PREVALENCE OF *STAPHYLOCOCCUS AUREUS* IN BOVINE RAW MILK, ANTIBIOGRAM, AND ASSOCIATED MILKING HYGIENE PRACTICES AMONG SMALL-SCALE FARMERS IN MAGU DISTRICT, MWANZA, TANZANIA

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THESIS IS SUBMITTED TO THE SCHOOL OF PUBLIC HEALTH, DEPARTMENT OF COMMUNITY HEALTH, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE MASTER OF PUBLIC HEALTH IN APPLIED EPIDEMIOLOGY AT AMREF INTERNATIONAL UNIVERSITY

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DECLARATION AND APPROVAL

Declaration by Candidate:

This research thesis is my original work and has not been presented to any other university/research institution for any award.

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DEDICATION

I dedicate this thesis to my beloved mother, Alena N. Muhairwa, who has always been there for me throughout my academic journey. Your support, love, and guidance have been a constant source of strength and motivation throughout this journey.

Furthermore, I extend my dedication to the small-scale farmers of the Magu district, Tanzania. Your collaboration, insights, and dedication to enhancing milk production practices have been invaluable. I sincerely hope that the outcomes of this research will contribute positively to your livelihoods and the dairy farming community of the Magu district.

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ABSTRACT

Background: *Staphylococcus aureus*, a common cause of foodborne illnesses, is transmitted from cows to humans through contaminated raw milk, reflecting the milking hygiene standards of the farm. Data on livestock-associated *Staphylococcus aureus* (LA-*S. aureus*) in Tanzania's bovine supply chain are scarce.

Objective: To assess the prevalence of *Staphylococcus aureus* in cows' raw milk, antibiogram, and associated milking hygiene practices among small-scale farmers in the Magu district.

Methods: The study employed a cross-sectional design, examining 410 raw milk samples from 48 farmers. Milking hygiene data were collected via questionnaires. *Staphylococcus aureus* was isolated using standard laboratory methods. Drug susceptibility was tested with the Kirby-Bauer disk diffusion method. Methicillin-resistant *Staphylococcus aureus* (MRSA) was identified using a cefoxitin disk, with results interpreted according to the Clinical Laboratory Standard Institute Guideline, 2022. Data analysis was conducted using R software, employing descriptive and inferential statistics.

Results: The study included predominantly male participants who reared indigenous cattle for dual production. *Staphylococcus aureus* prevalence was 23.9%, with 16.3% being MRSA displaying varying antibiotic resistance patterns. Penicillin exhibited the highest resistance at 45.9% (45/98), while ciprofloxacin showed the lowest at 1.1% (1/98).

Absence of gloves usage ($\chi2 = 111.7$, $p < 0.001$), poor udder cleaning practices ($\chi2 = 8.35$, $p = 0.0154$), poor utensil washing practices ($\chi2 = 10.44$, $p = 0.0054$), hand milking ($\chi2 = 111.7$, $p < 0.001$), and frequent use of antibiotics ($\chi2 = 14.06$, $p = 0.0071$) were significantly associated with *Staphylococcus aureus* contamination in raw milk.

Conclusion: The prevalence of *Staphylococcus aureus* was high with a significant proportion of isolates being MRSA, in raw milk from farmers. High antibiotic resistance underscores the need for improved antimicrobial stewardship. Poor milking hygiene calls for targeted interventions in raw milk production.

Recommendation: The veterinary and public health departments should educate farmers on appropriate milking hygiene practices.

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SYMBOLS, ABBREVIATIONS, AND ACRONYMS

%	Percentage
°C	Degree Centigrade/Celsius
AMIU	Amref International University
AMR	Antimicrobial Resistance
APHRC	African Population and Health Research Center
AST	Antimicrobial Susceptibility Test
CDAE	Capacity Development of Applied Epidemiologists
COPS	Coagulase Positive *Staphylococcus aureus*
CUHAS	Catholic University of Health and Allied Sciences
DED	District Executive Director
e	Margin of error
EDCTP	European Development and Clinical Trials Partnership
HOD	Head of Department
IHI	Ifakara Health Institute
km^2	Kilometer Squared

Lab	Laboratory
LA-*S.aureus*	Livestock Associated *Staphylococcus aureus*
LFO	Livestock Field Officer
mecA	Methicillin-Resistant Staphylococcal Cassette Chromosome mec
mL	Milliliter
MOH	Ministry of Health
Mr.	Mister
MRSA	Methicillin-resistant *Staphylococcus aureus*
n	Sample size
NBS	National Bureau of Statistics
p	Population size
PCR	Polymerase Chain Reaction
S. aureus	*Staphylococcus aureus*
SOP	Standard Operating Procedure
TALIRI	Tanzania Livestock Research Institute

TVLA	Tanzania Veterinary Laboratory Agency
WHO	World Health Organization
Z_{α}	Critical value
β	Beta
µg	Microgram
χ^2	Chi-Square

DEFINITION OF TERMS

Antimicrobial resistance: This occurs as microorganisms evolve to resist drugs, complicating infections, risking disease spread, severe illness, and death.

Beta-lactam: A key structural element in antibiotics like penicillin, vital for inhibiting bacterial cell wall synthesis.

Milking hygiene: These are pre-milking and post-milking procedures as well as the cleanliness of equipment used to milk the cows.

Methicillin-resistant *Staphylococcus aureus*: A *Staphylococcus aureus* clone carrying a gene that is resistant to methicillin and other beta-lactam drugs.

Bovine raw milk: Is the milk from cows that hasn't been pasteurized to kill bacteria that are harmful to people.

Small-scale farmers: An agricultural producer who engages in small-scale livestock, seafood/fish, or crop production.

Staphylococcus aureus: A spherically, gram-positive, non-motile, and common isolate of human and animal skin mucosa.

CHAPTER 1: INTRODUCTION

1.1 Overview

The purpose of the study was to assess the prevalence of *Staphylococcus aureus* in bovine raw milk, antibiogram, and associated milking hygiene procedures by small-scale farmers of Magu District, Mwanza, Tanzania. The main goal was to comprehend the possible *Staphylococcus aureus* contamination of raw milk and how it relates to milking procedures. By this study, criteria for milking hygiene were raised and new information regarding the safety and quality of raw milk produced locally was obtained. By addressing cow mastitis, antimicrobial resistance, and poor milking hygiene practices, the study contributes to protecting public health and ensuring the sustainability of the dairy industry.

1.2 Background of The Study

Over the past five decades, *Staphylococcus aureus* has gained attention due to its ability to adapt to antibiotic pressure, leading to a high burden of antibiotic resistance (Lee et al., 2018). Approximately 700,000 deaths yearly in the world result from infections by pathogens resistant to antimicrobials and 10 million by 2050 if enacted (Kraker et al., 2016; Shankar, 2016). The current statistics indicate a global linkage of 4.95 million deaths to antimicrobial resistance, with 1.27 million solely from antimicrobial resistance and 366,000 specifically from Eastern Sub-Saharan African countries (Wagenlehner & Dittmar, 2022). Failure to address this issue with urgency could lead to major global public health challenges (Kraker et al., 2016).

Staphylococcus aureus is widely reported as a common isolate in humans and animals (Ferradas et al., 2022; Rao et al., 2022). This opportunistic pathogen has evolved into virulent strains that are resistant to multiple drugs and are likely to cause zoonosis (Tibebu et al., 2021). MRSA has disastrous consequences for the well-being and welfare of animals, human health, and the dairy production economy (Karzis et al., 2021). *Staphylococcus aureus* has been detected in food-producing animals and food, such as milk and milk products globally, for over two decades and accounted for many food intoxications and enterotoxins in humans (Titouche et al., 2022; Kou et al., 2021).

Infections caused by *Staphylococcus aureus* in the host immune system include necrotic pneumonia, toxic shock syndrome, nosocomial and blood infection in humans, also mastitis and skin infections in animals (Kalayu et al., 2020; Kumar, 2016). Humans are at high risk of contracting the pathogen if they come into contact with or consume raw/undercooked milk and other contaminated food (Kalee et al., 2021). Milk from infected cow's udder, milkers' hands, or milking utensils during milking can be sources of bacteria shade in raw bovine milk (Khairullah et al., 2022).

The rapid development of antimicrobial resistance stems from various factors such as inadequate prescription practices driven by diagnostic challenges, patient demand, and financial incentives (Chan, 2017). Non-therapeutic use of antibiotics by unknowledgeable farmers due to chaotic animal health services, exposure of livestock workers to MRSA-carrying animals like pigs and cattle, and global mobility, have contributed to the spread of antibiotic-resistant pathogens (Kimera et al., 2020; Ruppé et al., 2019).

In a farm, the presence of *Staphylococcus aureus* in bovine raw milk is indicative of the farm's poor milking hygiene and sanitation standards (Nyokabi et al., 2021). A previous study in Tanzania's Njombe district revealed a prevalence of 22.6% *Staphylococcus aureus* in bovine raw milk, with 2.9% MRSA (Sanga et al., 2022). More previous studies have documented a high MRSA prevalence in Ethiopia, Cameroon, and South Africa, ranging from 42% to 72% (Founou et al., 2017). MRSA presence in farms is linked to antibiotic abuse (Selim et al., 2022).

A previous study in Morogoro reported how farmers' unhygienic practices like using cold water without detergent contributed to raw milk contamination (Kalee et al., 2021). Moreover, many Tanzanians opt for conveniently available raw milk from local small-scale farmers increasing the risk of *Staphylococcus aureus* contamination and food-borne illnesses (Gwandu et al., 2018; Mohammed et al., 2018). Despite this, a previous report in Arusha indicated that 65% of respondents admitted to consuming raw milk making the situation worse (Ngasala et al., 2015). Acknowledging the need for quality milk production in Tanzania, this study aims to analyze LA-*S. aureus* patterns, antibiograms, and milking practices which are limited (Mzee et al., 2021; Sanga et al., 2022).

1.3 Statement of The Problem

While the dairy industry is important for the economy and milk production, the safety of raw milk produced especially with bacterial contamination particularly *Staphylococcus aureus* is of veterinary and public health concern (Titouche et al., 2022). *Staphylococcus aureus* is a common isolate of raw milk and a leading cause of food-borne illnesses worldwide (Titouche et al., 2019). The pathogen is responsible for many other infections

with fatal outcomes such as severe dermatitis, toxic shock syndrome, blood and nosocomial infection in people, and udder infection in cows, (Kalayu et al., 2020; Tibebu et al., 2021). The devastating effects of MRSA have further complicated the situation (Karzis et al., 2021).

For example, Sanga et al. (2022) reported a prevalence of 2.9% MRSA and 22.6% *Staphylococcus aureus* in raw milk in Njombe, Tanzania, with 74% resistance to penicillin and 78% resistance to ampicillin. Ngasala et al. (2015) further reported in another study in Arusha that unhygienic milking practices by farmers contributed to the contamination of raw milk with pathogens. Hand milking and pre-milking hand washing with water only were identified as one of these unhygienic practices (Kalee et al., 2021).

Despite this, many Tanzanians still rely on raw milk from small-scale farmers in local markets which is convenient for them and drink it as purchased, increasing the risk of contracting *Staphylococcus aureus* (Gwandu et al., 2018; Mohammed et al., 2018). For example, according to the Mwanza Region Investment Guideline (2017), up to 24 million liters of surplus milk supplied in the Mwanza region were shown to be from small-scale farmers in Magu and Sengerema districts.

Recognizing the importance of quality milk production, the research aims to understand the patterns of LA-*S. aureus* in cows' raw milk, antibiogram, and milking hygiene practices which is scarce all over Tanzania (Founou et al., 2017; Mzee et al., 2023). This is important in the planning and implementation of contaminant-free milk production in Magu. Findings and recommendations are important for further reduction of *Staphylococcus aureus* and MRSA infections, zoonosis, and lethal impacts to humans.

1.4 Research Question

What is the relationship between the presence of *Staphylococcus aureus* in bovine raw milk, antibiogram, and associated milking hygiene practices among small-scale farmers in the Magu district of Mwanza, Tanzania?

1.5 Objectives

1.5.1 General Objective

To assess the prevalence of *Staphylococcus aureus* in bovine raw milk, antibiogram, and associated milking hygiene practices among small-scale farmers in the Magu district of Mwanza, Tanzania

1.5.2 Specific Objectives

i. To determine the presence of *Staphylococcus aureus* in bovine raw milk samples from small-scale farmers in the Magu district of Mwanza, Tanzania
ii. To determine an antibiogram of *Staphylococcus aureus* isolated from bovine raw milk samples from small-scale farmers in the Magu district of Mwanza, Tanzania
iii. To describe milking hygiene practices done by small-scale farmers in the Magu district of Mwanza, Tanzania
iv. To determine the association between hygienic practices and the presence of *Staphylococcus aureus* in bovine raw milk samples from small-scale farmers in the Magu district of Mwanza, Tanzania

1.6 Justification of The Problem

To establish effective preventive and control measures for producing high-quality, contaminant-free, and health-friendly milk, this study offers veterinary and public health insights into the prevalence of *Staphylococcus aureus,* antibiogram, and related hygienic measures for milking among small-scale farmers in Mwanza's Magu district, Tanzania.

1.7 Significance of The Study

The research has revealed the percentage prevalence of MRSA and *Staphylococcus aureus* in raw milk from cows. It has determined the milking hygiene practices associated with small-scale farmers in the Magu district of Mwanza, Tanzania. These findings provide valuable insight into the local LA-*S. aureus* epidemiology. This insight is crucial for planning and implementing effective prevention and control measures aimed at reducing or eradicating LA-*S. aureus* at the farm level.

Furthermore, the study has categorized appropriate antibiotics for the management of udder infection in cows, attributed to *Staphylococcus aureus* and MRSA, by assessing their susceptibility to resistance within the region. It has highlighted inadequate milking hygiene practices in the area, essential for reducing the risk of pathogen contamination in raw milk and preventing transmission to humans (zoonotic transmission) by addressing the source. This "One Health Approach" is vital for comprehensive health management encompassing animals, humans, and food security.

1.8 Scope of The Study

The purpose of the study was to find out how common *Staphylococcus aureus* was in cow raw milk samples, particularly MRSA, its antibiogram and to provide an overview of

the associated milking hygiene practices among small-scale farmers in Magu district, located in Mwanza, Tanzania. During the milking process, samples of raw cow's milk were collected for the study from individual cows. Only farmers who owned milking cows and expressed a willingness to be part of the study were enrolled.

However, the study was conducted in a relatively short time frame required to complete a master's degree program, potentially impacting the depth of exploration into certain aspects of the research, such as long-term trends and methodologies. Consequently, while the study provides valuable insights, it's essential to consider the potential limitations imposed by the short duration of the research process.

1.9 Assumptions

The study included small-scale farmers who were involved in rearing milking cows, whether they were of exotic, indigenous, or crossbreed varieties. Raw milk samples were obtained from individual healthy milking cows. A single questionnaire was administered to the willing small-scale farmers for data collection. The collected raw milk samples were obtained from cows intended for either home consumption or supply to local markets. Subsequently, these raw milk samples were transported to the Tanzania Veterinary Laboratory Agency (TVLA), for comprehensive microbiological examination.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Staphylococcus aureus, a gram-positive bacterium, is implicated in a spectrum of clinical infections acquired in diverse settings, including hospitals, communities, and livestock farms, and is notably associated with cow mastitis, a prevalent issue in dairy production globally (Crespo-Piazuelo & Lawlor, 2021; Selim et al., 2022; Taylor & Unakal, 2022). Over time, it has evolved into methicillin-resistant *Staphylococcus aureus* (MRSA), rendering treatment options limited and presenting a significant challenge for healthcare practitioners (de Águas., 2011; Lee et al., 2018). This strain, characterized by its virulence and resistance to β-lactams, underscores the necessity for heightened attention to highly pathogenic strains of *Staphylococcus aureus* (Tibebu et al., 2021).

Staphylococcus aureus infections in humans cover a range of manifestations, from simple abscesses to severe necrotizing pneumonia, while contamination of milk with this bacterium poses a notable risk of foodborne diseases (Khairullah et al., 2020; Li et al., 2017). In dairy production, such contamination leads to adverse consequences including decreased milk yield, milk condemnation, and culling or replacement of livestock, thereby impacting both economic viability and public health (Selim et al., 2022). Furthermore, treatment of MRSA infections presents challenges in achieving satisfactory outcomes for both people and animal patients, emphasizing the importance of detecting *Staphylococcus aureus* in samples of raw milk (Algammal et al., 2020; Keyvan et al., 2020).

Despite its nutritional value and commonality in human diets, milk provides an optimal environment for bacterial proliferation under favorable conditions, with contamination often linked to milking practices and utensil hygiene (Gagara et al., 2022; Tigabu et al., 2015). The essential routes of *Staphylococcus aureus* transmission to humans encompass infected udders resulting from mastitis, substandard milking hygiene practices, and the consumption of unpasteurized milk, underscoring the importance of considering these risk variables (Kashoma et al., 2016; Ngasala et al., 2015).

The emergence of MRSA strains is closely associated with the improper use of antimicrobials by farmers, often exacerbated by the absence of a well-organized delivery system for animal health services (Kimera et al., 2020). This phenomenon poses a considerable challenge for veterinarians due to the limited treatment options available for MRSA infections, thereby necessitating a multifaceted approach to mitigating antimicrobial resistance (Kashoma et al., 2016; Mdegela et al., 2021). The improper use of antibiotics, for both treatment purposes and stimulation of growth, has been implicated in the rise of antimicrobial resistance across various bacterial strains, including the development of MRSA (Mdegela et al., 2021).

A substantial percentage of participants in a previous study carried out in Central Ethiopia continued to consume raw milk despite having a high level of awareness of the health concerns involved, indicating a discrepancy between knowledge and behavior (Tigabu et al., 2015). In Tanzania, a previous study reported a concerning trend of consuming raw milk, exposing individuals to milk-borne illnesses and intoxication (Gwandu et al., 2018). There is a need to raise public awareness of milk-related health risks and improve hygiene practices for safe milk production (Ngasala et al., 2015).

2.2 Theoretical Framework

This study assessed how the quality of hygiene practices during milking directly affects the safety of milk with harmful bacteria, specifically, *Staphylococcus aureus* and MRSA, posing risks to human health when consumed. It emphasizes the interconnectedness of milking hygiene practices, bacterial contamination, and antibiotic resistance, crucial for food safety and public health from "one health perspective". The One Health perspective recognizes the interconnectedness of human, animal, and environmental health. It promotes interdisciplinary collaboration to address health challenges through surveillance, sustainable practices, and prevention. This approach is crucial for tackling emerging diseases, antibiotic resistance, and food safety. Figure 2.2 shows the theoretical framework of the study.

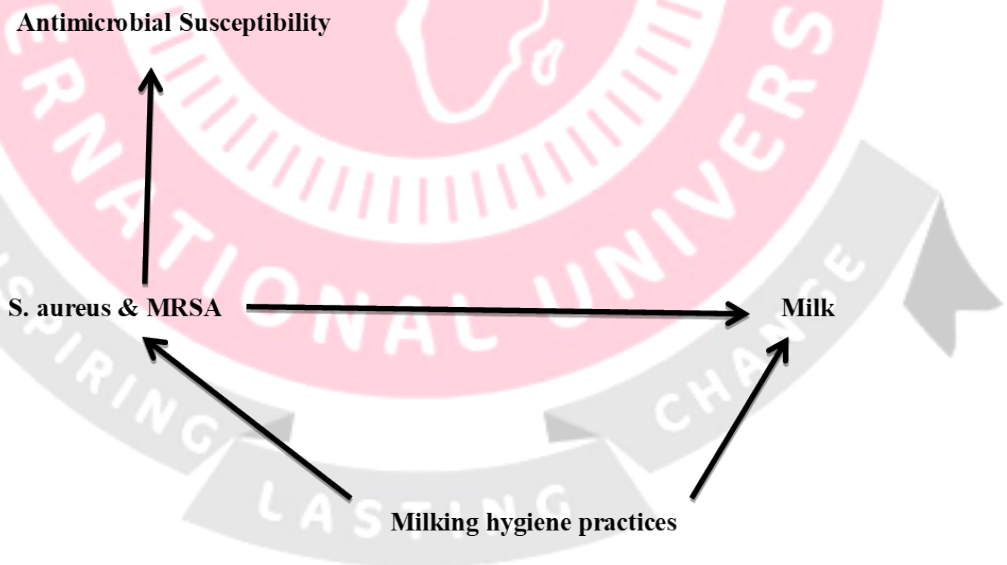

Figure 2.2: Theoretical Frame Work on the Impact of Milking Hygiene Practices on Milk Contamination with *Staphylococcus aureus* and MRSA, Antimicrobial Resistance, and Public Health.

2.3 Review of Related Literature

A considerable amount of literature has addressed the *Staphylococcus aureus* and MRSA prevalence worldwide, antimicrobial resistance patterns, and risk factors, particularly farmers' milking hygiene practices, providing insights into the bacteria contamination in bovine raw milk in Tanzania, Africa, and the world.

2.3.1 Prevalence of Staphylococcus aureus

Previous studies in several countries have documented the varying prevalence rates of *Staphylococcus aureus* in raw milk samples from different sources. For example, In Hefei, China, Wang et al. (2022) reported a *Staphylococcus aureus* prevalence of 72.5% (50/69) in raw milk samples from handcrafted dairy retail establishments, with 12% (6/50) identified as MRSA. Similarly, in Java, Indonesia, Tyasningsih et al. (2022) found a *Staphylococcus aureus* prevalence of 55.2% (138/250) in raw milk samples from dairy farms, with 20% (27/138) of these samples testing positive for MRSA. Although these results offer insightful information, more investigation is required to completely understand the occurrence of *Staphylococcus aureus* and MRSA in milk across various geographic locations.

Further previous studies reported varying prevalences of *Staphylococcus aureus ranging* from 15% to 70% in Turkey, Colombia, Egypt, Kenya, Zambia, Mozambique, Ethiopia, and Tanzania (Ágredo-Campos et al., 2023; Kalee et al., 2021; Keyvan et al., 2020; Nhatsave et al., 2021; Omwenga et al., 2021; Phiri et al., 2022; Sanga et al., 2022; Selim et al., 2022). Although these data are helpful, more research is needed to completely

understand the frequency and distribution of MRSA and *Staphylococcus aureus* in milk across different areas.

2.3.2 Antibiogram of Staphylococcus aureus

Previous studies in diverse geographic locations including, China, and Indonesia, have reported on the frequency of antibiotic resistance in isolates of *Staphylococcus aureus* from raw milk samples (Wang et al., 2022; Tyasningsih et al., 2022). Although, these studies provide valuable insights into resistance patterns, the need for more data in other regions, particularly Africa, where antibiotic resistance surveillance may not be as comprehensive is required.

For example, previous studies conducted in various regions of Ethiopia reported high resistance rates of *Staphylococcus aureus*. Borena et al. (2023) found resistance rates ranging from 87.8% to 98.5% for ampicillin in Ambo. In the West Shewa Zone, Banu and Gebremethin (2022) documented resistance rates of 31.7% to 92.42% for cefoxitin. Alembo and Tonjo Torka (2023) reported resistance rates of 23.9% to 83.33% for tetracycline in the Gamo zone. While these findings provide enlightenment on the status of antibiotic resistance by *LA-S aureus* in Ethiopia, further understanding of the underlying factors driving antibiotic resistance in Africa, is still a mystery.

Moreover, previous studies in Tanzania's Njombe, Morogoro, and Mbeya regions reported the resistance rates of *Staphylococcus aureus* for 28.2% - 93.5% penicillin, 4.4% - 17% cefoxitin, and 19.8% - 43.1% tetracycline (Kalee et al., 2021; Massawe et al., 2019; Mohammed et al., 2018; Sanga et al., 2022). Therefore, this highlights the significance of conducting further studies on antimicrobial resistance, including MRSA,

particularly in rural Tanzania where access to antibiotic stewardship programs is limited, potentially leading to an underestimation of resistance rates.

2.3.3 Milking Hygiene Practices

The existing literature, as evidenced by previous studies conducted in different regions of the world, has shed light on substantial deficiencies in hygiene practices and antimicrobial usage among dairy farmers. For instance, Ágredo-Campos et al. (2023) reported in a study of 150 dairy herds in Colombia that 31.3% (47/150) of milkers did not disinfect their hands before milking. Similarly, Gebremedhin et al. (2022) found in previous research in Holeta, Central Ethiopia, that a high number of farmers employed cold water and soap to clean their hands before milking, with only 16.9% (8/47) washing the udder before milking. This reflects the presence of poor milking hygiene practices complementing the quality of produced milk.

Moreover, in Asella, Ethiopia, a previous study reported that a high 36% (18/50) and 56% (28/50), number of farmers used cold water to clean utensils and hands before milking, with 60% (30/50), having poor barn hygiene (Deddefo et al., 2022). In a previous study in Bishoftu, Ethiopia, limited knowledge of antibiotic-resistant bacteria was reported among 84.6% (44/52) of farmers, (Tibebu et al., 2021). Further previous studies among Ethiopian farmers reported the use of plastic containers for milking, neglecting shed cleaning, and not isolating cows with mastitis (Mogotu et al., 2022). These findings from Ethiopia, consistent with observations from studies in other regions, underscore a widespread issue regarding milking hygiene practices that need to be addressed.

In Tanzania, a previous study reported comparable challenges in Morogoro, with dairy cows housed on dirty floors and milking utensils in poor condition (Kalee et al., 2021). The collective shreds of evidence from these studies emphasize the pressing requirement for additional research in dairy farming communities.

2.3.4 Staphylococcus aureus Vs. Milking Hygiene

Previous studies conducted in various regions of the world have reported a range of factors contributing to *Staphylococcus aureus* contamination in raw milk. For example, Ágredo-Campos et al. (2023) conducted a study in Colombia revealing that in-paddock milking practices increased *Staphylococcus aureus* prevalence by 33%, while effective hand disinfection, specific disinfectant use, and post-milking teat dipping reduced prevalence significantly by 92%, 40-43%, and 42%, respectively. This highlights the significance of improper milking hygiene in raw milk contamination with *Staphylococcus aureus.*

Similarly, a previous study in Ethiopia, reported how container-washing practices and materials were influencing *Staphylococcus aureus* contamination in raw milk, with synthetic plastic containers posing a higher risk compared to aluminum-coated containers (Alembo & Tonjo Torka, 2023). More, previous studies in Ethiopia reported the influence of factors such as barn cleanliness, udder washing practices, and milkers' awareness of hygiene protocols ($p<0.05$), in contamination of raw milk with the *Staphylococcus aureus* (Borena et al., 2023; Deddefo et al., 2023; Gebremedhin et al., 2022). This underscores the importance of milking hygiene practices in mitigating *Staphylococcus aureus* contamination in raw milk.

Furthermore, findings in Tanzania reported a significant correlation between the level of farm hygiene (p=0.009), hand washing without detergent (p=0.0008), and hand washing following milking with the presence of *Staphylococcus aureus* contamination in raw bovine milk (Kalee et al., 2021). Therefore, this study seeks to contribute to filling the continuous knowledge gap on the interaction effects between different hygiene practices and *Staphylococcus aureus* contamination in raw milk.

2.4 Identification of Knowledge Gap

In Tanzania, research on LA-*S. aureus* is limited, leaving crucial gaps in understanding its prevalence, drug resistance profiles, and milking hygiene practices in the bovine raw milk supply chain (Kalee et al., 2021; Massawe et al., 2019; Sanga et al., 2022). Similarly, more studies in Tanzania have emphasized the need for more extensive research to uncover *Staphylococcus aureus* origins on farms and associated antimicrobial genes (Mzee et al., 2021; Mohammed et al., 2018).

Addressing these knowledge gaps is crucial, especially considering the potential health risks posed by LA-*S. aureus* transmission between humans and animals. As a result, this study uses a "One Health approach" to analyze milking hygiene standards in Tanzania's Magu district and ascertain the frequency and antibiogram of *Staphylococcus aureus* isolates in cows' raw milk. By filling these gaps in knowledge, this research aims to inform targeted interventions that can effectively mitigate the spread of LA-*S. aureus* and improve public health outcomes in Tanzania's livestock sector.

2.5 Conceptual Framework

As previous studies showed, this work has addressed important gaps in understanding the occurrence, drug resistance profiles, and associated milking hygiene practices of LA-*S. aureus* in bovine raw milk and the related supply chain in Tanzania (Kalee et al., 2021; Massawe et al., 2019; Sanga et al., 2022). Despite existing research emphasizing the necessity for more investigations into its origins on farms, risk factors, and associated antimicrobial genes, limited studies have been conducted in Tanzania (Mohammed et al., 2018; Mzee et al., 2021).

To bridge these gaps, this study adopted a comprehensive "One Health approach" to assess the existence of *Staphylococcus aureus* isolates, including MRSA, in bovine milk and evaluate milking hygiene practices in the Magu district, Mwanza, Tanzania. By focusing on both dependent and independent variables, the study aimed to understand the intricate factors influencing milk contamination. Dependent variables, such as the existence of *Staphylococcus aureus* and MRSA in milk, served as key indicators of milk safety. Independent variables encompassed various aspects of milk quality, including udder health, cleanliness, milker hygiene, milking environment, utensil hygiene, and milk storage conditions.

Moreover, mediating variables, such as the education, experience, and knowledge of individuals involved in the milking process, were explored to understand their impact on milk contamination dynamics. Moderating variables, such as antimicrobial resistance, shed light on the pathways through which independent variables influence milk contamination. Covariate variables, such as the grazing environment were also considered to account for additional factors influencing the relationships between dependent and independent variables.

In essence, this study adopts a holistic approach to not only examine the direct relationships between bacteria presence and milk quality but also to uncover the complex interactions among various factors shaping milk contamination in Tanzania's dairy production system. By addressing these gaps, this study aims to inform targeted interventions that can mitigate the spread of LA-*S. aureus* and enhance public health outcomes in the Tanzanian livestock sector. Figure 2.5, shows the conceptual framework of the study.

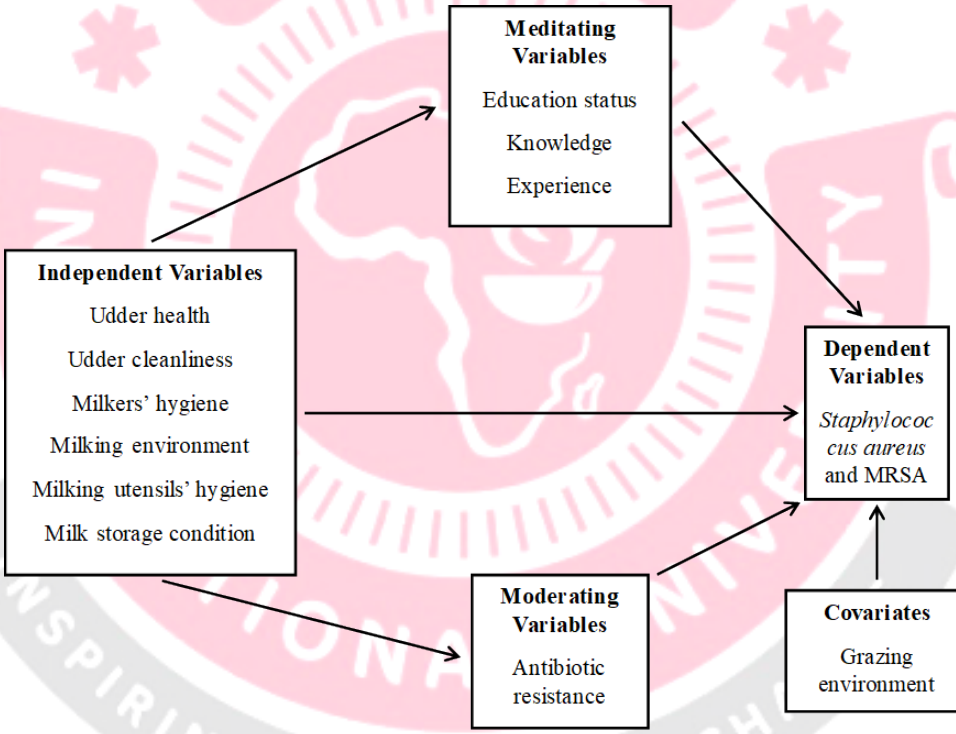

Figure 2.5: The Conceptual Framework on the Impact of Milking Hygiene Practices on Raw Milk Contamination with *Staphylococcus aureus* and MRSA, Antimicrobial Resistance, and Public Health

CHAPTER 3: METHODOLOGY

3.1 Introduction

This thesis methodology chapter examines the prevalence of *Staphylococcus aureus* in cow's raw milk and evaluates the related hygienic procedures used by small-scale farmers in Tanzania's Magu District. To give readers an idea of the research protocols followed throughout the study, it explains the research design, data collection methods, sample techniques, laboratory procedures, statistical analysis, ethical issues, and constraints related to the research that was conducted.

3.2 Research Design

This research employed a cross-sectional study design to assess the prevalence of *Staphylococcus aureus* in bovine raw milk and evaluate the milking hygiene practices among small-scale farmers in the Magu District of Mwanza, Tanzania.

3.3 Study Area

The study was conducted in the Magu district of Mwanza, Tanzania. Mwanza city, located in the northwestern part of Tanzania, sits between 1200 and 1400 meters above sea level, on the southern shores of Lake Victoria. Magu, one of the Mwanza region's seven districts, is divided into four divisions and twenty-five wards. Located 63.6 kilometers from Mwanza's city center, Magu borders Ukerewe to the north, Ilemela to the west, and Kwimba, Misungwi, and Busega Council to the south and east respectively.

Magu lies between longitudes 33° and 30° east of Greenwich, latitudes 20°10' and 2°50' south of the equator, with an average temperature of 29°C. The district covers a total area of 2671 km^2, of which Lake Victoria constitutes 1120 km^2 or 41.9%, while the remaining 1551 km^2, accounting for 59.1%, consists of islands. As per the Tanzanian census of 2022, the estimated population of the Magu district was 421,119 residents (National Bureau of Statistics, 2022). A previous survey in the Mwanza region reported the presence of an estimated 208,057 cattle in the Magu district (Mwanza Region Investment Guideline, 2017). Appendix 1, Figure A1 depicts the map of the study area and points to the households of which milk samples were obtained.

3.4 Study Population

3.4.1 Inclusion Criteria

This study enrolled small-scale farmers residing in the Magu District of Mwanza, Tanzania, who owned and managed milking cows, identifying themselves as either pastoralists or agro-pastoralists. These cows belonged to exotic or indigenous breeds and were raised within intensive, semi-intensive, or extensive livestock production systems for milk production purposes. Eligible participants were those with milking cows at the time of the study and who consented to participation, with the milk produced intended for either home consumption or local market supply.

3.4.2 Exclusion Criteria

Small-scale farmers who withdrew their consent for participation during the study, milking cows that were on medication, and samples with an indication of deterioration before microbiological evaluation were all excluded from the study.

3.4.3 Study Population Distribution

In this study, a total of 410 milking cows (340 local and 40 exotic) from 48 small-scale farmers, were sampled in 15 wards of the Magu district. Exotic cows were mostly Friesian and Ayrshire crossbreeds. The indigenous cows were Tanzanian short-horn zebu (TSHZ) and Ankole. Appendix 13, Figures A7 and A8, show TSHZ and Friesian breeds of cattle in the Magu district.

The wards included in the study were Bujora, Bukandwe, Isandula, Itumbili, Kabila, Kahangara, Kitongo Sima, Kongolo, Lubugu, Magu Mjini, Nyanguge, Nyigogo, and Sukuma. Over (50/410) milk samples were collected from the Nyanguge, Lubugu, and Kahangara wards, mainly local breeds, while fewer samples were obtained from the remaining wards. Table 3.4.3 below shows the distribution of collected milk samples per cattle breed and respective wards.

Table 3.4.3: Distribution of Samples Collected: Exotic Vs. Local Per Specific Ward

Ward	Breed		Total samples collected (n)
	Exotic	Local	
Bujora	3	13	16
Bukandwe	11	0	11
Chabula	7	4	11
Isandula	3	0	3
Itumbili	0	36	36
Kabila	0	30	30
Kahangara	0	56	56
Kisesa	2	11	13
Kitongo Sima	0	40	40
Kongolo	9	0	9
Lubugu	0	57	57
Magu Mjini	5	0	5
Nyanguge	0	88	88
Nyigogo	0	11	11
Sukuma	0	24	24
Total	**40**	**370**	**410**

n, Number of Samples

3.5 Sampling

3.5.1 Sample size Determination

The formula described used to calculate the sample size was adopted from (Thrusfield, 2005). It utilized a prevalence rate of 49% as reported by Kashoma et al. (2015), in Morogoro, Tanzania, a 95% confidence level, and a 5% margin of error. The critical

value Z_α was set at 1.96, corresponding to a population size of 208,057 cattle. The formula ($n = Z_\alpha^2 * p(1-p) / e^2$) was applied, resulting in a sample size (n) of 384.

However, a total of 410 milk samples were collected directly from individual cows across the Magu district of Mwanza, Tanzania, sourced from 48 small-scale farmers. The extra bovine raw milk samples were included as each sample was equally important ensuring utilization of all valuable data. Milk samples were utilized for analysis, focusing on milking hygiene factors specific to each cow and their potential association with the prevalence of *Staphylococcus aureus* during milking practices.

For example, a previously reported study in Kilombero and Mvomero municipalities, Morogoro region, Tanzania, where Kalee et al. (2021) obtained 397 bovine raw milk samples from individual cows in 20 dairy farms to examine their relationship with farm hygiene and antimicrobial use and prevalence of 49% *Staphylococcus aureus* to determine the sample size, as documented by a previous study in Morogoro (Kashoma et al., 2015).

3.5.2 Sampling Procedure

A multistage sampling method was utilized to select the samples. Initially, wards and small-scale farmers' households, where bovine raw milk samples were obtained, were conveniently selected based on the availability and willingness of the livestock field officers (LFOs) and farmers to participate in the study. All eligible milking cows that were milked or had been milked on a respective day were included in the sampling process, resulting in the collection of 410 milk samples from these cows sourced from 48 owner-farmers.

Kalee et al. (2021) reported a previous study in Morogoro, Tanzania, employing a similar multistage sampling technique but differing in the random selection of districts, wards, farmers, and individual cows compared to the current study.

3.6 Data Collection Instruments

3.6.1 Laboratory Setting

In the laboratory setting from March 28, 2023, to June 20, 2023, pivotal standard procedures such as media preparation, bacterial culture and subculture, gram staining, catalase, coagulase, and the Kirby Bauer disc diffusion test were thoroughly executed to assess and interpret results, with a specific focus on confirming the potential presence of *Staphylococcus aureus* and MRSA in bovine raw milk samples as per manufacturer's instructions and description by (Jahan et al., 2015; CLSI, 2022). These standardized protocols not only facilitated the testing process but also provided crucial visual cues, including colony appearance, color changes, and the presence or absence of reactions, essential for accurately identifying the bacteria.

3.6.2 Observation

Observations during laboratory tests, encompassing bacterial culture and subculture, gram staining, catalase, coagulase, and the Kirby Bauer disc diffusion test, played a critical role in elucidating results and confirming the potential presence of *Staphylococcus aureus* and MRSA in bovine raw milk samples. These observations were pivotal in discerning visual indicators such as colony appearance, color variations, and reaction outcomes, significantly contributing to the accurate identification process.

Conversely, observations of milking hygiene practices such as udder washing, milking area hygiene, the condition of milking utensils, calf feeding techniques, and documentation were carried out and recorded in the questionnaire. These observations provided valuable insights into the adherence of small-scale farmers to recommended hygiene protocols during the milking process.

3.6.3 Questionnaires

The Swahili version questionnaires were the primary instruments, administered to small-scale farmers who supplied bovine raw milk samples. The questionnaire involved both closed and open-ended questions. For the closed-ended questions that required observation, detailed instructions and clarifications were provided within the questionnaire to guide the interviewer on what to do with each question. They both targeted milking hygiene practices among small-scale farmers in the Magu district and played a pivotal role in gathering information, contributing to an understanding of practices and behaviors. Appendix 7, contains a Swahili version of the sample questionnaire used to gather milking hygiene data in field data.

3.7 Validity and Reliability

3.7.1 Validity

The questionnaires and laboratory tests were developed with a focus on content validity, ensuring alignment with research objectives. Experts in microbiology and veterinary medicine reviewed the instruments to validate their relevance. Face validity was addressed through research assistants during training, obtaining feedback to enhance the

clarity, comprehensibility, and relevance of questions. The instruments were adjusted for effective data collection.

3.7.2 Reliability

The data collection instruments' reliability was achieved through rigorous standardization and training of the team. Training sessions ensured personnel familiarity with protocols, minimizing variability. In the laboratory, calibration exercises maintained consistency in bacterial culture and tests. Standardized protocols and guidelines, ensured reliability by strictly following all standard operating procedures by the laboratory, ensuring uniformity.

3.8 Data Collection Procedure

3.8.1 Milk Sample Collection

Bovine raw milk samples were obtained from individual milking cows from sampled small-scale farmers, aseptically in gloved hands from all four teats after thoroughly washing the udder with warm water. The initial milk streams were discarded, and approximately 12mL of actual bovine raw milk was collected into sterile falcon tubes.

The falcon tubes or centrifuge tubes (CITOTEST® Ltd., Halmen City 226100, Hants, China, Ref 4610-1850, Lot 190018) were composed of 15ml volume, a diameter of 17x120mm, grey screw cap, conical bottom, polypropylene (PP) material, leakage-proof, printed with graduation intervals of 0.5ml, ranging from 1.5ml to 12ml, and in a sterile bulk pack. Each tube was marked with specific identification numbers, the date of collection, the ward, and the district where it was collected and transported in an ice-

packed cool box to the Tanzania Veterinary Laboratory Agency (TVLA) – Mwanza for microbiological examination.

3.8.2 Laboratory Procedures

At the TVLA, bovine raw milk samples underwent a series of laboratory tests to isolate and identify *Staphylococcus aureus* and MRSA. The tests included bacterial culture, gram staining, bacterial subculture, biochemical tests (catalase and coagulase), and the Kirby Bauer disc diffusion test for antimicrobial susceptibility following the standard procedures as described by (Jahan et al., 2015).

i. Media Preparation

The preparation of all three types of media (Mannitol Salt Agar, Muller Hinton Agar, and Blood Agar) was done according to the manufacturer's instructions and laboratory standard operating procedures as per manufacturer's instructions.

a. Blood Agar

The medium used was (Oxoid® Ltd., Basingstoke, Hants, United Kingdom, CM0055 Lot 3316403) which was composed of 10.0 g/l of powder, 10.0 g/l of peptone, 5.0 g/l of Sodium Chloride, and 15.0 g/l of Agar, with a final pH of 7.3 ± 0.2 at 25°C was used. The manufacturer's instructions were followed for preparation: A liter of distilled water was filled with 40 g of the powdered medium, well mixed, and heated to a boil to guarantee total dissolution. The medium was then autoclaved at 121°C for 15 minutes to disinfect it. The sterile medium was transferred into sterile Petri dishes after chilling to a temperature below 45°C and added with 5% sheep blood. Following two hours at

ambient temperature to allow the medium to harden, the plates were inverted and kept in an incubator set at 37°C for a full day.

b. Mannitol Salt Agar

The medium used was (LIOFILCHEM® S.r.l., Basingstoke, Hants, United Kingdom, REF610029 Lot 060622501) which was composed of 5 g/l pancreatic digest of casein, 5 g/l peptic digest of animal tissue, 1 g/l beef extract, 10 g/l D-Mannitol, 75 g/l Sodium chloride, 15 g/l Agar, and 0.025 g/l Phenol Red, with a final pH of 7.4 ± 0.1 at 25°C. The manufacturer's instructions were followed for preparation: A liter of distilled water was filled with 111 g of the powdered medium, well mixed, and heated to a boil to guarantee total dissolution. The medium was then autoclaved at 121°C for 15 minutes to disinfect it. The sterile medium was transferred into sterile petri dishes after chilling to a temperature below 45°C. Following two hours at ambient temperature to allow the medium to harden, the plates were inverted and kept in an incubator set at 37°C for a full day.

c. Muller Hinton Agar

The medium used was (Oxoid® Ltd., Basingstoke, Hants, United Kingdom, CM0337 Lot 3318556) which was composed of 300 g/l Beef, dehydrated infusion, 17.5 g/l Casein hydrolysate, 1.5 g/l Starch, and 17 g/l Agar, with a final pH of 7.3 ± 0.1 at 25°C. The manufacturer's instructions were followed for preparation: A liter of distilled water was filled with 38 g of the powdered medium, well mixed, and heated to a boil to guarantee total dissolution. The medium was then autoclaved at 121°C for 15 minutes to disinfect it. The sterile medium was transferred into sterile petri dishes after chilling to a

temperature below 45°C. Following two hours at ambient temperature to allow the medium to harden, the plates were inverted and kept in an incubator set at 37°C for a full day.

ii. Normal Saline Preparation

The solution was made by mixing 100 ml of sterile distilled water with 0.85 g of sodium chloride (Techno Pharmachem, Delhi, India, Cat. 2000122), stirring thoroughly, and autoclaving the mixture at 121°C for 15 minutes. After the mixture cooled down below 45°C, it was ready for use as per the manufacturer's instructions.

iii. Bacterial Culture

Samples of raw bovine milk were inoculated onto blood agar medium plates that had been enhanced with 5% sheep blood using sterile wire loops. These plates were incubated overnight at 37°C to isolate *Staphylococcus aureus* colonies. Identification of the colonies depended on colony appearance and beta-hemolytic patterns on blood agar media (Jahan et al., 2015).

iv. Gram Staining

Identified colonies of *Staphylococcus aureus* from an overnight culture on blood agar media plates were applied to a sterile microscope slide along with a drop of normal saline solution and a sterile inoculation loop. Staining was performed using the Gram Staining technique. Identification of *Staphylococcus aureus* depended on its staining characteristics and morphological appearance under the microscope (Jahan et al., 2015).

v. Bacterial Subculture

Identified colonies of *Staphylococcus aureus* from an overnight culture on blood agar media were sub-cultured on Mannitol salt agar and kept at 37°C for the entire night to produce a pure colony culture. Identification of *Staphylococcus aureus* was based on colony morphology on mannitol salt agar and mannitol fermentation ability (Jahan et al., 2015).

vi. Biochemical Test

a. Catalase Test

Using a sterile wire loop, a part of the colony isolates of *Staphylococcus aureus* obtained from an overnight culture on mannitol salt agar were transferred to a sterile glass slide. The suspension was mixed with a drop of 3% hydrogen peroxide, and the combination was emulsified. The development of gas bubbles indicated the presence of *Staphylococcus aureus* (Jahan et al., 2015).

b. Coagulase Test

Catalase-positive *Staphylococcus* were confirmed for the existence of *Staphylococcus aureus* using coagulase testing. Coagulase assays on slides were used for all the catalase-positive *Staphylococcus aureus*. The coagulase tube tests were only employed for those colonies that did not respond to slide coagulase tests.

Slide Coagulase Test

Using a sterile wire loop, a part of the colony isolates of *Staphylococcus aureus* obtained from an overnight culture on mannitol salt agar were transferred to a sterile glass slide. At two different locations, a drop of regular saline solution was added to produce a smooth, emulsified suspension. One area was filled with a loop that contained citrated rabbit plasma, which was then well mixed. Observation of any clumps confirmed the presence of the bacteria (Jahan et al, 2015).

Coagulase Tube Test

The coagulase tube test was employed for *Staphylococcus aureus* colonies on mannitol salt agar that did not respond to the slide coagulase test. Rabbit plasma was diluted at a 1:10 ratio with distilled water. *Staphylococcus aureus* positive and negative controls were included. Colonies to be tested were re-suspended in 0.5 ml of dilute plasma in a sterile test tube. For six hours, each test tube was kept at 37°C, and its gel formation was monitored hourly (Jahan et al., 2015).

vii. Antibiotic Susceptibility Test

Antibiotic susceptibility was assessed for confirmed coagulase-positive *Staphylococcus aureus* (CoPS) isolates using the Kirby-Bauer disk diffusion method, as outlined by the (Clinical and Laboratory Standards Institute, 2022) for a few popular antibiotics used in Tanzania, both by humans and by animals, according to research by (Sangeda *et al.,* 2021) was done.

For the antibiotic susceptibility test procedure, a sterile wire loop was used to pick up two to three pure *Staphylococcus aureus* colonies, which were then suspended in normal saline to create a turbid bacterium suspension equal to 0.5 of the McFarland standard solutions. The bacterial suspension was streaked onto Muller Hinton Agar to cultivate it. using a sterile cotton swab, air-dried, and cefoxitin (30µg), ciprofloxacin (5µg), clindamycin (2µg), erythromycin (15µg), gentamicin (10µg), penicillin (10µg), tetracycline (30µg), and trimethoprim/sulfamethoxazole (1.25/23.75µg) antibiotic disks were added to each plate. After that, the plates were incubated for 16 to 18 hours at 37°C.

Following incubation, a ruler was used to measure the *Staphylococcus aureus* inhibitory zone width surrounding each antibiotic disk. The results were analyzed, and the antibiotics were classified as resistant, susceptible, or intermediate, depending on the relevant criteria (Clinical and Laboratory Standards Institute, 2022). To identify MRSA, if the cefoxitin (30µg) disk-containing zone of inhibition was ≤21 mm, the isolate was considered MRSA. This cutoff value is consistent with CLSI, 2022 guidelines for MRSA detection using the cefoxitin disk diffusion method.

3.8.3 Questionnaires

i. Pre-Test

Before an actual survey of the field, research assistants, (one laboratory technician, one veterinarian, and two biotechnology interns), at Tanzania Veterinary Laboratory Agency (TVLA) were trained on March 31, 2023, and provided with a copy of the questionnaire, and were asked to review the questions for clarity, comprehensibility, and relevance. Due to limited funds, conducting a full-scale pilot study was not feasible, so the alternative

method was used to gather valuable feedback. The Research assistants examined the questionnaires individually and then reconvened to provide detailed feedback on any questions that were unclear or ambiguous. This collaborative approach allowed us to reconstruct and refine the problematic questions, ensuring that our study instruments were well-prepared for the actual fieldwork.

ii. Actual Field Survey

An actual field survey was conducted from April 2, 2023, to June 15, 2023. The team administering the printed questionnaires included four Research Assistants (one laboratory technician, one veterinarian, and two biotechnology interns), and myself by filling post-observation and responses from participants. In the field, a single questionnaire was administered to each small-scale farmer from whom bovine raw milk samples were obtained. Information on milking hygiene habits and related aspects was gathered using the Swahili version of the questionnaire, aiding in understanding the practices and behaviors of farmers in the field. Appendices 6 and 7 contain both English and Swahili versions of the sample questionnaire

3.9 Data Analysis and Presentation

The open-source program R, version 4.3.1, was used to analyze the data. The focus was on exploring associations between *Staphylococcus aureus,* antimicrobial resistance, and milking hygiene, employing various statistical methods, including bivariate analysis (chi-square tests), logistic regression by generalized linear mixed model (GLMM), and computation of proportions and percentages of variables.

The presence of *Staphylococcus aureus* and MRSA was presented by its prevalence, computed as the proportion of isolates found out of all the samples that were examined. This method allowed for the quantification of the frequency of *Staphylococcus aureus* within the sampled population and was well presented in a table offering a concise overview of and distribution.

Proportions of susceptibility of *Staphylococcus aureus* to antibiotics, including cefoxitin, ciprofloxacin, clindamycin, erythromycin, gentamycin, penicillin, tetracycline, and trimethoprim-sulfamethoxazole, were obtained by dividing the total number of *Staphylococcus aureus* isolates examined by the number of isolates sensitive to each drug, expressed as a percentage and well presented in a table format enabling a comparative analysis of their effectiveness.

Milking hygiene practices by small-scale farmers were evaluated by representing them as percentages in two distinct manners: first, as a general overview of hygiene practices, and second, about individual milk samples. Note that all open-ended questions on milking hygiene practices were categorized first before further analysis. The data, presented in a well-organized table format, enabled a comprehensive analysis of both trends.

Using a bivariate analysis through chi-square test, the relationship between *Staphylococcus aureus* and milking hygiene practices was evaluated. This statistical method assessed the impact of milking practices on the amount of *Staphylococcus aureus* in raw milk, and the results were neatly displayed in a table that made the relationship easy to understand.

A separate generalized linear mixed model (GLMM) was employed using the glmmTMB template model builder to further explore factors influencing *Staphylococcus aureus* occurrence in bovine raw milk samples.

The response variable, *Staphylococcus aureus* prevalence, was categorized as either detected or not detected. Conversely, predictor variables encompassed various factors including milking area hygiene, milk storage technique, post-milking udder treatment, milking utensils type, floor type, feeding technique, mastitis knowledge, mastitis screening, milking infected, laboratory-based results, hand washing practices, milk storage time, education level, grazing area, experience and presence of other animals. These variables were well presented in a table, offering a concise representation of their association.

Note that a comprehensive model incorporating all variables was initially developed. Subsequently, the step () function was utilized to select the best model, aiming to minimize the Akaike Information Criterion (AIC) value. The model assessment was conducted using the Dharma () package. Finally, odds ratios at a 95% confidence interval were determined based on the final model. This comprehensive approach ensured the selection of the most economical model while accurately assessing the factors influencing the occurrence of *Staphylococcus aureus*. Appendix 14, Figure A10, shows the results of the generalized linear mixed model (GLMM) assessment.

3.10 Ethical Considerations

The letter of approval to submit the research proposal for Ethical Review was provided by AMIU. The research proposal was then submitted to the Tanzania Livestock Research

Institute (TALIRI) / Research Ethics and Review Committee for approval. A research clearance certificate (Ref No. TLRI/RCC.23/005) was awarded.

The executive directors at Magu district were granted permission to collect data from their respective areas. Contact was established with livestock field officers (LFOs) in the designated areas, and those who expressed willingness to cooperate were granted permission to participate in the data collection process.

The goal of the study and its advantages were thoroughly explained to small-scale farmers before data collection. Participants were asked if they would like to participate, and those who declined were respected for their choice. Participants were given the assurance that they could opt out of the study at any time or decline to answer any questions. The report's substance was greatly influenced by the insights that the study participants provided. To protect the privacy and identity of study participants, with strict confidentiality.

When obtaining milk samples from cows, strict adherence to principles of animal welfare was maintained to ensure the well-being of the animals involved. To facilitate effective communication with participants, Swahili versions of the informed consent form and questionnaires were utilized during data collection activities. Appendices 2, 3, 5, and 7 contain an approval letter to proceed with ethical clearance from AMIU, a copy of the livestock research ethical clearance certificate awarded, an informed consent form, and a sample questionnaire used in the field.

3.11 Study Constraints and Limitations

The study was conducted within a limited time frame dictated by the requirements of completing a Master's degree which hindered comprehensive exploration of milking practices potentially overlooking seasonal variations and long-term trends. A more expansive, longitudinal investigation is warranted to discern evolving milking techniques and hygiene dynamics over extended periods.

The study faced limitations, notably a small sample size due to resource and logistical constraints. A larger sample could enhance insights and generalize findings to more small-scale farmers. However, this may impact statistical power, potentially limiting the detection of smaller effects or associations accurately.

The reliance on self-reported data through questionnaires presented challenges, introducing response biases like social desirability or recall bias. This could potentially affect the reliability and accuracy of the data, particularly regarding the recall of past milking practices.

The conduction of the study in the Magu district may limit its generalizability to other regions with differing socio-economic or cultural contexts. Extending results beyond Magu may be cautious due to unique constraints like limited lab facilities, hindering timely analysis of milk samples and potentially impacting overall research progress.

The use of a standard prevalence rate of 50% due to unavailable data on LA-*S. aureus* in the locality overlooks variances in farming practices, environment, and antibiotic resistance, potentially skewing results.

CHAPTER 4: RESULTS

4.1 Introduction

This section presents the findings on the prevalence of *Staphylococcus aureus* in raw cow's milk and evaluates the hygienic procedures used by small-scale farmers in Tanzania's Magu District. It outlines the analysis of data, including proportion percentages, bivariate chi-square tests, and multivariate logistic regression which are well presented in tables.

4.2 Socio-Demographic Characteristics of Small-Scale Farmers

The study involved 48 participants, predominantly 42 (87.5%) men compared to women 6 (12.5%). Among participants, 25 (52.1%) were identified as owners. About two-thirds 33 (68.8%) of participants had a primary level of education. The age range of participants varied from 18 to above 50 years old, with the largest age group being 18 to 30 years old, comprising 14 (29.7%) individuals. The mean age was 38 years, and despite this, 20 (41.75%) participants reported having more than 20 years of experience. On average, each person had 10.7 years of experience.

Three quarters 36 (75%), of the respondents, kept local breeds of cattle, 11 (29.9%) exotic, and only 1 (2.1%) had both breeds with a large number of 34 (70.8%) focusing on dual production. An extensive production system, 37 (77.1%), and grazing in communal grazing fields were most common among the farmers. Additionally, 38 (79.2%) kept other animals, such as dogs, pigs, sheep, goats, and chickens. Table 4.2 provides detailed findings on the socio-demographic characteristics of sampled farms.

Table 4.2: Socio-Demographic Characteristics of Sampled Small-Scale Farmers

Socio-demographic characteristics	Category	Total farmers = 48 n (%)
Sex	Female	6(12.5)
	Male	42(87.5)
Age (years)	18-30	14(29.7)
	31-40	9(18.8)
	41-50	12(25)
	>50	13(27.1)
Level of education	None	2(4.17)
	Primary	33(68.8)
	Secondary	7(14.6)
	Post-Secondary	6(12.5)
Ownership	Owner	25(52.1)
	Family member	1(2.08)
	Herd caretaker	22(45.8)
Experience (years)	<1	5(10.2)
	1-5	4(8.2)
	6-10	10(20.4)
	11-15	5(12.2)
	16-20	4(8.2)
	> 20	20 (40.8)
Cattle breeds kept	Exotic	11(22.9)
	Local	36(75)
	Both	1(2.1)
Production purpose	Beef	1(2.08)
	Milk	13(27.1)
	Dual	34(70.8)
Production system	Intensive	6(12.5)
	Semi-intensive	5(10.4)
	Extensive	37(77.1)
Grazing area	Communal grazing fields	40(83.3)
	Private grazing fields	2(4.17)
	Zero grazing	6(12.5)
Other animals kept (dogs, chickens, pigs, shoat)	Yes	38(79.2)
	No	10(20.8)

n, Number of Participants; %, Percentage

4.3 Prevalence of *Staphylococcus aureus*

Out of 410 bovine raw milk samples analyzed, 268 isolates were identified as *Staphylococcus* species. Among these, 98 were *Staphylococcus aureus*, constituting a prevalence of 23.9% (95% CI, 19.8% - 28.0%) with sixteen (16) being MRSA, constituting a prevalence of 16.3% (95% CI, 9.9% - 22.7%)

The prevalence of *Staphylococcus aureus* varied notably across different wards, highlighting diverse distribution patterns. The highest rates 66.7% (2/3) were observed in Isandula and the lowest 7.7% (1/13) in Kisesa. Consistent prevalence rates of 33.3% (12/36, 10/30, 1/3) were observed in the Itumbili, Kabila, and Kongolo wards showing consistency and offering insight into localized stability.

On the contrary, regarding MRSA prevalence, Magu Mjini, the more urbanized ward than the rest of the wards showed the highest rate at 40%, (2/5) while no MRSA isolates were detected in Bukandwe, Chabula, Bujora, Kahangara, Kisesa, and Kongolo. The average prevalence of MRSA in all wards was 3.9% (95% CI, 1.8% - 6.0%). Table 4.3 provides detailed findings on *Staphylococcus aureus* and MRSA among different wards in the Magu district.

Table 4.3: Distribution of *Staphylococcus aureus* and MRSA in Different Wards

Ward	Total collected sample per ward	*S. aureus* isolates prevalence n(%)	MRSA isolates prevalence n(%)
Bujora	16	5(31.3)	0(0)
Bukandwe	11	3(27.3)	0(0)
Chabula	11	4(36.4)	0(0)
Isandula	3	2(66.7)	1(33.3)
Itumbili	36	12(33.3)	3(8.3)
Kabila	30	10(33.3)	1(3.3)
Kahangara	56	5(8.9)	0(0)
Kisesa	13	1(7.7)	0(0)
Kitongo Sima	40	13(32.5)	1(2.5)
Kongolo	9	3(33.3)	0(0)
Lubugu	57	11(19.3)	2(3.5)
Magu Mjini	5	2(40)	2(40)
Nyanguge	88	20(22.7)	3(3.4)
Nyigogo	11	4(36.4)	1(9.1)
Sukuma	24	3(12.5)	2(8.3)
All wards	**410**	**98(23.9)**	**16(3.9)**

n, Number of Samples; %, Percentage

4.4 Antibiogram of *Staphylococcus aureus*

The antimicrobial profile of *Staphylococcus aureus* revealed the highest resistance rates against penicillin (10µg) at 45.9% (45/98), and tetracycline (30µg) at 33.7% (33/98).

Remarkably, ciprofloxacin (5µg) showed the lowest resistance at 1.1% (1/98), indicating its efficacy against the studied isolates. Erythromycin (15µg), cefoxitin (30µg), clindamycin (2µg), trimethoprim-sulfamethoxazole (1.25/23.75µg), and gentamycin (10µg) exhibit a notable resistance rate.

Out of 98 *Staphylococcus aureus* isolates, sixteen (16.3%) were resistant to cefoxitin (30µg) making them MRSA and exhibited resistance to penicillin (10µg). Resistance to ciprofloxacin (5µg) and gentamicin (10µg) was lower, observed in only 1.1% (1/9) isolate. Notable resistance to other antibiotics was also observed.

Among the strains resistant to clindamycin (2µg) and trimethoprim-sulfamethoxazole (1.25/23.75µg), five out of six resistant strains were MRSA. Furthermore, the sole strain resistant to ciprofloxacin (5µg) also was an MRSA. Importantly, all MRSA isolates were resistant to beta-lactam (β-lactam) drugs. Table 4.4 provides detailed findings on the antibiogram of *Staphylococcus aureus* isolates and MRSA.

Table 4.4: Antibiogram of *Staphylococcus aureus* and MRSA Isolates

Antimicrobial	Antimicrobial Susceptibility Test (AST) Results			
	%Susceptible (n=98)	%Intermediate (n=98)	%Resistant (n=98)	%MRSA (n=16/98)
Cefoxitin (30µg)	82(83.7)	0(0)	16(16.3)	16(16.3)
Ciprofloxacin (5µg)	90(91.8)	7(7.1)	1(1.1)	1(1.1)
Clindamycin (2µg)	78(79.6)	14(14.3)	6(6.1)	5(5.1)
Erythromycin (15µg)	66(67.3)	11(11.2)	21(21.4)	8(8.2)
Gentamycin (10µg)	93(94.9)	2(2.0)	3(3.1)	1(1.1)
Penicillin (10µg)	53(54.1)	0(0)	45(45.9)	16(16.1)
Tetracycline (30µg)	59(60.2)	6(6.1)	33(33.7)	4(4.1)
Tri-Sulfa (1.25/23.75µg)	89(90.1)	3(3.1)	6(6.1)	5(5.1)

AST, Antimicrobial Susceptibility Test; n, Number of Isolates; %, Percentage; µg, Microgram

4.5 Milking Hygiene Practices

4.5.1 Pre-Milking Hygiene Practices

All 48 participants were assessed for milking hygiene practices, with more than two-thirds, 34 (70.8%), maintaining good milking areas, indicating high standards of hygiene. The milking area floor was predominantly composed of soil, with 37 (77.1%) respondents using this type of flooring. Regarding calf-feeding techniques, 39 (81.3%) of small-scale farmers practiced residual suckling.

The number of milkers was fairly evenly split, with 27 (56.2%) having 0-1 milkers, and 21 (43.8%) having more than one. Hand washing before milking was inconsistent, as

only 12 (25%) used water and detergent, with none opting for gloves as an alternative. Regarding cow udder hygiene, 17 (35.4%) did not practice udder washing at all.

Three quarters 36 (75%) of the participants used plastic containers for milking and storage, while half of them 24 (50%) cleaned them with either warm water or water with detergent, with the remaining half using cold water only. Knowledge of udder infection (mastitis) was evident among over half of the participants, with 25 (52.1%) demonstrating awareness of the condition. Table 4.5.1 provides detailed findings on the pre-milking hygiene practices among farmers in the Magu district.

Table 4.5.1: Pre-Milking Hygiene Practices Among Sampled Farmers in Magu District

Milking practice	Category	Total farmers = 48 n (%)
Milking area hygiene	Good	34(70.8)
	Poor	14(29.2)
Milking area floor-type	Concrete	11(22.9)
	Soil	37(77.1)
Calf feeding technique	Residual suckling	39(81.3)
	Bucket feeding	8(16.7)
	Weaned	1(2.1)
Number of milkers'	0-1	27(56.2)
	Above 1	21(43.8)
Hand washing practice	Water only	29(60.4)
	Water and soap	12(25)
	Don't wash hands	7(14.6)
Use of gloves	Yes	0(0)
	No	48(100)
Udder wash	Cold water	20(41.7)
	Warm water	11(22.9)
	Don't wash	17(35.4)
Type of utensils	Metal canes	6(12.5)
	Plastic containers	36(75)
	Mixed types (Calabash, Plastic, and metal cane)	6(12.5)
Utensils washing practice	Cold water	24(50)
	Warm water	11(22.9)
	Water and detergent	13(27.1)
Mastitis recognition	Yes	25(52.1)
	No	23(47.9)

n, Number of Participants; %, Percentage; -, To;

4.5.2 Milking Hygiene Practices

The hand-milking technique was predominantly used, with all 48 (100%) respondents employing it. The majority, 43 (89.6%), stored milk at room temperature, but most, 20 (39.6%), exceeded the recommended storage duration of 2 hours, posing a potential risk to milk quality, which is mostly for supply 33 (68.8%).

Only a quarter of 12 (25%) of respondents performed the post-milking udder treatment. Notably, 4 (8.3%) farmers continued to milk infected cows randomly (without consideration), thereby increasing the risk of contamination. Additionally, 28 (58.3%) reported conducting mastitis screening for their cows. A notable number, either never 18 (37.5%) or rarely 28 (60.4%), used laboratory-based results for drug use decisions.

The frequency of antibiotic use varied considerably. Only 1 (2.08%) reported frequent usage, while 9 (18.8%) reported using them more frequently. Regarding adherence to drug withdrawal time, two-thirds 32 (66.7%) of respondents reported compliance, while one-third 16 (33.3%) did not adhere to this practice. Veterinary Specialists 42 (87.5%) were identified as the primary personnel responsible for drug prescriptions. A reliable number of respondents 32 (66.7%) also observed drug withdrawal times. Table 4.5.2 provides detailed findings on the milking hygiene practices among farmers in the Magu district.

Table 4.5.2: Milking Hygiene Practices Among Sampled Farmers in Magu District

Milking hygiene practice	Category	Total farmers = 48 n (%)
Milking technique	Hand milking	48(100)
	Machine milking	0(0)
Milk storage technique	Room temperature	43(89.6)
	Refrigerator	5(10.4)
Milk storage time before supply or use	<2	29(60.4)
	2-24	18(37.5)
	>24	2(2.1)
The practice of milking infected cow	Before healthy cows	5(10.4)
	After healthy cows	12(25)
	Don't milk	27(56.2)
	Randomly	4(8.3)
Post-milking udder treatment	Yes	12(25)
	No	36(75)
Mastitis screening	Yes	28(58.3)
	No	20(41.7)
Use of lab-based results	Never	18(37.5)
	No, rarely	29(60.4)
	Yes, always	1(2.08)
Frequency of antibiotic use	More frequently (at least once a week)	1(2.08)
	Frequently (at least once in 2 to 4 weeks)	9(18.8)
	Rarely (at least once in 1 to 2 months)	1(2.08)
	Very rare (at least once in 3months)	20(41.7)
	Never used	17(35.4
Observation of drug withdrawal time	Yes	32(66.7)
	No	16(33.3)
Drug prescription personnel	Veterinary Specialist	42(87.5)
	Owner	3(6.25)
	Herd caretaker	1(2.08)
	Vet or Owner	1(2.08)
	None	1(2.08)

n, Number of Participants; %, Percentage; <, Less Than; > Greater Than; -, To

4.5.3 Distribution of Pre-Milking Hygiene Practices by Milk Samples or Individual Cow

Of the 410 milk samples that were collected, 236 (57.6%) demonstrated good hygiene levels in the milking areas, while 174 (42.4%) exhibited poor hygiene conditions. Soil emerged as the predominant flooring type in these areas, representing 369 (90%) of the cases. Residual suckling was observed in 375 (91.5%) milk samples and was a predominantly feeding practice.

The data on milkers' distribution revealed that 273 (66.6%) milk samples originated from farms employing more than one milker. Interestingly, 194 (47.3%) farmers whose milk samples were collected confessed to neglecting hand washing despite none of the farmers mentioned utilizing gloves while milking. Note that, 53 (12.9%) milk samples were obtained from farms where the udders were not washed before milking.

Plastic containers were the predominant choice for utensils, with 375 (77.8%) milk samples coming from various locations. Among these, 243 (59.3%) milk samples were reported from respondents washing them with cold water. Only 62 (15.1%) farmers were milk samples obtained claimed to be capable of identifying mastitis symptoms. Table 4.5.3 provides detailed findings on the distribution of milking hygiene practices by milk samples collected.

Table 4.5.3: Distribution of Pre-Milking Hygiene Practices by Bovine Milk Samples

Milking hygiene practice	Category	Total milk samples (410), n(%)
Milking area hygiene	Good	236(57.6)
	Poor	174(42.4)
Milking area floor-type	Concrete	41(10)
	Soil	369(90)
Calf feeding technique	Residual suckling	375(91.5)
	Bucket feeding	34(8.3)
	Weaned	1(0.2)
Number of milkers'	0-1	137(33.4)
	Above 1	273(66.6)
Hand washing practice	Water only	117(28.5)
	Water and soap	99(24.2)
	Don't wash hands	194(47.3)
Use of gloves	Yes	0(0)
	No	410(100)
Udder wash	Cold water	200(48.8)
	Warm water	157(38.3)
	Don't wash	53(12.9)
Type of utensils	Metal canes	33(8.0)
	Plastic containers	319(77.8)
	Mixed types (Calabash, Plastic, and metal cane)	58(14.2)
Utensils washing practice	Cold water	243(59.3)
	Warm water	62(15.1)
	Water and detergent	105(25.6)
Mastitis recognition	Yes	228(55.6)
	No	182(44.4)

n, Number of Participants; %, Percentage; -, To;

4.5.4 Distribution of Milking Hygiene Practices by Milk Samples or Individual Cows

Out of the 410 collected milk samples, hand-milking was the predominant technique, accounting for all samples (100%) collected. The majority, 398 (97.1%), of milk samples were from participants who stored their milk at room temperature. Regarding storage duration, there was variation observed, with 297 (72.4%) milk samples from participants who stored milk for less than 2 hours.

Milk samples from farmers who randomly milked udder-infected cows accounted for 20 (4.9%) of the total. A reliable portion of milk samples, 331 (80.7%), were obtained from respondents who did not implement post-milking udder treatment for cows. Among participants, 114 (27.8%) milk samples were from farmers who practiced mastitis screening. Only 13 (3.2%) milk samples were from farmers who relied on lab-based results to treat their cows.

Only 3 (0.7%) of milk samples were obtained from farmers who had never used antibiotics on their milking cows. It's notable that the majority, 269 (65.6%) of milk samples, were obtained from farmers who were keen on observing drug withdrawal times and adhered to this practice. Among them, 259 (63.2%) milk samples were from farmers who relied on a veterinary specialist for drug prescriptions. Table 4.5.4 provides detailed findings on the distribution of milking hygiene practices by milk samples collected.

Table 4.5.4: Distribution of Milking Hygiene Practices by Bovine Milk Samples

Milking hygiene practice	Category	Total milk samples (410), n (%)
Milking technique	Hand milking	410(100)
	Machine milking	0(0)
Milk storage technique	Room temperature	398(97.1)
	Refrigerator	12(2.9)
Milk storage time	<2	297(72.4)
	2-24	111(27.1)
	>24	2(0.5)
The practice of milking infected cow	Before healthy cows	48(11.7)
	After healthy cows	70(17.1)
	Don't milk	272(66.3)
	Randomly	20(4.9)
Post-milking udder treatment (Teat dip)	Yes	79(19.3)
	No	331(80.7)
Mastitis screening	Yes	114(27.8)
	No	296(72.2)
Use of lab-based results	Never	200(48.8)
	No, rarely	197(48.0)
	Yes, always	13(3.2)
Frequency of antibiotic use	More frequently (at least once a week)	3(0.7)
	Frequently (at least once in 2 to 4 weeks)	64(15.6)
	Rarely (at least once in 1 to 2 months)	3(0.7)
	Very rare (at least once in 3months)	212(51.7)
	Never used	128(31.2)
Observation of drug withdrawal time	Yes	269(65.6)
	No	141(34.4)
Drug prescription personnel	Veterinary Specialist	259(63.2)
	Owner	22(5.4)
	Herd caretaker	13(3.2)
	Vet or Owner	17(4.1)
	None	1(0.2)

n, Number of Participants; %, Percentage; <, Less Than; > Greater Than; -, To

4.6 Milking Hygiene Vs. Staphylococcus aureus

4.6.1 Association Between Pre-Milking Hygiene Practices and Presence of Staphylococcus aureus in Raw Milk: Chi-square Test

Upon conducting bivariate analysis using a Chi-square test at a 95% confidence interval to assess the statistical associations between selected individual cow pre-milking hygiene practices factors by 410 bovine raw milk samples for the presence of *Staphylococcus aureus* the following results were observed:

There was a significant association between the absence of gloves use ($p<0.001$), poor udder washing ($p=0.0154$), and poor utensil washing practices ($p=0.0054$) with the presence of *Staphylococcus aureus* in raw cow milk, indicating a strong correlation with milk contamination. However, no significant associations were found with other pre-milking hygiene practices as shown below. Table 4.6.1 provides detailed findings on the association between pre-milking hygiene practices and *Staphylococcus aureus* contamination in raw milk by chi-square test.

Table 4.6.1: Pre-Milking Hygiene Practices Associated with *Staphylococcus aureus* Contamination in Bovine Raw Milk: Chi-square Test

Milking Practice	Category	Isolate status (*S. aureus*)		Total	Chi-square (X^2)	Df.	P. Value
		Yes	No				
Milking area hygiene	Good	55(13.40)	131(32)	236(57.6)	0.0454	1	0.8312
	Poor	43(10.5)	181(44.1)	174(42.4)			
Milking area floor type	Concrete	15(3.7)	26(6.3)	41(10)	3.2912	1	0.0696
	Soil	83(20.2)	286(69.8)	369(90)			
Calf feeding technique	Bucket feeding	13(3.2)	21(5.1)	34(8.3)	4.4689	2	0.1070
	Residual suckling	85(20.7)	290(70.3)	375(91.5)			
	Weaned	0(0)	1(0.2)	1(0.2)			
Number of milkers	0-1	37(9)	100(24.4)	137(33.4)	1.1390	1	0.2860
	Above 1	61(14.9)	212(51.1)	273(166.6)			
Hand washing practice	Water only	33(8)	84(20.5)	117(28.5)	1.7441	2	0.4181
	Water and soap	21(5.1)	78(19)	99(24.2)			
	Don't wash hands	44(10.7)	150(36.6)	194(47.3)			
Use of gloves	**Yes**	**0(0)**	**0(0)**	**0(0)**	**111.7**	**1**	**p<0.001**
	No	**98(23.9)**	**312(76.6)**	**410(100)**			
Udder wash	**Cold water**	**42(10.2)**	**158(38.5)**	**200(48.8)**	**8.3506**	**2**	**0.0154**
	Warm water	**35(8.5)**	**122(29.7)**	**157(38.8)**			
	Don't wash	**21(5.1)**	**32(7.8)**	**53(12.9)**			
Type of utensils	Metal canes	9(2.2)	24(5.9)	33(8)	0.2778	2	0.8703
	Plastic containers	76(18.30)	243(59.3)	319(77.8)			
	Mixed types (Calabash, Plastic, and Metal cane)	13(3.20	45(11)	58(14.6)			
Utensils washing practice	**Cold water**	**47(11.5)**	**196(47.8)**	**243(59.3)**	**10.442**	**2**	**0.0054**
	Warm water	**24(5.8)**	**38(9.3)**	**62(15.1)**			
	Water and detergent	**27(6.6)**	**78(19)**	**105(25.6)**			
Mastitis recognition	Yes	55(13.4)	173(42.2)	228(55.6)	3.23e-07	1	0.9995
	No	43(10.5)	139(33.9)	182(44.4)			

Df., Degree of Freedom; P. Value, Probability Value; X^2, Chi-square test; %, Percentage; -, To

4.6.2 Association Between Milking Hygiene Practices and The Presence of Staphylococcus aureus in Raw Milk: Chi-Square Test

Further bivariate analysis was conducted for the selected milking hygiene practices, employing a Chi-square test at a 95% confidence interval, to establish statistical associations between selected individual cows' milking hygiene practice factors among 410 bovine raw milk samples for the presence of *Staphylococcus aureus* and upon interpretation of results, the following associations were observed:

There was a significant association between hand milking technique ($p<0.001$), absence of mastitis screening ($p=0.0036$), and the frequency of antibiotic use ($p=0.0071$) with the presence of *Staphylococcus aureus* in raw milk. However, no significant associations were found with other milking hygiene practices as shown below. Table 4.6.2 provides detailed findings on the association between milking hygiene practices and *Staphylococcus aureus* contamination in raw milk by chi-square test

Table 4.6.2: Milking Hygiene Practices Associated with *Staphylococcus aureus* Contamination in Bovine Raw Milk: X^2-Test

Milking Practice	Category	Isolate status (*S. aureus*)		Total	Chi-square (X^2)	Df.	P. Value
		Yes	No				
Milking technique	**Hand milking**	**98(23)**	**312(77)**	**410(100)**	**111.7**	1	**<0.001**
	Machine milking	**0(0)**	**0(0)**	**0(0)**			
Milking storage technique	Room temperature	93(22.6)	305(97.1)	398(97.1)	1.2566	1	0.2623
	Refrigerator	5(1.2)	7(1.7)	12(2.9)			
Milk storage time	<2	70(17.1)	227(55.4)	297(72.4)	0.7531	2	0.6862
	2-24	28(6.8)	83(20.2)	111(27.1)			
	>24	0(0)	2(0.5)	2(0.5)			
Milking infected cows	Before healthy cows	15(3.7)	33(8)	48(11.7)	5.2603	3	0.1537
	After healthy cows	20(4.9)	50(12.2)	70(17.1)			
	Don't milk	56(13.7)	216(52.7)	272(66.3)			
	Randomly	7(1.7)	13(3.2)	20(4.9)			
Post-milking udder treatment	Yes	26(6.30)	53(12.9)	79(19.3)	3.7744	1	0.0520
	No	72(17.6)	259(63.2)	331(80.7)			
Screening for mastitis	**Yes**	**39(9.5)**	**75(18.3)**	**114(27.8)**	**8.4562**	1	**0.0036**
	No	**59(14.4)**	**237(57.8)**	**296(72.2)**			
Use of lab-based results	Never	39(9.5)	161(39.3)	200(48.8)	4.9794	2	0.0829
	No, rarely	54(13.2)	143(38.9)	197(48.0)			
	Yes, always	5(1.2)	8(2)	13(3.2)			
Frequency of antibiotic use	**More frequently (weekly)**	**1(0.2)**	**2(0.5)**	**3(0.7)**	**14.062**	**4**	**0.0071**
	Frequently (2 to 3 weeks)	**14(3.4)**	**50(12.2)**	**64(15.6)**			
	Rarely (1 to 2 months)	**2(0.5)**	**1(0.2)**	**3(0.7)**			
	Very rare (> 3 months)	**63(15.4)**	**149(36.3)**	**212(51.7)**			
	Never used	**18(4.4)**	**110(26.8)**	**128(31.2)**			
Observation of drug withdrawal time	Yes	69(16.8)	200(48.8)	269(65.6)	1.0495	1	0.3056
	No	29(7.1)	112(27.3)	141(34.4)			
Drug prescription personnel	Veterinary Specialist	82(20)	259(63.2)	341(83.2)	5.8797	4	0.2083
	Owner	4(1)	22(63.2)	26(6.3)			
	Herd caretaker	2(0.5)	13(3.17)	15(3.6)			
	Vet or Owner	8(2)	17(4.1)	25(6.1)			
	None	2(0.5)	1(0.2)	3(0.7)			

Df., Degree of Freedom; P. Value, Probability Value; X^2, Chi-Square; %, Percentage; -, To; >, greater than

4.6.3 Association Between General Milking Hygiene Practices and Staphylococcus aureus Contamination: Logistic Regression Analysis

Further, analysis was done using logistic regression to establish the association between general milking hygiene practices and *Staphylococcus aureus* contamination in raw milk by generalized linear mixed model (GLMM). Notably, cattle grazing in private fields exhibited significantly lower contamination odds, with a 0.23 times lower risk compared to those grazing in community fields. Individuals not undergoing mastitis screening demonstrated significantly higher contamination odds, with a 3.05 times higher risk compared to those who did not.

Furthermore, infrequent use of lab-based results was associated with higher contamination odds, with a 2.67 times higher risk. Regarding hygiene practices, hand washing with water and soap or water only significantly lowered contamination odds, with risks 0.22 and 0.19 times lower, respectively, compared to those who didn't wash their hands. Table 4.6.3 provides general milking hygiene practices associated with *Staphylococcus aureus* contamination in bovine raw milk by logistic regression analysis.

Table 4.6.3: General Milking Hygiene Practices Associated with *Staphylococcus aureus* Contamination in Bovine Raw Milk: Logistic Regression Analysis

Predictors	Odds Ratio (OR)	Confidence Interval (CI)	P. Value
(Intercept)	2.09	0.26 -16.48	0.485
Storage [Refrigerator]	1		
Storage [Room temperature]	0.24	0.04 -1.43	0.118
Grazing area [Community fields]	1		
Grazing area [Private fields]	**0.23**	**0.05 - 0.98**	**0.046**
Grazing area [Zero grazing]	0.21	0.04 -1.08	0.063
Mastitis screening [Yes]	1		
Mastitis screening [No]	**3.05**	**1.35 – 6.89**	**0.007**
Lab-based results [Never]	1		
Lab-based results [No, Rarely]	**2.67**	**1.51 – 4.73**	**0.001**
Lab-based results [Yes, Always]	1.91	0.53 – 6.91	0.322
Hand washing [Don't wash hands]	1		
Hand washing [Water and soap]	**0.22**	**0.009 – 0.55**	**0.001**
Hand washing [Water only]	**0.19**	**0.08 – 0.43**	**<0.001**

CI, Confidence Interval; OR, Odds Ratio; P. Value, Probability Value; <, Less Than

CHAPTER 5: DISCUSSION

5.1 Introduction

This section provides a thorough overview of the conclusions and revelations from the research on the "Prevalence of *Staphylococcus aureus* in bovine raw milk and associated milking hygiene practices among small-scale farmers in Magu District of Mwanza, Tanzania." The study addressed a varied set of objectives, aiming to shed light on various dimensions of raw milk safety, antibiotic resistance, and small-scale farmers' hygiene practices. This discussion looks deeper into each aspect, providing a more detailed analysis of our findings in comparison to other studies, and discussing their implications for veterinary, public health, and food security.

5.2 Prevalence of *Staphylococcus aureus* and MRSA

The prevalence rates of *Staphylococcus aureus* and MRSA in raw milk samples of the current study are consistent with several global studies, although variations in detection methods across studies may influence reported rates. In comparison to the current *Staphylococcus aureus* prevalence of 23.9% (95% CI, 19.8 - 28.0), previous studies reported similar prevalences ranging from 21.3% to 24.9% in Tanzania and Ethiopia (Sanga et al., 2022; Alembo & Tonjo Torka, 2023; Deddefo et al., 2022). Similarly, the present MRSA prevalence of 16.3% (95% CI, 9.9 - 22.7) is comparable to those previously reported in China and Indonesia (Tyasningsih et al., 2022; Wang et al., 2022). This indicates the presence of low-quality and contaminated milk in both production and distribution, posing potential risks to human health.

Contrastingly, previous studies reported higher *Staphylococcus aureus* prevalences ranging from 31.2% to 72.5% in China, Indonesia, Turkey, Kenya, Mozambique, and Tanzania (Kalee et al., 2021; Keyvan et al., 2020; Nhatsave et al., 2021; Omwenga et al., 2021; Wang et al., 2022). Similarly, compared to the current findings, elevated MRSA prevalences of 33.3% to 35.7% were reported in Turkey and Egypt, while lower MRSA prevalences of 2.9% and 4.2% were found in the Njombe and Morogoro regions of Tanzania (Keyvan et al., 2020; Selim et al., 2022; Mohammed et al., 2018; Sanga et al., 2022). These disparities highlight the severity of the situation and underscore the urgent need for interventions and further research to address issues concerning milk contamination with *Staphylococcus aureus* and MRSA.

Some previous studies cited in current research utilized different sampling techniques, such as collecting milk from bulk containers or pre-screening for subclinical mastitis, which may impact the concentration of detected bacteria (*Staphylococcus aureus*) and introduce variability in prevalence rates (Keyvan et al., 2020; Sanga et al., 2022; Wang et al., 2022). Additionally, while the current study relied on conventional detection methods, other studies employed advanced techniques like polymerase chain reaction (PCR), potentially influencing the reported prevalence rate (Kalee et al., 2021; Mohammed et al., 2018; Nhatsave et al., 2021; Sanga et al., 2022). Thus, to improve sensitivity and specificity in identifying *Staphylococcus aureus* and MRSA in raw milk samples, future studies could benefit from taking into account sophisticated molecular techniques like PCR.

Despite these variations, while the current study contributes valuable insights into bacteria prevalence in raw milk, acknowledging methodological differences is crucial for contextualizing the findings within the broader literature in advancing the understanding of milk contamination and supporting evidence-based risk reduction strategies.

5.3 Antibiogram of *Staphylococcus aureus*

The antibiogram of *Staphylococcus aureus* isolates showed resistance to all tested antibiotics: 45.9% penicillin, 33.7% tetracycline, 21.4% erythromycin, 16.3% cefoxitin, 6.1% clindamycin, 6.1% trimethoprim-sulfamethoxazole, 3.1% gentamycin and 1.1% ciprofloxacin indicating a diverse range of resistance properties for tested isolates. The antimicrobial profile of *Staphylococcus aureus* observed in the current study is comparable to previous studies conducted in China, Turkey, Ethiopia, and Tanzania (Borena et al., 2023; Keyvan et al., 2020; Sanga et al., 2022; Wang et al., 2022).

The current resistance of *Staphylococcus aureus* to gentamycin, cefoxitin, and tetracycline is comparable to previous reports of 2.2%, 17%, and 30.2% in Indonesia, Turkey, Tanzania, and Ethiopia (Keyvan et al., 2020; Sanga et al., 2022; Tyasningsih et al., 2022). Contrastingly, previous studies reported high resistance rates of *Staphylococcus aureus* including 92.4% to cefoxitin, 83.3% to tetracycline, 13.2% to trimethoprim-sulfamethoxazole, 20.7% to ciprofloxacin, 69.8% to penicillin, and 67.9% to clindamycin in Oromia, Ethiopia, Hefei, China, and Turkey (Borena et al., 2023; Keyvan et al., 2020; Omwenga et al., 2021; Wang et al., 2022). The occurrence of antibiotic-resistant *Staphylococcus aureus* and MRSA necessitates region-specific antimicrobial stewardship initiatives.

Conversely, *Staphylococcus aureus* resistance in the current study is comparable to previous reports of 19.8% for tetracycline, 8% for clindamycin, 1.6% for trimethoprim-sulfamethoxazole, and 0% for ciprofloxacin in Tanzania (Kalee et al., 2021; Mohammed et al., 2018; Sanga et al., 2022). Similarly, compared to previous reports of 13% resistance to tetracycline in Ethiopia, the resistance of *Staphylococcus aureus* in the current study is high (Gebremedhin et al., 2022). This highlights the variability in antimicrobial resistance patterns of *Staphylococcus aureus* isolates across different geographical locations, emphasizing the importance of regional context in understanding antibiotic resistance trends.

Ensuring methodological consistency is essential for the reliability of comparative analyses. The current study, similar to the previous cited, adopted the Kirby Bauer Disc Diffusion method (Kalee et al., 2021; Massawe et al., 2019; Mohammed et al., 2018; Nhatsave et al., 2021; Omwenga et al., 2021; Sanga et al., 2022). This standardized methodology facilitates more meaningful result comparisons. However, it is crucial to acknowledge that, while the method offers consistency, variations in environmental factors, sample populations, and laboratory practices can contribute to differences in reported resistance rates.

The collective adoption of the Kirby Bauer Disc Diffusion method, employed in the present study as well, improves overall comparability and enhances the validity of observed resistance patterns. The continual application of this method maintains a standardized approach, enhancing the reliability of comparisons. These outcomes emphasize the lasting importance of surveillance, collaborative research efforts, and the application of customized strategies to effectively address antimicrobial resistance.

5.4 Milking Hygiene Practices

The assessment of milking hygiene practices in the current study offers valuable insights into the diverse landscape of dairy farming practices in comparison to previous studies. The exclusive reliance on 100% hand milking in the current study is consistent with previous studies, which also reported exclusive hand milking in Tanzania and Ethiopia. (Deddefo et al., 2022; Kalee et al., 2021). This shows the prevailing preference for traditional hand-milking methods across various regions in Africa. In contrast, to the current study, 94% of dairy farmers used machine milking in Colombia (Ágredo-Campos et al., 2023).

The current study reported that more than three-quarters (77.1%) of farmers preferred soil-type floors, which is comparable to a previous report from Morogoro that found 84.9% of farmers preferred soil floors for their milking cows (Kalee et., al 2021). This might be due to the cheaper maintenance of concrete floors. This preference might be due to the cheaper maintenance costs compared to concrete floors. Furthermore, 75% of farmers in the current study were committed to good farm hygiene practices, in contrast to the previous study in Morogoro where 90.6% of milking cows were reported to be living on dirty or muddy floors (Kalee et al., 2021). These differences show how environmental factors affect milking practices, stressing the importance of adopting region-specific actions.

Moreover, the current study reported that 60.4% of milkers washed their hands with water only, while 14.6% did not wash their hands at all. This is comparable to previous studies in Tanzania, Ethiopia, and Colombia, which reported 45%, 96.6%, and 54%,

respectively, neglecting proper hand washing and disinfection before milking (Republic et al., 2022; Ágredo-Campos et al., 2023; Alembo & Tonjo Torka, 2023). This might be due to negligence or unawareness of the importance of proper hand hygiene in producing uncontaminated milk. These similarities highlight common challenges, possibly due to limited access to soap or inadequate awareness about the importance of thorough hand hygiene. In contrast, the use of gloves was observed in 41.3% of cases in a previous study in Colombia (Colombia Ágredo-Campos et al., 2023). This might be due to variations in hand hygiene practices before milking across different regions.

Looking deeper into udder washing practices in the current study, 35% of farmers did not wash cow udders before milking, and 41.7% used cold water. These findings are comparable to previous studies in Tanzania and Ethiopia, which reported similar patterns of unhygienic udder practices with varying percentages (100%, 16.78%, 56%), (Deddefo et al., 2022; Gebremedhin et al., 2022; Kalee et al., 2021). The consistency in these practices highlights a widespread issue of poor udder hygiene, which can significantly impact milk quality and safety. Addressing these practices through targeted education and interventions is crucial for improving dairy hygiene standards and ensuring the health of both cows and consumers.

In comparison to the current study, the practice of washing utensils with cold water is comparable to a previous report in Ethiopia (Deddefo et al., 2022). This similarity may be due to a lack of access to hot water and detergent or limited awareness of the importance of using hot water and detergent for proper sanitation and the removal of bacteria and contaminants. Furthermore, a previous report in Morogoro indicated that 78.1% of milking utensils were in poor condition (Kalee et al., 2021). In contrast, other studies in

Ethiopia reported that over half (57.9% and 60%) of farmers used cold water but included detergent for washing milking utensils (Alembo & Tonjo Torka, 2023; Borena et al., 2023). This indicates the presence of poor washing practices that jeopardize the milk quality produced. The use of plastic containers was nearly three quarter 75% and 70% for both the current study and the previous report in Ethiopia making them comparable (Borena et al., 2023). This similarity might be due to the cost-effectiveness and ease of availability of plastic containers.

Both the current study and a previous study in Colombia reported the screening of milking cows, however, disparities persist, with higher screening rates of 91.3% in Colombia compared to 58.3% in the current study (Ágredo-Campos et al., 2023). This difference may be due to varying levels of awareness, resources, and implementation of dairy farming practices between the two regions. The study further reveals a lack of knowledge on udder infection, with at least half of the population showing similar results to a previous report in Ethiopia (Tibebu et al., 2021). This may be due to insufficient education and outreach efforts regarding udder health and milk hygiene practices in these regions. Hence, demanding profound awareness campaigns to farmers on udder infection and its effects on milk production.

Meanwhile, the current study reported varying frequencies of antibiotic use, with 41.7% rarely using antibiotics and 18.8% admitting to very frequent use (at least once weekly). In comparison to a previous report in Morogoro, Tanzania, both studies indicated that over half of the participants adhered to antibiotic withdrawal times (66.7% and 90.62%), respectively (Kalee et al., 2021). This adherence may be attributed to increasing awareness among farmers about the importance of antibiotic stewardship and following

guidelines to prevent antibiotic residues in milk and ensure food safety. This highlights the evolving nature of antibiotic practices in dairy production.

Furthermore, in alignment with previous literature emphasizing the pivotal role of veterinary guidance in antibiotic use, the current study reported that 87.5% of participants utilized veterinary services (Tibebu et al., 2021). This highlights the widespread recognition among farmers of the importance of veterinary expertise in ensuring proper antibiotic usage, which includes dosage, duration, and adherence to withdrawal periods to safeguard animal health and food safety. This approach underscores the critical role the veterinary specialists play in ensuring judicious antibiotic use, thereby preventing antibiotic residues in milk and safeguarding both animal and human health (Mogotu et al., 2022).

The questionnaire method, employed in both the current study and the cited literature, offers a standardized approach for data collection, enabling a comprehensive understanding of dairy farming hygiene practices (Deddefo et al., 2023; Kalee et al., 2021; Tibebu et al., 2021; Alembo & Tonjo Torka, 2023; Borena et al., 2023; Gebremedhin et al., 2022; Republic et al., 2022). Customized interventions are essential for tackling regional inequalities and improving hygiene practices in dairy farming, thereby promoting safe milk production and sustainable practices. Survey findings underscore the need for targeted initiatives and educational schemes to address challenges and enhance milk product safety and quality in small-scale dairy farming settings (Mogotu et al., 2022).

5.5 *Staphylococcus aureus* Vs. Milking Hygiene Practices

The significant associations identified in the current study regarding the absence of glove usage, poor utensil, and udder washing practices, hand milking technique, absence of mastitis screening, and frequent use of antibiotics underscore crucial factors contributing to *Staphylococcus aureus* contamination in raw milk. These results are consistent with previous research findings, which emphasize the pivotal role of proper farm hygiene practices in mitigating *Staphylococcus aureus* contamination in bovine raw milk (Banu & Geberemedhin, 2022; Deddefo et al., 2022).

In the current study, the role of proper hand-washing practices in reducing the odds of *Staphylococcus aureus* contamination was highlighted by the association of hand washing with a lower contamination risk. This finding is comparable to a previous study in Colombia, which observed a strong association between hand milking technique, absence of glove usage, and the presence of *Staphylococcus aureus* (Ágredo-Campos et al., 2023). The similarity arises because both studies emphasize the critical role of hand hygiene in preventing *Staphylococcus aureus* contamination in milk. This underscores the imperative for stringent hand hygiene and disinfection measures during milking to mitigate contamination risks, as corroborated by conclusions from research.

However, the current study is comparable to a previous study reported in Ethiopia, as both studies highlight the significance of udder washing in reducing *Staphylococcus aureus* contamination in milk (Deddefo et al., 2022; Gebremedhin et al., 2022). This similarity underscores the consistent impact of maintaining proper udder hygiene throughout the milking process. Furthermore, the association between the absence of

mastitis screening and *Staphylococcus aureus* contamination in raw milk, with farmers not conducting screenings facing significantly higher contamination risks, is comparable in both the current study and the previous report in Ethiopia (Tibebu et al., 2021; Borena et al., 2021). This similarity suggests a lack of proactive disease management in small-scale dairy farming, highlighting the importance of comprehensive hygiene protocols to enhance milk quality in both current and previous studies.

The frequent use of antibiotics in milking cows observed in the current study showed a higher association with *Staphylococcus aureus* contamination in raw milk, which is comparable to a previously reported study in Ethiopia (Alembo & Tonjo Torka, 2023). This similarity may be due to the habitual use of antibiotics by farmers in milking cows. It raises concerns about antibiotic use and emphasizes the significance of using antibiotics sparingly and implementing alternative methods of disease control to reduce the emergence of resistant strains in dairy farming. Additionally, infrequent utilization of lab-based results was found to be associated with heightened *Staphylococcus aureus* contamination risk in the current study, which is comparable to a previous report in Ethiopia (Alembo & Tonjo Torka, 2023). This similarity might be due to the lack of regular monitoring and reliance on laboratory testing for early detection and management of contamination. Thus, this emphasizes the crucial necessity of regular monitoring to mitigate contamination risks and ensure milk safety.

Furthermore, in comparison to the current study, previous research reported similar findings regarding the impact of environmental factors and monitoring practices on *Staphylococcus aureus* contamination in raw milk. Cattle grazing in private fields exhibited significantly lower contamination odds compared to those in community fields

in a previous report in Colombia (Ágredo-Campos et al., 2023). This similarity might be due to the presence of *Staphylococcus aureus* contamination on grazing fields underscoring the profound environmental impact on contamination dynamics.

Methodological differences between the current study and existing literature may contribute to variations in results. While the current study utilized bivariate analysis via chi-square tests and logistic regression, many studies in the literature employed chi-square tests, univariate, and multivariate logistic regression. These differences allow for varying levels of adjustment for confounding variables. By employing different combinations of these methods, both the current study and previous literature aimed to uncover associations and predict outcomes. However, the varied methodologies may lead to slight discrepancies in the strength and interpretation of these associations (Ágredo-Campos et al., 2023; Banu & Geberemedhin, 2022).

However, despite these disparities, the findings collectively underscore the critical role of proper hygiene practices, disease management strategies, and antibiotic stewardship in ensuring milk safety and quality in dairy farming. Further research aimed at exploring possible explanations for these associations and evaluating the effectiveness of interventions is warranted. Such research will help address identified gaps and improve dairy farming practices.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Introduction

This chapter offers the study's conclusions in line with specific objectives and recommendations from the study findings.

6.2 Conclusions

There is high contamination of cows' raw milk with *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) which emphasizes the critical need for improved hygiene practices in dairy farming. This contamination poses health risks to consumers and suggests potential compromises in milk quality. Enhancing cleanliness and sanitation procedures in dairy farms is crucial to guaranteeing the safe production of raw milk. Examples of these practices include inadequate cleaning of the udder and washing utensils in cold water.

A high antimicrobial resistance rate signals an alarming trend of ongoing resistant strains of *Staphylococcus aureus* and MRSA which pose a noteworthy threat due to their resistance to many antibiotics. This resistance undermines the effectiveness of common antibiotics, posing challenges in treating bacterial infections. There is an urgent need for comprehensive strategies to address antimicrobial resistance in dairy farming, including using antibiotics sparingly and putting other control methods in place to lessen the spread of resistant bacteria.

The poor milking hygiene practices, such as the lack of udder cleaning and the use of cold water for utensil washing, reflect a concerning neglect of cleanliness and sanitation

measures within dairy farming operations in the Magu district. These practices are crucial for maintaining the safety and quality of cows' raw milk. Inadequate udder cleaning can lead to the transfer of harmful bacteria, including *Staphylococcus aureus*, into the milk during the milking process. Similarly, using cold water for utensil washing may not effectively remove bacteria and other contaminants, increasing the risk of milk contamination.

The significant associations observed between poor udder and utensil washing practices, absence of glove use, lack of mastitis screening in cows, hand milking, and frequent antibiotic use highlight critical concerns in dairy farming. These factors demonstrate the urgent need for better hygienic measures, appropriate screening procedures, and prudent antibiotic use to maintain the safety and quality of milk products. They also lead to increased bacterial contamination and antimicrobial resistance in cows' raw milk.

The room temperature storage and infrequent cleaning lower contamination odds, while the absence of screening increases risks of *Staphylococcus aureus* contamination in raw milk of bovines, emphasizing the need for better education and protocols. Hand-washing practices significantly influence the danger of contamination. These results highlight how crucial maintaining good hygiene is and education in preventing *Staphylococcus aureus* contamination in milk production, urging ongoing efforts to improve standards in the dairy industry.

6.3 Recommendations

1. The Public Health (PH) and Veterinary Departments should collaborate to conduct regular inspections and training programs for dairy farmers. These programs should

emphasize proper hygiene practices, including udder and utensil washing, and encourage the use of gloves during milking to reduce bacterial contamination.

2. These departments should also prioritize disseminating information on the benefits of boiling milk to both farmers and consumers. Boiling milk effectively eliminates pathogens, reducing the occurrence of foodborne illnesses and promoting overall public health.
3. Policy implications regarding food safety standards in dairy farming should be considered. To guarantee the safety of raw milk, authorities should enact laws requiring the adoption of sanitary milking procedures and the use of suitable disinfectants.
4. Policymakers should focus on promoting responsible antibiotic use in dairy farming by implementing regulations that restrict antibiotic use to therapeutic purposes only and encourage the adoption of alternative disease prevention measures.

2 Future research should explore the specific characteristics and mechanisms of *Staphylococcus aureus* and MRSA strains found in dairy farming environments. This research will provide insights into developing targeted interventions to control and prevent bacterial contamination in raw milk.

3 Research is required to evaluate the success of educational initiatives on antibiotic stewardship and antimicrobial resistance in dairy producing communities. Understanding the impact of these campaigns will inform future strategies for combating antimicrobial resistance effectively.

4 Collaboration among government agencies, public health organizations, agricultural associations, and consumer groups is vital. By working together, we can promote

milk boiling, disseminate food safety education, and raise awareness about antimicrobial resistance. This collective effort strengthens surveillance, response, and sustainability across food systems, thus safeguarding public health.

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APPENDICES

Appendix 1: Study Area

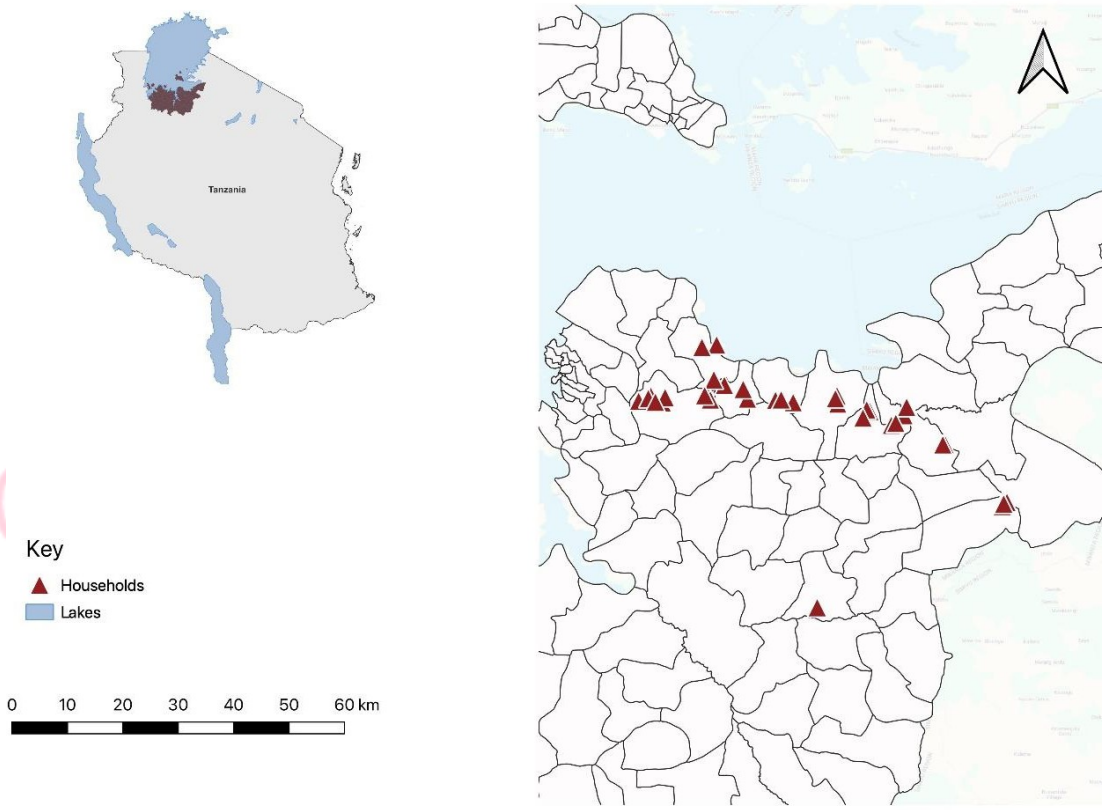

Figure A1: Study Area Map: Distribution of Sampled Small-Scale Farmers' Households, Magu District, Mwanza, Tanzania

Appendix 2: Letter of Approval for Ethical Review (AMIU)

OFFICE OF THE DEAN, **GRADUATE SCHOOL**

February. 15 2023

Ngassa Doris Richnrd SHS/MPH/4995-1/2022

Proposal Title: Prevalence of *Stuph5lucuccus Aureus* in Bovine Raw Milk and Associated Milking Hygiene Practices Among Small Scale Farmers in Magu District of Mwanza. Tanzania.

Following your full proposal presentation on September 8th 2022, and subsequent review of your revised proposal, Graduate School has approved your work for submission for ethical review before the commencement of fieldwork. Ethical approval is a mandatory requirement for all research prmess.

You are advised to seek ethical approval from your country Institutional Research Board (IRB) and share the copy of approval letter with Graduate School.

You are required to update Graduate School of your progress after every three months by submitnng progress reporis uxing the forms attached.

Dr. Dancan Lungu
Dean, Graduate School & Lead Enterprise Development

CC: HOD Community Health

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Appendix 3: Ethical Clearance Form (TALIRI)

FORM NO. 10

THE UNITED REPUBLIC OF TANZANIA
MINISTRY OF LIVESTOCK AND FISHERIES
TANZANIA LIVESTOCK RESEARCH INSTITUTE (TALIRI)

Director General, Tanzania Livestock Research Institute, Block No. 39, .
P.O. Box, 834, Dodoma, Email: or

REF NO. TLRI/RCC.23/005

RESEARCH CLEARANCE CERTIFICATE

1. This certificate is hereby presented to:

   Dr. Doris Richard Ngassa.

   Title of **the** proposed **research:**

   Prevalence of *Staphylococcus* aureus in bovine raw milk and associated milking hygiene practices among small-scale farmers in Magu District of Mwanza, Tanzania.

2. **General objective** of the Research:

   To assess prevalence of *Staphylococcus aureus* in bovine raw milk and associated milking hygiene practices among small-scale farmers in Magu District of Mwanza, Tanzania.

3 Study areas:

   This research will be conducted in 1 Region of Tanzania — Mwanza (Magu District).

4. Starting date: 09th February, 2023

5. Ending date: 10th February, 2024

DIRECTOR GENERAL
arch Institute

Signature of the Director General _____ **Date** 26/02/2023

Appendix 4: Informed Consent Form (English Version)

Farm or household name/code……
Written Informed Consent on "My milking practice"
Information to be addressed to the participant:

Before choosing to take part in the study, please carefully read the instructions, and don't hesitate to ask any questions you may have for more information:

Hello, I am **Doris Ngassa**, a graduate student at Amref International University (AMIU) conducting a study on; "*Prevalence of Staphylococcus aureus in bovine raw milk and associated milking hygiene practices among small-scale farmers in Magu district of Mwanza, Tanzania*" funded by European Development and Clinical Trials Partnership and Capacity Development of Applied Epidemiologists (EDCTP-CDAE).

Acknowledging the significance of small-scale farming in the dairy supply chain in Tanzania and Mwanza, the study aims to assess the bacterial (*Staphylococcus aureus*) status of raw bovine milk produced and the hygiene practices used in milking by small-scale farmers in the region (Magu district) and does not intend to cause you any discomfort.

As a small-scale farmer engaged in dairy farming, you will be asked questions about your milking practices for about 30 minutes, and answers recorded on a questionnaire. With your permission, milk samples from all cows for milking today on your farm will be taken to the Tanzania Veterinary Laboratory Agency (TVLA) for diagnosis.

Participating in the study carries no predicted hazards, however, the information gathered will be used to prepare a report that is essential for organizing and putting into practice bacterial (*Staphylococcus* aureus) control strategies for contaminant-free milk production at the farm level.

Recognizing the value of your rights, you are free to decide whether or not to participate in the study. Throughout the study, you will have the freedom to ask any questions you may have, to refuse to answer any that you do not want to, to take any photo you choose with your consent, and to leave the study at any moment. You won't suffer any consequences for not taking part. We will maintain the privacy of any information that identifies you.

In case of any questions or queries on the study and rights as a participant, you will be provided with my contact and contact from a member of the TALIRI Ethics and Review Committee.

Agreement:
I give permission to take part in today's "**My milking practice**" research. I have been given a copy of this description and I willingly consent to take part.

Participant…… Signature…..…….. Date ……
Researcher…… Signature……. Date……

Appendix 5: Informed Consent Form (Kiswahili Version)

Jina/Namba ya utambulisho ya shamba au makazi…….
Fomu ya ridhaa kwa maandishi: "Mbinu zangu za ukamuaji"

Maelekezo muhimu kwa mshiriki:
Tafadhali sikiliza kwa makini maelezo yafuatayo kabla ya kuamua kushiriki na kuwa huru kuuliza swali lolote na kwa wakati wowote kwa ajili ya ufafanuzi:
Salamu, jina langu ni Doris Ngassa, ninasoma shahada ya uzamili katika chuo kikuu cha kimataifa cha Amref (AMIU) niko hapa kufanya utafiti kuhusu, "*Ueneaji wa vijidudu aina ya Staphylococcus aureus katika maziwa mabichi/freshi ya ng'ombe na uhusishwaji wa mbinu za ukamuaji miongoni mwa wakulima wadogo wa wilayani Magu, Tanzania"* na kudhaminiwa na EDCTP-CDAE.

Kwa kuzingatia umuhimu wa ufugaji wa ng'ombe wa maziwa wa kiwango kidogo katika mnyororo wa usambazaji wa maziwa ndani ya Mwanza na Tanzania; utafiti unalenga kutathmini hali ya uwepo wa vijidudu (*Staphylococcus aureus*) katika maziwa yanayozalishwa na mbinu za ukamuaji za wakulima wadogo wa wilayani Magu bila kukuletea usumbufu wowote.

Wewe kama mkulima mdogo unayejihusisha na uzalishaji wa maziwa ya ng'ombe, utaulizwa maswali kwa takribani dakika 30 juu ya mbinu unazotumia katika ukamuaji na majibu yako kurekodiwa katika dodoso. Kwa ruhusa/ridhaa yako sampuli za maziwa zitachukuliwa kwa ng'ombe wako kwa ajili ya kukamua leo na kupelekwa maabara ya veterinari (TVLA) kwa uchunguzi.

Hakuna athari yoyote inayotarajiwa kutokana na kushiriki kwako katika tafiti hii bali taarifa zitakazozalishwa zitatumika kuandaa ripoti ambayo ni muhimu katika kupanga na kutekeleza hatua za udhibiti wa vijidudu hivi (*S. aureus*) ili kuzalisha maziwa yasiyo na uchafuzi katika ngazi ya shamba.

Kwa kutambua umuhimu wa haki zako kama mshiriki, uamuzi wa kuwa sehemu ya tafiti hii ni juu yako. Uko huru kuuliza chochote, kutokujibu swali lolote na kujiondoa kwa wakati wowote ule kwenye utafiti. Hautapungukiwa na chochote kwa kutoshiriki. Uchukuaji wa picha yoyote ni kwa ridhaa yako. Kila taarifa zote zitakazokutambulisha zitahifadhiwa kwa usiri mkubwa na kwa kufuata sheria zitakazokulinda kama mshiriki.

Ikiwa kuna chochote hakijaeleweka kuhusu utafiti huu na kuhusu haki zako za msingi kama mshiriki utapewa mawasiliano yangu na mawasiliano ya mwanachama kamati ya maadili ya utafiti na ukaguzi ya Kituo cha utafiti wa magonjwa ya mifugo (TALIRI) kwa ufafanuzi zaidi.

Makubaliano:
Kwa hiari yangu, nimeridhia kushiriki katika utafiti wa leo kuhusu "**Mbinu zangu za ukamuaji**" na nimepokea nakala ya fomu hii.

Mshiriki……	Sahihi…..……	Tarehe ……..
Mtafiti……...	Sahihi…….	Tarehe…….....

Appendix 6: Sample Collection Form (English Version)

(*Use a separate form for each sampled household/farm*)

S/N		
1.	Name of sample household	
2.	Location (GPS Co-ordinates)	
3.	Date of interview [DD/MM/YYYY]	
4.	Region/District/Ward	Mwanza/Magu/…….....
5.	The contact number of the respondent	
6.	Age of the respondent	
7.	The respondent's gender	i. Male ii. Female
8.	The respondent's educational background	i. None ii. Primary iii. Secondary iv. Post-secondary (College/University)
9.	What is your role on the farm?	i. Owner (Includes family member) ii. Herd caretaker iii. Hired manager iv. Ordinary worker v. Other (Specify)…… ……
10.	How long have you been keeping/attending the cattle?	
11.	How many cattle are there in total on the farm? The hygienic practices were generally observed as opposed to the use of the questionnaires.	
12.	Do you own any particular breed of cattle?	i. Exotic ii. Indigenous/local iii. Cross-breed iv. Different breeds (Specify)…… ……
13.	What purpose do you keep the cattle for?	i. Milk production ii. Beef production iii. Dual (i & ii) iv. Other

		(Specify)…… ……
14.	How many milking cows are there among all cattle?	
15.	What farming system do you use?	i. Extensive system ii. Semi-intensive system iii. Intensive system
16.	Where do you commonly graze your cattle?	i. Communal grazing field ii. Private grazing fields iii. Zero grazing iv. Other (Specify)…… ……
17.	Apart from grazing pastures, do you give any other feeds to your cattle?	i. Yes (Specify) ii. No Specify…… ……
18.	Where do you get drinking water for your cattle?	
19.	Do you keep other animals apart from cattle?	i. Yes (Specify) ii. No Specify…… ……
20.	How is the general hygienic condition of the farm? (Observe)	i. Good (dry, cow dung-free) ii. Poor(overly wet, mud, cow dung)
21.	Where do you milk your cows? (Observe)	i. Milking parlor (Special area for milking) ii. In the pens iii. Anywhere
22.	What kind of flooring is there in the milking area? (Observe)	i. Soil ii. Concrete
23.	Do you clean the milking area? How many times a week?	i. Yes, always ii. Yes, rarely Specify…….. …….
24.	How is the hygienic condition of the milking area? (Observe)	i. Good (dry, cow dung-free) ii. Poor (overly wet, mud, cow dung)
25.	What is the daily milking count of cows? (On a specific day)	
26.	Which method of milking do you employ? (Observe)	i. Machine milking ii. Hand milking
27.	How many milkers perform the hand-milking process? (On a specific day)	

28.	Before milking, what do you use to wash your hands? (Observe)	i. Use water only ii. Use water and soap iii. Don't wash hands
29.	Do you use gloves when milking? (Observe)	i. Yes ii. No
30.	Are cows' udders washed before milking? (Observe)	i. Yes ii. No
31.	If yes, what do you use? (Observe)	i. Coldwater ii. Warm water iii. Don't wash hands
32.	Are udders dried post-washing before milking? (Observe)	i. Yes ii. No
33.	Do you use separate towels? (Observe)	i. Yes ii. No
34.	What do you use to clean the milking utensils including storage containers? (Observe)	i. Only use cold water ii. Only use warm water iii. Only use hot water iv. Use water and detergent
35.	What is type of milking utensils used including milk storage containers? (Observe)	i. Calabash (Wooden/milk gourd) ii. Plastic container iii. Glass bottle iv. Metal cane v. Different types (Specify)…… ……
36.	How much time do you keep milk in storage before selling it?	
37.	What is the use of collected milk?	i. Home Consumption ii. Supply to local market iii. Both (i & ii) iv. Other (Specify)…… …….....
38.	In which form do you supply/sell the milk?	i. Whole/Fresh milk ii. Sour milk iii. Both (i & ii)
39.	What is the state of whole/fresh milk supplied?	i. Raw ii. Boiled iii. Both (i & ii)
40.	Do you boil milk for sour milk production?	i. Yes ii. No
41.	At what state do you consume whole/fresh milk at home?	i. Raw ii. Boiled iii. Both (i & ii)
42.	How do you store excess milk?	i. Room temperature ii. Refrigeration
43.	Do you do any post-milking udder	i. Yes

	treatment? (Teat dipping), (Observe)	ii. No
44.	Have you ever heard of udder infection?	
45.	Can you recognize cows with udder infection?	i. Yes ii. No (**If no, go to Question 50**)
46.	If yes, how do you know?	i. Changes in milk (appearance & and consistency) ii. Changes in udder (size, temperature, consistency) iii. Other (Specify)…… ……
47.	Apart from physical changes in milk and udder, do you use any other way to detect udder infection?	i. Yes ii. No Specify…… ……
48.	If not, who helps you identify udder infection?	Specify……..…… ……
49.	Do you milk these cows with udder infection?	i. Yes ii. No
50.	When do you milk these infected cows?	i. Before healthy cows ii. After healthy cows iii. Randomly (No consideration)
51.	For cows with udder infection, what is the fate of their obtained milk?	i. Discard ii. Home Consumption iii. Retail Supply/Sell iv. Feed to calves v. Other (Specify)…… ……
52.	Do you screen milking cows for udder infection?	i. Yes (How often? week, month, etc.) ii. No **(If no go to Question 58)** Specify…… ……
53.	Who is responsible for the screening?	i. Myself/Family member ii. Veterinarian/Livestock officer (LFO) iii. Another farmer iv. Herd caretaker v. Other (Specify)…… ……
54.	Have you treated your milking cows recently? If yes, specify the condition:	i. Yes ii. No Specify the

		condition…….…… ……
55.	Which medication did you use? Name the drugs/antibiotics, (If necessary request to see it)	i. Specify a. …… b. …… c. …… ii. Not known iii. Not applicable
56.	Who prescribes the antibiotics for your milking cows?	i. Myself ii. Veterinarian/Livestock officer (LFO) iii. Another farmer iv. Herd caretaker v. Other (Specify)……... ……
57.	Who administers the antibiotics for your milking cows?	i. Myself ii. Veterinarian/Livestock field officer (LFO) iii. Another farmer iv. Herd caretaker v. Other (Specify)…..... ……
58.	If not a Veterinarian or Livestock Field Officer, Why?	i. Unavailable veterinary services ii. Unaffordable veterinary services iii. Knowledge/Experience of disease symptoms iv. Other (Specify)
59.	Do you use laboratory-based results to guide yourself on the type of antibiotic to use?	i. Yes (Always) ii. No (Rarely) iii. Never
60.	How frequently do you use antibiotics on your cattle?	i. More frequently (at least once a week) ii. Frequently (at least once in 2 to 4 weeks) iii. Rarely (at least once in 1 to 2 months) iv. Very rare (at least once in 3months) v. Never used
61.	For what purpose do you use antimicrobials for your milking cows?	i. Growth promotion ii. Therapeutics iii. Prophylaxis iv. Others (Specify)……... ……
62.	Do you observe the antibiotic	i. Yes

	withdrawal period for your milking cows?	ii. No
63.	How do you know the antibiotic withdrawal period?	i. Instructions on the label ii. Instructions from Veterinarian/ Livestock field officer (LFO) iii. Instructions from drug seller iv. Other (Specify)…… ……
64.	Have you ever received any training on production or biosecurity?	
65.	Identify any sampled milking cow that is on treatment or has received treatment with antibiotics in less than 2 weeks.	Specify here……..... …… …… ……...
66.	Date of commencing or terminating treatment	i. Starting date: …….. ii. Ending date: ……..…… iii. Not remember
67.	**For official use:** Identify the obtained samples from the household by their assigned numbers.	**Specify the assigned numbers**.

Appendix 7: Sample Collection Form (Kiswahili Version)

(*Tumia fomu moja kwa kila kaya*)

S/N		
1.	Jina la kaya	
2.	Eneo (GPS-Coordinates)	
3.	Tarehe ya mahojiano	
4.	Mkoa/Wilaya/Kata	Mwanza/Magu/…….....
5.	Mawasiliano ya mhojiwa	
6.	Umri wa mhojiwa	
7.	Jinsi ya mhojiwa	i. Kike (Ke) ii. Kiume (Me)
8.	Kiwango cha elimu cha mhojiwa	i. Hajasoma ii. Elimu ya msingi iii. Elimu ya sekondari iv. Chuo
9.	Nini jukumu lako katika shamba?	i. Mmiliki ii. Muangalizi/Mchungaji wa mifugo iii. Meneja mwajiriwa iv. Mfanyakazi wa kawaida v. Nyingine (Orodhesha)…… ……....
10.	Kwa muda gani sasa umekuwa ukifuga/ukiangalia ng'ombe?	
11.	Kuna jumla ya ng'ombe wangapi shambani/nyumbani?	
12.	Ni aina gani ya ng'ombe unaofuga?	i. Kisasa ii. Kienyeji iii. Chotara iv. Aina tofauti (Orodhesha)…… ……..
13.	Unawafuga kwa ajili ya matumizi gani?	i. Uzalishaji wa maziwa ii. Uzalishaji wa nyama iii. Uzalishaji wa maziwa na nyama (i&ii) iv. Nyingine (Orodhesha)……. ……...
14.	Kati ya ng'ombe wote, wangapi ni wa maziwa/kukamua?	
15.	Unatumia mfumo gani katika ufugaji?	i. Unawaacha wakale majani kwenye malisho ya jumuiya ii. Zote kuwaletea majani na kuwaachia wakale kwenye malisho ya jumuiya

		iii. Unawafungia ndani na kuwaletea majani
16.	Unawalisha/unawachunga ng'ombe wako wapi?	i. Mashamba ya malisho ya jamii ii. Mashamba ya malisho ya binafsi iii. Unawaletea majani nyumbani iv. Nyingine (Orodhesha)……. ……..
17.	Unawapa ng'ombe wako chakula kingine tofauti na majani?	i. Ndio (Orodhesha) ii. Hapana Orodhesha ……. ……...
18.	Unatoa wapi maji ya kunywa kwa ajili ya ng'ombe?	
19.	Unafuga mifugo/wanayama wengine tofauti na ng'ombe?	i. Ndio (Orodhesha) ii. Hapana Orodhesha……. …….....
20.	Vipi hali ya usafi shambani? (Tazama)	i. Nzuri (kavu, bila kinyesi kutapakaa) ii. Mbaya (unyevu kupitiliza, tope, kinyesi kutapakaa)
21.	Unakamua wapi ng'ombe wako? (Tazama)	i. Eneo maalum kwa ajili ya kukamulia ii. Kwenye boma/banda iii. Popote tu
22.	Ni aina gani ya sakafu ipo katika eneo la kukamulia? (Tazama)	i. Mchanga ii. Zege
23.	Unasafisha eneo la kukamulia? Mara ngapi kwa wiki? Taja	i. Ndio, kila mara ii. Ndio, kwa nadra Orodhesha…..... …….
24.	Vipi hali ya usafi katika eneo la kukamulia? (Tazama)	i. Nzuri (kavu bila kinyesi kutapakaa) ii. Mbaya (unyevu kupitiliza, tope, kinyesi kutapakaa)
25.	Unakamua ng'ombe wangapi kwa siku? (Rejea siku husika)	
26.	Unatumia mbinu gani kukamua? (Tazama)	i. Mashine ya kukamulia ii. Kukamua kwa mikono
27.	Kama ni kukamua kwa mikono, ni wakumuaji wangapi hukamua? (Rejea siku husika)	
28.	Unatumia nini kunawa mikono kabla ya kukamua? (Tazama)	i. Maji tu ii. Maji na sabuni iii. Sinawi mikono
29.	Unatumia glavu wakati wa kukamua? (Tazama)	i. Ndio ii. Hapana

30.	Je, viwele vya ng'ombe huoshwa kabla ya kukamuliwa? (Tazama)	i. Ndio ii. Hapana
31.	Kama ni ndio, unatumia nini? (Tazama)	i. Maji tu ii. Maji ya uvuguvugu iii. Haoshi kabisa
32.	Je, viwele vya ng'ombe hukaushwa kabla ya ng'ombe kukamuliwa? (Tazama)	i. Ndio ii. Hapana
33.	Unatumia taulo tofauti kukausha viwele vya ng'ombe tofauti? (Tazama)	i. Ndio ii. Hapana
34.	Unatumia nini kusafisha vyombo vya kukamulia na kuhifadhia maziwa?	i. Maji ya baridi tu ii. Maji ya uvuguvugu tu iii. Maji ya moto tu iv. Maji na dawa ya kuulia vijidudu
35.	Unatumia aina gani ya vyombo vya kukamulia na kuhifadhia maziwa? (Tazama)	i. Kibuyu ii. Vyombo vya plastiki iii. Chupa za kioo iv. Vyombo vya metaliki v. Aina tofauti (Orodhesha)…… ……..
36.	Unahifadhi maziwa kwa muda gani kabla ya kutumia/kupeleka sokoni?	
37.	Maziwa yanayokamuliwa ni kwa ajili ya matumizi gani?	i. Matumizi ya nyumbani ii. Kusambaza/kuuza sokoni iii. Yote (i & ii) iv. Matumizi mengine (Orodhesha)……. ……..
38.	Unasambaza/unauza maziwa ya aina gani?	i. Maziwa freshi/mabichi ii. Maziwa mgando iii. Yote (i & ii)
39.	Unasambaza maziwa yakiwa katika hali gani?	i. Yasiyochemshwa/Bila kuchemshwa ii. Yaliyochemshwa iii. Yote (i & ii)
40.	Unachemsha maziwa kabla ya kugandisha?	i. Ndio ii. Hapana
41.	Unakunywa maziwa yakiwa katika hali gani?	i. Yasiyochemshwa/Bila kuchemshwa ii. Yaliyochemshwa iii. Yote (i & ii)
42.	Unahifadhi vipi maziwa ya ziada?	i. Joto la chumba ii. Jokofu/Friji
43.	Unafanya matibabu yoyote ya kiwele baada ya kukamua?	i. Ndio ii. Hapana

	(Kuzamisha kwenye dawa ya kuulia vijidudu)	
44.	Umeshawahi kusikia kuhusu ugonjwa/ maambukizi ya kiwele?	
45.	Unaweza kutambua ng'ombe mwenye ugonjwa/maambukizi ya kiwele?	i. Ndio ii. Hapana (**Nenda swali la 50**)
46.	Kama ni ndio, unamtambuaje?	i. Mabadiliko katika maziwa (muonekano & uthabiti) ii. Mabadiliko katika kiwele (ukubwa, joto, uthabiti) iii. Nyingine (Orodhesha)……. ……...
47.	Ukiachilia mbali mabadiliko katika kiwele na maziwa, je kuna njia zozote nyingine hutumia kutambua maambukizi ya/ ugonjwa wa kiwele?	i. Ndio ii. Hapana Ziorodheshe…… ……..
48.	Kama ni hapana, je ni nani hukusaidia kutambua ugonjwa/maambukizi ya kiwele kwa ng'ombe wako?	Orodhesha…....…….…. ……...
49.	Je ng'ombe wenye ugonjwa/ maambukizi ya kiwele hukamuliwa?	i. Ndio ii. Hapana
50.	Ng'ombe hawa wenye ugonjwa/maambukizi hukamuliwa muda gani?	i. Kabla ya ng'ombe wasio na ugonjwa/maambukizi ya kiwele ii. Baada ya ng'ombe wasio na ugonjwa/ maambukizi ya kiwele iii. Yoyote (Bila uzingatiaji wowote)
51.	Unafanyia nini maziwa yaliyopatikana kutoka kwa ng'ombe wenye ugonjwa/ maambukizi ya kiwele?	i. Mwaga ii. Matumizi ya nyumbani iii. Kusambaza/kuuza sokoni iv. Kunyweshwa ndama v. Mengine (Orodhesha)……. ……..
52.	Huwa mnawachunguza ng'ombe kama wana maambukizi ya kiwele?	i. Ndio (Mara ngapi? mwezi, mwezi n.k) ii. Hapana Orodhesha…… ……..
53.	Nani hufanya uchunguzi wa ng'ombe kama wana maambukizi ya kiwele?	i. Mmiliki ii. Daktari wa mifugo/ Afisa mifugo iii. Mfugaji mwingine

		iv. Muangalizi wa mifugo v. Mwingine (Orodhesha)…… ……...
54.	Umewatibu wanyama wako hivi karibuni? Kama ni ndio kipindi gani kilichopita?	i. Ndio ii. Hapana Orodhesha…… ……..
55.	Ulitumia dawa gani? Orodhesha dawa ulizotumia (Kama kuna ulazima, omba kuona dawa husika)	i. Orodhesha a. …… b. …… c. …… ii. Sijui
56.	Nani huwaandikia dawa za kutumia ng'ombe wako?	i. Mmiliki ii. Daktari wa mifugo/ Afisa mifugo iii. Mfugaji mwingine iv. Muangalizi wa mifugo v. Mwingine (Orodhesha)……..…..... ……...
57.	Nani huwapa dawa/ antibiotiki ng'ombe wako?	i. Mmiliki ii. Daktari wa mifugo/ Bwana mifugo iii. Mfugaji mwingine iv. Muangalizi wa mifugo v. Mwingine (Orodhesha)……..... ……...
58.	Kama ni mmiliki, ni kwanini?	i. Kutokuwepo kwa huduma za mifugo ii. Kushindwa kumudu huduma za mifugo iii. Maarifa/ ujuzi kuhusu dalili za magonjwa iv. Nyingine (Orodhesha)…….…… ……...
59.	Je, unatumia majibu ya vipimo vya maabara kukuongoza katika kuchagua aina ya dawa/antibiotiki ya kutumia?	i. Ndio (Kila mara) ii. Hapana (Kwa nadra) iii. Sijawahi
60.	Ni mara ngapi hutumia dawa/antibiotiki kwa ng'ombe wako?	i. Mara kwa mara (walau mara moja kwa wiki) ii. Mara kwa mara (walau mara moja kwa wiki mbili mpaka nne) iii. Mara chache (walau mara moja kwa mwezi mpaka miezi miwili) iv. Mara chache sana (walau mara moja kwa miezi mitatu) v. Sijawahi kutumia
61.	Unatumia antibiotiki kwa ajili ya…?	i. Kuchangia katika ukuaji ii. Matibabu

		iii. Kinga Nyingine (Orodhesha)……..…… ……..
62.	Unazingatia kipindi cha dawa kukaa mwilini?	i. Ndio ii. Hapana
63.	Unatambuaje kipindi cha dawa kukaa mwilini?	i. Maelekezo kwenye kipeperushi cha dawa ii. Mtaalamu wa mifugo iii. Maelekezo kutoka kwa muuzaji wa dawa iv. Nyingine (Orodhesha)……. …….
64.	Ulishawahi kupata mafundisho yoyote kuhusu uzalishaji au kinga ya bayolojia?	
65.	Orodhesha ng'ombe aliyechukuliwa sampuli na amepata matibabu karibuni (ndani ya wiki mbili).	Orodhesha hapa…… ……. …….
66.	Tarehe ya kuanza au kumaliza dozi	i. Tarehe ya kuanza: ……. ii. Tarehe ya kumaliza: ……. iii. Sikumbuki
67.	**Kwa matumizi ya kiofisi:** Andika namba ya utambulisho kwa kila sampuli iliyochukuliwa hapa	**Orodhesha hapa:**

Appendix 8: Laboratory Procedures and Field Pictures

Figure A8.1: Media Preparation Procedures by Researcher in the Laboratory: (A) Weighing, (B) Dilution and Proper Mixing in Distilled Water, (C) (B) Autoclaving, (D) Pouring and Cooling into Petri Dishes.

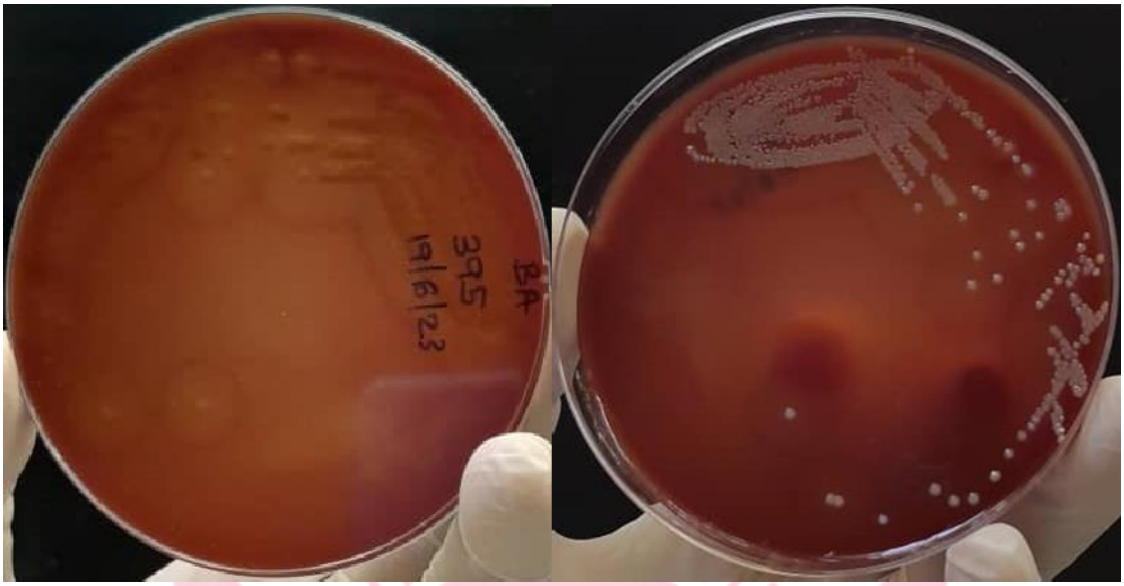


Figure A8.2: Bacteria Culture Results Showing Staphylococci Isolates: (A) Beta (β) Hemolysis, and (B) Golden Yellow Colonies Growth on Blood Agar Media

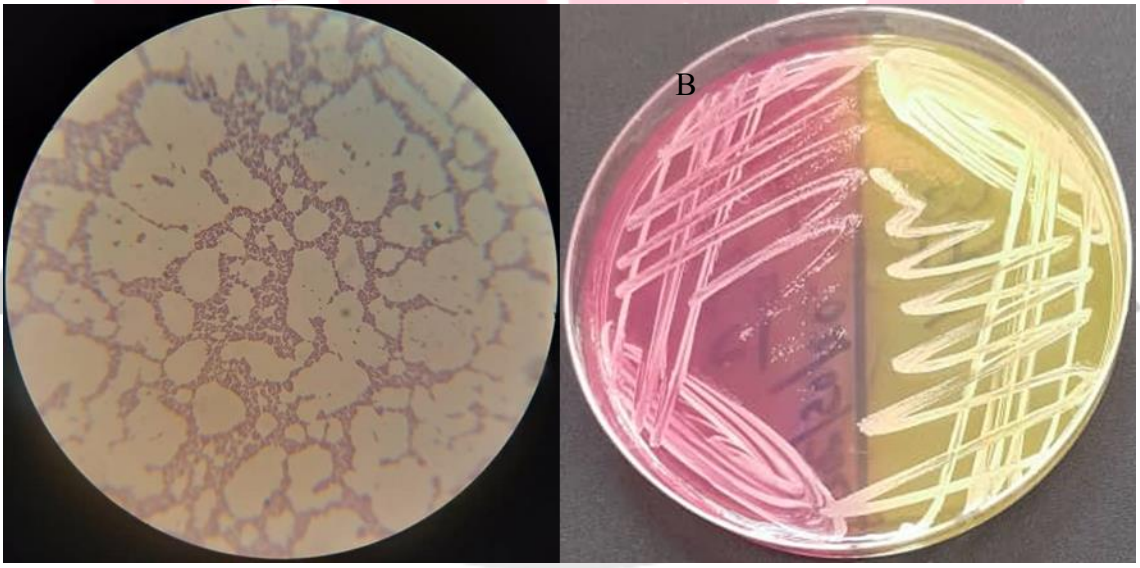


Figure A8.3: Gram Staining and Bacteria Subculture Results Indicating Presence of Staphylococci Isolates: (A) Microscopic View of Gram-Positive Cocci in Clusters, (B) Non-Mannitol Fermenter (Pink), and (C) Mannitol Fermenter (Yellow)

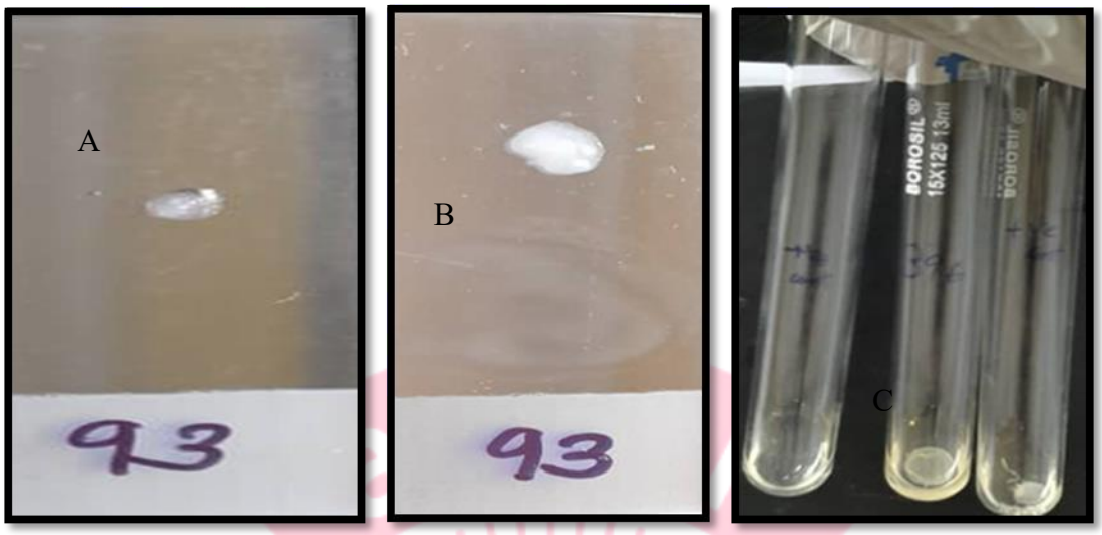


Figure A8.4: Biochemical Test Results for Staphylococcal Species Isolates: (A) Catalase Test (Bubble Formation), (B) Slide Coagulase Test (Clot Formation), and (C) Tube Coagulase Test (Gel Formation)

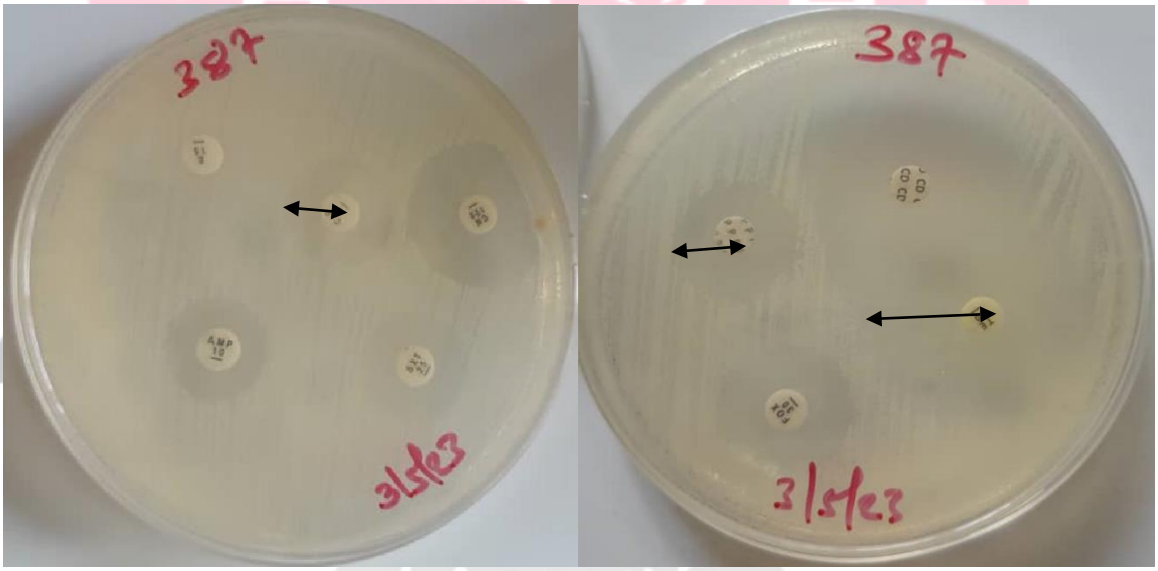


Figure A8.5: Antimicrobial Susceptibility Test (AST) Results of *Staphylococcus aureus* Isolates: (S) Susceptible, (I) Intermediate, and (R) Resistant Categories to Antibiotic Discs on Muller Hinton Agar as per (Clinical and Laboratory Standards Institute, 2022)

Figure A8.6: Indigenous Tanzanian Short-Horn Zebu (Tshz) Extensively Reared: (A) Soil/Grass Floor Inside and Outside Pen, (B) Hand Milking Practice Anywhere, (C) Mixed Milking Utensils (Calabash & Plastic Containers), and (D) Residual Suckling Calf, at Kitongo Sima Ward, Magu

Figure A8.7: Exotic Friesian Cows Kept Under Intensive Zero-Grazing Rearing: (A) Dry -Concrete Floor (B) Milking Parlor, at Kongolo Ward, Magu

Appendix 9: Generalized Linear Mixed Model (GLMM) Assessment Results

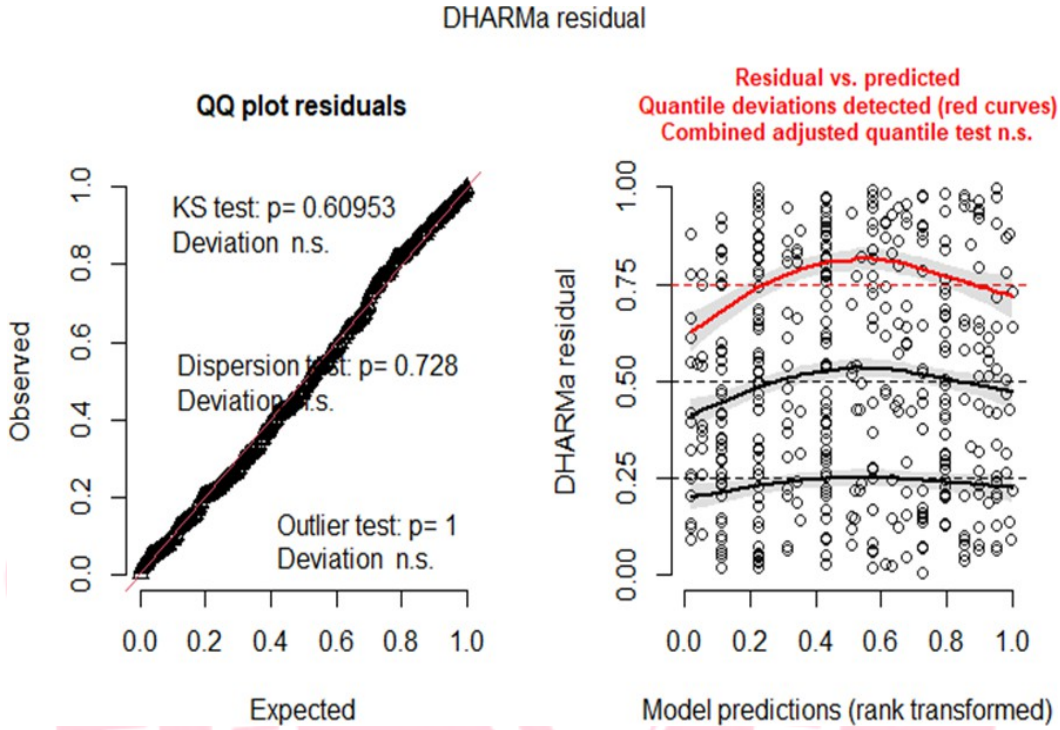
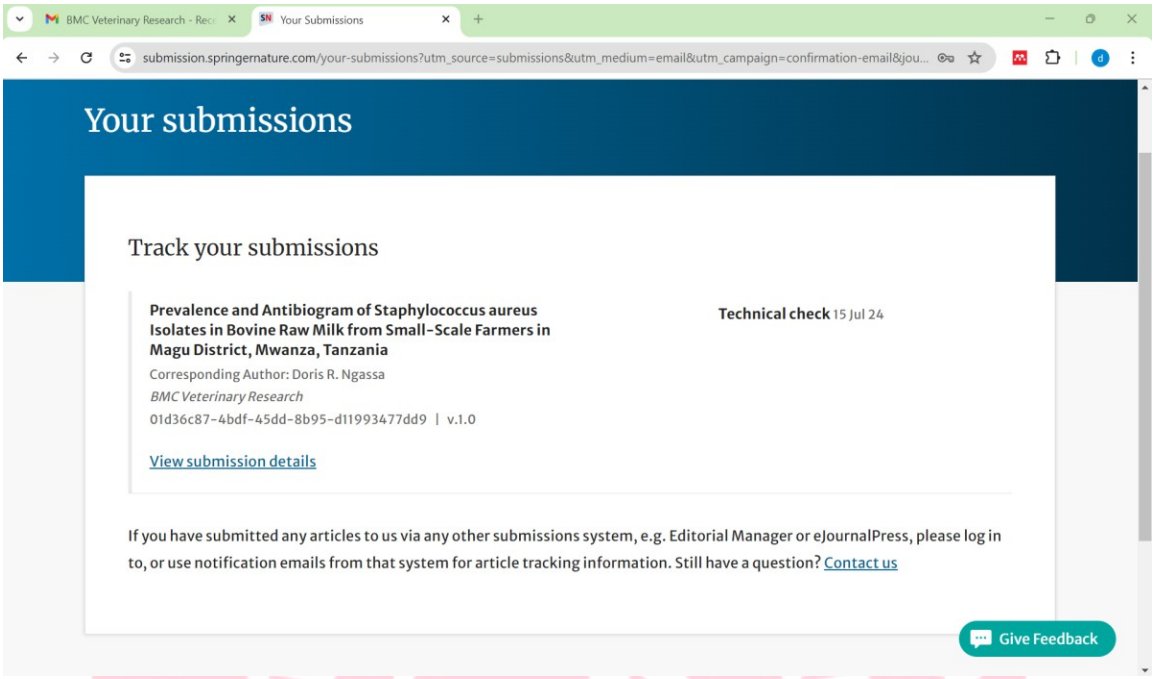
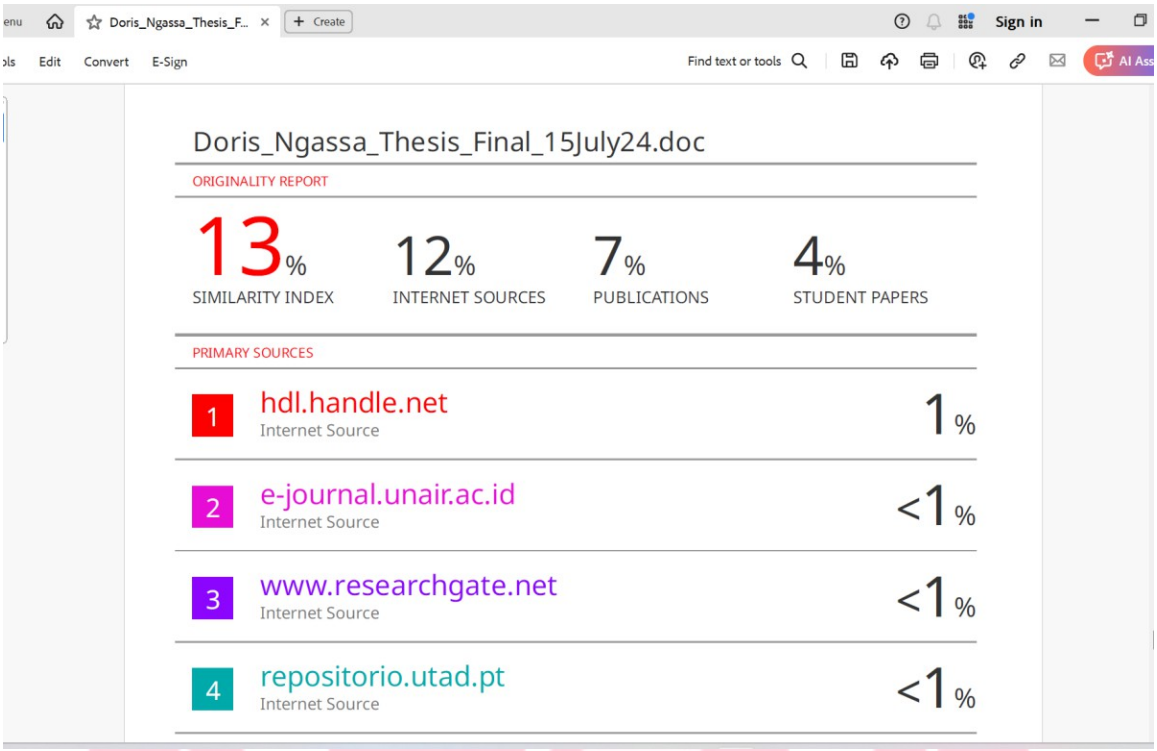


Figure A9: The Generalized Linear Mixed Model (GLMM) Assessment Results

Appendix 10: Evidence of Publication



Appendix 11: Percentage Plagiarism



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