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ASSESSMENT OF KEEPING QUALITY AND MICROBIAL LOAD OF *Staphylococcus aureus* IN CATTLE MILK, PROCESSING FACILITIES, AND ENVIRONMENT IN NORTHERN OYO STATE, NIGERIA

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ABSTRACT

Milk is a vital nutritional resource but is highly perishable and prone to contamination by pathogens like *Staphylococcus aureus*, posing significant public health risks. In Northern Oyo State, where dairy production is predominantly traditional, poor hygiene practices and limited sanitation infrastructure exacerbate these risks. This study addresses gaps in understanding the keeping quality and microbial contamination of raw milk in the region, providing data to improve safety standards. A cross-sectional study was conducted from August 2023 to January 2024, collecting 1,200 samples from five Local Government Areas (LGAs). These samples included raw milk, swabs from milk handlers, and environmental sources. The keeping quality of milk was assessed using the methylene blue reduction test (MBRT) and total bacterial count (TBC) was used to determine microbial load. *S. aureus* was isolated using standard culture methods. Results revealed that 81.4% of raw milk samples demonstrated "very good" keeping quality, while 4.8% were categorized as "very bad." TBCs across all samples exceeded international safety standards, indicating significant microbial contamination. The prevalence of *S. aureus* was 2.7%, with most isolates originating from milk handlers, emphasizing poor hygiene practices as a critical factor. These findings highlighted the urgent need for improved sanitation, handler training, and proper milk storage protocols to enhance dairy safety and protect public health. This study provides foundational data to guide interventions aimed at reducing microbial contamination in Nigeria's dairy sector.

Keywords: Milk quality, *Staphylococcus aureus*, Microbial contamination, Northern Oyo State, Dairy safety

INTRODUCTION

Milk, a highly nutritious and widely consumed food product, plays an essential role in global diets, serving as a significant source of proteins, vitamins, and minerals. It also contributes to the livelihoods of millions involved in dairy farming, processing, and distribution (Smith et al. 2022). However, milk's rich nutritional content makes it highly perishable and susceptible to contamination by microorganisms, some of which pose serious public health risks (Bharathy et al. 2015). One of the most notable pathogens in milk is *Staphylococcus aureus* (*S. aureus*), a bacterium capable of producing harmful toxins that may lead to foodborne illnesses (Zhang et al. 2022; Odetokun et al. 2023). Addressing the dual challenges of maintaining milk quality and ensuring its safety is therefore crucial for both consumer health and the economic sustainability of the dairy industry. Globally, efforts to ensure milk safety have led to the establishment of international guidelines such as the Codex Alimentarius standards, which emphasize stringent microbial quality criteria. However, many developing countries, including Nigeria, face significant challenges in meeting these standards due to limited resources, infrastructure, and awareness.

In developing countries like Nigeria, dairy production is predominantly carried out under traditional, smallholder systems, particularly in rural regions (Cole et al. 2024) such as Northern Oyo State. These systems are characterized by low levels of mechanization, poor access to sanitation infrastructure, and limited awareness of best hygiene practices. Consequently, milk often becomes contaminated during critical points in the production chain, including milking, processing, storage, and transportation (Kümmel et al. 2016; Ghali-Mohammed et al. 2022). *S. aureus* contamination, in particular, is a major concern as it can occur through infected animals, handlers with poor hygiene practices, or unsanitary environments and equipment (Odetokun et al. 2024). Such contamination will not only compromise milk quality but also endangers public health through the consumption of unsafe dairy products.

The prevalence of *S. aureus* in milk and its microbial load serve as critical indicators of hygiene and safety within dairy production systems. Beyond public health risks, contamination impacts milk's organoleptic properties, shelf life, and marketability (El-Sayed et al. 2022). Previous studies have linked high microbial loads in milk to factors such as poor milking practices, inadequate cleaning of utensils and facilities, and suboptimal storage conditions (Garedew et al. 2012). Identifying these contamination sources and assessing microbial loads are vital steps in developing effective interventions to improve milk safety and quality (Deddefo et al. 2023). Beyond health risks, milk contamination adversely affects the livelihoods of smallholder farmers and rural communities who depend on dairy production as their primary source of income. Contaminated milk reduces marketability, leading to economic losses and eroding consumer trust in local dairy products.

Northern Oyo State, with its significant cattle population and vibrant Fulani pastoral communities, serves as a major hub for dairy production in Nigeria. Despite the region's contributions to the national dairy supply, limited studies have systematically examined milk quality and the prevalence of *S. aureus* within the production chain. Previous studies have highlighted the prevalence of microbial contamination in dairy production, including research from other regions of Nigeria that identified significant lapses in hygiene practices (Olatoye et al. 2018; Ghali-Mohammed et al. 2022). However, studies focusing on *S. aureus* contamination and

milk quality in Northern Oyo State remain limited, leaving critical knowledge gaps in addressing the region's unique challenges. Understanding these parameters is essential for addressing the underlying challenges and enhancing the region's dairy production standards. This study assesses milk quality and the prevalence of *S. aureus* in raw milk, milk processors, and environmental samples in Northern Oyo State. The findings aim to inform the development of targeted interventions, promote better hygiene practices, and enhance dairy product safety. Ultimately, this research aspires to contribute to the sustainability of dairy farming in the region while safeguarding public health.

MATERIALS AND METHODS

Study design

A cross-sectional study involving questionnaire administration and raw milk, milk processors, and environmental samples collection for the isolation of *S. aureus* was carried out from August 2023 to January 2024.

Study area

This study was carried out in the Northern part of Oyo state with notable towns that include Ogbomoso, Iseyin, Saki, Igboho and Kisi. Its capital is Ibadan, the third most populous city in the country. The state is located in the south West Region of Nigeria. It covers 28,454 square kilometres. It is bounded in the north by Kwara state and in the south by Ogun state, and it is bounded by Osun in the east while it is bounded in the west by the Republic of Benin with the latitude 7° North and longitude 4° East bisected by the state into four nearly equal parts. In this region of Oyo state, animal husbandry is common, including cattle rearing, involving Fulani pastoralists who are stakeholders in dairy production.

Sample size and milk sampling

The number of raw milk samples collected was determined using the formula for cross-sectional studies: $n = Z_{\alpha}^2 \times P_{(exp)} (1- P_{exp})/d^2$ where n is the required sample size for the number of milk samples to be collected across the study area. $Z_{\alpha}^2 = 1.96$ (is the multiplier for a 95 % confidence interval from a two-tailed significance level of 0.05 based on the standard normal distribution). P_{exp} = is the expected prevalence of the population. In this study, an expected prevalence of 50 % (Thrusfield, 2007), $Z_{1-\alpha/2}$ at 1.96, and an absolute error margin of 3 % were used in the calculation of the sample size for the various milk, human, and environmental samples, the sample size was calculated as 1066 using a confidence level of 3 %. A 12 % contingency was added to make up for non-response making a sample of at least 1193. In this study, a total of 1200 samples were obtained with 240 from each LGA.

Collection of samples

Five (5) out of the 10 LGAs in the northern Oyo state were randomly selected for sampling by simple balloting. A total of 1200 samples were collected across the selected five local government areas comprising raw milk (freshly-voided mid-stream) samples (n = 400), swabs (n = 400) – nasal/hands/armpit, environmental samples (n = 400) –water, dust, milking utensils. Using sterile universal bottles for raw milk collection while milk processors and environmental samples were collected using sterile swab sticks as previously described by Badawy et al. (2022). The farm owners have consented and those that were agreed to participate in the study were selected for sampling. The samples were collected for three months from August 2023 to January 2024.

The collected samples were immediately transported in the cold chain to the Food Safety and Zoonoses Laboratory, Department of Veterinary Public Health and Preventive Medicine, University of Ilorin, Ilorin, for processing and analyses.

Determination of keeping quality of milk

The methylene blue reduction test (MBRT) is a rapid economical and easiest method of determining microbiological milk quality. The MBRT is used to detect the presence of reductase enzymes, which influence the "keeping quality" of milk. The principle involved the enzymatic reduction of methylene blue by a metabolically active organism turning the blue colour of methylene blue to colourless (Anwer et al. 2019). The materials required for this test include methylene blue dye, test tubes, a water bath set at 37°C, and milk samples. All test tubes and rubber stoppers involved are sterilized in an autoclave at 121 °C 15llb. One ml of the methylene blue thiocyanate solution was added into sterile test tubes, then 10 ml of milk was added into each test tube and shaken to mix the dye with milk in the test tubes. Tubes were placed in the water bath immediately for incubation at 37 °C as previously described (Anwer et al. 2019). The procedure involves setting up three test tubes: a positive control containing 10 mL of known mastitic milk and 1 mL of methylene blue dye, a negative control with 10 mL of distilled water and 1 mL of dye, and a test sample containing 10 mL of milk sample and 1 mL of dye. These tubes were incubated in a water bath at 37 °C for six hours, during which colour changes in the test tubes were closely monitored at regular intervals. This test is based on the fact that the enzymatic activities of the microbes lead to the exhaustion of oxygen present in the milk. The exhaustion of the oxygen lead to decolourization of the dye solution added to the milk (Anwer et al. 2019). The primary focus of the test is the time taken for complete discoloration of the dye, which indicates the milk's quality.

Determination of milk contamination using the total aerobic plate count

Enumeration of total bacterial count in cow raw milk was carried out on the plate count agar (Oxoid Ltd., Hampshire, UK) plates as previously described by (Barrow & Feltham, 1993). The preparation of this agar was done according to the manufacturer's specifications. One millilitre of each milk sample was serially diluted using peptone water. One millilitre of a six-fold dilution was spread on prepared plates. All plates were incubated at 37 ºC for 24h. Bacterial counts were expressed as a log of colony-forming units per ml (log cfu/mL).

Isolation of *Staphylococcus aureus*

This was carried out as previously described (Odetokun et al. 2018). Mueller Hinton Broth was prepared according to the manufacturer's instructions and supplemented with 5% NaCl. Nine millilitres of this broth were dispensed into sterile sample bottles for sample collection. One millilitre of milk sample was collected into each bottle and labelled properly. The samples collected were transported under an ice pack at 4°C in a cool box to the Bacteria Zoonoses Laboratory of the Department of Veterinary Public Health and Preventive Medicine, University of Ilorin. The peptone water-containing samples were incubated at 37°C for 24 hours. 50 µl of pre-enriched samples were inoculated on mannitol salt agar (MSA), and these were incubated at 37 °C for 24 hours under aerobic conditions.

A discrete colony of *S. aureus* on MSA appeared tiny with a golden yellow colour, and this was selected and sub-cultured on Blair Parker Agar (BPA) supplemented with egg-yolk

tellurite supplement and incubated at 37 °C for 24 hours. The discrete colony of *S. aureus* on BPA appeared tiny with hazy surroundings. Presumptive *S. aureus* on BPA was purified on blood agar (Oxoid, Hampshire, UK) incubated at 37 °C for 24 hours. Pure colonies were stored in 20 % Mueller Hinton glycerol broth at -20°C until needed.

Identification of *S. aureus* was based on cultural, Gram staining/microscopy, and biochemical characteristics, including catalase test, oxidase test, haemolysis on sheep blood agar, DNase test, and coagulase test (slide and tube) (Cheesbrough, 2006).

Ethical clearance

The University of Ilorin Faculty of Veterinary Medicine ethical review committee approved the proposal with approval number UERC/FVM/UIL/PGS/20/68VA001 before the commencement of the study.

Data management and analysis

According to Khattak et al. (2013), the quality of milk can be assessed based on the time taken for complete reduction. Milk that completes reduction within 15 minutes was categorized as "very bad", while milk completing reduction in 30 to 60 minutes was considered "bad". Milk requiring 1.5 to 2 hours for complete reduction was classified as "poor", whereas milk taking 3 to 4 hours was deemed "doubtful". "Good-quality" milk completed a reduction in 4.5 to 6 hours, and milk requiring more than 6 hours for complete reduction was classified as "very good". Results of bacterial counts indicating the quality of milk sold in the study area were expressed as log colony-forming units per ml (log cfu/mL) and compared with standards. A comparison of bacterial counts across zones was tested using ANOVA using SPSS v.22 with significant values set at $p < 0.05$. The prevalence of *S. aureus* was expressed in percentages.

RESULTS AND DISCUSSION

Table 1. shows that 325 (81.4%) of the 400 fresh raw milk samples collected in the study area to determine the milk quality, were ''very good'' milk, from which Oorelope recorded the highest 71 (17.8%) and ATISBO 59 (14.8%). 30 (7.1%) of the milk collected was evaluated as ''Good milk'', the highest 12 (3.0%) was recorded by ATISBO and Oorelope recorded the lowest to be 1 (0.3%). ''Doubtful milk'' represented 8 (2.1%) with Irepo and Oorelope LGAs recording the highest 3 (0.8%) and the lowest 1 (0.35%), respectively. The high percentage of "very good milk" (81.4%) in the study area signifies favourable milk-keeping quality. This outcome may be attributed to improvements in the training of milk processors, the adoption of better handling practices, and increased awareness of hygienic milk production among farmers, as highlighted in a prior study (Ghali-Mohammed et al. 2022).

Furthermore, 16 (4.1%) of the total sample of milk evaluated represented ''poor milk'' in the study area, both Irepo and Oorelope had the highest 6 (1.5%) in and the lowest 1 (0.3%) in the area, respectively. 2 (0.5%) represented ''bad milk'' which was found only in ATISBO LGA. Lastly, 19 (4.8%) were evaluated as "very bad milk''. The highest 7 (1.8%) were found in Irepo and the lowest 3 (0.8%) was found in both ATISBO and Saki-West LGAs. In this study, 81.4% of "very good milk" signified good keeping quality of milk from the study area which was higher than the 78.0% and 65.0% in Oke-Ogun and Ibarapa respectively (Olatoye et al. 2018) and 54.0% recorded in Abuja (Okpalugo et al. 2008). The 4.1% recorded for poor milk in the study area was lower than the 89% reported by Anwer et al. (2018) in Pakistan. The high percentage of very good milk in the study area signifies good milk keeping quality and may be unconnected with the training of milk processors, adoption, improvement, and maintenance or close to a high standard of farmers' knowledge, attitudes, and practices in milk handling and production (Ghali-Mohammed et al. 2022).

Table 1: Keeping quality of milk in the five selected Local Government Areas in Northern Oyo State, Nigeria

Local Government Area	Very bad n (%)	Bad n (%)	Poor n (%)	Doubtful n (%)	Good n (%)	Very good n (%)	Total
Saki-West	0 (0.0)	0 (0.0)	3 (0.8)	2(0.5)	10 (2.5)	65 (16.3)	80
ATISBO	3 (0.8)	2 (0.5)	2 (0.5)	2 (0.5)	12 (3.0)	59 (14.8)	80
Irepo	7 (1.8)	0 (0.0)	6 (1.5)	3 (0.8)	2 (0.5)	62 (15.5)	80
Saki-East	3 (0.8)	0 (0.0)	4 (1.0)	0 (0.0)	5 (1.3)	68 (17.0)	80
Oorelope	6 (1.5)	0 (0.0)	1 (0.3)	1 (0.3)	1 (0.3)	71 (17.8)	80
Total	19 (4.8)	2 (0.5)	16 (4.1)	8 (2.1)	30 (7.1)	325 (81.4)	400

Very bad milk (15 mins); Bad milk (30 mins – 1 hour); Poor (1 – 2 hours); Doubtful (3 – 4 hours); Good (4 – 6 hours); Very good milk (> 6 hours)

Total Bacterial count (TBC)

The microbial safety of any food product is determined by its level of microbial loads. Table 2. shows that the range of bacteria counts obtained in this study ranges between 8.4 ± 0.36 and 7.5 ± 0.48. The highest microbial load was found in Irepo, and the lowest was found in Saki-West. The bacteria count obtained from milk within Saki-West, ATISBO, and Irepo were significant ($P< 0.05$), while the counts obtained in Saki-East and Oorelope were not significant ($P> 0.05$). In this study, all the counts obtained in the study area are higher than the normal standard limit (5×10^6) of the International Microbiological Criteria for Dairy Products (IMCDP) Codex Alimentarius Commission (2020).

The high TBC might likely be a result of a lack of washing of the teats before milking, the transfer of microorganisms from milkers or other people handling the milk among other factors associated with microbial contamination of milk at the farm level as previously reported. This corroborated with the previous reports by Elmoslemany et al. (2009) and Twagirayezu et al. (2021), that poor hygiene of udder and teat surface and from uncleaned and unsensitized milking equipment could result from the transferring of environmental organisms into the milk which can be associated with foodborne outbreaks. This may be a result of lapses in milk sanitation as well as conducive ambient temperature and relative humidity for the growth of bacteria accompanied by the lack of milk refrigeration in the situation of long distances of transportation.

The presence of a high bacteria load in this study signifies a high level of contamination that may be connected with low or poor hygiene during the milking of the cows by milk processors/Fulani herdsmen which highlighted the critical

need for real improvement. The observed bacterial load exceeding international standards not only reflects lapses in hygiene but also underscores a significant public health concern. Consumption of contaminated milk poses risks of gastrointestinal infections and foodborne illnesses, particularly in vulnerable populations like children and the elderly. In addition, most of these milk processors are illiterate and do not care or mind the prevention of contamination of milk from the type of water, utensils and equipment used during milking. The contaminated environment in which the milking takes place also contributes to milk contamination. The high total bacterial count values found in this study are quite similar to earlier reports in the milk of Nigeria breeds of cattle in fresh milk (Olorunnisomo et al. 2014) and in raw milk (Omoshaba et al. 2018).

Table 2: Total bacterial count of milk in five selected local government areas in Northern Oyo State, Nigeria

Location	No of Samples collected	No of Positive Samples	TBC (log cfu/ml)
Saki-West	80	49	7.5 ± 0.48^{a}
ATISBO	80	47	7.8 ± 0.56^{b}
Irepo	80	18	8.4 ± 0.36^{c}
Saki-East	80	19	7.9 ± 0.55^{ab}
Oorelope	80	17	7.8 ± 0.48^{ab}

TBC: Total bacterial count. The superscript on the results of the TBC from each of the local government areas indicates the significance of the count. Those with the same alphabet on superscript are Non-Significant, while those with different alphabet are Significant.

Prevalence of *Staphylococcus aureus*

The result in Table 3 reveals that out of 1200 samples collected in the study area, 32 were positive for *S. aureus* giving an overall prevalence of 2.7 %. This is in agreement with the 3 % reported in milk and dairy products in Zaria, Nigeria (Usman et al. 2016; Esonu et al. (2021) and Greece (Papadopolous et al. 2018) but higher than the 2 % reported in Turkey (Taban et al. 2021). It is, however, lower than the 3.6% reported by Oludairo et al. (2020) in other parts of Oyo State, Nigeria. Many factors can be responsible for variations in prevalence such as sample size, sampling season, geographical location, and isolation method used as previously reported (Bharathy et al. 2015; Jamali et al. 2015; Alghizzi et al. 2021). Also, the isolation of pathogens in milk could be a result of the dirty hands of milk processors, inadequate storage conditions, unhygienic sanitary conditions, and contamination during processing and distribution/retail points (Odetokun et al. 2023). Other factors causing the observed prevalence were the state of an animal before slaughter (Abebe et al. 2016) and during milk collection (Hogue et al. 2018).

Table 3: Prevalence of *Staphylococcus aureus* isolated from Milk, Herdsmen, and Environment at Northern Oyo, Nigeria

Variables	Number of samples collected	Number of negative samples	Number of positive Isolates n(%)	Chi-square	P-value
Swabs from herders					
Saki-West	80	71	9 (11.3)	1.204	0.878
Saki-East	80	77	3 (3.8)		
Oorelope	80	65	15 (18.8)		
Irepodun	80	80	0 (0.0)		
ATISBO	80	80	0 (0.0)		
Raw Milk					
Saki-West	80	79	1 (1.3)	0.008	1.000
Saki-East	80	80	0 (0.0)		
Oorelope	80	79	1 (1.3)		
Irepodun	80	80	0 (0.0)		
ATISBO	80	80	0 (0.0)		
Environmental samples					
Saki-West	80	78	2 (2.5)	0.020	0.999
Saki-East	80	80	0 (0.0)		
Oorelope	80	80	0 (0.0)		
Irepodun	80	80	0 (0.0)		
ATISBO	80	79	1 (1.3)		
Total	1,200	1,168	32 (2.7)		

Table 3 reveals different percentages of the isolates in each local government in the study area based on swab samples collected from the herders, raw milk and environmental samples. Most of the *S. aureus* were isolated from the herders, with the highest [15 (18.8%)] recorded in Oorelope LGA, followed by Saki-West (11.3%) and Saki-East (3.8%). The number of isolates recovered from the raw milk and environmental samples was generally low. The high number of isolates from the herders could be a result of poor hygiene of the handlers. Poor storage conditions, contamination during the processing and unsanitary conditions during processing as revealed by Odetokun et al. (2023) could lead to milk contamination. The high percentage of *S. aureus* among the handlers could also mean that there is a high possibility of the raw milk being contaminated by the herders milking cows at the farm level. Though the isolation rate of *S.*

aureus from the environment was low, the possibility of raw milk getting contaminated should not be ruled out from the environment and materials used in milking (Kamal et al. 2013).

Several studies have reported varying rates of *S. aureus* in raw bovine milk from various geographical regions. The vary difference could be attributed to the different isolation techniques utilized in the various studies. A study in Morogoro Municipality involving raw milk samples from retail shops in 2015 reported 41% *S. aureus* (Mohammed et al. 2018). Another study involving three dairy farms located within a similar climatic region in Tanzania reported a prevalence of 49% *S. aureus* in raw milk (Kashoma & Medardus, 2015). Massawe et al. (2019) found 15% of *S. aureus* in raw milk samples from farmers and retail markets in Mbozi and Mbeya rural Tanzania. A study in Algeria reported a prevalence of 41.8% in raw bovine milk samples collected from five farms (Chaalal et al. 2014). A higher prevalence of 51.2% of *S. aureus* from cows with subclinical mastitis was reported in Ethiopia where crossbred cattle were more infected with mastitis than local cattle (Abebe et al. 2016). Among the reasons contributing to milk contamination by *S. aureus* mentioned in these studies include poor hygiene and farm management practices, improper washing of milking utensils/containers and hands or using untreated borehole water for sanitation (Ateba et al. 2010).

This study faced several limitations that may influence the generalization of its findings. Firstly, the cross-sectional design only provided a snapshot of milk keeping quality and *S. aureus* prevalence, which might not capture seasonal variations in contamination levels or milk production practices. Secondly, the reliance on traditional culture-based methods for bacterial identification, while effective, may have excluded other potential pathogens or underestimated the prevalence of *S. aureus* due to its limitations in sensitivity compared to molecular techniques. Additionally, logistical constraints limited the geographical scope to selected local government areas in Northern Oyo State, potentially excluding variations in milk quality across the broader region. Lastly, the study also relied on self-reported practices from milk processors, which may be subject to recall bias or social desirability bias, potentially skewing the interpretation of hygiene practices. Despite these challenges, the findings provided a valuable understanding of the microbial safety and quality of milk in the study area and highlighted critical areas for intervention and future research.

CONCLUSION

This study highlighted the microbial quality and contamination risks associated with raw milk and dairy production in Northern Oyo State, Nigeria. While a significant proportion (81.4%) of milk demonstrated "very good" keeping quality, the total bacterial counts across all samples exceeded international safety standards, indicating persistent hygiene challenges in the dairy production chain. The prevalence of *Staphylococcus aureus* was relatively low (2.7%), but its isolation from milk handlers underscored the critical role of human hygiene practices in contamination. These findings emphasized the urgent need for improved sanitation measures, handler training, and enhanced awareness of milk safety protocols among smallholder farmers and processors. Addressing these issues will not only safeguard public health but also strengthen the economic sustainability of the dairy sector. Future research should focus on seasonality, advanced detection methods, and scalable interventions to mitigate contamination and elevate milk production standards in Nigeria.

REFERENCES

Abebe, R., Hatiya, H., Abera, M., Megersa, B., & Asmare, K. (2016). Bovine mastitis: prevalence, risk factors and isolation of *Staphylococcus aureus* in dairy herds at Hawassa milk shed, South Ethiopia. *BMC Veterinary Research*, 12, 1-11.

Acton, D. S., Tempelmans Plat-Sinnige, M. J., van Wamel, W., de Groot, N., & van Belkum, A. (2009). Intestinal carriage of *Staphylococcus aureus*: how does its frequency compare with that of nasal carriage and what is its clinical impact?. *European Journal of Clinical Microbiology and Infectious Diseases*, 28, 115-127. DOI: 10.1007/S10096-008-0602-7.

Alghizzi, M., & Shami, A. (2021). The prevalence of *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* in milk and dairy products in Riyadh, Saudi Arabia. *Saudi Journal of Biological Sciences*, 28(12), 7098-7104.

Anwer, S. S., Zaki, S. M., Rasul, S. A., Hassan, R. J., Ahmad, I. J., & Qader, A. J. (2018). Detection of kid's milk quality using methylene blue reduction test. *International Journal of Environment, Agriculture and Biotechnology*, 3(4), 1450-1456. https://doi.org/10.22161/ijeab/3.4.41

Ateba, C. N., Mbewe, M., Moneoang, M. S., & Bezuidenhout, C. C. (2010). Antibiotic-resistant *Staphylococcus aureus* isolated from milk in the Mafikeng Area, North West province, South Africa. *South African Journal of Science*, 106(11), 1-6.

Badawy, B., Elafify, M., Farag, A. M. M., Moustafa, S. M., Sayed-Ahmed, M. Z., Moawad, A. A., Algammal, A. M., Ramadan, H., & Eltholth, M. (2022). Ecological Distribution of Virulent Multidrug-Resistant *Staphylococcus aureus* in Livestock, Environment, and Dairy Products. *Antibiotics*, 11(11), 1651. https://doi.org/10.3390/antibiotics11111651

Barrow G. I., & Feltham. (1993). *RKA Cowan and Steel's manual for the identification of medical bacteria* (third). Cambridge University Press.

Bharathy, S., Gunaseelan, L., Porteen, K., & Bojiraj, M. (2015). Prevalence of *Staphylococcus aureus* in raw milk: Can it be a potential public health threat. *International Journal of Advanced Research*, 3(2), 801-806.

Chaalal, W., Aggad, H., Saidi, N., & Kihal, M. (2014). Prevalence and antimicrobial resistance of Methicillin-resistant *Staphylococcus aureus* isolated from raw meat and bovine milk in Algeria. *Advances in Environmental Biology*, 8(1), 1-5.

Cheesbrough, M. (2006). *District Laboratory Practice in Tropical Countries* (Part 2). Cambridge University Press.

Cole, J., Adetona, M. A., Basiru, A., Jimoh, W. A., Abdulsalami, S., Ade-Yusuf, R. O., ... & Eltholth, M. (2024). Climate Change, Land Use, and the Decline in Traditional Fulani Cattle Practices: Drivers of Antimicrobial Resistance in Kwara, Nigeria. *Challenges*, 15(4), 41.

Deddefo, A., Mamo, G., Asfaw, M., & Amenu, K. (2023). Factors affecting the Microbiological Quality and Contamination of Farm Bulk Milk by *Staphylococcus aureus*

in Dairy Farms in Asella, Ethiopia. *BMC Microbiology*, 23(1), 65. https://doi.org/10.1186/s12866-022-02746-0

Elmoslemany, A, M., G. Keefe, I. Dohoo, and B. Jayarao. (2009). Risk factors for bacteriological quality of bulk tank milk in Prince Edward Island dairy herds. Part 2: Bacteria count-specific risk factors. *Journal of Dairy Science*, 92, 2644-2652. http://doi.org/10.3168/jds.2008-1813.

El-Sayed, A. S., Ibrahim, H., & Farag, M. A. (2022). Detection of potential microbial contaminants and their toxins in fermented dairy products: A comprehensive review. *Food Analytical Methods*, 15(7), 1880-1898. https://doi.org/10.1007/s12161-022-02253-y

Esonu, D. O., Ismail, S., Ajala, A., Yusuf, S. M., Otolorin, R. G. (2021). Occurrence and anti microbial susceptibility patterns of *Staphylococcus aureus* and *Salmonella species* in fresh milk and milk products sold in Zaria and environs, Kaduna State, Nigeria. *Sahel Journal of Veterinary Sciences*, 18(2), 1-8.

Garedew, L., Berhanu, A., Mengesha, D., & Tsegay, G. (2012). Identification of gram-negative bacteria from critical control points of raw and pasteurized cow milk consumed at Gondar town and its suburbs, Ethiopia. *BMC Public Health*, 12, 1-7.

Gebremedhin, E. Z., Ararso, A. B., Borana, B. M., Kelbesa, K. A., Tadese, N. D., Marami, L. M., & Sarba, E. J. (2022). Isolation and identification of *Staphylococcus aureus* from milk and milk products, associated factors for contamination, and their antibiogram in Holeta, Central Ethiopia. *Veterinary Medicine International*, 2022(1), 6544705. https://doi.org/10.1155/2022/6544705

Ghali-Mohammed, I., Odetokun, I. A., Raufu, I. A., & Adetunji, V. O. (2022). Handling practices and contamination of raw milk sold for consumption in markets of Kwara State, Nigeria. *Sokoto Journal of Veterinary Sciences*, 20(1), 50–58. https://doi.org/10.4314/sokjvs.v20i1.7

Hoque, M. N., Das, Z. C., Rahman, A. N. M. A., Haider, M. G., & Islam, M. A. (2018). Molecular characterization of *Staphylococcus aureus* strains in bovine mastitis milk in Bangladesh. *International Journal of Veterinary Science and Medicine*, 6(1), 53-60.

Jamali, H., Paydar, M., Radmehr, B., Ismail, S., & Dadrasnia, A. (2015). Prevalence and antimicrobial resistance of *Staphylococcus aureus* isolated from raw milk and dairy products. *Food Control*, 54, 383-388.

Kamal, R. M., Bayoumi, M. A., & Abd El Aal, S. F. (2013). MRSA detection in raw milk, some dairy products and hands of dairy workers in Egypt, a mini-survey. *Food Control*, 33(1), 49-53.

Kashoma, I., & Medardus, J.J. (2015). Prevalence and antimicrobial susceptibility profiles of *Staphylococcus aureus* from cow's milk, nasal and environmental swabs in selected dairy farms in Morogoro, Tanzania. *Tanzania Veterinary Journal*, 30(2), 61 – 75.

Khattak, B., Iqbal, H., Sherwani, S. K., & Shah, M. A. (2013). Microbial analysis and quality control of milk collected from various districts of Khyber Pakhtunkhwa. *The International Journal of Pharmaceutical Research and Bio-Science*, 2(4), 243-252.

Kreausukon, K., Fetsch, A., Kraushaar, B., Alt, K., Müller, K., Krömker, V., & Tenhagen, B. A. (2012). Prevalence, antimicrobial resistance, and molecular characterization of methicillin-resistant *Staphylococcus aureus* from bulk tank milk of dairy herds. *Journal of Dairy Science*, 95(8), 4382-4388.. https://doi.org/10.3168/jds.2011-5198.

Kümmel, J., Stessl, B., Gonano, M., Walcher, G., Bereuter, O., Fricker, M., ... & Ehling-Schulz, M. (2016). *Staphylococcus aureus* entrance into the dairy chain: tracking *S. aureus* from dairy cow to cheese. *Frontiers in Microbiology*, 7, 1603.

Massawe, H.F., Mdegela, R.H., & Kurwijila, L.R. (2019). Antibiotic resistance of *Staphylococcus aureus* isolates from milk produced by smallholder dairy farmers in Mbeya Region, Tanzania. *International Journal of One Health*, 5(3), 31–37.

Mohammed, J., Ziwa, M. H., Hounmanou, Y. M. G., Kisanga, A. & Tuntufye, H. N. (2018). Molecular typing and antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* isolated from bovine milk in Tanzania. *International Journal of Microbiology* 2018(3), 1 – 6.

Nange, & Katrine. (2019). Which is the largest city in Africa: Lagos vs. Ibadan city. *Legit. ng-Nigeria news. Retrieved 2021-03*, *7*.

Odetokun, I. A., Adetona, M. A., Ade-Yusuf, R. A., Adewoye, A. O., Ahmed, A. N., Ghali-Mohammed, I., Al-mustapha, A. I., and Fetsch, A. (2023). *Staphylococcus aureus* contamination of animal-derived foods in Nigeria: a systematic review, 2002-2022. *Food Safety and Risk*, 10(6), 2. https://doi.org/10.1186/s40550-023-00106-y.

Odetokun, I. A., Adewoye, A. O., Adetona, M. A., Ade-Yusuf, R. O., Raza, A., Ogundijo, A. O., & Ghali-Mohammed, I. (2024). *Staphylococcus aureus* contamination of Milk and Cheese: Assessment of Consumers and Vendors Knowledge, Attitudes and Practices in Ilorin, Nigeria. *UMYU Scientifica*, 3(2), 211-222.

Odetokun, I. A., Ballhausen, B., Adetunji, V. O., Ghali-Mohammed, I., Adelowo, M. T.,Adetunji, S. A., & Fetsch, A. (2018). *Staphylococcus aureus* in two municipal abattoirs in Nigeria: Risk perception, spread and public health implications. *Veterinary Microbiology*, 216, 52–59. https://doi.org/10.1016/j.vetmic.2018.01.022.

Okpalugo, J., Ibrahim, K., Izebe, K.S., and Inyang, U. S. (2008). Aspects of microbial quality of some milk products in Abuja, Nigeria. *Tropical Journal of Pharmaceutical Research*, 7(4), 1169-1177.

Olatoye, O., Amosun, A., Ogbu, U., & Okunlade, Y. (2018). Bulk tank somatic cell count and associated microbial quality of milk from selected dairy cattle herds in Oyo State, Nigeria. *Italian Journal of Food Safety*, 7(2), 7130. https://doi.org/10.4081/ijfs.2018.7130

Olorunnisomo, O.A., Ososanya, T. O., & Adedeji, O. Y. (2014). Homogenisation of milk and its effect on sensory and physio-chemical properties of yoghurt. *African Journal of Food Science*, 8(9), 465-470.

Oludairo, O.O., Olatoye, O.I., Awoniyi, O.E., Adejumobi, O.A. & Aiyedun, J.O. (2020). Prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) in cattle milk from dairy herds in Oyo State, Nigeria. *Journal of Veterinary and Biomedical Sciences*, 2(2), 72–83.

Omoshaba, E. O., Ojo, O. E., Sofela, O., & Onifade, O. I. (2018). Prevalence and antibiotic resistance patterns of methicillin-resistant *Staphylococcus aureus* in raw milk and soft cheese (wara) sold in Abeokuta, Nigeria. *Sokoto Journal of Veterinary Sciences*, 16(1), 1-8.

Papadopoulos, P., Papadopoulos, T., Angelidis, A. S., Boukouvala, E., Zdragas, A., Papa, A., Hadjichristodoulou, C., & Sergelidis, D. (2018). Prevalence of *Staphylococcus aureus* and of methicillin-resistant *S. aureus* (MRSA) along the production chain of dairy products in north-western Greece. *Food Microbiology*, 69, 43–50. https://doi.org/10.1016/j.fm.2017.07.016

Smith N. W., Fletcher A. J., Hill J.P., and McNabb W.C. (2022). Modeling the Contribution of Milk to Global Nutrition. *Frontiers in Nutrition*, 8, 716100. https://doi.org/10.3389/fnut.2021.716100

Taban, B. M., Hassankhani, A., & Aytac, S. A. (2021). Investigation of *mecA*-and *mecC*-positive *Staphylococcus aureus* from raw milk and traditional artisanal dairy foods. *International Journal of Food Properties*, 24(1), 954-964.

Thrusfield M. (2007). *Veterinary Epidemiology, third edition. Blackwell Publishing, Oxford, UK.*

Twagirayezu, J. B. . ., Musanayire, V. ., Murerwa, L. ., Mouiche, M. M. M. ., Hakizimana, J. N. . ., & Nyabinwa, P. . (2021). Prevalence of Subclinical Mastitis and its Effects on Reproductive Performance in Dairy Cows during the Postpartum Period in Gasabo District, Rwanda. *International Journal of Veterinary Sciences Research*, 6(1), 1–13. https://doi.org/10.18488/journal.110.2021.61.1.13

Usman, R. Z., Mustapha, B. M., & Mohammed, F. I. (2016). Isolation and identification of Methicillin Resistant *Staphylococcus aureus* (MRSA) from traditionally fermented milk "nono" and yoghurt in Zaria metropolis, Nigeria. *Food Science and Quality Management*, 2(2), 1-21.

Zhang, Z., Song, Y., Ma, L., Huang, K., & Liang, Z. (2022). Co-Occurrence of *Staphylococcus aureus* and *Ochratoxin* A in Pasteurized Milk. *Toxins*, 14(10), 718. https://doi.org/10.3390/toxins14100718