/200) using phenotypic and genotypic characterization methods. The susceptibility to vancomycin was observed in all isolates, and they had a multiple antibiotic resistance (MAR) index of >0.4.

Conclusion: This investigation showed the colonization of healthy dairy cows by multidrug-resistant MRSA.

Keywords: Cow, Dairy, Kebbi, Methicillin-resistant *Staphylococcus aureus*

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1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterium that can cause mild to serious infections and even death in humans and animals. Because of the possibility of MRSA strains being transmitted between humans and animals, the epidemiology of this infection is remarkably diverse (Parisi et al., 2016). *S. aureus*, particularly those resistant to methicillin/oxacillin, are mostly associated with mastitis in dairy cows; however, these pathogens are found in apparently healthy cows, too (Basanisi et al., 2017). These strains of MRSA are found on the teats of milking cows, particularly in dairy cows with subclinical mastitis, and can contaminate milk without causing changes in milk quality, enabling certain strains of MRSA to circulate easily during the milking of cows. Studies have reported an increase in infection with MRSA associated with dairy milk (Feßler et al., 2010; Vanderhaeghen et al., 2010), dairy products (Basanisi et al., 2017), and MRSA transmission between dairy farmers and dairy industry workers (Antoci et al., 2013). Transmission of MRSA and other pathogens has been reported due to contact between humans and animals, dairy processing, and exposure to environmental pollutants (Marshall and Levy, 2011; Meamar et al., 2021; Yakubu et al., 2022). MRSA disease transmission cases have become a significant concern for dairy farming and the dairy industry (Antoci et al., 2013). MRSA in cow's milk can be increased by poor dairy farm management and inappropriate antibiotic use (Joshi et al., 2014). Cases of nosocomial-associated and MRSA isolates found among individuals in the community without previous history of hospitalization have been reported in other studies.

Additionally, strains associated with livestock and pets have also been reported recently (Yakubu et al., 2022). Veterinarians, dairy processing workers, breeders, and people who come into close contact with animals have been implicated as major influencers of the colonization of dairy cows by livestock-associated (LA)-MRSA (Paterson et al., 2012; Yakubu et al., 2022). LA-MRSA infections in humans, which are caused by milk and milk products, can range from mild to serious skin and soft tissue infections (Kadlec et al., 2009; Lakhundi & Zhang, 2018). The danger of MRSA infection cases to human health can be reduced by investigating the spread of MRSA sourced from animal-derived foods such as milk and dairy products (Paterson et al., 2012). There is little information regarding MRSA in dairy cattle in the study area, Kebbi State, and in the region comprising three states, namely Sokoto, Kebbi, and Zamfara. Hence, this study was designed to investigate the occurrence and antimicrobial resistance profiles of MRSA in dairy cows.

2. Materials and Methods

Study area

Birnin Kebbi, a city in North-Western Nigeria, lies between 12.450 N and 4.20 E latitude. Kebbi State is an agrarian community with a viable environment due to its high soil fertility, large arable land, and economically viable rivers, all protected by a pleasant tropical climate. Agriculture has been the State's primary source of income and, indeed, its economic backbone. Millet, maize, cassava, guinea corn, potatoes, beans, rice, and vegetables are the main food crops in the district.

Sample size determination

Two hundred milk samples were collected randomly from four farms within the State using proportionate sample allocation. The size of the samples collected was determined using an estimated reported prevalence of 4.8% (Umaru et al., 2017) as described by Thrusfield (2018):

$$N=t^2 \times Pexp(1-Pexp)/d^2$$

$$N=1.962 \times 0.048 \times (1-0.048)/0.025$$

$$N=70$$

Study design and sample collection

A cross-sectional study was conducted between May and July 2019 in Kebbi State to isolate and identify MRSA in raw milk collected from healthy cows and to estimate its prevalence based on PCR detection. A total of 200 dairy cows from four dairy farms were randomly selected, and the cooperation of the farm owners was sought before sampling. After a quarter of the udder had been washed with tap water and dried (to remove dirt from it), the teat end was swabbed with cotton soaked with 70% alcohol. Approximately 10 mL of milk samples were aseptically collected from the mammary gland of lactating dairy cows using clean sample collection bottles. The collected samples were shipped in an ice pack to the Centre for Advanced Medical Research and Training (CAMRET), Usmanu Danfodiyo University Sokoto, for bacterial culture and identification.

Bacteriological isolation and phenotypic detection of MRSA

Bacterial isolation and identification were conducted using standard bacteriological methods. Briefly, a loopful of milk samples was inoculated directly onto a freshly prepared mannitol salt agar (Oxoid, Basingstoke, UK) and incubated at 35°C for 24 h. Presumed *S. aureus* isolates were identified using colonial morphology, Gram staining, catalase, and coagulase. Two to three suspect colonies of the Staphylococcus species were sub-cultured on nutrient agar (Oxoid) slant and stored until used. *S. aureus* was confirmed using a PCR assay of the species-specific endonuclease gene. Recovered cultures of *S. aureus* were cultured onto MRSA screening agar (ORSAB or oxacillin resistance screening agar base) to detect MRSA phenotypes. Suspect MRSA isolates with characteristic deep blue coloration were considered MRSA.

Antimicrobial susceptibility test

The susceptibility of *S. aureus* and MRSA isolates was tested against 7 commonly used antimicrobials at both human and veterinary settings in Kebbi State. The antimicrobials used include penicillin (P, 10 IU), erythromycin (E 15µg), gentamicin (GEN, 10µg), oxytetracycline (OXT, 30µg), neomycin (N, 30µg), sulfonamide (SUL, 23.75µg), and vancomycin (VAN, 30µg). Clinical breakpoints of each antimicrobial agent were measured and categorized based on the guidelines of the Clinical Laboratory Standards Institute (CLSI) (2021).

Genotypic detection of MRSA

Genomic DNA extraction using the crude boiling method and PCR detection of the mecA gene using appropriate primers and PCR cycling conditions was conducted as described in Gaddafi et al. (2021).

Data analysis

Data were presented in the tables. The Chi-squared analysis was done using Invivostat version 4.1 to establish the association between MRSA colonization and the farms sampled.

3. Results

Staphylococcus aureus and MRSA in dairy cattle farms

Of 200 raw milk samples examined, 45% (90/200) were positive for *S. aureus* based on culture, biochemical, and molecular characterization. The highest isolation rate was in Farm D, with a prevalence of 64% (32/50), followed by Farm A, with 48% (24/50). Farms C and B had a prevalence of 40% (20/50) and 28% (14/50), respectively. A statistically significant association ($P \leq 0.05$) was established between the occurrence of *S. aureus* and farms, with farm D more likely to have been colonized by *S. aureus* than the rest of the farms (Table 1). The Chi-squared test was performed within (each farm) and between farms for the statistical association of *S. aureus* based on breed (exotic and indigenous breed). Within farms, the prevalence of *S. aureus* in indigenous breeds was 68% (32/47) and 0% (0/3) in exotic dairy cattle. A significant association ($P \leq 0.05$) was observed between the occurrence of *S. aureus* and breed. Indigenous breeds were more likely to harbor *S. aureus* than exotic breeds. Similarly, carriage of *S. aureus* was more in indigenous breeds of cow than exotic breeds. There was no association ($P \geq 0.05$) between the occurrence of *S. aureus* and breed because there was no sample taken for the exotic cattle.

In this study, phenotypic detection of MRSA on ORSAB showed a prevalence of 18% (36/200). The highest isolation rate was in farm A, with a prevalence of 24% (12/50), followed by farm B at 18% (9/50), farms C at 14% (7/50), and D at 16% (8/50). No significant association ($P \geq 0.05$) was observed between the occurrence of MRSA and the farms (Table 1). Also, MRSA results from dairy farms A, B, C, and D, based on breed, all showed no statistically significant association between colonization and breeds of dairy cattle samples. PCR amplification of all 36 positive samples on ORSAB to detect the mecA gene showed 100% positivity (Figure 1). Hence, the overall molecular detection rate of MRSA from dairy farms studied was 18% (36/200).

Antimicrobial resistance profiles of MRSA from dairy farms

Antimicrobial susceptibility tests revealed that MRSA phenotypes showed varying levels of resistance against the panels of antimicrobial agents. Out of 36 MRSA isolates recovered from dairy farms, resistance to oxytetracycline, neomycin, and gentamicin was observed in 22 (61%) and 23 isolates (63.9%), respectively. Twenty-four MRSA isolates (66.7%) were resistant to erythromycin. The highest level resistance of MRSA isolates was observed in penicillin (27 isolates, 75%), and the lowest resistance recorded was against sulfonamide (20 isolates, 55.6%) (Figure 2). Susceptibility to vancomycin was observed in all isolates. A total of 8 isolates were found to show multidrug resistance (MDR), with ERY-

Table 1. Distribution of Staphylococcus aureus and MRSA from dairy cattle farms in Kebbi State (N=200)

Grouping Factor		Samples, No.	S. aureus Isolates, No.	χ^2	df	P	MRSA, No.	χ^2	df	P
Farm	A	50	24				12	1.90	3	0.59
	B	50	14				9			
	C	50	20				7			
	D	50	32	13.82	3	0.003	8			
Total		200	90				36			

OXY-NEO-PEN-SUL-GEN being the most common pattern, and the multiple antibiotic resistance (MAR) index is ≥0.4 (Figure 3; Table 2).

4. Discussion

S. aureus and MRSA are mastitis pathogens and major problems in the dairy industry worldwide. The risk of foodborne-related zoonosis because of MRSA from dairy cattle is low because milk is usually pasteurized before consumption. Nonetheless, cases of mastitis in cattle-caused MRSA strains are an important problem in veterinary medicine because of limited treatment options. Additionally, the detection rate of *S. aureus* from dairy cows is usually low due to the intermittent shedding patterns in milk. This situation also makes the comparison between studies cumbersome because of differences in the types of specimens, volumes of inoculum, pre-enrichment, and detection methods (Schnitt and Tenhagen, 2020; Foroutan et al., 2022). In this study, however, a prevalence of 45% (90/200) of *S. aureus* isolation was recorded from milk samples of dairy cattle farms. The prevalence recorded in this study is higher than the prevalence recorded in previous studies, where the authors reported prevalence rates of 30.9%, 4.8%, 12.63%, 29.7%, 32.14%, 12.9%, and 9.7% in Plateau, Zaria and Kaduna, Zaria and Kaduna, Pokhara Nepal, Wolayta Sodo Ethiopia, Asella Ethiopia, South Italy, and Nasarawa, respectively (Suleiman et al., 2012; Umaru et al., 2017; Umaru et al., 2014; Tessema, 2016; Abunna et al., 2016; Basanisi et al., 2017; Aliyu et al., 2020).

In agreement with our study, reports by Daka and Yihdego (2012), Desissa et al. (2012), and Mohanta and Mazumder (2015) showed comparable prevalence rates of 48.75%, 44%, and 47.86% in the Hawassa area, Ethiopia, Debre-Zeit, Ethiopia and Southern Assam, India, respectively. In the same vein, the prevalence of MRSA observed in this study was much lower than those reported in other studies, where prevalence rates of 60%, 83.75%, 74.5%, and 64.1% were reported in Dehradun, Northeast India, Karnal North India, and Morogoro Municipality Tanzania (Pant et al., 2013; Sharma & Brinty, 2014; Sarkar et al., 2014; Mohammed 2015). The variations in the reported prevalence of MRSA and that observed in this study could be attributed to variations

Table 2. Antibiotic resistant pattern of MRSA isolates from dairy farms in Kebbi State (n=27)

Antibiotic-resistant Pattern	Isolates, No.	Multiple Antibiotic Resistance Index
ERY-NEO-PEN-SUL-GEN	1	0.71
ERY-PEN-GEN	1	0.42
ERY-OXY-NEO-PEN-GEN	2	0.71
ERY-PEN-SUL-GEN	2	0.57
PEN-SUL-GEN	2	0.42
ERY-OXY-NEO-PEN	4	0.57
ERY-OXY-NEO-PEN-SUL	7	0.71
ERY-OXY-NEO-PEN-SUL-GEN	8	0.86

ERY: erythromycin; OXY: oxytetracycline; NEO: neomycin; PEN: penicillin; SUL: sulfonamide; GEN: gentamycin.

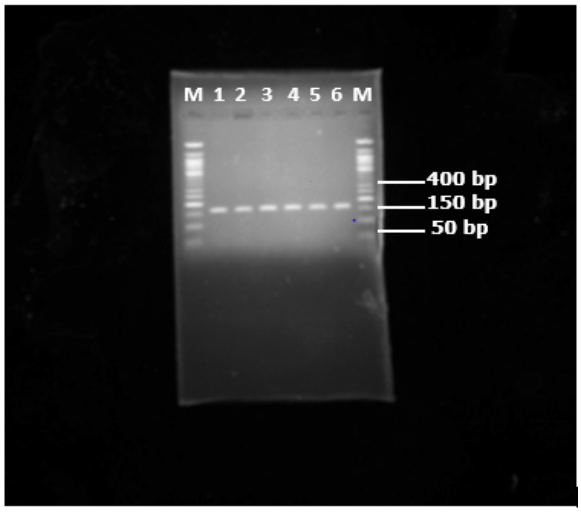

Figure 1. The 163-bp mecA Gene

Lane 1-5: Methicillin-resistant staphylococcus aureus (MRSA) isolates; Lane 6: positive control; Lane M: 100-bp molecular DNA ladder

in the sensitivity of detection methods, the number of samples collected, production systems, farmers/owners' knowledge, farm practices, animal health delivery systems, antimicrobial use, and demographics of the study area. Furthermore, Methicillin-resistant *S. aureus* is recognized as a contagious mastitis pathogen that enters the mammary gland via the teat canal. In most dairy farms, a major predominant clone has been circulating between multiple cows and is disseminated from cow to cow within a herd. This issue indicates that the primary risk of transmission is during the milking process via the hands of milkers, udder clothes, and milking utensils (Keefe, 2012; Schnitt & Tenhagen, 2020).

MRSA prevalence from dairy cattle farms in this study is 18% based on culture on ORSAB media. The prevalence observed in this study is higher than what was reported by Umaru et al. (2017), Usman et al. (2016), Okpo et al. (2016), and Aliyu et al. (2020), who reported prevalence rates of 12.14%, 12.6%, 8.70%, and 5% in Zaria, other parts of Kaduna and Nasarawa, respectively. The variation in the prevalence rates could be attributed to the difference in a geographical region or to the fact that their samples were drawn from a commercial milk vendor, while in this study, it was directly from the udder in which environmental contamination is a possibility. On the contrary, a higher prevalence of MRSA has been reported in different countries by authors who reported prevalence rates of 56%, 55.26%, 32%, and 25.53% from dairy products in Turkey, Kenya, Algeria, and Bangladesh, respectively (Njage et al., 2013; Gundogan & Avci, 2014; Jahan et al., 2015; Chaalal et al., 2016). The paucity of indigenous studies on MRSA from dairy cattle and dairy products makes it difficult to compare and assess the MRSA status in dairy cattle in Kebbi State. This study is the first comprehensive research conducted to carefully examine the presence of MRSA in dairy cattle in different areas of Kebbi State, Nigeria, where these animals are readily available for dairy production.

MRSA was detected in all the farms sampled in this study. The recovery of MRSA isolates from dairy cattle milk posed a significant public health problem because cow milk forms part of the daily delicacies of locals living in the study area. The findings of this study also support the narration that dairy products are a major source of MRSA transmission to humans. There was no statistically significant association (P≥0.05) between the recovery rate of MRSA based on breed and farm in this study. This finding can be attributed to the similar and uniform husbandry practices within the study area across all the dairy farms.

Dairy cattle farms from this study also displayed a varying resistance pattern, with penicillin having the highest resistance rate of 75% (27/36), erythromycin

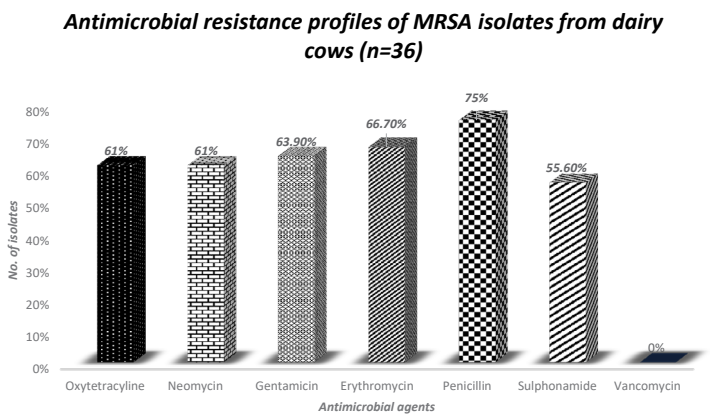

Figure 2. Antibiotic-resistance profiles of MRSA isolates (n=36) from dairy farms in Kebbi State

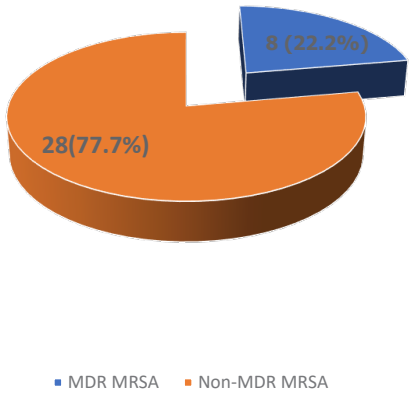

Figure 3. Multidrug resistance (MDR) MRSA from dairy cattle farms in Kebbi State

and gentamycin with a resistance of 66% (24/36) and 63.9% (23/36), respectively. In comparison, oxytetracycline and neomycin have the same resistance rate of 61% (22/36), and sulfonamide has the least resistance rate at 55.6% (20/36). The high resistance of these isolates to penicillin, gentamicin, and oxytetracycline agreed with other findings (Njage et al., 2013; Rodrigues et al., 2017), where a significant level of resistance of MRSA to these antimicrobials was reported in Brazil and Kaduna, Nigeria, respectively.

Also, this study reported a significant level of MRSA susceptibility (100%) to vancomycin. This result was not an unexpected finding because vancomycin is hardly used in treating livestock in the study area. In agreement with these outcomes, Suleiman et al. (2012) and Rodrigues et al. (2017) suggested that the apparent lack of utilization of vancomycin in veterinary settings within the study area is the result of the elevated level of susceptibility to vancomycin. Our findings from this study further agree with Alian et al. (2012) and Gaddafi et al. (2021) in Iran and Zuru, who reported zero resistance rate and 100% susceptibility to vancomycin. This finding, however, differed from the reports of Usman et al. (2016) and Umaru et al. (2014), where 66.7% and 42.6% of MRSA isolates from milk and milk products were found to be resistant to vancomycin. This finding may result from human contamination of milk and milk products during processing and because of the indiscriminate use of antimicrobial agents in dairy farms in the study area.

The mecA gene detection by PCR assay is considered the gold standard for determining MRSA from either food, clinical or environmental samples. All the phenotypically positive MRSA isolates used in this study harbored the mecA gene. This finding agrees with Gaddafi et al. (2021) and Gaddafi et al. (2022), who reported that all the phenotypic MRSA isolates from livestock and poultry harbored the mecA gene. Furthermore, Umaru et al. (2014), Rodrigues et al. (2017), and Aliyu et al. (2020) reported the presence of mecA in phenotypically positive MRSA isolates. This result differed from the reports of Usman et al. (2016), in which the mecA gene was not detected in MRSA phenotypes from milk products in the Kaduna metropolis, Nigeria. Similarly, in Malaysia, Shamila-Syuhada et al. (2016) reported the absence of the mecA gene in 15 MRSA phenotypes from raw milk. The development of antimicrobial resistance in this study might be because of prolonged and regular use of antimicrobials in dairy farms owing to poor biosecurity.

5. Conclusion

In this study, a significant association was observed between phenotypic resistance to methicillin and genotypic detection of the mecA gene. The resistance profiles of the MRSA isolates showed resistance to penicillin, gentamycin, and tetracycline. This finding poses a significant public health risk because these antimicrobials are used in Nigeria therapeutically in human or veterinary medicine or for promoting growth in animal production.

Ethical Considerations

Compliance with ethical guidelines

All experiments were done in accordance with the guidelines for the use and care of animals approved by the Research Ethics Committee of the Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto (UDUS/FAREC/03/2019).

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Authors' contributions

Conceptualization and Supervision: Yusuf Yakubu, Abdulkadir Usman Junaidu, and Mohammed Bashir Bello; Methodology: Mohammed Sani Gaddafi; Investigation and Writing-original draft: Mohammed Sani Gaddafi; Writing–review & editing: Asinamai Athliamai Bitrus and Bashir Garba; Data collection: Mohammed Sani Gaddafi, Aliyu Ibrahim Musawa, and Habiba Lawal; Data analysis: Mohammed Sani

Gaddafi; Funding acquisition and Resources: All authors.

Conflict of interest

The authors declare no conflict of interest

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