Thesis Ref. No___________

ADDIS ABABA UNIVERSITY
COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE
DEPARTMENT OF VETERINARY MICROBIOLOGY, PARASITOLOGYY AND POULTRY HEALTH.

**MASTER OF SCIENCE PROGRAMME IN ONE HEALTH.**

# ASSESSMENT OF *STAPHYLOCOCCUS AUREUS* AND METHICILLIN-RESISTANT *S. AUREUS* (MRSA) IN DAIRY FARMS OF SHEGER CITY, OROMIA, ETHIOPIA: A ONE HEALTH APPROACH

**Msc THESIS**

BY

MAKIDA ELIAS

**JUNE, 2025**

**BISHOFTU, ETHIOPIA**

# ASSESSMENT OF *STAPHYLOCOCCUS AUREUS* AND METHICILLIN-RESISTANT *S. AUREUS* (MRSA) IN DAIRY FARMS OF SHEGER CITY, OROMIA, ETHIOPIA: A ONE HEALTH APPROACH

***A thesis submitted to the school of graduate studies of Addis Ababa University in partial fulfilment of the requirements for the degree of Master of Science In One Health.***

**BY**

**MAKIDA ELIAS**

**JUNE, 2025**

**BISHOFTU, ETHIOPIA**

## ADDIS ABABA UNIVERSITY

## COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

DEPARTMENT OF VETERINARY MICROBIOLOGY, PARASITOLOGYY AND POULTRY HEALTH.

The thesis entitled "Assessment of *Staphylococcus aureus* and Methicillin-Resistant *S. aureus* (MRSA) in Dairy Farms of Sheger City, Oromia, Ethiopia: A One Health approach" was prepared by Makida Elias under the supervision of the MVSc research advisors. As the advisors, we have carefully read and assessed the thesis, and we recommend that it be approved as fulfilling the thesis requirement for the Master of Science in One Health degree.

Submitted by**: Makida Elias** ______________ 09/06/2024

Name of Student Signature Date

Approved for submittal to a thesis assessment committee

**1. Dr.Fufa Abunna** ( DVM, MSc, PhD) __________________ 09/06/2025

Advisor Signature Date

**2. Dr.Beteley Tekola** (MSc, PhD) ___________________09/06/2025

Co- Advisor Signature Date

**3. Hailegebriel Bedada** (DVM, MSc, Asso.prof) _________________09/06/2025

Department Head of VMPPH Signature Date

## ADDIS ABABA UNIVERSITY

## COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

DEPARTMENT OF VETERINARY MICROBIOLOGY, PARASITOLOGYY AND POULTRY HEALTH

As members of the examining board for MVSc open defense, we have carefully read and reviewed Makida Elias thesis, titled **"Assessment of *Staphylococcus aureus* and Methicillin-Resistant *S. aureus* (MRSA) in Dairy Farms of Sheger City, Oromia, Ethiopia: A One Health approach"** Based on our careful examination of the thesis, we accepted that it be approved as satisfying the requirement for the thesis of a Master of Science in One Health degree.

___________________________ _____________ _____________

External Examiner Signature Date

___________________________ ______________ _____________

Internal Examiner Signature Date

___________________________ ______________ ______________

Chairperson Signature Date

# *DEDICATION*

*This thesis is dedicated to my beloved daughter, Seena Motuma, whose innocent smile, love, and presence have been my greatest source of strength and inspiration throughout this journey.*

*May this work be a symbol of what perseverance, education and love can achieve.*

## STATEMENT OF THE AUTHOR

I, Makida Elias, hereby state that I am the writer of this thesis and have properly credited all the sources of information used. The Addis Ababa University College of Veterinary Medicine and Agriculture has accepted this thesis as partial fulfilment of the requirements for an MSc degree. This thesis is now housed in the university or college library and will be accessible to borrowers according to the library's policies.

I declare that this thesis is not being submitted to or accepted for credit towards a degree, diploma, or certificate from any other academic institution.

Short quotations from this thesis may be used without further permission, provided the source is properly cited. If the department head or college dean believes this work will promote research, they may grant permission to quote from it extensively or to reproduce it in whole or in part. However, permission from the author is required for all other situations.

Name: Makida Elias      Signature: __________

College of Veterinary Medicine and Agriculture, Bishoftu

Date of Submission: June/09/2025

**TABLE OF CONTENTS PAGE**

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF ANNEXES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| AMR | Antimicrobial resistance |
| ARB | Antimicrobial-resistant bacteria |
| ARGs | Antimicrobial resistance genes |
| CLSI | Clinacal and laboratory standard institute |
| FAO | Food And Agricultural Organization |
| IMs | Intramammary infection |
| LMICs | low- and middle-income countries |
| MDR | Multi Drug Resistance |
| MecA | Methicillin Resistance Gene A |
| MecE | Methicillin resistance Gene C |
| MGE | Mobile genetic elements |
| MRSA | Methicillin resistance *staphylococcus aureus* |
| MSSA | Methicillin susceptible *staphylococcus aureus* |
| PBP | Penicillin-Binding protein |
| PCR | Polymerase chain reaction |
| SCCmec | Staphylococcal CassettenChromosome mec |
| VFG | Virulence factor gene |
| WHO | World Health Organization |
| WOAH | World Organisation for animal health |

# ACKNOWLEDGEMENT

First and foremost, I am deeply grateful to the Almighty Allah for enabling me to do my work effortly.

My sincere gratitude goes to my advisors Dr. Fufa Abunna and Dr. Beteley Tekola for their invaluable directions, support, and insightful comments on the scientific and editorial aspects of this study. I greatly appreciate the materials they provided and their continuous supervision all along the course of the investigation.

I would also like to extend my special thanks to employees at the Animal Health Institute, Mr. Abdi Ahmed, Mr. Abebe Olani, Mrs. Mekdes Tamiru and especially to Dr.Tesfaye Rufael for their valuable advice, encouragement and support.

I am grateful to Addis Ababa University, ACEWM and One Health NORHED Project (Norway) for giving me the opportunity to enroll in this postgraduate program and for providing me with the necessary funding.

Last but not least a great appreciation goes to my collegue Dr.Gerade Abdujamil for his support and encouragement through out my work.

## ABSTRACT

Antimicrobial resistance (AMR), particularly involving *S. aureus* and methicillin-resistant *S. aureus* (MRSA), presents a growing public health concern in low- and middle-income countries such as Ethiopia. A cross-sectional study was carried out to investigate the occurrence, antimicrobial resistance profiles and environmental risk factors of *S. aureus* and MRSA in dairy farms in Sheger City, Oromia Region, using a One Health approach. A total of 312 sample comprising 212 udder milk, 40 hand swabs, and 60 environmental swab were collected from December 2024 to May 2025. Bacterial isolation was performed using conventional culture methods and confirmed by MALDI-TOF MS and antimicrobial susceptibility was tested using the disk diffusion method according to CLSI, (2022) guidelines. Out of the 312 samples, 65 (20.83%) were found to be positive for *S. aureus*, and 31 (9.9%) were identified as MRSA. The prevalence of *S. aureus* was 23.11% in udder milk, 27.5% in hand swabs, and 8.3% in environmental swabs. MRSA was detected only in udder milk samples, with a prevalence of 14.62%. All isolates exhibited multidrug resistance (MDR), with 100% resistance to amoxicillin, penicillin, tetracycline, clindamycin, and cloxacillin. High susceptibility was observed for ciprofloxacin, gentamicin, and norfloxacin according to Interpretive categories and zone diameter breakpoint (CLSI, 2022). Significant associations were found between *S. aureus* prevalence and poor hygiene practices, including inadequate handwashing, udder cleaning, and substandard drainage systems.The detection of *S. aureus* and MRSA particularly the exclusive occurrence of MRSA in milk samples raises serious concerns regarding milk safety and the potential for zoonotic transmission. The widespread multidrug resistance among isolates further emphasizes the need for targeted interventions. These findings call for urgent improvements in farm hygiene, promotion of prudent antimicrobial use and implementation of integrated AMR surveillance systems to mitigate the risks to animal and public health.

**Keywords**: *Antimicrobial resistance, Dairy farm, MRSA, One health, S .aureus, Sheger city*

# 1. INTRODUCTION

## 1.1. Background

Antimicrobial resistance (AMR) is a major global public health concern, causing serious challenges in sub-Saharan African countries (Elton *et al*., 2020; Gebretekle *et al*., 2020). Increasing rates of AMR reduce the effectiveness of antibiotics, leading to increased morbidity, mortality and healthcare costs associated with bacterial infections (Global Action Plan on Antimicrobial Resistance, 2015; IACG Report, 2019). AMR is largely inhanced by the misuse of antimicrobials, a problem especially severe in low- and middle-income countries (LMICs) due to constrained diagnostic infrastructure, poor AMR monitoring, and weak Infection Prevention Control (IPC) strategies (Escher *et al*., 2021; Gebretekle *et al*., 2018, 2020). These constraints often result in empiric antibiotic prescribing based on syndromic approaches rather than microbiological evidence, further accelerating resistance selection.

In addition to human medicine, the widespread use of antimicrobials in livestock sector highly contributes to the emergence and dissemination of antimicrobial-resistant bacteria (ARBs) (WHO, 2014). infact, over 11.6 million kg of antimicrobials were used in U.S. animal agriculture in 2018 alone (FDA, 2021). Such large usage creates a significant reservoir of antimicrobial resistance genes (ARGs) and pathogens that are resistant in both animals and their surrounding environment (Kimera *et al*., 2020). These reservoirs can contaminate soil, water, and the food chain, thereby increasing the risk of transmission to humans through contact or consumption of animal food derived products (Hendriksen *et al*., 2019). Additionally, virulence factor genes (VFGs) and mobile genetic elements (MGEs) enhances the persistence, dissemination, and pathogenicity of antimicrobial-resistant organisms. (de Nies *et al*., 2021).

Among priority pathogens, *staphylococcus aureus* is notable for its ability to rapidly developmental of antimicrobial resistance genes including resistance to methicillin. Methicillin-resistant *Staphylococcus. aureus* (MRSA) is particularly concerning as it exhibits resistance to all β-lactam antibiotics,(beta-lactam rings) thereby significantly inhabiting or restricting treatment options (McMillan *et al*., 2016). Globally, there are three primary types of MRSA that have emerged: Healthcare-associated (HA-MRSA), community-associated (CA-MRSA), and more recently, livestock-associated MRSA (LA-MRSA). LA-MRSA is particularly alarming because of its zoonotic potential and multidrug resistance, presenting health risks to both livestock and individuals in close contact with them (Chukwunonso *et al*., 2018; Carfora *et al*., 2016).

LA-MRSA has been detected in various animal species, animal derived food products and humans, highlighting its high ability to persist and spread within animal populations (Larsen *et al*., 2016; Fetsch *et al*., 2021; Silva *et al*., 2022; Graveland *et al*., 2011). Importantly, the ability to spread from animals to humans, importantly among farm workers and through contaminated environments, emphasizes the need for surveillance using a One Health framework an approach that confess the interconnectedness of human, animal and environmental health (Chen and Wu, 2021; Cuny *et al*., 2015).

In disk diffusion testing, cefoxitin is regarded as the most applicable surrogate marker for MRSA, owing to its high sensitivity, specificity and its powerful ability to enhance the *mecA* gene. Nevertheless, polymerase chain reaction (PCR) detection of the *mecA* gene remains the gold standard for validating MRSA isolates (Anand *et al*., 2009; Sathyamoorthy *et al*., 2022).

Many of the drugs used to treat human disease are also commonly employed in veterinary medicine (Vestergaard *et al*., 2019). Macrolides ,tetracyclines, aminoglycosides, beta-lactams, fluoroquinolones, sulfonamides and rifamycins are commonly employed for *staphylococcal* infection control in both humans and animals (Tong *et al*., 2015). Moreover, MRSA strains have also demonstrated resistance to many antibiotics commonly used for the treatment of bacterial infections (Ruchi *et al*., 2016). The One Health approach, emphasizing the interconnectedness of human-animal-environment health, has been highlighted as crucial for detecting disease transmission and implementing more effective control measures (Guardabassi *et al*., 2020). Effective and targeted strategies are still required to reduce the burden of infectious diseases and prevent the impact of antimicrobial resistance in both livestock and humans. Implementing a One Health approach is key to improving animal well-being, maintaining food safety, and preserving public health. A key concern is the risk of MRSA transmission between animals and humans, along with host-switching events that can give rise to novel pathogenic or drug-resistant strains (Fitzgerald, 2012; Spoor *et al*., 2013)**.**

MRSA occurred after the use of methicillin to clinical practice. The biggest challenge occurred by MRSA globally is decreased susceptibility to other antibiotics including the beta-lactam drugs. At this time MRSA rapidly spread to entire human community and in livestock. However,colonization rates are notably high in certain groups, such as children, hospitalized elderly patients and young women. Factors such as overcrowding, sharing personal items, poor hygiene, living in densely populated areas, and intravenous drug use further contribute to the spread of the infection (Chukwunonso *et al*., 2018**).**

Several studies have been carried out in Ethiopia to investigate MRSA infections in different regions of the country,(Gebremeskel *et al*., 2022; Tefera *et al*., 2021; Girmay *et al*., 2020; Mekuriya *et al*., 2022; Tibebu *et al*., 2021; Desta *et al*., 2022; Asmelash *et al*., 2016; Tassew *et al*., 2018). The prevalence of MRSA has been reported to be higher in America compared to Europe. Different studies reported that prevalence rates of greater than 70% in South Korea and Vietnam, and less than 50% in Portugal, Greece and Italy (Chukwunonso *et al*., 2018**).**

In Egypt, a study shows prevalence of *S. aureus* was 17.2%. In another study, 70–73% of *S. aureus* strains isolated from various foods were resistant to β-lactam such as Penicillin and Ampicillin (Beyene *et al*., 2017). In Ethiopia, overall prevalence of *S. aureus* with different study showed that 15.3% originating from raw cows' milk, 25% from swabs of milkers' hand, 20% from swabs of milking bucket and 10% from swabs of drying towel.(Regasa *et al*., 2019)

In study done in South Africa the prevalence of MRSA was 5.7–7% in commercial farms. In other African country study, the prevalence of MRSA were higher in Ethiopia (60.3%) in Nigeria 28.57%, in Morocco 15% and low prevalence in Kenya 7.8% were recorded (Lozano *et al*., 2016). However, information on prevalence of *S. aureus* and MRSA in udder milk, milkers hand and in farm environment and environmental risk factors such as hygiene practices and waste management that facilitate the occurrence and spread of MRSA in dairy farms has not been extensively studied in Ethiopia and specifically in Sheger city.

## 1.2. Problem Statement

The emergence and wide spread of antimicrobial resistant bacteria, particularly methicillin-resistant *S.aureus* (MRSA), have become a significant public health concern globally (Elton *et al*., 2020; Gebretekle *et al*., 2020). In Ethiopia, where the dairy sector is rapidly expanding, the crisis posed by *S. aureus* and MRSA in dairy farms remains under explored.The misuse of antimicrobials in livestock, in addition with poor hygiene, inadequate waste management and weak infection prevention measures, may render the occurrence and dissemination of these resistant pathogens. Despite their potential to impact animal health, farm workers, milk consumers and the environment, few studies have examined the microbiological profiles, resistance patterns and environmental risk factors of *S. aureus* and MRSA in Ethiopian dairy farms, (Muluneh *et al*., 2023; Reta and Alemayehu, 2020; Beyene *et al*., 2021).

In particular, Sheger City in Oromia, a major area for dairy production, lacks data on the prevalence and antimicrobial resistance of MRSA across different farm related sources, including udder milk, farm personnel and environmental surfaces. Moreover, little is known about how sanitation, hygiene practices and waste management influence the presence and spread of these pathogens. This knowledge gap hinders the development of effective interventions and policies grounded in a One Health approach to mitigate antimicrobial resistance risks in the livestock sector.

## 1.3. Research Questions

The general question of this research was:

- ✓ What is the prevalence of *S. aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) in dairy farms in Sheger City, Oromia, Ethiopia?
- ✓ What are the risk factors associated with the spread of *S. aureus* and MRSA in the dairy farming sector in Sheger City?

- ✓ How do antimicrobial susceptibility profiles of MRSA strains isolated from dairy farms compare across different sources (udder milk, farm personnel, environmental surfaces)?
- ✓ What sanitation and hygiene practices are currently implemented in dairy farms, and how do they influence the occurrence *of S. aureus* and MRSA?

## 1.4. Objective of the Study

The general objective of this research was:

To investigate the occurrence, antimicrobial resistance profiles and risk factors associated with *S. aureus* and methicillin-resistant *S. aureus* (MRSA) in dairy farms from a One Health perspective in Sheger city,oromia,Ethiopia.

The specific objectives of this research was:

- ➢ to isolate and identify *S. aureus* and MRSA from various sources on dairy farms, including udder milk, farm environmental and farm personnel.
- ➢ to evaluate the antimicrobial susceptibility profiles of isolated MRSA strains and
- ➢ to assess sanitation, hygiene and waste management practice contributing to occurrence *of S.aureus* and MRSA.

## 1.5. Significance of Study

The findings of this study will offer crucial insights into the epidemiology, antimicrobial resistance profiles and environmental and management-related risk factors associated with *S aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) in dairy farms within Sheger City, Ethiopia.

Understanding the occurrence and distribution of *S. aureus* and MRSA in the dairy farming environment is essential for several reasons.

- ✓ it provides baseline data that can inform national and regional AMR surveillance systems and policy frameworks.

- ✓ the study will help to identify specific farm practices and environmental conditions that may facilitate the transmission and persistence of these pathogens across human, animal and environmental interfaces an approach that is strongly aligned with the One Health paradigm.
- ✓ the research will generate evidence to inform the development of targeted interventions, including improved hygiene practices, judicious antibiotic use and enhanced biosecurity measures in dairy farms.
- ✓ the study will serve as a valuable resource for veterinarians, public health officials, policymakers and dairy farm managers by providing localized data on antimicrobial resistance patterns and critical control points in dairy production systems.
- ✓ It also contributes to the scientific literature on AMR in Ethiopia, where current data are sparse, especially in peri-urban dairy farming settings like Sheger City.

# 2. LITERATURE REVIEW

## 2.1. Epidemiology

### *2.1.1. Etiology*

*S. aureus* is a Gram-positive, cocci-shaped bacterium that typically appears in grape-like clusters. It is capable of growing in media containing up to 10% sodium chloride and forms distinctive golden-yellow colonies hence the name *aureus*, meaning "golden." This facultative anaerobe can thrive in a temperature range of 18°C to 40°C, making it adaptable to various environmental conditions. Identification tests include catalase positivity (for all pathogenic *Staphylococcus* species), coagulase positivity (specific to *S. aureus*), novobiocin sensitivity (to distinguish from *S. saprophyticus*) and mannitol fermentation positivity (to distinguish from *S. epidermidis*) (Rasigade and Vandenesch, 2014; Lowy, 1998). Methicillin-resistant *S. aureus* (MRSA) strains carry the *mecA* gene within the *staphylococcal* chromosomal cassette *mec* (SCCmec), which encodes PBP2a, a penicillin-binding protein with low affinity for β-lactam antibiotics. As a result, MRSA strains resist nearly all β-lactams, including methicillin, nafcillin, oxacillin, and cephalosporins (CDC, 2003; Rasigade *et al*., 2014).

### *2.1.2. Host Range*

*Staphylococcus* species are ubiquitous organisms distributed all over the world (Pal *et al*., 2021). The primary ecological niches of *S. aureus* are the nasal passages and skin of humans and other warm-blooded animals (Pal *et al*., 2020). It colonizes the upper respiratory, urogenital tracts, and gastrointestinal tracts of about 20% to 30% of humans (Becker *et al*., 2014). They inhabit the distal nasal passages, external nares, mucous membranes, teats, udder, and skin, especially near mucocutaneous borders such as the perineum, external genitalia of milking animals (Crosby *et al*., 2016). They act as the most crucial reservoir and source of contamination (Berge and Baars, 2020). Infections caused by *S. aureus* have been reported in humans as well as in various animal species, including poultry (Pal, 2007).

### *2.1.3. Transmission*

*S. aureus* can be transmitted among animals, among humans and bidirectionally between animals and humans, highlighting its zoonotic and anthropozoonotic potential. Transmission typically occurs through direct contact, particularly via the hands, with colonized or infected individuals or animals, as well as through contact with contaminated equipment, surfaces, or environmental materials (Pal, 2007). The most common transmission pathways include the transfer from an infected mammary gland to an uninfected gland via fomites, such as milking equipment, or the milker's hands, uncontrolled animal traffic between different farms and handling or eating food contaminated with *S. aureus*. Individuals or animals colonized or infected with *S. aureus* can release the bacterium from their nasal cavities and skin into the environment, implying that transmission through the air may be a possible infection route. Vectors such as the housefly (*Musca domestica*) have also been implicated in the transmission of *S.aureus*, serving as mechanical carriers that facilitate the spread of the pathogen between animals, humans and contaminated environments (Cuny *et al*., 2010; Catry *et al*., 2010).

### *2.1.4.Clinical manifestation.*

In dairy cattle, *S. aureus* is a leading cause of mastitis, both acute and chronic. Acute infections involve abnormal milk and visible inflammation, while chronic infections are often subclinical and harder to treat, leading to high somatic cell counts (SCC) and frequent culling (Watts, 1988; Bardiau *et al*., 2016; Piepers *et al*., 2009). Biofilm formation and intracellular persistence enable evasion of both immune response and antibiotic treatment (Bayles *et al*., 1998; Almeida *et al*., 1996). *S. aureus* also contributes to contagious mastitis and is able to cause foodborne illness through contaminated raw milk (Fisher *et al*., 2018; Johler *et al*., 2015).

### *2.1.5.Pathogenicity*

Bacterial pathogens have developed intricate mechanisms to sense, respond to, and adapt to the harsh conditions present in the mammalian host during infection. This capacity allows them to invade the host, establish themselves, and survive despite the host’s immune responses and antimicrobial treatments. *S. aureus* produces several enzymes that contribute to its virulence. One such enzyme is coagulase, which converts plasma fibrinogen to fibrin, allowing the bacteria to coat themselves and potentially evade phagocytosis. Additionally, the enzyme hyaluronidase breaks down hyaluronic acid in tissues, facilitating the spread of *S. aureus* within the host. The bacteria also produce DNase (deoxyribonuclease), which degrades DNA, as well as lipase, which digests lipids, and staphylokinase, which dissolves fibrin. This bacteria is also known to produce β-lactamases for drug resistance, esterase, elastase, and phospholipase enzymes that facilitate colonization and pathogenicity. Other virulence factors of S. *aureus* include leucocidin, which causes cytolytic destruction of phagocytes of some animal species and toxic shock syndrome toxins (TSST) which induces excessive lymphokine production, resulting in tissue damage (Quinn *et al*., 2011). Depending on the strain, *S. aureus* can secrete other toxins, which constitute the major virulence factors. These toxins can be categorized into three groups such as exfoliative toxins, superantigens and other toxins that act on cell membranes including alpha toxin, beta toxin, gamma toxin, delta toxin, and other bicomponent toxins, such as Panton-Valentine toxin or leucocidin (PVL) (Foster,2005;Kong, *et al*., 2016 ;Kashif *et al*., 2019). Protein A, which has a key role in the immune evasive strategies, is attached to staphylococcal peptidoglycan pentaglycine bridges by the transpeptidase sortase A.

### *2.1.6. Mechanism of Pathogenicity*

Although *S.aureus* is a common inhabitant of the skin and mucous membranes, any disruption of the skin barrier or colonization in individuals with weakened immune systems can create opportunities for this bacterium to invade and cause infection. The disease process can be mediated through two mechanisms; the production of toxins and the colonization that causes tissue invasion and destruction (Yilmaz and Aslantas 2017)

**Adhesion and colonization:** *S. aureus* can up-regulate a variety of virulence factors, enhancing its ability to adhere to and colonize the nasal passages, damage skin, and infect other surfaces, which can lead to serious bloodstream infections. A crucial component in this process is teichoic acid, a polymer present on the surface of the bacterium. Research by Weidenmaier *et al*. (2004) has shown that teichoic acid facilitates adhesion and colonization, significantly contributing to the pathogenicity of *S. aureus*.

**Invasion:** *S. aureus* disrupts the skin barrier through the secretion of exfoliative toxins and hemolysins, which create pores in skin cell membranes. Additionally, it produces various enzymes that damage tissue. This invasion is often triggered when the immune system is compromised, when there is a breach in the physical barrier, or during periods of localized inflammation (Otto, 2004).

**Evasion:***S. aureus* employs multiple strategies to evade the immune system. It secretes anti-opsonizing proteins, such as chemotaxis inhibitory protein, which impede phagocytosis by neutrophils. Additionally, Protein A on the bacterial surface has antiphagocytic properties. The bacterium produces Panton-Valentine leukocidin (PVL), which lyses leukocytes, and secretes superantigens like enterotoxin and TSST1. These superantigens disrupt the normal immune response by inducing strong, polyclonal stimulation and expansion of T cells, ultimately leading to the deletion or suppression of these cells into an anergic state, as noted by Haas *et al*. (2004). Moreover, PVL genes can be transmitted via bacteriophages, enhancing their spread between organisms (Jaggi *et al.,* 2007).

### *2.1.5. Predisposing/Risk Factors*

Factors influencing *S. aureus* infection include host immune status, antibiotic exposure, hospital presence, poor farm hygiene and contact with colonized animals. Notably, livestock-associated MRSA (LA-MRSA) ST398 has shown high prevalence in intensive farms and demonstrates resistance to multiple antibiotics (Lowy, 2003; Cuny *et al*., 2010; Van Belkum *et al*., 2009)

### *2.1.6. Zoonotic Transmission*

*S. aureus* is a common commensal bacterium found in humans and some animal species, including livestock and pets. Some strains like MRSA, have zoonotic potential meaning they can be transmitted between animals and humans. This livestock-associated MRSA (LA-MRSA) has been identified in pigs and can infect farmers and veterinarians who come into close contact with these animals (Mithu *et al*., 2024). This transmission can lead to infections in humans that are untreatable due to antibiotic resistance (Daniel *et al*., 2021a).

### *2.1.7. Environmental Persistence*

*S. aureus* is capable of surviving in diverse environmental conditions such as in manure, water and airborne particles.This environmental resilience allows it to spread beyond direct contact with infected animals or humans. For example, MRSA can stay in air for several days, enhancing indirect transmission through contaminated surfaces or aerosols (Mithu *et al*., 2024). The environmental existence of *S. aureus* can be worsened by some agricultural practices, like the routine use of antibiotics in livestock, which may collaborate to the emergence and survival of antibiotic resistant strains (Daniel *et al*., 2021b).

### *2.1.8. Public Health Implications*

MRSA poses significant threats to public health, with potential for severe infections in both clinical and community settings. The emergence of LA-MRSA increases the hardship of managing zoonotic infections, especially in LMICs like Ethiopia where integrated surveillance and control measures are constrained (Daniel *et al*., 2021a; Shibabaw *et al*., 2021).

### *2.1.9. Mechanisms of Antimicrobial Resistance*

One of the most agitating character of *S. aureus*, especially methicillin-resistant strains (MRSA), is their ability to resist multiple classes of antibiotics. This multidrug resistance is enhanced by various genetic and biochemical mechanisms, including the acquisition of resistance genes, alteration of target sites and the production of enzymes that inactivate antimicrobial agents. These mechanisms make the treatment of MRSA infections increasingly hard and contribute to its persistence in both animal and human populations (Chambers and DeLeo, 2009).

The hallmark of MRSA is the mecA gene, which encodes penicillin-binding protein 2a (PBP2a). This altered PBP has decreased affinity for β-lactam antibiotics, including methicillin, oxacillin and most cephalosporins, thereby allowing cell wall synthesis to continue even in the presence of these drugs (Lowy, 2003; Rasigade *et al*., 2014). The mecA gene is carried on a mobile genetic element known as the *staphylococcal* cassette chromosome mec (SCCmec). Various SCCmec types (I–XIII) have been described, differing in size, gene content and antibiotic resistance profiles. In livestock, SCCmec type IV and V are mostly associated with community and livestock-associated MRSA strains, which often show resistance to multiple antibiotics beyond β-lactams (Cuny *et al*., 2010).

In dairy farm environments, the development and spread of antimicrobial resistance may be increased by the reduntant use of antibiotics for mastitis treatment, the administration of subtherapeutic doses in feed and lack of hygiene and biosecurity practices. MRSA strains isolated from livestock and farm environments often show cross-resistance to commonly used antibiotics such as tetracycline, erythromycin and chloramphenicol, limiting therapeutic options and posing zoonotic risks (Mithu *et al*., 2024 ).

The environmental presence of resistance genes in water, soil and dust especially around livestock housing demonstrates the potential for gene transfer among bacteria in the microbiome. These environmental reservoirs act as amplifiers of resistance genes that can be transmitted to both animal and human microbiota through direct contact or via food products (Daniel *et al*., 2021b).

### *2.1.10. Treatment and Control of MRSA Infection*

The widespread use of antibiotics in both humans and animals has rehabilitated to the emergence of multidrug-resistant methicillin-resistant *S. aureus* (MDR-MRSA). Dwelling MDR-MRSA infections through a One Health approach necessitates co-ordinated efforts among public health professionals, epidemiologists,microbiologists and both veterinary and medical clinicians. Recently , MRSA strains posed resistance to the majority of commercially available antibiotics.Handling antibiotic resistance in both humans and animals needs ongoing investigation and surveillance of resistant strains, including routine screening and antimicrobial susceptibility testing (Disc diffusion method) (Bua *et al*., 2018).

The misuse of broad-spectrum antibiotics for treating MRSA infections should be strongly avoided. Instead, optimal treatment should be supported by the results of antibiotic susceptibility testing (Pitkälä *et al*., 2004). New antibiotics, such as telavancin and tedizolid, are regarded to be more effective in the treatment of MRSA infections (Gajdác and Albericio 2019). Additionally, other strategy involves identifying molecules that can restrict bacterial efflux mechanisms, thereby restoring the effectiveness of existing antibiotics (Usai *et al*., 2019). To prevent the spread of MRSA within the environment, hospitals and veterinary authorities must encourage comprehensive precautionary measures. These include strict adherence to general hygiene practices, standardized infection control procedures and routinely environmental disinfection (Burke and Rose, 2014).

## 2.2. Application of Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry in Microorganisms Identification

These days, laser desorption/ionization (LDI), electrospray ionization (ESI), surface-enhanced laser desorption/ionization (SELDI), and matrix-assisted laser desorption/ionization (MALDI) are the most widely utilized ionization techniques for studying chemical structures in biological systems. With the introduction of soft ionizationin the late 1980s, protein analysis rapidly advanced and transformed mass spectrometry.Complete bacterial cells may be distinguished utilizing MALDI in combination with a time of flight (TOF) analyzer, as demonstrated by the innovative application of mass spectrometry in microbiology in the late 1990s. Owing to its proven success, the MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry) system has advanced significantly and is now considered a valuable tool for the rapid and accurate microbiological characterization of bacteria, fungi, viruses, and even parasitic worms.(Torres-Sangiao *et al*., 2021).

For the quick, accurate, and economical characterization and identification of microorganisms, matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS) is a useful technique. In addition to being ideal for precise microbial identification at the genus and species levels, it also has the potential to be used for strain typing and identification. It employs distinct mass spectral fingerprints, which are the distinct signatures of every microorganism (Croxatto *et al*., 2012). Based on the cellular proteome, a representation of the organism's metabolic by products and gene products, the analytical method known as MALDI-TOF mass spectrometry examines a plethora of proteins, primarily ribosomal ones, found in microorganisms weighing between 2,000 and 20,000 Daltons. These proteins are ionised into charged molecules by the addition or removal of one or more protons, allowing for the determination of the mass-to-charge (m/z) ratio (Vrioni *et al*., 2018).

The sample to be examined is mixed with an energy-absorbing material called a "matrix."The trapped sample crystallizes as well once the matrix dries. Subsequently, the material is ionised by a laser beam, yielding single protonated ions. Following their acceleration at a specific potential and separation from one another, the time it takes for each ion to transit the length of the flight tube also known as the time of flight, or TOF is used to calculate the m/z ratio of these ions (Vrioni *et al*., 2018). The "Peptide Mass Fingerprint" (PMF) is a distinct mass spectrum created using the TOF data. Next, this PMF, which features peaks specific to genera and species that set them apart from other types of microorganisms, is contrasted with a database. A family, genus, and species level identification of the unknown organism can be done by comparing the PMF of the unknown microbial isolates to known microbial isolates that have been stored in the database (Nomura, 2015; Vrioni *et al*., 2018). Numerous microorganisms, such as bacteria, fungi and viruses, have been discovered using MALDI-TOF MS. MALDI-TOF offers potential applications in a wide range of fields,including biodefense, environmental monitoring, medical diagnostics, and food quality management, due to its rapid microbe characterization capabilities. When identifying microorganisms, MALDI-TOF MS offers a high-throughput, reasonably priced substitute for conventional laboratory biochemical and molecular identification technologies (Croxatto *et al*., 2012).

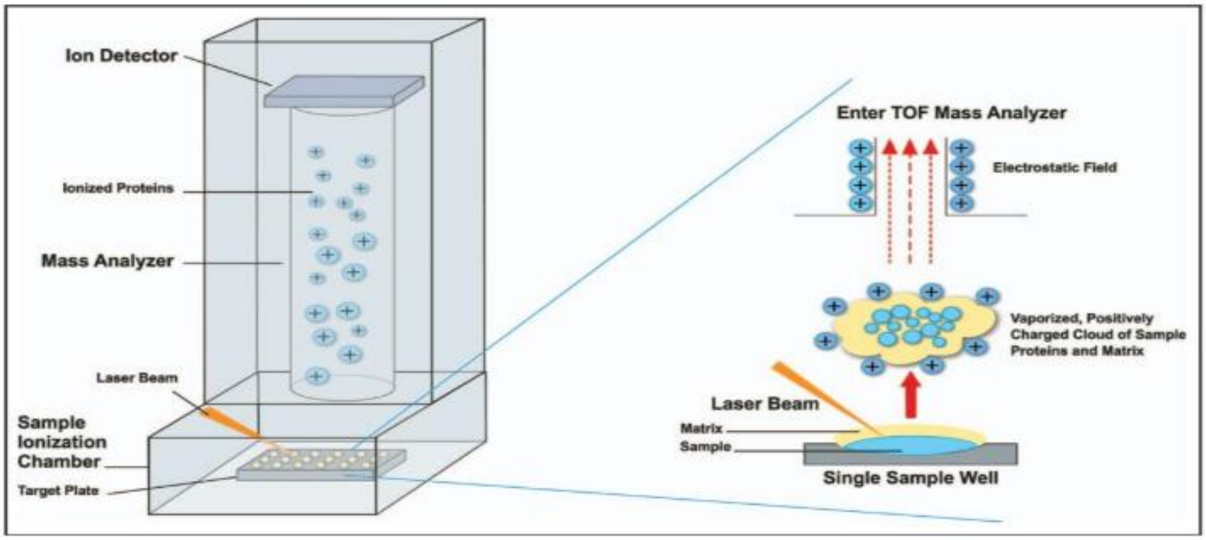


**Figure 1:** MALDI-TOF mass spectrometer

**Source**: Patel (2015).

## 3. MATERIALS AND METHODS

### 3.1. Study Area

The study was conducted in Koye Fache subcity(Wadesa and Tulu Dimtu) and Gelan subcity (Gelan and sida Awash ). These areas lie on the southeastern and southern outskirts of Addis Ababa, respectively and have become important centers for small- to medium-scale dairy production due to their proximity to the capital and growing demand for animal-source foods. Koye Fache is located at approximately 8.9000° N latitude and 38.8500° E longitude, with an elevation of around 2,300 meters above sea level. It is situated in the southeastern periphery of Addis Ababa and is characterized by mixed farming systems where dairy farming plays a central economic role. Gelan lies slightly south of Koye Fache, at approximately 8.8667° N latitude and 38.8167° E longitude, with an elevation of about 2,250 meters above sea level. It is positioned along the main road connecting Addis Ababa to Bishoftu and Adama, which enhances market access and facilitates the distribution of dairy products. Both areas experience a temperate highland climate, with bimodal rainfall.The main rainy season occurs from June to September, and a shorter rainy season from March to May.The moderate climate, combined with fertile soils and access to water, supports intensive livestock production.

The total livestock population of Sheger city is estimated to be 335,042 cattle, 30,848 goats, 155985 sheep, 1727 horses, 34,134 donkeys, 57,33282 and poultry according to Sheger city Agriculture Bureu (2025).Gelan subcity consists of 136 initiative farms with total of 55,399 dairy cows and koye Fache subcity consists of 133 initiatives farms with 5,857 dairy cows

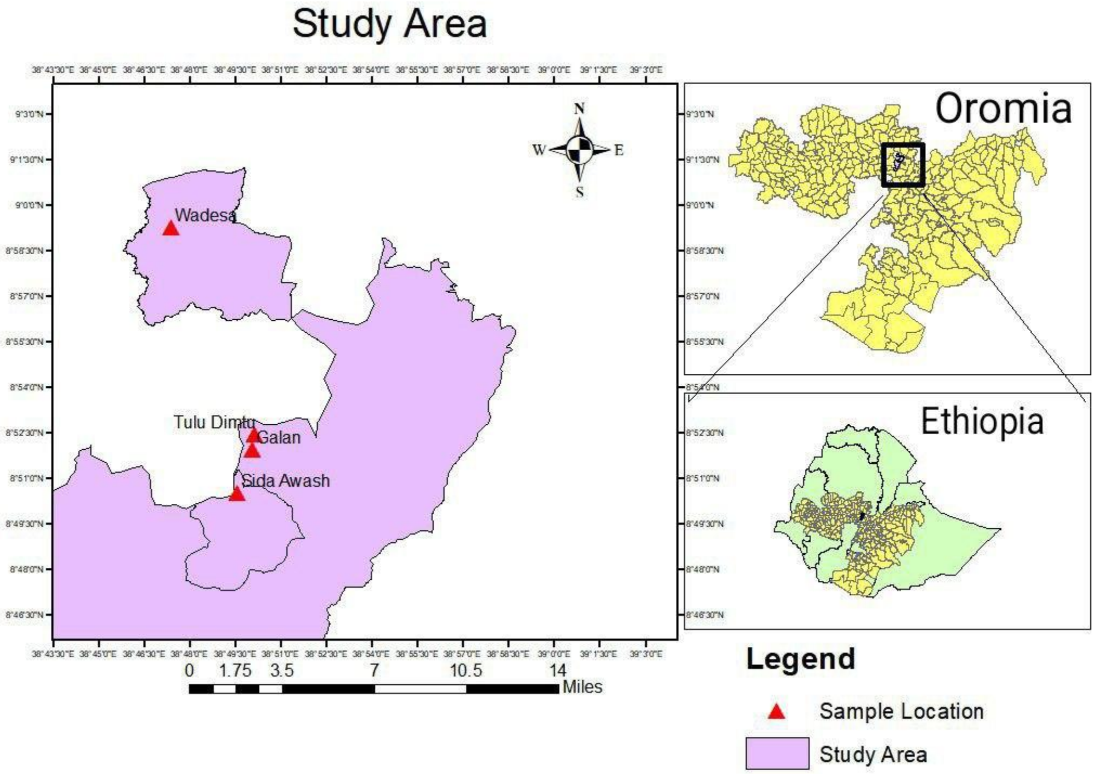


**Figure 2**: Map of Study Area

### 3.2. Study Population

The study population was milking dairy cows from dairy farms and owners in Gelan and koye Fache subcity of Sheger city who are currently producing milk. In Gelan subcity ,136 registered dairy farms were found during the time of sample collection. The number of milking cows in these farms are estimated to be 55,399 (Animal Production and Health Office, 2025). Koye face subcity 133 registered dairy farms were found during the time of sample collection. The number of milking cows in these farms are estimated to be 5,857 (Animal Production and Health Office, 2025)

### 3.3. Study Design

A cross-sectional study was conducted from December 2024 to May 2025 to investigate the occurrence, antimicrobial resistance profiles and environmental risk factors associated with *S.*

*aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) in dairy farms from a One Health perspective.

### 3.4. Sample Size Determination

Sample size was determined using the formula described previously (Thrusfield 2005) with 95% confidence, desired absolute precision of 5% and an expected 16.6% prevalence of *S. aureus* in dairy farm reported by Regasa *et al*. (2019).

$$N= \frac{1.96^2 Pexp(1 - Pexp)}{d^2}$$

here; N = required sample size, P = expected prevalence, and d = desired absolute precision.Therefore, the estimated sample size was 212 dairy cows, and the sample size was proportionally distributed in each district based on the number of dairy cows. Additionally, forty-four (40) swab samples from milkers' hands and sixty (60) swab samples from farm environment was collected by purposive sampling technique. Overall, 312 samples was collected and subjected to microbiological testing.

### 3.5. Sampling Technique.

Four weradas (Gelan, Sida Awash, Wadesa and Tulu Dimtu) were selected using a simple random sampling technique from Representative subcity and 20 dairy farms 5 from each was selected from these subcities using systematic random sampling technique based on the list of Woredas and dairy farm owners. The sampling frame used for selection of the dairy farms was a list of house-holds registered by the Sheger city Farmers and Urban Agricultural Commission. The number of samples was proportionally allocated to each subcity based on the number of farms available in each Woreda.

### 3.6. Sample Collection

Approximately 5 mL of milk was aseptically collected from each cow using pre-sterilized collection tubes. Prior to sampling, the tubes were thoroughly sterilized to maintain sample integrity and prevent contamination. In addition to the udder milk samples, hand swabs and environmental swabs was also collected from farm workers and farm environments respectively using Sterilized cotton buds which were then placed in small containers enriched with Phosphate Buffer Solution (PBS). These containers helped to maintain the viability of the collected samples during transportation to the laboratory, which was completed within 24 hr of sample collection.These swab samples were also placed in containers enriched with PBS to preserve their integrity during transportation to the Microbiology Laboratory, college of veterinary medicine and agriculture , Addis Ababa University, in ice box containing ice pack within 3-4 hr of collection. The use of strict aseptic techniques, along with appropriate collection containers and solutions, ensured the preservation, quality, and reliability of the collected samples.

### 3.7. Bacterial Isolation and Identification

One milliliter of each collected sample was enriched in 10 mL of Tryptone soya broth (Himedia, India) at 37 °C for 18–24 hrs. Next, bacterial cultures were streaked individually on blood agar with 5% sheep blood (Oxoid, UK). Colonies exhibiting beta-hemolytic patterns on blood agar were subsequently inoculated onto mannitol salt agar, (MSA) (Himedia, India) a selective medium for *S. aureus*. The plates were incubated aerobically at 37 °C for 18–24 hours. Colonies exhibiting a golden-yellow coloration on mannitol salt agar (MSA) were considered presumptive *S.aureus*. A bacterium showing clusters resembling grapes and golden-yellow colonies was streaked over nutrient agar (HiMedia, India) and cultured at 37 °C for 24 hours. Finally, the automated device known as Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS, Bruker Daltonik, Germeny) was used to confirm the representative (pure) colonies, which were typical grapes like arrangement and golden-yellow colour from nutrient agar at national Animal health institute,Sebeta, Ethiopia

### 3.8. MALDI-TOF MS Based Identification of *Staphylococcus aureus*

The method for species identification was carried out using matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonik) according to the company's instructions (Bizzini *et al.*, 2010). In short, representative colonies were placed in 300 µl of deionized water, vortexed, and precipitated with 900 µl of ethanol (96% vol/vol) after all putative isolates were sub-cultured on nutrient agar (HIMEDIA, INDIA) and incubated at 37 °C for 24 hours overnight. Following a 2-minute centrifugation at 15,000 rpm, the pellet was reconstituted using a solution of 25 µl formic acid (70% vol/vol) and 25 µl acetonitrile. After two minutes of centrifugation at 15,000 rpm,1µl of the supernatant was spotted on an MSP 96 target plate that had been polished with Steel BC. The plate was then left to air dry at room temperature. For every designated BTS QC spot, 1 µl of BTS was applied, and it was left to air dry. After covering the entire area with a droplet of 1µl of matrix solution (CHCA), the region was once more fully dried at room temperature. Lastly, analysis was carried out using biotyper software after inserting the MALDI target into the MALDI-TOF mass spectrometer

### 3.9. Antimicrobial Sensitivity Test

After identifying bacterial isolates with MALDI-TOF (Bruker Daltonik), susceptibility of antimicrobial testing was done using the disc diffusion method (Kirby Bauer technique) (Khalili *et al.*, 2012). Thirteen (13) antimicrobial discs were prioritized including penicillin G (10µg), Ciprofloxacin (5µg) , Trimethoprim-Sulphamethoxazole (25µg), Ampicillin(10µg), Tetracycline(30µg), Gentamycin (10µg), Clindamycin (2µg) cefoxitin (30µg), Amoxacillin 30 µg, norfloxacillin 10 µg, ceftriaxone 30µg,streptomycin 10 µg and Amoxicillin 2 µg based on their availability and distribution to nearby veterinary clinics and pharmacies. Representative colonies of isolates were suspended in sterile 0.85% NaCl solution and adjusted to a turbidity of 0.5 McFarland units using a densitometer. A sterile cotton swab was immersed in the bacterial suspension and uniformly spread across the surface of Mueller-Hinton agar. Antimicrobial discs were applied and plates were incubated at 37°C for 18 hours. Zone diameters were measured and interpreted as sensitive, intermediate, or resistant according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2022) (Annex 4.). *S. aureus* isolates resistant to three and above antimicrobial classes were considered multidrug-resistant and cefoxitin susceptibility testing was employed with 30 µg of cefoxitin disks to identify methicillin resistance, following the Clinical Laboratory Standards Institute (CLSI, 2019) criteria.

### 3.10. Inclusion and Exclusion Criteria

The inclusion criteria for participation in the study included farms with one or more lactating cows.Additionally, dairy farm owners who were willing to supply milk and milkers who were willing to participate in the study and provide necessary information via questionnaire were included. Conversely, exclusion criteria comprised non-lactating cows and cows not raised under intensive management systems. Furthermore, farmers who were absent during the study or declined to participate, farm owners with hearing impairments unable to provide required information, and those unable to complete questionnaire interviews were excluded from the study.

### 3.11. Questionnaire Survey

A pre-structured questionnaire survey was conducted to determine the status of hygienic, sanitation and waste management activities in dairy farms such as house cleaning, udder cleaning, hand washing and other conditions that were thought to influence the occurrence of *S.aureus*. A total of 20 individual farm owners/farm workers from each study area were interviewed to generate data on waste management status. The farms were classified into intensive (if cows are managed under confinement with supplementation of feeding and watering) and sem-intensive (if animals are partly confined and allowed to graze freely or under paddocking, supplementation of diet in addition to natural pasture (Biffa *et al*., 2005).

### 3.12. Data Analysis

All collected raw data were compiled, coded and entered into a Microsoft Excel 2007 spreadsheet and transferred to STATA Version 12 software for statistical analysis. Descriptive and inferential statistical techniques were used to analyze the collected data. Descriptive statistics, including percentages, were calculated. Chi-square tests ($\chi^2$), P-values, and logistic regression analyses were performed to assess the association between potential risk factors and the occurrence of the isolates, as well as to determine the strength of these associations using Odds ratio (OR) with 95% confidence interval (CI). P-values $< 0.05$ were considered as statistically significant.

### 3.13. Ethical Clearance

Before commencing the study, a request explaining the goal of the investigation was presented to the animal research ethics and review committee of the Addis Ababa University College of Veterinary Medicine and Agriculture and for Human it was presented to Aklilu Lemma institute of Pathobiology-IRERC, Addis Ababa university After obtaining an acceptance certificate from the committee (Annex 5),bearing reference No**.ALIPB-IRERC / 155/2017/24** and **VM/ERC/04/79/17/05** the research work was conducted.

# 4. RESULTS

## 4.1. Overall Prevalence

Out of the total of 312 different examined samples of udder milk, hand swab and environment swab, 65 samples (20.83%) were found to be contaminated with *S. aureus* and 31 samples (9.9%) were confirmed to be MRSA. The site-based prevalence of MRSA was 10(12.82%), 4(6.4%), 9(10.97%) and 8(10.81%) in Gelan, Sida Awash, Tulu Dimtu and Wadesa respectively. The difference in the prevalence of MRSA among the study sites was not statistically significant (P > 0.05)(Table 1). This might be due to similarity in type of intensive farming system.

The prevalence of *S.aureus* was 49(23.11%),11(27.5%) and 5(8.3%) in udder milk, hand swab and environment swab, respectively and MRSA among sample types was detected only in udder milk with prevalence of 31(14.62%). However, there was no statistically significant association in the isolation rate of *S. aureus* and MRSA among the different sample types (P >0.05) (Table 1).

**Table 1**: Prevalence of *S. aureus* and MRSA Among the Sample Types and Study sites

| **Sample source** | **Variables** | **No.of examined.** | **No.of positive** | **Prevalence of *s.aureus* (%)** | **MRSA** | $\chi^2$ | **P-value** |
|---|---|---|---|---|---|---|---|
| **Sample type** | Udder milk | 212 | 49 | 23.11 | 31(14.62%) | 7.6 | 0.106 |
| | Hand swab | 40 | 11 | 27.5 | 0 | | |
| | Environment waste | 60 | 5 | 8.3 | 0 | | |
| **Study site** | Galan | 78 | 15 | 19.23 | 10(12.82%) | 7.1 | 0.070 |
| | Sida awash | 78 | 9 | 11.54 | 4(6.4%) | | |
| | Tulu dimtu | 82 | 21 | 25.62 | 9(10.97%) | | |
| | Wadesa | 74 | 20 | 27.03 | 8(10.81%) | | |

The odds of finding *S. aureus* in environmental samples was 98.5 lower than in hand swabs and it was Statistically significant (P < 0.05). (Table 2).

**Table 2**: Bivariate Logistic Regression results of *S. aureus* among Different Sample types.

| **Sample type predictor** | ***S.aureus***<br>**OR(95%CI)** | **P-value** |
|---|---|---|
| Hand swab | reference | |
| Udder milk | 0.72(0.37-1.7) | 0.551 |
| Farm Environment | 0.015(0.08-0.8) | 0.015 |

### 4.2. Risk Factors for the Occurence of *S. Aureus*

The prevalence of *S. aureus* in hand swab samples from workers with poor hygiene practices was found to be 50%. The difference in the prevalence of *S. aureus* among Hygiene of worker categories was statistically significant (P < 0.05). However there was no significance association (p>0.05) of occurrence of *S .aures* with use of antiseptic.(Table 3)

A significant association was observed between the occurrence of *S. aureus* in milk and the practice of handwashing before and between each milking session and udder cleaning (p < 0.05). However, there was a significant association between drainage system (p<0.05) but there was no statistical significance between feed storage with the occurrence of *S. aureus* (p > 0.05) in Farm environment (Table 3).

**Table 3:** Risk Factors for Isolation Rate of *S. aureus* and MRSA in Hand swab, Udder milk and Farm environment..

| **Associated Factors** | | **Total Samples Examined (n)** | **Positive Samples for *S. aureus* n (%)** | $\chi^2$ | **P-value** |
|---|---|---|---|---|---|
| **Hygiene of worker** | Good | 6 | 0(0.0) | 10.3 | 0.006 |
| | Satisfactory | 14 | 1(7.14) | | |
| | Poor | 20 | 10(50%) | | |
| **Use of antiseptic** | Yes | 25 | 6(24%) | 0.4 | 0.522 |
| | No | 15 | 5(33.33%) | | |
| **Hand washing before milking** | Always | 207 | 60(28.99%) | 11.6 | 0.001 |
| | Sometimes | 5 | 5(100%) | | |
| **Hand washing between each milking** | Rarely | 187 | 53(28.34%) | 4.0 | 0.045 |
| | Sometimes | 25 | 12(48%) | | |
| **Udder cleaning** | Always | 205 | 59(28.78%) | 10.3 | 0.001 |
| | sometimes | 7 | 6(85.71%) | | |
| **Drainage system** | Good | 39 | 1(2.56) | 0.1 | 0.0465 |
| | Poor | 21 | 4(19.05) | | |
| **Feed storage** | Sheds | 47 | 4(8.5) | 0.1 | 0.955 |
| | Bunkers | 13 | 1(7.7) | | |

### 4.3. In vitro Antimicrobial Susceptibility Pattern of *S. aureus* Isolates

The antimicrobial susceptibility profile of the 65 tested isolates reveals a concerning pattern of resistance to several commonly used antibiotics. Complete resistance (100%) was observed against Amoxicillin-Clavulanic Acid (AMC), Penicillin (P), Tetracycline (TET), Clindamycin (DA), and Cloxacillin (AX), indicating these antibiotics are ineffective against the tested organisms.Conversely, excellent susceptibility (100%) was seen with Ciprofloxacin (CIP), Ampicillin (AMP), Norfloxacin (NOR), and Gentamicin (CN), suggesting these agents remain highly effective options for treatment.Ceftriaxone (CRO) demonstrated variable effectiveness, with 40% of isolates being susceptible, 40% intermediate, and 20% resistant. This mixed pattern suggests that while Ceftriaxone may still be useful in some cases, resistance is emerging.Similarly, Sulphamethoxazole-Trimethoprim (SXT) showed good effectiveness, with 80% susceptibility and 20% resistance, indicating it could still serve as a treatment option depending on the context.Streptomycin (S) showed moderate activity, with 40% susceptibility and a high resistance rate of 60%, limiting its reliability.Cefoxitin (FOX) displayed nearly equal susceptibility (52.3%) and resistance (47.7%), suggesting limited clinical utility without prior susceptibility testing.

**Table 4:** Antimicrobial Resistance Patterns

| **Antimicrobial agent** | **Interpretation categories** | | |
|---|---|---|---|
| | **S n(%)** | **I n(%)** | **R n(%)** |
| AMC(30 µg) | 0 | 0 | 65(100%) |
| P(10µg) | 0 | 0 | 65(100%) |
| CRO(30 µg) | 26(40) | 26(40) | 13(20%) |
| CIP(10 µg) | 65(100%) | 0(%) | 0(0%) |
| S(10 µg) | 26(40%) | (0%) | 39(60%) |
| SXT(25 µg) | 52(80%) | 0 | 13(20%) |
| TET (30µg) | 0 | 0 | 65(100%) |
| AMP (10µg) | 13(20%) | 0 | 52(80%) |
| NOR (10µg) | 65(100%) | 0 | 0 |
| FOX (30µg) | 34(52.31%) | 0 | 31(47.7%) |
| DA (2µg) | 0 | 0 | 65(100%) |
| AX (2µg) | 0 | 0 | 65(100%) |
| CN(10 µg) | 65(100) | 0 | 0 |

Abbreviations: n, number; %, percent, AMC,amoxacillin; P,penicillin; CRO,ceftriaxone; CIP,ciprofloxacillin; S,streptomycin; SXT,sulphamethoxaole; TET,tetracycline AMP,ampicillin; NOR,norfloxacillin; FOX,cefoxitin; DA,clindamycin; AX,amoxicilli; CN; gentamycin

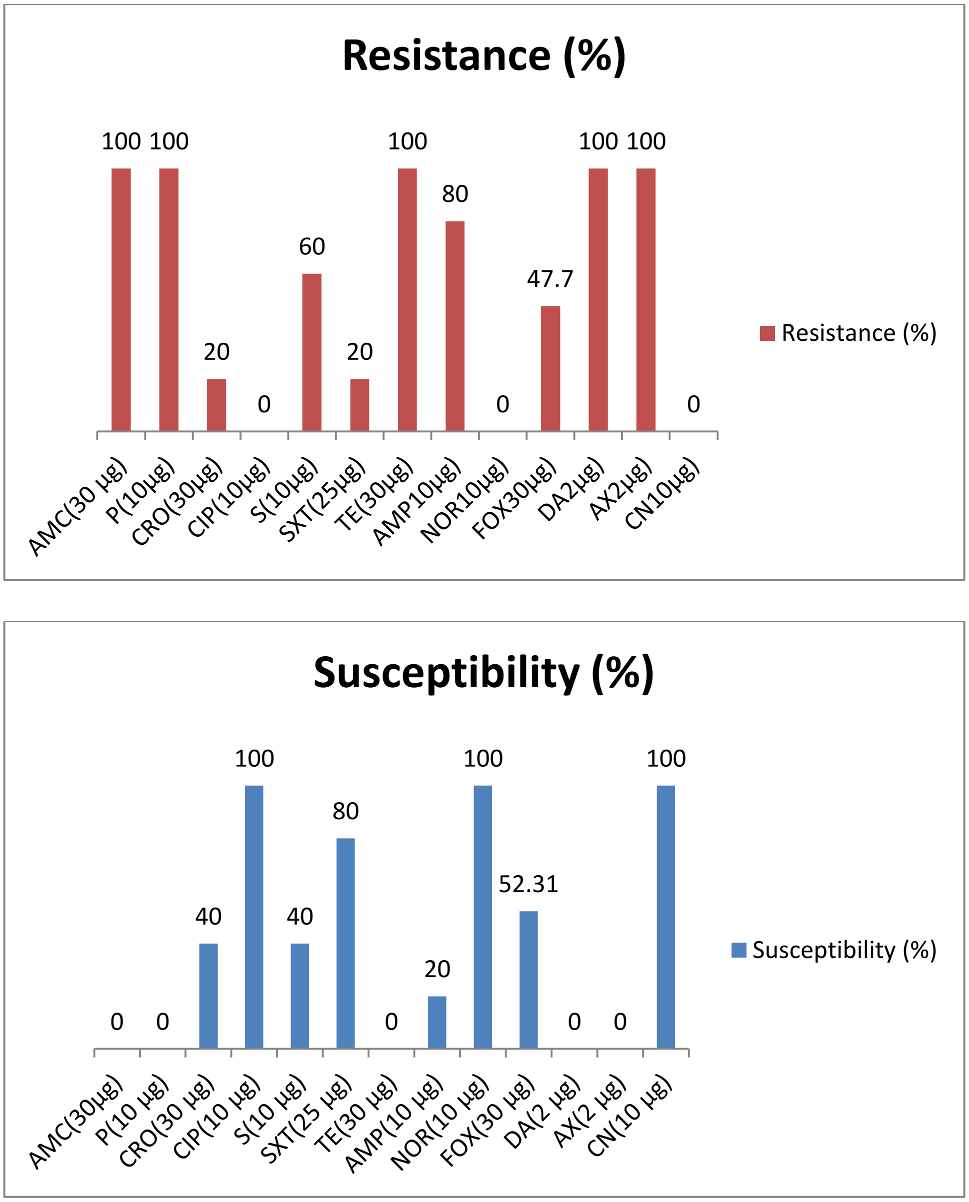


**Figure 3:** Antimicrobial Resistance Patterns

### *4.3.1. Multi-drug resistance*

The result shown in Table 5. indicated that 65 (100%), 39(60%),30 (46%), and 13(20%), of *S. aureus* isolates showed resistance to three,four,five and six antibiotics, respectively. In general, 65 isolates of *S. aureus* (100%) showed multi-drug resistance to three and more than three antimicrobial drugs.

**Table 5:** The isolates of *S. aureus* in the study area exhibit a multidrug resistance pattern

| No.of antibiotics | Resistance pattern | No.of isolates | Percentage(%) |
|---|---|---|---|
| **Three** | B-Lactams,TET,lincosamides | 65 | 100 |
| **Four** | B-Lactams,TET,lincosamides,Aminoglycosides | 39 | 60 |
| **Five** | B-Lactams,TET,lincosamides, Aminoglycosides,cephalosporins | 25-30 | 38-46 |
| **Six** | +sulfonamides | 13 | 20 |

### 4.4. Descriptive Statistics of Questionaire Survey on Sanitation and Waste Management in Study Area.

The socio-economic characteristics of the study sites are presented in Table 6.The study showed that 40% of the surveyed dairy farms were owned by female households while 60% of them were owned by male household. The average age of the dairy farmers was 41.5 years (ranging from 24 to 59 years). The study also showed that higher percentage of respondents have attended secondary school. Besides dairying, about 35% are employed temporarily. Larger percentages (65%) of the respondent have dairy farm experience less than one year and they all run intensive type of dairy farming.

### *4.4.1. Socio-Demographic Characteristics of Dairy Farmers*

**Table 6:** Socio-Demographic Characteristics of Dairy Farmers

| **Variable** | **Categories** | **Respondent No.** | **Percentage(%)** |
|---|---|---|---|
| **Educational status.** | Illiterate | 1 | 5 |
| | Primary | 5 | 25 |
| | Secondary | 6 | 30 |
| | Tertiary | 3 | 15 |
| | University | 5 | 25 |
| **Gender** | Male | 12 | 60 |
| | Female | 8 | 40 |
| **Age of Respondent:** | 24-38 | 6 | 30 |
| | 39-48 | 11 | 55 |
| | 50-59 | 3 | 15 |
| | >59 | 0 | 0 |
| **Employment Status:** | Temporary | 7 | 35 |
| | Permanent | 0 | 0 |
| | Small business | 3 | 15 |
| | Dairy only | 10 | 50 |
| **Experience in Dairy Farming:** | <1 year | 13 | 65 |
| | 1-5 years | 5 | 25 |
| | 6-10 years | 2 | 10 |
| **Type of Dairy Farming:** | Intensive | 20 | 100 |
| | Extensive | 0 | 0 |

### *4.4.2. Sanitation and Hygiene Practices*

A majority of farms (60%) clean animal sheds daily with the remaining 40% doing so weekly.while frequent cleaning reduces pathogen buildup,the absence of monthly or rare cleaning practices is positive.However, 60 % of farms do not use disinfectant during cleaning and among those that do (40%), 65% apply them rarely,with only 30 % disinfecting daily. (Table 7). This reliance on physical cleaning without chemical disinfections raises concerns about residual microbial contamination. Pest control measures were used by 55% of farmers, with 75% applying them daily. (Table 7) This regular application contributes to improved farm hygiene by reducing pest populations such as flies and rodents that can act as mechanical vectors for pathogens like *S. aureus* and MRSA.

Floor-based bedding was the predominant system used by 80% of the farms, while only 20% utilized sand bedding. In terms of bedding management, 70% of farmers reported changing bedding daily, and 30% did so weekly, with no reports of monthly or rare changes. (Table 7) Although frequent bedding changes can help reduce bacterial load and moisture, floor-based system especially those made of organic materials still tend to harbor higher levels of pathogens like *S. aureus* and MRSA due to their absorbent nature. In contrast, sand bedding, though less commonly used, is inorganic and less hospitable to bacterial survival.

**Table 7:** Sanitation and Hygiene Practices in Dairy farm

| Variable | Category | Respondent (n=20) | Percentage(%) |
|---|---|---|---|
| How animal sheds cleaned? | Daily | 12 | 60 |
| | Weekly | 8 | 40 |
| | Monthly | 0 | 0 |
| | Rarely | 0 | 0 |
| What materials are used for bedding? | Straw | 0 | 0 |
| | Sand | 4 | 20 |
| | Rubber mats | 0 | 0 |
| | Floor | 16 | 80 |
| How often is bedding changed? | Daily | 14 | 70 |
| | Weekly | 6 | 30 |
| | Montly | 0 | 0 |
| | Rarely | 0 | 0 |
| disinfectants used for cleaning animal shed | Yes | 8 | 40 |
| | No | 12 | 60 |
| How often are disinfectants used? | Daily | 6 | 30 |
| | Montly | 1 | 5 |
| | Weekly | 0 | 0 |
| | Rarely | 13 | 65 |
| What pest control measures are used on the farm? | Insecticides | 4 | 20 |
| | Rodent control | 0 | 0 |
| | None | 5 | 25 |
| | Others | 11 | 55 |
| How often are pest control measures applied? | Daily | 15 | 75 |
| | Weekly | 0 | 0 |
| | Montly | 0 | 0 |
| | Rarely | 5 | 25 |

### *4.4.3. Milking Hygiene and Milk Handling Practices*

A majority of respondents (75%) consistently washed udders before milking, with the remaining 25% practicing intermittent washing (Table 8). This suggests a general awareness of udder hygiene to prevent contamination. However, handwashing practices were less: 60% of farmers always washed their hands before milking, while 40% did so only occasionally. This discrepancy implies potential gaps in understanding the role of hand hygiene in reducing microbial transfer during milking. While udder cleaning is prioritized, the inconsistent handwashing raises concerns about cross-contamination risks, particularly given the direct contact between hands, udders, and equipment.

Cleaning frequency for milking equipment was largely satisfactory, with 75% of respondents cleaning after each use and 25% doing so daily. However, the choice of disinfectants revealed a critical shortcoming: 70% relied on soap and water, while only 30% used alcohol-based disinfectants. Chlorine-based agents, (Table 8) which are widely recommended for effective microbial control, were not utilized. This indicates a lack of awareness or access to appropriate disinfectants, potentially compromising the sterility of equipment and increasing the risk of bacterial proliferation in milk.

Milk storage practices posed significant risks to quality. A concerning 60% of farms stored milk at ambient temperature, while only 40% used refrigeration (Table 8). Ambient storage accelerates spoilage and bacterial growth, especially in the absence of preservatives (none reported).This practice likely reflects limited access to refrigeration infrastructure or insufficient training on cold-chain management. Conversely, all farms transported milk in sealed containers, a positive step to prevent contamination during transit. However, only 45% reported regular cleaning of transport vehicles, with 55% neglecting this practice. Dirty vehicles undermine the benefits of sealed containers, as residual contaminants in transport environments can compromise milk safety.

**Table 8:** Milking Hygiene Practices Among Dairy Farmers

| Variable | Category | Respondent (n=20) | Percentage (%) |
|---|---|---|---|
| **Are udders washed before milking?** | Always | 15 | 75 |
| | Sometimes | 5 | 25 |
| | Rarely | 0 | 0 |
| | Never | 0 | 0 |
| **Are hands washed before milking?** | Always | 12 | 60 |
| | Sometimes | 8 | 40 |
| | Raraely | 0 | 0 |
| | Never | 0 | 0 |
| **How often is milking equipment cleaned?** | After each use | 15 | 75 |
| | Daily | 5 | 25 |
| | Weekly | 0 | 0 |
| | Rarely | 0 | 0 |
| **Disinfectant used for cleaning** | Chlorine-based | 0 | |
| | Alcohol-based | 6 | 30 |
| | Soap and water | 14 | 70 |
| **Milk storage before sale or transport?** | Refrigeration | 8 | 40 |
| | Ambient temperature | 12 | 60 |
| | Use of preservatives | 0 | 0 |
| **How is milk transported?** | Sealed containers | 20 | 100 |
| | Open containers | 0 | 0 |
| | Others (Specify): | 0 | 0 |
| **Are transport vehicles cleaned regularly?** | Yes | 9 | 45 |
| | No | 11 | 55 |

### *4.4.4. Worker Hygiene and Biosecurity*

Only 50% of the farms provided hygiene training to workers, and just 65% of workers practiced regular handwashing before and after milking critical measures for preventing the spread of *pathogens*. Although 85% of farms supplied protective gear such as gloves and aprons, only 30% implemented quarantine measures for new animals, increasing the risk of introducing pathogens into the herd. On a more positive note, 75% of farms enforced visitor restrictions and basic biosecurity protocols.(Figure 4) These findings highlight significant gaps in worker hygiene and animal introduction practices that could undermine efforts to control bacterial transmission in dairy farm environments.

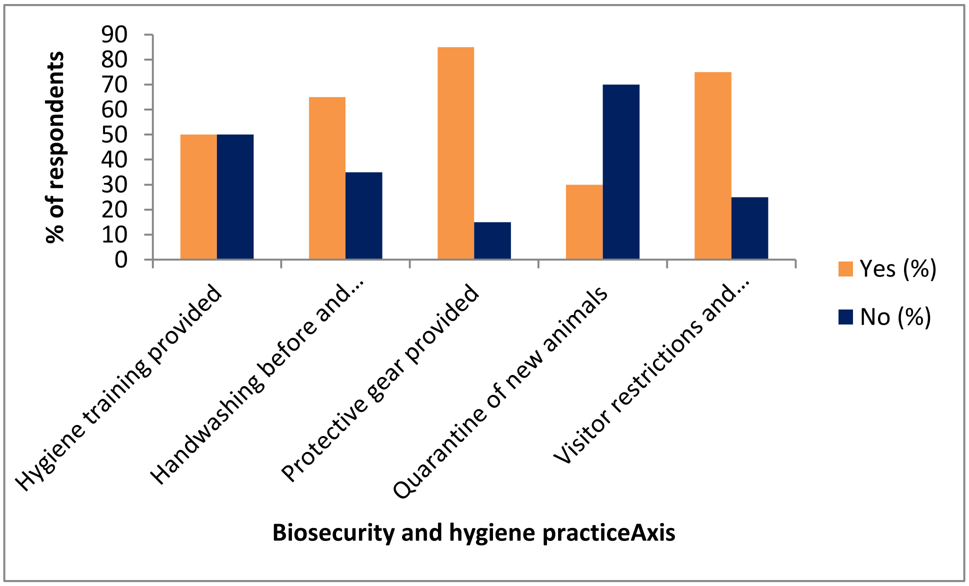


**Figure 4:** Worker Hygiene and Biosecurity practices in Dairy Farm

### *4.4.5. Antibiotic Use and Awareness*

A majority of farmers (80%) relied on veterinarians to treat sick cattle, while 20% self-administered treatments.(Table 9) This indicates reasonable trust in professional veterinary care, though self-administration raises concerns about inappropriate antibiotic use. Moreover, 40% of farmers admitted to using antibiotics as growth promoters in feed, a practice strongly discouraged by global health authorities due to its role in driving AMR. Among these, tetracyclines (45%) and unspecified "others" (50%) dominated, suggesting potential misuse of broad-spectrum or non-recommended antibiotics. The high "others" category suggests further investigation to identify specific antibiotics, as unregulated use could exacerbate resistance. While 75% of farmers claimed to always follow veterinary prescriptions, 25% did so only intermittently, risking under- or over-dosing. Encouragingly, 75% reported strictly adhering to antibiotic withdrawal periods before selling milk, but 5% (1 farm) did not, posing a direct risk of antibiotic residues entering the food chain. This non-compliance, though small, suggesting the need for stricter enforcement and monitoring. (Table 9)

Antibiotics were used "occasionally" by 60% of farms and "rarely" by 35%, with only 5% using them frequently. While limited frequency is positive, the reliance on antibiotics (even occasional) without alternatives remains problematic. Alarmingly, 70% of farmers did not maintain records of antibiotic use (type, dosage, duration, or withdrawal periods).(Table 9)

Only 30% of farmers were fully aware of antibiotic resistance, while 50% had partial awareness, and 20% lacked knowledge entirely.(Table 9) This knowledge gap is critical, as uninformed practices (e.g., using antibiotics for growth promotion) directly contribute to AMR. Additionally, 65% of farmers did not use alternative treatments (e.g., herbal remedies, probiotics), indicating over-reliance on antibiotics even probably for preventable conditions

**Table 9:** Antibiotic Use and Awareness among Dairy Farmers

| Variable | Category | Respondent (n=20) | Percentage |
|---|---|---|---|
| Who treats your cattle when they are sick? | Veterinarian | 16 | 80 |
| | Self- administration | 4 | 20 |
| | Traditional healer | 0 | 0 |
| | Others (Specify): | 0 | 0 |
| Do you use antibiotics as additives for growth promotion in feed? | Yes | 8 | 40 |
| | No | 12 | 60 |
| If yes, which antibiotics do you commonly use as growth promoters | Tetracyclines | 9 | 45 |
| | Penicillins | 1 | 5 |
| | Sulfonamides | 0 | 0 |
| | Fluoroquinolones | 0 | 0 |
| | Cephalosporins | 0 | 0 |
| | Others | 10 | 50 |
| Do you follow a veterinarian's prescription when using antibiotics? | Always | 15 | 75 |
| | Sometimes | 5 | 25 |
| | Rarely | | |
| | Never | | |
| Are withdrawal periods for antibiotics strictly followed before selling milk? | Yes | 15 | 75 |
| | No | 5 | 5 |
| How often are antibiotics used on the farm? | Rarely | 7 | 35 |
| | Occasionally | 12 | 60 |
| | Frequently | 1 | 5 |
| Do you keep records | Yes | 6 | 30 |

| | | | |
|---|---|---|---|
| of antibiotic use (type, dosage, duration, withdrawal period)? | No | 14 | 70 |
| Are you aware of antibiotic resistance? | Yes | 6 | 30 |
| | No | 4 | 20 |
| | Somewhat | 10 | 50 |
| Do you use alternative treatments | Yes | 7 | 35 |
| | No | 13 | 65 |

*4.4.6. Milk Quality and Safety Practices*

Only 30% of farms conducted milk quality testing such as checking for antibiotic residues or bacterial load prior to sale. Among those encountering milk that failed quality tests, 30% sold it at a reduced price, while the remainder used unspecified disposal or handling methods. Furthermore, only 50% of respondents reported receiving feedback on milk quality from consumers or processors. (Table 10) These practices reflect limited quality control measures and weak feedback mechanisms, which can compromise public health and contribute to the spread of antimicrobial resistant bacteria such as *S.aureus* and MRSA through the dairy value chain.

**Table 10:** Milk Quality Control and Feedback Practices in Dairy Farms

| **Variable** | **Category** | **Respondent(n=20)** | **Percentage(%)** |
|---|---|---|---|
| Is milk tested for quality (e.g., bacterial load, antibiotic residues) before sale? | Yes | 6 | 30 |
| | No | 14 | 70 |
| What happens to milk that fails quality tests? | Discarded | 0 | 0 |
| | Sold at a lower price | 6 | 30 |
| | Used for animal feed | 0 | 0 |
| | Others | 14 | 70 |
| Do you receive feedback from consumers or milk processors about milk quality? | Yes | 10 | 50 |
| | No | 10 | 50 |

### *4.4.7 . Farm Infrastructure and Environment*

Regarding farm infrastructure, 75% of farms had concrete floors, while 25% used dirt floors, which are more difficult to clean and may harbor pathogens. All farms relied on natural ventilation, with no mechanical airflow systems in place. Additionally, all farms were located within one kilometer of another, indicating high farm density and an increased risk of disease transmission, including the spread of *S.aureus* and MRSA. These environmental and infrastructural factors may contribute to the persistence and dissemination of pathogens within and between farms.(Figure 5)

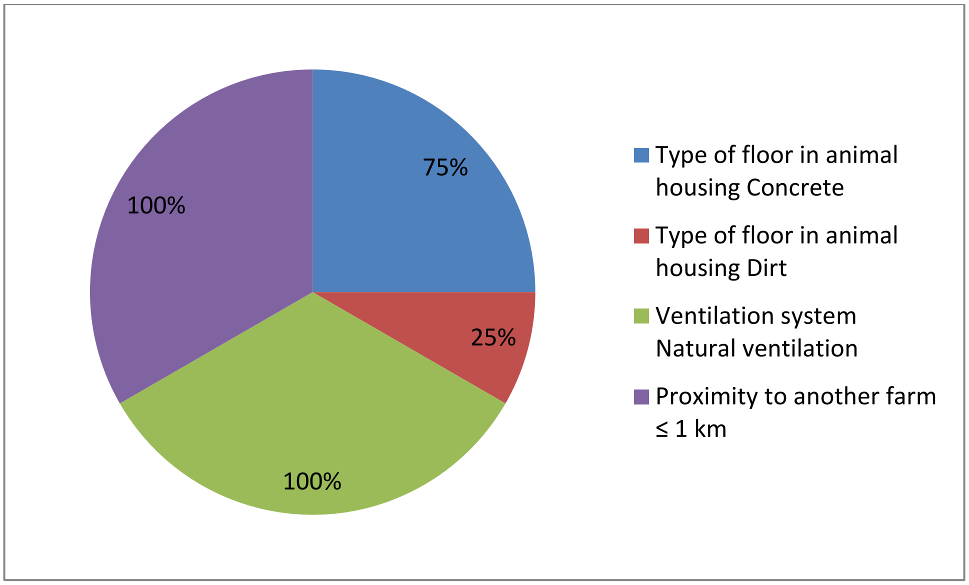


**Figure 5:** Farm Infrastructure and Environment

## 5. DISCUSSIONS

The transmission of pathogenic bacteria from livestock to humans through milk presents a worrying public health concern, with serious implications for farm workers, veterinarians and the community. The increasing incidence of *S. aureus* infections in addition with the prevalence of MRSA in livestock, accentuates the magnitude of its impact on public health (Pantosti, 2012; Silva *et al.*, 2022). Dairy cows, can act as reservoirs for MRSA, enhancing its transmission through milk and other animal derived products. This transmission pathway poses a direct risk to consumers, with milk serving as a potential vehicle for MRSA infection. In this context, Methicillin-resistant *S. aureus* (MRSA) has emerged as a significant global public health concern, affecting humans, animals and the environment over the past few decades (Ganai *et al*., 2016; Begum *et al*., 2007). One of the contributing factors to the intensifying concern is the misuse of antibiotics in dairying may contribute to the development and spread of antibiotic resistant MRSA.

In this study, a total of 312 samples collected from udder milk, hand swabs and farm environmental swabs across four dairy sites (Gelan, Sida Awash, Tulu Dimtu and Wadesa) were examined for the presence of *S. aureus* and methicillin-resistant *S. aureus* (MRSA). The overall prevalence of *S. aureus* was found to be 20.83%, while MRSA was detected in 9.9% of the samples. Among the various sample types, *S. aureus* was most frequently isolated from hand swabs (27.5%), followed by udder milk samples (23.11%), and was least prevalent in the farm environment (8.3%).Interestingly, MRSA was detected only in udder milk, with a prevalence of 14.62% among the milk samples.

When analyzed by study site, the prevalence of MRSA was 12.82% in Gelan, 6.4% in Sida Awash, 10.97% in Tulu Dimtu, and 10.81% in Wadesa. Although slight differences were observed, these variations were not statistically significant ($P > 0.05$), indicating a relatively uniform distribution of MRSA across the dairy farms studied.

These findings reveal a moderate burden of *S. aureus* and MRSA contamination in dairy production settings and highlight the udder as a primary reservoir for MRSA.

The prevalence of *S. aureus* in the present study is higher than other studies conducted at Mukaturi and Sululta 16.6%, (Regasa *et al*., 2019) Sebeta 19.6%,(Ayele., 2017) Iran 6.61% (Rahi *et al*., 2020) and Egypt 19%, (Seedy *et al*., 2017) North west India 19.84%. (Sharma *et al* ., 2017).These variation might be due to difference in sample size, isolation techniques, awareness and skills of the farm workers, geographic regions and variation in study subjects for example In the case of Iran, the samples were collected from buffaloes and camels. This finding is comparable to the prevalence reported at Alage Veterinary College Dairy Farm in Ethiopia (21.2%) by Bekele *et al*. (2016), in Uganda (20.3%) by Asiimwe *et al*. (2017), and in China (22.3%) by Liu *et al*. (2018).

However, our result is lower than the prevalence conducted in Addis Ababa 50% (Beyene *et al*., 2017) Hawassa 51.2%, (Abebe *et al*., 2016 ) Bishoftu 28.65%, (Hika *et al* ., 2017) Hawassa 78%, (Daka *et al* 2012) Mekele 32.81%, (Girmay *et al*., 2020) China 46.2%, (Wang *et al*., 2018) Italy 47.2% (Cortimiglia *et al*., 2016) and India 54.3%. (Bhati *et al*, 2018). These variations may be attributed to differences in farm management systems, types of samples analyzed for instance, bulk tank milk was used in the study from Italy, while only individual milk samples were examined in the study from Hawassa (Daka *et al*., 2012 and diagnostic methods employed, such as the use of PCR testing in studies conducted in Mekele and India.

Recently, the isolation of MRSA in animal and human have become a worldwide concern.(Tessema, 2017). The current study revealed 9.9% Prevalence of MRSA.This finding was inline with a study conducted in Mekelle 4.5%,(Kalayu *et al*., 2020) Tanzania 4.4% (Mohammed *et al* ., 2018) and Italy 3.8%, but much lower than in Sebeta 100%.(Ayele *et al*., 2017). The occurrence of MRSA in dairy farms is primarily attributed to subclinical mastitis, which facilitates its transmission into raw milk. This creates an opportunity for MRSA to spread from milking halls and the contaminated hands of infected milkers to raw milk, as well as to other humans and animals.In addition, the presence of substandard hygienic procedure practiced by milkers are the most important probable reasons for occurrence of MRSA (Rahi *et al*., 2020). The identification of MRSA in dairy farms in this study highlights the need to raise awareness among milkers about safe milk collection practices and the importance of adhering to proper hygiene procedures to prevent cross-contamination. Additionally, regular monitoring and judicious administration of antimicrobials are essential to control the spread of MRSA.

The prevalence of *S. aureus* from cow milk in this study was 23.11%.. This is consistent with previous reports of 21% positivity in raw cow milk from the central highlands of Ethiopia (Tigabu *et al*., 2015) and 45 - 25% positivity in the Hawassa, Addis Ababa and Sebeta town (Daka *et al*., 2012; Lemma *et al*., 2021; Ayele *et al.*, 2017). However, the prevalence was slightly higher than the 15.29% -17.3% reported in Ambo and Bako, Addis Ababa, Bishoftu, the West Shewa Zone ( Borena *et al*., 2023; Mekuria *et al*., 2013; Feyissa *et al*., 2023; Gizaw, and Duguma., 2014), but lower than the 50% prevalence previously reported in Addis Ababa ( Beyene *et al*., 2017). The variation in *S. aureus* prevalence between the present study and previous reports may stem from differences in research methodologies, including study design, sample source, sampling techniques, sample size and type, as well as the laboratory detection methods employed. These factors likely contribute to the discrepancies observed across different studies.

On the other hand, the isolation rate of *S. aureus* in milkers' hand swabs was (27.5%) .The present study was higher than that reported in Mukaturi and Sululta (25%) (Regasa *et al* ., 2019), but lower than the rate reported in India (41.2%) (Bhati *et al*., 2018). The observed variation may be attributed to differences in diagnostic techniques, as the study in India utilized PCR, which is more sensitive than conventional methods.

The odds of finding *S. aureus* in environmental samples was 98.5 lower than in hand swabs and it was Statistically significant ($P < 0.05$). The findings of this study indicate that the hygiene status of dairy workers is a significant risk factor for the presence of *S. aureus* on hand swabs. Among workers with poor hygiene, 50% tested positive for *S. aureus*, compared to only 7.14% of those with satisfactory hygiene, and none with good hygiene. This difference was statistically significant ($\chi^2 = 10.2642$, $p = 0.006$), highlighting that poor personal hygiene considerably increases the risk of *S. aureus* contamination. Poor hand-washing practices before milking can increase the risk of intramammary *S. aureus* infection, and people working on dairy farms are more likely to contract the infection (Azevedo *et al.*, 2016)

In contrast, the use of antiseptics did not show a statistically significant association with the presence of *S. aureus*. Workers who used antiseptics had a 24% positivity rate, while those who did not had a 33.33% rate ($\chi^2 = 0.4096$, $p = 0.522$). Although antiseptic users appeared to have lower contamination rates, the difference was not statistically significant. This may be due to improper use, inconsistency, or inefficacy of the products used. Oliver *et al*. (2005) demonstrated that antiseptics can effectively reduce microbial load when applied correctly and consistently, but their effectiveness diminishes without good hygiene practices.

Poor handwashing practices further contributed to the spread of *S. aureus* and methicillin-resistant *S. aureus* (MRSA). Individuals who only sometimes washed their hands before milking had a 100% positivity rate, and those who rarely washed hands between cows also showed significantly higher prevalence.These findings support the role of hands as mechanical vectors that facilitate pathogen transmission during milking.The FAO (2022) also emphasizes hand hygiene, including glove use and routine washing, as essential to preventing intramammary infections and zoonotic transmission.

Additionally, udder hygiene was identified as a key factor. Inconsistent udder cleaning was associated with an 85.71% infection rate, significantly higher than the 28.78% seen in farms with consistent cleaning practices.These results are consistent with previous research. Saini *et al*. (2012) reported that disinfecting teats before milking can lower the incidence of *S. aureus* infections by up to 50%. Similarly, Kivaria *et al*. (2006) associated poor udder hygiene with the occurrence of clinical mastitis. Ruegg (2021) also highlighted the significance of pre-milking teat dipping and proper udder preparation in minimizing the risk of *S. aureus* and MRSA .

In this study, the association between environmental management practices specifically drainage systems and feed storage methods and the occurrence of *S aureus* was assessed. A statistically significant relationship was observed between drainage quality and the presence of *S. aureus* ($p = 0.0465$). Farms with poor drainage had a markedly higher prevalence of *S. aureus* (19.05%) compared to those with good drainage (2.56%). The odds ratio (OR = 0.112) further indicates that the odds of detecting *S. aureus* were substantially lower in farms with adequate drainage. These findings suggest that poor drainage creates conditions favorable for bacterial persistence and indirect transmission.

Although *S. aureus* is mainly transmitted via direct contact and milking equipment, environmental hygiene plays a supporting role in its control. This is in agreement with Melchior *et al*. (2006), who reported that poorly drained, moist environments can enhance the persistence of *S. aureus* through biofilm formation, which leads to chronic infections. Fox (2009) similarly reported that farms with poor sanitation and drainage showed a higher prevalence of *S. aureus* up to 25% compared to farms with good drainage (<5%). In a Tanzanian study, Kivaria *et al*. (2006) observed a prevalence of *S. aureus*-associated mastitis of 21.5% in farms with suboptimal drainage, compared to just 8.3% in those with proper environmental hygiene and infrastructure.

The observed 100% resistance of the isolates to Penicillin G aligns with several previous studies, including Ayele *et al.* (2017) who reported 98.85% resistance in Sebeta town; Pati and Mukherjee (2016) with 96.0% in the Northern Plains of India; Daka *et al*. (2012), Girmay *et al*. (2020), Derib *et al*. (2017), and Ayana *et al*. (2017) who all reported 100% resistance in the Hawassa area, Shire town, Wolaita Sodo, and Bishoftu town, respectively; Mathenge *et al*. (2017) with 99.6% in Nairobi, Kenya; Abera *et al*. (2010) with 94% in Gondar; Zewdu *et al*. (2021) with 100% in central Ethiopia; and Mekonnen *et al*. (2018). These consistently high resistance rates may be attributed to the prolonged and unregulated use of β-lactam antibiotics in both veterinary and human healthcare.

The observed 100% resistance to amoxicillin-clavulanic acid (AMC**)** in this study contradicts with the previous studies reported by (Demissie *et al*., 2019) (43.6%). Meta District and agrees with Garedew *et al*. (2020) (78.6%) Mekelle. The trend may reflect extended use of broad-spectrum antibiotics in farm settings, coupled with poor regulatory enforcement disagrees with the report of Pati and Mukherjee (2016) (37.0%) in Northern Plains of India.Tetracycline (TE) resistance in this study (100%) is also mirrored by earlier work. In Bishoftu, Daka *et al*. (2020) reported a 78.9%, while Tolossa *et al*. (2023) found over 75% resistance among isolates, However,100.0% susceptibility to Tetracycline was reported by Sori *et al*. (2011) in Jimma town. Although less commonly studied in veterinary isolates, resistance to clindamycin

(DA)which also showed 100% resistance in the present study,contradicts resistance rates ranging from 43% (Abera *et al*., 2014) to 57.1% (Tadesse *et al*., 2020)

The complete susceptibility to ciprofloxacin (CIP) aligns with prior studies by Mekonnen *et al*. (2018) (95.8%) in Addis Ababa, Abera *et al*. (2010) ( 96.4%) Gondar, Zewdu *et al*. (2021) (90.5%) ,central Ethiopia. All of the *S. aureus* isolates(100%) were susceptible to Norfloxacin which was in line with the finding of Sori *et al*. (2011) who reported 100.0% sensitivity to Norfloxacin in Jimma town.

Gentamicin (CN) also showed excellent performance in the current study, matching the results by Abebe and Ashenafi (2016) 98% susceptibility, Debre Zeit. Similarly, Mekonnen *et al*. (2018) observed 93.7% susceptibility in Addis Ababa.Sulphamethoxazole-Trimethoprim (SXT) showed good effectiveness, with 80% susceptibility and 20% resistance. This finding is consistent with Tassew *et al*. (2018) (77%) susceptibility, Mekelle, Northern Ethiopia.In contrast, Omar *et al*. (2024) reported resistance levels of up to 40% in Uganda.

Streptomycin (S) showed moderate activity, with 40% susceptibility and a high resistance rate of 60%. Several Ethiopian studies corroborate these findings. Tassew *et al*. (2018) reported a 66% resistance rate among *S. aureus* isolates Mekelle. This nation wide trend may reflect the long-term, unregulated use of streptomycin in veterinary and mixed-use (human-animal) settings, especially in rural and peri-urban areas where alternative drugs are less accessible. A study by Abebe *et al*. (2020) in the Oromia region found even higher resistance levels exceeding 70%, particularly among isolates from backyard dairy farms where antibiotics are frequently administered without veterinary guidance. Beyond Ethiopia, studies in neighboring East African countries echo these concerns. Omar *et al*. (2024) reported streptomycin resistance rates of 55–70% in *S. aureus* isolates from cattle in Kenya, Uganda, and Tanzania using metagenomic surveillance.

In the current study, *S. aureus* isolates exhibited nearly equal levels of susceptibility (52.3%) and resistance (47.7%**)** to Cefoxitin (FOX), a second-generation cephamycin antibiotic often used as a surrogate marker for detecting methicillin resistance (MRSA). This finding is consistent with reports by Tassew *et al*. (2018) and Omar *et al*. (2024), who documented a 48% prevalence in Uganda. These comparable results suggest a widespread occurrence of

MRSA in livestock production systems across Ethiopia and the region, likely driven by the misuse of β-lactam antibiotics and inadequate biosecurity measures. Notably, resistance to Cefoxitin is not unique to Ethiopia, indicating a broader regional or even global trend in MRSA resistance patterns The 47.7% resistance of *S. aureus* to Cefoxitin observed in this study was lower than the rates reported in previous studies, such as Ayele *et al*. (2017) in Sebeta town, and Aliyu *et al*. (2019) and Yakubu *et al*. (2020) in Nasarawa State, Nigeria, all of whom reported 100% resistance to Cefoxitin. However, Mekonnen *et al*. (2018) reported 100% sensitive *S. aureus* isolates to Cefoxitin in North-Western Ethiopia and Sudhanthiramani *et al*. (2015) reported low magnitude of Cefoxitin resistance (4.65%) in Tirupathi, India.

The widespread and often inappropriate use of antibiotics in both human and veterinary healthcare has played a major role in the emergence and increment of antimicrobial resistance among pathogens (Mokgophi *et al*., 2021; Qamar *et al*., 2020). This may explain the elevated resistance levels of *S. aureus* to commonly used and economically accessible antibiotics.

*S.aureus* is widely known for its ability to swiftly develop resistance to a broad range of antibiotic classes (Parvin *et al*., 2021). The development of resistance to multiple antimicrobial agents heightens both the severity and risk associated with infections it causes. Furtherrmore, the appearance and dissemination of methicillin-resistant strains linked to livestock (LA-MRSA) have become a serious public health problem (Tamendjari *et al*., 2021). In the current study, antimicrobial susceptibility testing performed in vitro demonstrated that all *S. aureus* isolates (100%) were resistant to three or more antibiotics, indicating multidrug resistance (MDR). This outcome is in line with earlier findings by Girmay *et al.* (2020) in Shire Town, Beyene *et al*. (2017) in Addis Ababa, and Weldeselassie *et al*. (2020) in Mekelle, who documented MDR rates of 100.0%, 100.0%, and 93.75%, respectively.

The present study evaluated key aspects of sanitation, hygiene, antibiotic use and general farm management among dairy farmers in the study area. The findings showed that 60% of respondents clean animal sheds daily and 70% change bedding daily showing good sanitation practices.These findings are similar to those of Megersa *et al*. (2020), who observed similar routine cleaning practices among urban dairy farmers in Ethiopia, though they renowed variability depending on farmers' awareness and training. Contrary studies from smallholder farms in Europe, such as Fessler *et al*. (2010), reported more structured hygiene practices due to strict regulations and typical veterinary oversight.

However, the use of disinfectants in this study was limited, with only 40% of farmers using them and just 30% applying them daily. This agrees with the observations of  Oliver *et al.* (2005), who found that lack of  knowledge and access to disinfectants often impede their regular use in low resource settings. Despite the inadequate use of disinfectants, pest control was practiced more , with 75% of farmers applying measures daily. This is larger than pest control rates reported in European farms where Van Duijkeren *et al*. (2010) noted that inadequate pest management contributed to the spread of resistant pathogens, including MRSA, in Netherland.

Water and waste management practices also presented mixed results.100% of respondents relied on tap water, 40% stored it in open containers and 80% had never tested their water for quality. The  trends were noted by Megersa *et al*. (2020), who disclosed low awareness and lack of regular water testing among Ethiopian dairy farmers. According to EFSA (2009), lack of proper storage and untesting water is a major risk factor for microbial contamination.

The study also showed concerning trends in antibiotic use. Although 80% of farmers relied on veterinarians for treatment, 40% admitted to using antibiotics as growth promoters and 70% did not keep proper records. Only 30% of respondents were fully aware of antibiotic resistance. These findings are inline with those of Tenhagen *et al*. (2009), who found poor documentation and awareness in German livestock farms, although at lower rates than observed in this study. The World Health Organization (WHO, 2017) has strongly prohibited the use of antibiotics for growth promotion, citing its contribution to antimicrobial resistance (AMR).

In terms of milking practices, the study showed that 75% of farmers washed udders before milking, and 60% always washed their hands. However, only 40% stored milk under refrigeration. These findings are inline with those of Megersa *et al*. (2020), who highlighted good milking hygiene but limited access to refrigeration facilities. In contrast, dairy farms in developed countries, such as those in the United States, have near universal use of cold chain storage, highly decreasing microbial risks (Oliver *et al*., 2005).

Biosecurity practices were suboptimal in the study area. Only 30% of farms quarantined new animals, and 50% of workers received hygiene training. Van Duijkeren *et al*. (2010) emphasized that quarantine procedures and regular training are critical in reducing disease transmission, including MRSA, especially in densely populated farming areas.

Overall, while the surveyed farms demonstrated relatively good hygiene and some responsible veterinary practices, significant gaps remain in biosecurity, antibiotic stewardship, and awareness of AMR. The findings call for improved farmer education, stricter regulatory oversight, and the implementation of best practices to mitigate public health risks associated with dairy farming in the region.

## 6. LIMITATIONS

A limitation of this research is that molecular characterization and resistance gene detection among animal, human and environment samples were not carried because of lack of resources in terms of money and primers.

# 7. CONCLUSION AND RECOMMENDATIONS

The results of this study showed a moderate prevalence of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) in dairy farms across Sheger city. The findings highlighted that udder milk is a major reservoir for MRSA, while hand swabs showed the highest incidence of *S. aureus.* Poor hygiene practices among dairy workers and inadequate environmental management, particularly drainage systems, contributed to higher contamination rates.The threatening levels of antibiotic resistance observed emphasizes the necessary need for better antibiotic stewardship in dairy farming.

Based on the conclusions of this study, the following recommendations are proposed:

- enforce regular training programs on proper handwashing and udder cleaning techniques for dairy farmers
- enhance the use of antiseptics and ensure proper application to decrease contamination risks.
- Invest in good drainage systems and overall farm sanitation to minimize the persistence of pathogens in the dairy farm environment.
- Enforce quarantine procedures for new animals and ensure regular veterinary management to reduce disease transmission.
- Encourage responsible use of antibiotics, avoiding their use as growth promoters, and ensure adherence to veterinary prescriptions.
- Raise awareness among farmers regarding the implications of antimicrobial resistance on public health and the importance of adhering to best practices.
- Promote collaboration between veterinary, public health, and environmental sectors to combat antibiotic resistance.

# 8. REFERENCES

Abebe, E., Gugsa, G., and Ahmed, M. (2020). Review on major food-borne zoonotic bacterial pathogens. *Journal of Tropical. Medicine*,**1,** 1–19

Abebe, R. and Ashenafi, H. (2016). Bacteriological quality and antimicrobial susceptibility of *Staphylococcus aureus* isolated from raw milk samples collected from dairy farms in Debre Zeit, Ethiopia. *African Journal of Microbiology Research*, **10**(17), 574–579.

Abebe, R., Hatiya, H., Abera, M., Megersa, B., & Asmare, K. (2016). Bovine mastitis: Prevalence, risk factors and isolation of *Staphylococcus aureus* in dairy herds at Hawassa milk shed, South Ethiopia. *BMC Veterinary Research*, ***12***(1), 270

Abera M, Demie B, Aragaw K, Regassa F, Regassa A (2010). Isolation and identification of *Staphylococcus aureus* from bovine mastitic milk and their drug resistance patterns in Adama town, Ethiopia. *Journal of Veterinary Medicine Animal Health*, **2**(3):29–34.

Abera, B., Kibret, M., and Mulu, W. (2014). Knowledge and beliefs on antimicrobial resistance among physicians and nurses in hospitals in Amhara Region, Ethiopia. *BMC Pharmacology and Toxicology*, **15**(1), 26

Aliyu, Y., Abdullahi, I. O., Whong, C. M. Z., and Olayinka, B. O. (2019). Antibiotic resistant phenotypes of *Staphylococcus aureus* isolated from fresh and fermented milk in parts of Nasarawa State, Nigeria. *Africa Journal of Microbiology. Resource*. 1**3**, 446–456.

Almeida RA, Matthews KR, Cifrian E, Guidry AJ and Oliver SP. (1996): *S. aureus* invasion of bovine mammary epithelial cells. *J Dairy Sci* **79**:1021–1026.

Anand, K., Agrawal, P., Kumar, S. and Kapila, K. (2009). Comparison of cefoxitin disc diffusion test, oxacillin screen agar, and PCR for *mecA* gene for detection of MRSA. *Indian Journal of Medical Microbiology*, **27**(1), 27–29.

Asiimwe B, Baldan R, Trovato A, and Cirillo D. (2017). Prevalence and molecular characteristics of *Staphylococcus aureus*, including methicillin resistant strains, isolated from bulk can milk and raw milk products in pastoral communities of South-West Uganda. BMC *Infectious Disease*, **17**(1):422-4

Asmelash, Tesfaye, Mesfin., Nigatu, Addisu., Dagnachew, Mersha., Chalie, and Tsegaye, Shewaneh. (2016). Isolation, identification and drug resistance patterns of methicillin-resistant *Staphylococcus aureus* from mastitic cow's milk from selected dairy farms in and around Kombolcha, Ethiopia. *Journal of Veterinary Medicine and Animal Health*, ***8***(1), 1–10.

Ayana, H. W., Mekonnen, B. T., Bulle, A. S., and Berecha, M. S. (2017). Isolation and identification of methicillin-resistant *Staphylococcus aureus* from mastitic dairy cows in Bishoftu town, Ethiopia. *Africa Journal of Microbiology Research*.**11**, 1606–1613

Ayele Y, Gutema F, and Edao B. (2017). Assessment of *Staphylococcus aureus* along milk value chain and its public health importance inSebeta, Central Oromia, Ethiopia. *BMC Microbiology*,**17**(1):141.

Azevedo, C., Pacheco, D., Soares, L., Romão, R., Moitoso, M., Maldonado, J., and Simões, J.(2016). Prevalence of contagious and environmental mastitis-causing bacteria in bulk tank milk and its relationships with milking practices of dairy cattle herds in São Miguel Island (Azores). *Tropical animal health and production*, **48**, 451-459.

Bardiau M, Caplin J, Detilleux J, Graber H, Moroni P, Taminiau B, and Mainil J. (2016). Existence of two groups of *Staphylococcus aureus* strains isolated from bovine mastitis based on biofilm formation, intracellular survival, capsular profile and agr-typing. *Veterinary Microbiology*, **185**,1–6

Bayles K, Wesson C, Liou L, Fox L, Bohach G, and Trumble W. (1998).Intracellular *Staphylococcus aureus* escapes the endosome and induces apoptosis in epithelial cells. *Infectious Immunology*, **66**,336–342.

Becker, K., Heilmann, C.and Peters, G (2014). Coagulase-negative staphylococci. *Clinical Microbiology Review*, **27**, 870-926.

Begum, H. A., Uddin, M. S., Islam, M. J., Nazir, I. M. K., and Rahman, M. T. (2007). Detection of biofilm producing coagulase positive *Staphylococcus aureus* from bovine mastitis, their pigment production, hemolytic activity and antibiotic sensitivity pattern. *Journal of Bangladesh Society for Agricultural Science and Technology*, ***4***, 97–100.

Bekele, M., Mamo, G., Mulat, S., Ameni, G., Beyene, G., and Tekeba, E. (2016). Epidemiology of bovine tuberculosis and its public health significance in Debre-Zeit intensive dairy farms, Ethiopia. *Journal of Biomedical and Nursing*, **2**(2), 8–18.

Berge, A. C., and Baars, T. (2020). Raw milk producers with high levels of hygiene and safety. *Epidemiology and Infection*, **148**, e14

Beyene, T., Endalamaw, D., Tolossa, Y. H., Feyisa, A., and Hunduma, D. (2021). Antimicrobial resistance in Ethiopia: A systematic review of the literature covering twenty years (2000–2020). *One Health, **13***, 10031

Beyene, Tesfaye, Hayishe, Hamid, Gizaw, Fikadu, Beyi, Aklilu Feleke, Abunna, Fufa, Mammo, Getachew, and Grace, Delia. (2017). Prevalence and antimicrobial resistance profile of *Staphylococcus* in dairy farms, abattoir, and humans in Addis Ababa, Ethiopia. *BMC Research Notes, **10***(1), 171

Bhati, T., Gaurav, K., Khichar, V., and Kataria, A. (2018). Prevalence of *Staphylococcus aureus* isolated from mastitic milk, udder surfaces and milkers' hands from different farms in Bikaner, Rajasthan. *Indian Journal of Animal Research*, ***52***(5), 867–870

Biffa D, Debela E, Beyene F.(2005). Prevalence and risk factors of mastitis in lactating dairy cows in Southern Ethiopia. *International Journal of Applied Research Veterinary medicine Medicine* ,**3**,1–10.

Bizzini, A., Durussel, C., Bille, J., Greub, G., and Prod'Hom, G. (2010). Performance of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of bacterial strains routinely isolated in a clinical microbiology laboratory. *Journal of Clinical Microbiology*, **48**(5), 1549-1554

Borena, B. M., Gurmessa, F. T., Gebremedhin, E. Z., Sarba, E. J., and Marami, L. M. (2023). *Staphylococcus aureus* in cow milk and milk products in Ambo and Bako towns,Oromia, Ethiopia: prevalence, associated risk factors, hygienic quality, and antibiogram. *International Microbiology*, **1**,15.

Bua A, Usai D, Donadu MG. (2018). Antimicrobial activity of Austroeupatorium inulaefolium (HBK) against intracellular and extracellular organisms. *Natural Product Research* ,**2** (23), 2869–2871.

Burke SL and Rose WE. (2014). New pharmacological treatments for methicillin-resistant *Staphylococcus aureus* infections. *Expert Opin Pharmacotherapy*,**15**,483–491

Carfora V, Giacinti G, Sagrafoli D, (2016): *S. aureus* in dairy sheep and in-contact humans: an Intra-Farm Study. *American Dairy Science Asso*ciation, **99**(6),4251–4258.

Catry, B., Van Duijkeren, E., Pomba, M., Greko, C., Moreno, M.,Pyorala, S., and Ruzauskas, M. (2010). Reflection paper on MRSA in food-producing and companion animals.

Epidemiology and control options for human and animal health. *Epidemiology and Infection*, **138**(5): 626-644.

Centers for Disease Control and Prevention (CDC). (2003). *Methicillin-resistant S. aureus infections in correctional facilities—Georgia, California, and Texas, 2001–2003*. *Morbidity and Mortality Weekly Report (MMWR), **52***(41), 992–996.

Chakravarty, S. and Massé, E. (2019). RNA-dependent regulation of virulence in pathogenic bacteria. *Frontiers in Cellular and Infection Microbiology*, **9**,337.

Chambers H, Deleo F (2009). Waves of resistance: *staphylococcus aureus* in the antibiotic era. *National Rev Microbiology,* **7**(9),629–641.

Chen C, and Wu F (2021).Livestock-associated methicillin-resistant *S. aureus* (LA-MRSA) colonisation and infection among livestock workers and veterinarians: a systematic review and meta-analysis. *Occupational Environmental Medicine*,**78**(7),465–471

Chukwunonso, Ekene, Veronica, Blessing, Toyo, Precious, & others. (2018). Methicillin-resistant *Staphylococcus aureus*. *International Journal of Medical Research & Health Sciences, **7***(1), 122–127.

CLSI. (2019). Performance standards for antimicrobial susceptibility testing, 29th ed. In: CLSI Supplement M100. Wayne, PA: *Clinical and Laboratory Standards Institute*; 2019.

CLSI. (2022). Performance standards for antimicrobial susceptibility testing (32nd ed., CLSI supplement M100). Clinical and Laboratory Standards Institute.

Cortimiglia C, Luini M, Bianchini V.(2016). Prevalence of *Staphylococcus aureus* and of methicillin-resistant *S. aureus* clonal complexes in bulk tank milk from dairy cattle herds in LombardyRegion (Northern Italy). *Epidemiology Infectious*. **144**(14)

Crosby, H., Kwiecinski, J., Horswill, A., Roy, J., and City, I.(2016). *Staphylocoocus aureus* aggregation and coagulation mechanisms and their function in host-pathogen interactions. *Advance in Applied Microbiology,* **96**, 1-41.

Croxatto, A., Prod'hom, G., and Greub, G. (2012).Applications of MALDI-TOF massspectrometry in clinical diagnostic microbiology. *FEMS Microbiology Reviews*, **36**(2), 380-407.

Cuny C, Wieler LH, Witte W.(2015). Livestock-associated MRSA: the impact on humans. *Antibiotics*,**4**(4),521–543.

Cuny, C., Friedrich, A., Kozytska, S., Layer, F., Nübel, U, and Ohlsen, K.(2010). Emergence of MRSA in different animal species. *International Journal of Medical Microbiology*, **300**(2):109-117.

Daka, D., G/silassie, S., and Yihdego, D. (2012). Antibiotic-resistance *Staphylococcus aureus* isolated from cow's milk in the Hawassa area, South Ethiopia. *Annals of Clinical Microbiology and Antimicrobials*, **11**, 26

Daniel Crespo-Piazuelo and Peadar G. Lawlor., (2021a).Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) prevalence in humans in close contact with animals and measures to reduce on-farm colonization. *Iran Veterinary J*ournal,**74**,21

Daniel, D. S., Lee, S. M., & Veeraraghavan, B. (2021b). Antimicrobial resistance: a global concern. *Current Infectious Disease Reports*, **23**(11), 1–10.

de Nies, L., Lopes, S., Busi, S. B., Chan, B. K. and Mazel, D. (2021). Antibiotic resistance determinants in environmental resistomes: Identification, linkage, and the role of mobile genetic elements. *Current Opinion in Microbiology*, ***62***, 1–9.

Demissie, T., Alemayehu, H., Fentahun, T., and Getaneh, A. (2019). Prevalence and antimicrobial susceptibility pattern of *Staphylococcus* species isolated from bovine mastitis in Meta District, East Hararghe Zone, Eastern Ethiopia. *Journal of Veterinary Medicine and Animal Health*, **11**(10), 147–153.

Derib, B. T., Birhanu, B. T., Tessema, T. S., & Wubshet, A. K. (2017). Isolation and identification of methicillin resistant *Staphylococcus aureus* (MRSA) from bovine mastitic milk in and around Wolaita Sodo, Southern Ethiopia. *Open Access Journal of Veterinary Science and Research*, **2**(3), 1–11.

Desta, Kassu, Aklillu, Elias, Gebrehiwot, Yimtubezinash, Asrat, Daniel, Tesfaye, Mulugeta, Woldeamanuel, Yohannes, and Schultsz, Constance. (2022). High levels of methicillin-resistant *Staphylococcus aureus* carriage among healthcare workers at a teaching hospital in Addis Ababa, Ethiopia: First evidence using *mecA* detection. *Infection and Drug Resistance*, ***15,*** 3135–3147

EFSA (European Food Safety Authority). (2009). Scientific Opinion on a Quantitative Microbiological Risk Assessment of MRSA in the Food Chain. *EFSA Journal*, **7**(10), 1383

Elton L, Thomason MJ, Tembo J, Velavan TP, Pallerla SR, Arruda LB, (2020): Antimicrobial resistance preparedness in sub-Saharan African countries. *Antimicrob Resist Infect Control* ,**9**(1),145.

Escher, M., Scaioli, G., Merten, S., Ezat, W. P. S., & Tang, S. (2021). Antibiotic use and resistance in LMICs: A scoping review of primary care perspectives. *Biomedical Medicine Journal of Global Health*, ***6***(3), e005245.

FDA. (2021). 2020 Summary Report on Antimicrobials Sold or Distributed for Use in Food-Producing Animals. U.S. Food and Drug Administration, Center for Veterinary Medicine.

Fessler, A. T., Olde Riekerink, R. G., Rothkamp, A., Kadlec, K., Sampimon, O. C., Lam, T. J. G. M., and Schwarz, S. (2010). Characterization of methicillin-resistant *Staphylococcus aureus* isolates from food and food-producing animals in Germany. *Journal of Antimicrobial Chemotherapy*, **65**(3), 619–625.

Fetsch A, Etter D, Johler S.(2021). Livestock-associated methicillin-resistant *Staphylococcus aureus* current situation and impact from a one health perspective. *Current Clinical Microbiology Report*. **8**(3),103–113.

Feyissa, N., Alemu, T., Birri, D. J., and Dessalegn, A. (2023). Isolation, identification, and determination of antibiogram characteristics of *Staphylococcus aureus* in milk and dairy products in Addis Ababa and its surroundings, Ethiopia. *BMC Microbiology*, **23**(1), 1-13

Fisher EL, Otto M, and Cheung G. (2018): Basis of virulence in enterotoxin- mediated *staphylococcal* food poisoning. *Front Microbiology* **9**:436.

Fitzgerald, Julian R. (2012). Livestock-associated *S. aureus*: Origin, evolution and public health threat. *Trends in Microbiology*, ***20***(4), 192–198.

Food and Agriculture Organization of the United Nations (FAO). (2022). Wash your hands frequently! This is good for food safety and good for health. Open Knowledge FAO Food and Drug Administration ,U.S: Accessed on 22 May 2025).

Foster, T.J. (2005). Immune evasion by Staphylococci. *Nature Reviews Microbiology*, **3**, 948-958

Fox, L. K. (2009). Prevalence, incidence and risk factors of heifer mastitis. *Veterinary Microbiology*, **134**(1–2), 82–88.

Gajdács M, and Albericio F.(2019). Antibiotic resistance: from the bench to patients. *Antibiotics*, **8**:129.

Ganai, A. W., Wani, S. A., Wani, M. H., Hussain, I. and Malik, A. H. (2016). Detection of *mecA* gene of methicillin resistant *Staphylococcus aureus* by PCR assay from raw milk. *Indian Journal of Animal Sciences, **86***(5), 508–511.

Garedew, L., Berhanu, M., & Tsegay, G. (2020). Bacteriological quality and antimicrobial susceptibility pattern of raw cow milk in and around Mekelle, Tigray, Ethiopia. *Ethiopian Veterinary Journal,* **24**(2), 65–78.

Gebremeskel, Fekadu T., Alemayehu, Tinsae, and Ali, Mohammed M. (2022). Methicillin-resistant *S aureus* antibiotic susceptibility profile and associated factors among hospitalized patients at Hawassa University Comprehensive Specialized Hospital, Ethiopia. *IJID Regions,* **3**, 129–134

Gebretekle GB, Haile Mariam D, Abebe Taye W, Mulu Fentie A, Amogne Degu W, and Alemayehu T, (2020).Half of Prescribed Antibiotics Are Not Needed: A Pharmacist Led Antimicrobial Stewardship Intervention and Clinical Outcomes in a Referral Hospital in Ethiopia. *Front Public Health* ,**8**, 109.

Gebretekle GB, Haile Mariam D, Abebe W, Amogne W, Tenna A, Fenta TG, (2018).Opportunities and barriers to implementing antibiotic stewardship in low and middle-income countries: Lessons from a mixed-methods study in a tertiary care hospital in Ethiopia. *PLoS One* ,**13**(12)

Girmay W, Gugsa G, and Taddele H, (2020). Isolation and Identification of Methicillin-Resistant *Staphylococcus aureus* (MRSA) from Milk in Shire Dairy Farms, Tigray, Ethiopia. *Veternary Medicine International*, **1** ,1–7.

Gizaw, F., and Duguma, R. (2014). Staphylococcus: Epidemiology and its drug resistance in cattle, food Chains and humans in central Ethiopia. Bishoftu, Ethiopia: Addis Ababa University

Global Action Plan on Antimicrobial Resistance.(2015). World Health Organization; 2015a. Available from: https://www.who.int/antimicrobial-resistance/global- action- plan/en/. [Accessed 22 May 2025].

Graveland H, Duim B, and van Duijkeren E, (2011). Livestock-associated methicillin-resistant *S. aureus* in animals and humans. *International Journal of Medicine Microbiology*. **301**(8),630–634.

Guardabassi, Luca, Butaye, Patrick, Dockrell, David H., Fitzgerald, Julian R., and Kuijper, Ed J. (2020). One Health: A multifaceted concept combining diverse approaches to prevent and control antimicrobial resistance. *Clinical Microbiology and Infection, **26***(12), 1604–1605

Haas, P., De Haas, C. and Kleibeuker, W. (2004). N-terminal residues of the chemotaxis inhibitory protein of *Staphylococcus aureus* are essential for blocking formylated peptide receptor but not C5a receptor. *Journal of Immunology*, **173**(9), 5704-5711.

Hendriksen, R. S., Munk, P., Njage, P., van Bunnik, B., McNally, L., Lukjancenko, O. and Aarestrup, F. M. (2019). Global monitoring of antimicrobial resistance based on metagenomics analyses of urban sewage. *Nature Communications, **10***, 1124.

Hika, W. A., Biruk, T. M., Ashenafi, S. B., and Mekonnen, S. B. (2017). Isolation and identification of methicillin-resistant *Staphylococcus aureus* from mastitis dairy cows in Bishoftu town, Ethiopia. *African Journal of Microbiology Research*, **11**(44), 1606–1613

IACG (Interagency Coordination Group on Antimicrobial Resistance). (2019). No Time to Wait: Securing the Future from Drug-Resistant Infections. Report to the Secretary-General of the United Nations.

ISO 6888-3:(2003). Microbiology of food and animal feeding stuffs horizon-tal method for the detection and identification of Staphylococci. Part 3. Geneva, Switzerland. *Journal of Food Microbiology*,**64**,361–66.

Jaggi, P., Paule, S.M., Peterson, L.R. and Tan, T.Q. (2007). Characteristics of *Staphylococcus aureus* Infections, Chicago Pediatric Hospital. *Emerging Infectious Diseases*, **13**(2), 311-314.

Johler S, Weder D, Bridy C, Huguenin MC, Robert L, Hummerjohann J, and Stephan R. (2015). Outbreak of *staphylococcal* food poisoning among children and staff at a Swiss boarding school due to soft cheese made from raw milk. *Jornal of Dairy Science* ,**98**, 2944–2948.

Kalayu AA, Woldetsadik DA, Woldeamanuel Y, Wang S-H, Gebreyes WA and Teferi T. (2020). Burden and antimicrobial resistance of *S. aureus* in dairy farms in Mekelle, Northern Ethiopia. *BMC Veterinary Resource*, **16**(1), 20

Kashif, A., McClure, J.A., Lakhundi, S., Pham, M., Chen, S., Conly, J.M. and Zhang, K. (2019). *Staphylococcus aureus* ST398 Virulence is Associated with Factors Carried on Prophage ΦSa3. *Frontiers in Microbiology*, **10**, 2219.

Khalili, H., Soltani, R., Negahban, S., Abdollahi, A. and Gholami, K. (2012). Reliability ofMdisk diffusion test results for the antimicrobial susceptibility testing of nosocomial gram-positive microorganisms is E-test method better. *Iranian Journal of Pharmaceutical Research*, **11**(2), 559

Kimera, Z. I., Mshana, S. E., Rweyemamu, M. M., Mboera, L. E. G. and Matee, M. I. N. (2020). Antimicrobial use and resistance in food-producing animals and the environment: An African perspective. *Antibiotics, **9***(12),919.

Kivaria, F., M., Noordhuizen, J., P. T. M., and Kapaga, A. M. (2006). Risk indicators associated with subclinical mastitis in smallholder dairy cows in Tanzania. *Tropical Animal Health and Production*, **38(**6), 429–436.

Kong, C., Neoh, H. and Nathan, S. (2016). Targeting *Staphylococcus aureus* Toxins: A Potential form of Anti Virulence Therapy. *Toxins,* **8,** 72.

Larsen J, Stegger M, Andersen PS, (2016). Evidence for human adaptation and foodborne transmission of livestock-associated methicillin-resistant *Staphylococcus aureus, Clinical Infectious Disease*, **63**(10),1349–1352

Lemma, F., Alemayehu, H., Stringer, A., and Eguale, T. (2021). Prevalence and antimicrobial susceptibility profile of *Staphylococcus aureus* in milk and traditionally processed dairy products in Addis Ababa, Ethiopia. BioMed Research International

Liu, B., Sun, H., Pan, Y., Gao, Y., Liu, Q., Zhang, X., and Liu, J. (2018). Prevalence, resistance pattern, and molecular characterization of *Staphylococcus aureus* isolates from healthy animals and sick populations in Henan Province, China. *Gut Pathogens*, ***10***(1), 31.

Lowy, F. D, (2003): Antimicrobial resistance, the example *of Staphylococcus aureus*. The *Journal of Clinical Investigation*, **111**(9),1265-1273.

Lowy, F. D**.** (1998). *Staphylococcus aureus* infections. *The New England Journal of Medicine*, ***339*** (8), 520–532

Lozano, Carmen, Gharsa, Haythem, Ben Slama, Khaled, Zarazaga, Margarita, and Torres, Carmen. (2016). *Staphylococcus aureus* in animals and food: Methicillin resistance, prevalence and population structure. A review in the African continent. *Microorganisms*, ***4***(12), 1–19.

Mathenge, J. M., Mbaria, J. M., Okemo, P. O., Ng'ang'a, P. M., & Gicheru, M. M. (2017). Multiple drug resistance *Staphylococcus aureus* isolated in foods of animal origin in Nairobi, Kenya. *East African Medical Journal*, ***94***, 180–185.

McMillan, K., Moore, S. C., McAuley, C. M., Fegan, N., and Fox, E. M**.** (2016). Characterization of *Staphylococcus aureus* isolates from raw milk sources in Victoria, Australia. *BMC Microbiology*, ***16***(1), 1–12.

Megersa, M., Tadesse, E., and Alemayehu, R. (2020). Dairy Farmers' Knowledge, Attitudes, and Practices towards Milk Hygiene and Public Health Risks in Addis Ababa, Ethiopia. *Veterinary Medicine International*, **10,** 5

Mekonnen, S. A., Lam, T. J. G. M., Hoekstra, J., Rutten, V. P. M. G., Tessema, T.S., Broens, E. M. (2018). Characterization of *Staphylococcus aureus* isolated from milk samples of dairy cows in small holder farms of North-Western Ethiopia. *BMC Veterinary Resource* ,**14**, 1–8.

Mekuria, A., Asrat, D., Woldeamanuel, Y., and Tefera, G. (2013). Identification and antimicrobial susceptibility of Staphylococcus aureus isolated from milk samples of dairy cows and nasal swabs of farm workers in selected dairy farms around Addis Ababa, Ethiopia. *African Journal of Microbiology Research*, **7**(27), 3501-3510.

Mekuriya, Eyob, Manilal, Anish, and Aklilu, Aemiro, (2022). Methicillin-resistant *Staphylococcus aureus* colonization among medicine and health science students, Arba Minch University, Ethiopia. *Scientific Reports*, **12**(1), 1–11

Melchior, M. B., Fink-Gremmels, J., and Gaastra, W. (2006). Biofilms: A role in recurrent mastitis infections? *Veterinary Journal*, **171**(3), 398–407.

Mithu, S. R., Islam, M. Z., Nahar, A., and Hasan, M. N. (2024). Livestock-associated MRSA: Prevalence, risk factors, and control strategies. *One Health Advances*, **2**(1), 1–12.

Mohammed, J., Ziwa, M. H., Mahuton, Y., Hounmanou, G., Kisanga, A., and Tuntufye, H. N. (2018). Molecular typing and antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* isolated from bovine milk in Tanzania. *International Journal of Microbiology*, **414**

Mokgophi, T. M., Gcebe, N., Fasina, F., and Adesiyun, A. A. (2021). Antimicrobial resistance profiles of Salmonella isolates on chickens processed and retailed at outletsof the informal market in Gauteng Province, South Africa, *Pathogens* **10**, 1–15.

Muluneh, C., Gebremichael, S., and Moges, F. (2023). Prevalence and antimicrobial susceptibility pattern of *Staphylococcus aureus*, including MRSA isolates, from bovine milk in Ethiopia: A systematic review and meta-analysis. *Veterinary Medicine and Science*, **9**(1), 256–270.

Nomura, F. (2015). Proteome-based bacterial identification using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS): A revolutionary shift in clinical diagnostic microbiology. *Biochimica Biophysica Acta(BBA)-Proteins and Proteomics*, **1854** (6), 528-537

Oliver, S. P., Jayarao, B. M., and Almeida, R. A. (2005). Foodborne Pathogens in Milk and the Dairy Farm Environment: Food Safety and Public Health Implications. *Foodborne Pathogens and Disease*, **2**(2), 115–129.

Omar KM, Kitundu GL, Jimoh AO, Namikelwa DN, Lisso FM, Babajide AA, Olufemi SE, and Awe OI. (2024). Investigating antimicrobial resistance genes in Kenya, Uganda and Tanzania cattle using metagenomics, *Peer Journal* **12**,171

Otto, M. (2004). Quorum sensing control in *staphylococci* a target for antimicrobial drug therapy. FEMS Microbiology Letters, **241**(2), 135-41.

Otto, M. (2008). *Staphylococcal* Biofilms. Current Topics in Microbiology and Immunology, **322**, 207-228.

Pal, M. (2007): Zoonoses. 2nd Edition. Satyam Publishers, Jaipur,India.

Pal, M., Kerorsa, G., Megersa, L. and Kandi V.(2020). Epidemiology, pathogenicity, animal infections, antibiotic resistance, public health significance, and economic impact of *S. aureus*: a comprehensive review. *American Journal of Public Health Research*, **8**, 14-21.

Pal, M., Gutama, K.P. and Koliopoulos, T.(2021). *Staphylococcus aureus*, an important pathogen of public health and economic importance: A comprehensive review. *Journal of Emerging Environmental Technologies and Health Protection, **4**(2),17-32.*

Pantosti, A (2012). Methicillin-resistant *Staphylococcus aureus* associated with animals and its relevance to human health, *Frontier Microbiology*, **3**,127

Parvin, S., Ali, Y., Talukder, S., Nahar, A., Chowdhury, E. H., Rahman, T. (2021). Prevalence and multidrug resistance pattern of methicillin resistant *S.aureus* isolated from frozen chicken meat in Bangladesh. *Microorganisms*, **9**, 1–16

Patel, R. (2005). Biofilms and antimicrobial resistance. *Clinical Orthopedics and Related Research*, **5**, 41-47

Piepers S, De Vliegher S, de Kruif A, Opsomer G, and Barkema H.( 2009). Impact of intramammary infections in dairy heifers on future udder health, milk production, and culling. *Veterinary Microbiology*, **134**,113– 120.

Pitkälä A, Haveri M, Pyörälä S, Myllys V, Honkanen-Buzalski T(2004). Bovine mastitis in Finland prevalence, distribution of bacteria, and antimicrobial resistance. *Journal Dairy Science*, **87**, 2433–2441.

Qamar, A., Ismail, T., and Akhtar, S. (2020). Prevalence and antibiotic resistance of Salmonella spp. in South Punjab-Pakistan. *PLoS ONE*, **15**, 1–15

Quinn, P.J., Markey, B.K., Leonard, F.C., FitzPatrick, E.S., Fanning, S. and Hartigan, P.J. (2011). *Staphylococcus Species*: *Veterinary Microbiology and Microbial Disease*. 2nd Edition. Dublin,159-165.

Rahi A, Kazemeini H, Jafariaskari S, Seif A, Hosseini S, Safarpoor Dehkordi F.(2020). Genotypic and phenotypic-based assessment of antibioticresistance and profile of *staphylococcal* cassette chromosome mec in the methicillin resistant *Staphylococcus aureus* recovered from raw milk.*Infectious Drug Resistant*, **30**(13),273–283.

Rasigade, J. P., Vandenesch, F., and Etienne, J. (2014). New epidemiology of *Staphylococcus aureus infections*: emergence of community-acquired methicillin-resistant strains. **44** (8), 289–295.

Rasigade, JP., and Vandenesch, F. (2014). *Staphylococcus aureus*: A pathogen with still unresolved issues. *Infection, Genetics and Evolution*, **21**, 510–514.

Regasa S, Mengistu S, Abraha A. (2019): Milk safety assessment, isolation and antimicrobial susceptibility profile of *Staphylococcus aureus* in selected dairy farms of Mukaturi and Sululta Towns, Oromia Region, Ethiopia. *Veterinary Medicine International,* **2**,1–11

Reta, M. ., and Alemayehu, T. (2020). Methicillin-resistant *S aureus* in Ethiopia: A systematic review and meta-analysis. *BMC Infectious Diseases, **20***, 599.

Ruchi, Tiwari., Yadav, Surendra Kumar, and Shanker, Shiv. (2016). Methicillin-resistant *S aureus* isolated from wounds of livestock and companion animals of Uttar Pradesh, India: A preliminary study. *International Journal of Pharmacology*, ***12***(8), 821–829.

Ruegg, P. L. (2021). Managing cows, milking, and the environment to minimize mastitis. *Proceedings of the National Mastitis Council Annual Meeting*, 40–50.

Saini, V., McClure, J. T., Léger, D., and Barkema, H. W. (2012). Prevalence of and risk factors for anti-microbial resistance in *Staphylococcus aureus* isolated from dairy cows in Canada. *Journal of Dairy Science*, **95**(3), 1553–1566.

Sathyamoorthy, Ranjitha, Vinay, Prasanna, D'Souza, Aaron O. and Vinod, Rachana. (2022). Comparison of phenotypic and genotypic characterization methods for the detection of methicillin-resistant *Staphylococcus aureus*. *Cureus*, ***14***(1)

Seedy, F. R. E., Samy, A. A., Salam, H. S. H., Khairy, E. A.,and Koraney, A. A. (2017). Polymerase chain reaction detection of genes responsible for multiple antibiotic resistance *Staphylococcus aureus* isolated from food of animal origin in Egypt. *Veterinary World*, **10**(10), 1205–1211.

Sharma, P., Dahiya, D. K., Balda, S., and Rathee, M. (2017). Prevalence and antibiotic resistance of methicillin-resistant *Staphylococcus aureus* in milk and milk products in India. *Veterinary World*, **10**(6), 738–742.

Shibabaw, A., Abate, D., Lakew, A. and Mihret, A. (2021). Antimicrobial resistance profiles of *Staphylococcus aureus* isolated from clinical samples in Ethiopia: A systematic review and meta-analysis. *PLOS ONE*, **16**(6), 10

Silva, V., Almeida, F., Maltez, L., Pereira, J. E., Oliveira, C., Mendo, S., and Capelo, J. L. (2022a). Insights into livestock-associated MRSA: Epidemiology, molecular characteristics, and zoonotic implications. *Antibiotics, **11***(2), 178.

Sori, H., Zerihun, A., and Abdicho, S. (2011). Dairy cattle mastitis in and around Sebeta, Ethiopia. *Journal of Animal and Veterinary Advances*, **10**(6), 738–743.

Spoor, Laura E., McAdam, Paul R., Weinert, Lucy A., Rambaut, Andrew, Hasman, Henrik, Aarestrup, Frank M. and Fitzgerald, Julian R. (2013). Livestock origin for a human pandemic clone of community-associated methicillin-resistant *Staphylococcus aureus*. *mBio*, ***4***(4), e00356–13

Sudhanthiramani, S., Swetha, C. S., and Bharathy, S. (2015). Prevalence of antibiotic resistant *Staphylococcus aureus* from raw milk samples collected from the local vendors in the region of Tirupathi, India. *Veterinary World*. **8**, 478–481

Tadesse, T., Tessema, T. S., & Beyene, G. (2020). Prevalence and antimicrobial resistance profile of methicillin-resistant *Staphylococcus aureus* isolated from bovine mastitis milk in selected dairy farms of Addis Ababa, Ethiopia. *BMC Veterinary Research*, **16**(1), 1–7.

Tamendjari, S., Bouzebda, F. A., Chaib, L., Aggad, H., Ramdani, M., and Bouzebda,Z. (2021). Antibiotic resistance of *Staphylococcus aureus* isolated from raw cow and goat milk produced in the Tiaret and Souk Ahras areas of Algeria. *Veterinary World*, **14**,1929–1934.

Tassew, A., Aki, A., and Legesse, K. (2018). Isolation, identification and antimicrobial resistance profile of *Staphylococcus aureus* and occurrence of methicillin-resistant *S. aureus* isolated from mastitic lactating cows in and around Assosa Town, Benishangul Gumuz Region, Ethiopia. *Journal of DairyVeterinary & Animal Research,* ***6***(6), 180.

Tefera, Sisay, Awoke, Tadesse, and Mekonnen, Desalegn. (2021). Methicillin and vancomycin-resistant *S. aureus* and associated factors from surgical ward inpatients at Debre Markos referral hospital, northwest Ethiopia. *Infection and Drug Resistance*, **14**, 3053–3062

Tenhagen, B. A., Alt, K., Pfeffer, M., and Wiehle, L. (2009). Methicillin-resistant *Staphylococcus aureus* in Dairy Herds in Germany. *Deutsche Tierarztliche Wochenschrift*, **116**(9), 316–320.

Tessema, F. (2017). Review on the prevalence and drug resistance patterns of *Staphylococcus aureus* in food producing animals, their products and humans. *Internal Journal of Biological Sciences and Medicine,* **1**, 5.

Thrusfield M. Veterinary Epidemiology.(2005): 3rd ed. Cambridge, USA: Blackwell Science Ltd, **225**–228 p.

Tibebu, Lemma, Belete, Yitayih, Tigabu, Ermias, & Tsegaye, Wondimagegn. (2021). Prevalence of *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* and potential risk factors in selected dairy farms at the interface of animal and human in Bishoftu, Ethiopia. *Veterinary Medicine: Research and Reports*, **12**, 241–251

Tigabu, E., Kassa, T., Asrat, D., Alemayehu, H., Sinmegn, T., Adkins, P. R., and Gebreyes,W. (2015). Phenotypic and genotypic characterization of *Staphylococcus aureus* isolates recovered from bovine milk in central highlands of Ethiopia. *African Journal of Microbiology Research*, **9**(44), 2209-2217.

Tolossa, Y. H., Fentie, T., and Kassa, T. (2023). Bacterial pathogens and their antimicrobial susceptibility patterns isolated from bovine mastitis milk in central Ethiopia. *Veterinary Medicine International*, **4**,1

Tong, Steven Y. C., Davis, Joshua S., Eichenberger, Emily, Holland, Thomas L. and Fowler, Vance G. (2015). *S. aureus* infections: Epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiology Reviews*, ***28***(3), 603–661

Torres-Sangiao, E., Leal Rodriguez, C., and García-Riestra, C. (2021). Application and perspectives of MALDI–TOF mass spectrometry in clinical microbiology laboratories. *Microorganisms*, **9**(7), 1539.

Usai, D., Donadu, M. G., and Bua, A**.** (2019). Enhancement of antimicrobial activity of pump inhibitors associating drugs. *Journal of Infection in Developing Countries*, **13**(2), 162–164

Van Belkum, A., Melles, D., Nouwen, J., van Leeuwen, W., Van Wamel, W. and Wertheim, H.,(2009). Co-evolutionary aspects of human colonisation and infection by *S. aureus*.Infectious, *Genetetic and Evolution*, **9**(1): 32-47

Van Duijkeren, E., Hengeveld, P. D., Albers, M., Pluister, G. N., Jacobs, P., Heres, L., and Wagenaar, J. A. (2010). Prevalence of Methicillin-resistant *S. aureus* in Pigs and Pig Farmers in the Netherlands. *Journal of Clinical Microbiology*, **48**(11), 4207–4211.

Vestergaard, Martin, Frees, Dorte, and Ingmer, Hanne. (2019). Antibiotic resistance and the MRSA problem. *Microbiology Spectrum*, ***7***(2), 7.2.

Vrioni, G., Tsiamis, C., Oikonomidis, G., Theodoridou, K., Kapsimali, V., and Tsakris, A.(2018). MALDI-TOF mass spectrometry technology for detecting biomarkers of antimicrobial resistance: current achievements and future perspectives. *Annals of translational medicine*, **6**(12)

Wang W, Lin X, and Jiang T. (2018). Prevalence and characterization of *Staphylococcus aureus* cultured from raw milk taken from dairy cows with mastitis in Beijing, *China. Front Microbiol*ogy, **9,** 1123.

Watts JL. (1988). Etiological agents of bovine mastitis. *Veterinary Microbiology* ,**16**,41–66.

Weidenmaier, C., Kokaikun, J. and Kristian, S. (2004). Role of teichoic acids in *Staphylococcus aureus* nasal colonization, a major risk factor in nosocomial infections. *Nature Medicine*, **10**(3), 243-245.

Weldeselassie, M., Gugsa, G., Kumar, A., Tsegaye, Y., Awol, N., Ahmed, M. (2020). Isolation and characterization of *Staphylococcus aureus* from food of bovine origin in Mekelle, Tigray, Ethiopia. *Open Microbiology Journal*, **14**, 234

World Health Organization (WHO) (2017). Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. Geneva, Switzerland: WHO. (Accessed on 22 May 2025).

World Health Organization (WHO). (2014). Antimicrobial Resistance, Global Report on Surveillance.( Accessed on 22 May 2025).

Yakubu, A., Abdullahi, I. O., Whong, C. Z., and Olayinka, B. (2020). Prevalence and antibiotic susceptibility profile of *Staphylococcus aureus* from milk and milk products in Nasarawa State, Nigeria. Sokoto *Journal of Veterinary Science.* **18,**1–12.

Yilmaz, E.S. and Aslantas, O. (2017). Antimicrobial resistance and underlying mechanisms in *Staphylococcus aureus* isolates. Asian Pacific *Journal of Tropical Medicine,* **10**(11): 1059-1064.

Zewdu, E., Abdissa, R., and Aleme, H. (2021). Bacterial profile and antimicrobial susceptibility patterns of isolates from bovine mastitis milk in selected areas of central Ethiopia. *Veterinary Medicine International*, **20**,1

## 9. ANNEXES

**Annex 1:** Research Informed Consent Form

**S/No.: _________________CardNo.:_______________FullName:________________**

I the above mentioned confirm that the document describing the purpose, benefits, risks, and confidentiality of the research study on Assessment of *Staphylococcus aureus* and Methicillin-Resistant *S. aureus* (MRSA) in Dairy Farms of Sheger City, Oromia, Ethiopia: A One Health approach has been read and explained to me. During the process I will be encouraged to ask questions, understood that I have the right to withdraw from the study at any time and have been informed that other people will not know my results as it is coded by numbers rather than names of people. I realized that there are no personal benefits to me apart from the clinical services related to my hand swab examination. In addition to the above mentioned I have no objection if part of the hand swab specimen is shipped to other country for further examination and agreed to use this sample for the same experiment in the future. Therefore, with full understanding of the importance of the study, I hereby confirm that I have voluntarily participated on the research.

Signature______________
Name of the professional taking consent_______ Signature________ Date_______

**Annex 2:** Questionner Survey

### A. Socio-Demographic Characteristics of Dairy Farmers

1. Respondent Name: ____________________________
2. Address: ____________________________________
3. Educational Status:
   ☐ Illiterate ☐ Grade 1-6 ☐ Grade 7-12 ☐ Grade >12
4. Gender:
   ☐ Male ☐ Female
5. Age of Respondent:
   ☐ <20 ☐ 20-30 ☐ 31-40 ☐ 41-50 ☐ >50
6. Employment Status:
   ☐ Temporary ☐ Permanent
7. Experience in Dairy Farming:

☐ <1 year ☐ 1-5 years ☐ 6-10 years ☐ >10 years

8. Type of Dairy Farming:

☐ Intensive ☐ Semi-intensive ☐ Extensive

9. Location: ____________________________________

10. Questionnaire Code: __________________________

**B. Sanitation Practices**

1. How often are animal sheds cleaned?

☐ Daily ☐ Weekly ☐ Monthly ☐ Rarely

2. What materials are used for bedding?

☐ Straw ☐ Sand ☐ Rubber mats ☐ Others (Specify): __________________

3. How often is bedding changed?

☐ Daily ☐ Weekly ☐ Monthly ☐ Rarely

4. Are disinfectants used for cleaning animal sheds?

☐ Yes ☐ No

5. How often are disinfectants used?

☐ Daily ☐ Weekly ☐ Monthly ☐ Rarely

6. What pest control measures are used on the farm?

☐ Insecticides ☐ Rodent control ☐ None ☐ Others (Specify): ____________

7. How often are pest control measures applied?

☐ Weekly ☐ Monthly ☐ Rarely

**C. Water and Waste Management**

1. What is the main water source for animals?

☐ Tap water ☐ River ☐ Underground water ☐ Others (Specify): _________________

2. How is water stored on the farm?

☐ Tanks ☐ Open containers ☐ Others (Specify): ____________

3. Are water storage containers cleaned regularly?

☐ Yes ☐ No

4. Is water tested for quality?

☐ Yes ☐ No

5. How is manure disposed of?

☐ Composting ☐ Direct application to fields ☐ Dumping ☐ Others (Specify): ________

6. How long is manure stored before disposal or use?

☐ <1 week ☐ 1-4 weeks ☐ >4 weeks

7. Is the manure storage area isolated from the main farm?

☐ Yes ☐ No

8. Is there a drainage system for liquid waste?

☐ Yes ☐ No

9. Is wastewater treated before disposal?

☐ Yes ☐ No

## D. Milking Practices

1. Are udders washed before milking?
   ☐ Always ☐ Sometimes ☐ Rarely ☐ Never
2. Are hands washed before milking?
   ☐ Always ☐ Sometimes ☐ Rarely ☐ Never
3. How often is milking equipment cleaned?
   ☐ After each use ☐ Daily ☐ Weekly ☐ Rarely
4. What disinfectant is used for cleaning milking equipment?
   ☐ Chlorine-based ☐ Alcohol-based ☐ Soap and water ☐ Others (Specify): __________
5. How is milk stored before sale or transport?
   ☐ Refrigeration ☐ Ambient temperature ☐ Use of preservatives ☐ Others (Specify): _____________
6. How is milk transported?
   ☐ Sealed containers ☐ Open containers ☐ Others (Specify): ________________
7. Are transport vehicles cleaned regularly?
   ☐ Yes ☐ No

## E. Worker Hygiene and Biosecurity

1. Do workers receive hygiene training?
   ☐ Yes ☐ No
2. How often is hygiene training provided?
   ☐ Annually ☐ Biannually ☐ Never
3. Are workers provided with protective gear (e.g., gloves, boots)?
   ☐ Yes ☐ No
4. How often do workers wash their hands during the workday?
   ☐ Before and after milking ☐ After handling manure ☐ After breaks ☐ Rarely
5. Are visitors restricted from direct animal contact?
   ☐ Yes ☐ No
6. Are visitors required to follow biosecurity protocols?
   ☐ Yes ☐ No
7. Are new animals quarantined before joining the herd?
   ☐ Yes ☐ No

## F. Antibiotic Use in Dairy Farms

1. Who treats your cattle when they are sick?
   ☐ Veterinarian ☐ Self-administration (farmer treats animals) ☐ Traditional healer
   ☐ Others (Specify): _________________________
2. Do you use antibiotics as additives for growth promotion in feed?
   ☐ Yes ☐ No
3. If yes, which antibiotics do you commonly use as growth promoters?

☐ Tetracyclines ☐ Penicillins ☐ Sulfonamides ☐ Fluoroquinolones ☐ Cephalosporins
☐ Others (Specify): ________________________

4. Do you follow a veterinarian’s prescription when using antibiotics?
☐ Always ☐ Sometimes ☐ Rarely ☐ Never
5. Are withdrawal periods for antibiotics strictly followed before selling milk?
☐ Yes ☐ No
6. How often are antibiotics used on the farm?
☐ Rarely ☐ Occasionally ☐ Frequently
7. Do you keep records of antibiotic use (type, dosage, duration, withdrawal period)?
☐ Yes ☐ No
8. Are you aware of antibiotic resistance?
☐ Yes ☐ No ☐ Somewhat
9. Do you use alternative treatments (e.g., herbal remedies, probiotics) instead of antibiotics?
☐ Yes ☐ No

**G. Farm Infrastructure and Environment**

1. What type of flooring is used in animal sheds?
☐ Concrete ☐ Dirt ☐ Rubber mats ☐ Others (Specify): ____________________
2. What is the ventilation system in animal sheds?
☐ Natural ☐ Mechanical ☐ None
3. What is the distance to the nearest dairy farm?
☐ <1 km ☐ 1-5 km ☐ >5 km

**H. Milk Quality and Safety**

1. Is milk tested for quality (e.g., bacterial load, antibiotic residues) before sale?
☐ Yes ☐ No
2. What happens to milk that fails quality tests?
☐ Discarded ☐ Sold at a lower price ☐ Used for animal feed
☐ Others (Specify): ___________
3. Do you receive feedback from consumers or milk processors about milk quality?
☐ Yes ☐ No

**I. General Farm Management**

1. How often does a veterinarian visit the farm?
☐ Monthly ☐ Quarterly ☐ Only when needed
2. What is the source of animal feed?
☐ Produced on-farm ☐ Purchased commercially ☐ Others (Specify): _________________
3. Are animals vaccinated?
☐ Yes ☐ No

4. If yes, against which diseases? ________________________

### J. Physical Observation Checklist (For Researcher)

1. Cleanliness of animal sheds:
☐ Satisfactory ☐ Unsatisfactory
2. Condition of milking equipment:
☐ Clean ☐ Dirty
3. Presence of pests:
☐ Yes ☐ No
4. General hygiene of workers:
☐ Satisfactory ☐ Unsatisfactory

### K. Swab Sampling Protocol (For Researcher)

1. Location sampled: ________________________
2. Time of sampling: ________________________
3. Sample ID: ________________________

**Annex 3:** Standard protocol for *S. aureus* identification with MALDI_TOF

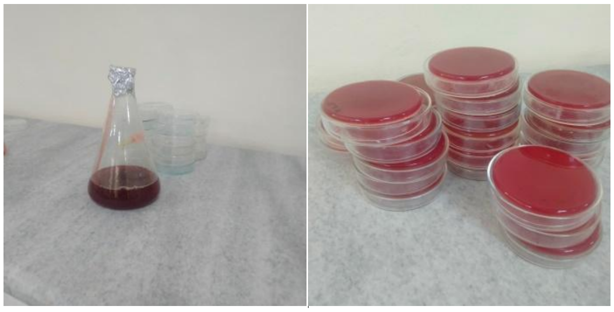
1. An Eppendorf tube was filled with 300 μl of water that was suitable for HPLC analysis.
2. After mixing HPLC-grade water with isolated pure colonies from the cultivated plate, a full suspension was made.
3. The suspensions were mixed by centrifugation at 15000 rpm for two minutes after adding 900 μL of pure ethanol.
4. With a pipette, the supernatant was removed.
5. To ensure there was no leftover ethanol, the tubes were centrifuged for two minutes at 15,000 rpm.
6. For five minutes, the tubes were allowed to air dry at ambient temperature.
7. Following the addition of 25 μL of 70% formic acid, the pellets were reconstituted.
8. Centrifugation was performed for two minutes at 15000 rpm after adding 25 μL of 100% acetonitrile.
9. A single, clean MALDI-target plate was coated with 1μl supernatant, which was thenallowed to air dry for fifteen minutes.
10. At the designated BTS QC point, 1 μL of BTS was applied, and the mixture was allowed to dry completely.
11. Every sample position and BTS QC position had 1μL of CHCA matrix solution applied over them.
12. The areas were allowed to air dry at ambient temperature.
13. A unique bar code was scanned, and then the target plate was placed into the mass Spectrometer

**Annex 4.** The antimicrobial disc used to measure the zone of inhibition of *S. aureus* Susceptibility, together with its interpretations

| **Specific antibiotic disc** | **Symbol** | **Interpretive categories and zone diameter breakpoint** | | |
|---|---|---|---|---|
| | | S | I | R |
| Amoxacillin | AMC $_{30\,\mu g}$ | ≥21 | - | ≤19 |
| Ampicillin | AMP $_{10\,\mu g}$ | ≥29 | - | ≤28 |
| Amoxicillin | AX $_{2\,\mu g}$ | ≥29 | - | ≤28 |
| ciprofloxacillin | CIP $_{10\mu g}$ | ≥21 | 16-20 | ≤15 |
| Gentamycin | CN $_{10\,\mu g}$ | ≥15 | 13-14 | ≤12 |
| Ceftriaxone | CRO $_{30\,\mu g}$ | ≥25 | | ≤1219 |
| Clindamycin | DA $_{2\,\mu g}$ | ≥21 | 15-20 | ≤1214 |
| Cefoxitin(oxacillin) | FOX $_{30\,\mu g}$ | ≥22 | | ≤21 |
| Norfloxacillin | NOR $_{10\,\mu g}$ | ≥17 | 13-16 | ≤12 |
| Penicillin | P $_{10\,\mu g}$ | ≥29 | | ≤28 |
| Streptomycin | S $_{10\,\mu g}$ | ≥15 | | ≤12 |
| Sulfamethoxazole | SXT $_{25\,\mu g}$ | ≥16 | 11-15 | ≤10 |
| Tetracycline | TE $_{30\,\mu g}$ | ≥19 | 15-18 | ≤14 |

S=susceptible I =intermediate R=resistance

**Annex 5**: Laboratory work results and instrument used photos



Media prepared

Bacteria inoculation

**MALDI TOF MS**

Run Identifier: 250325-1241-1011027837

Run Creation Date/Time: 2025-03-25T13:08:24.881

*Result overview table—continued from previous page*

| Sample Name | Sample ID | Organism (best match) | Score Value | Organism (second-best match) | Score Value |
|---|---|---|---|---|---|
| A8<br>(+)(B) | 6807<br>(Standard) | Staphylococcus sciuri | 1.87 | **No Organism Identification Possible** | [illegible] |
| A9<br>(+++)(A) | 6808<br>(Standard) | Proteus mirabilis | 🟩 | Proteus mirabilis | 🟩 |
| A10<br>(+++)(A) | 6809<br>(Standard) | Proteus mirabilis | 🟩 | Proteus mirabilis | 🟩 |
| A11<br>(+)(B) | 6810<br>(Standard) | Proteus mirabilis | 1.80 | Proteus mirabilis | 1.77 |
| A12<br>(+++)(A) | 6811<br>(Standard) | Proteus mirabilis | 🟩 | Proteus mirabilis | 🟩 |
| B1<br>(+)(B) | 6812<br>(Standard) | Proteus mirabilis | 1.77 | Proteus mirabilis | 1.76 |
| B2<br>(-)(C) | 6813<br>(Standard) | **No Organism Identification Possible** | [illegible] | **No Organism Identification Possible** | [illegible] |
| B3<br>(+++)(A) | 6814<br>(Standard) | Proteus mirabilis | 🟩 | Proteus mirabilis | 🟩 |
| B4<br>(+++)(A) | 6815<br>(Standard) | Enterococcus faecalis | 🟩 | Enterococcus faecalis | 🟩 |
| B5<br>(-)(C) | 6816<br>(Standard) | **no peaks found** | [illegible] | **no peaks found** | [illegible] |
| B6<br>(+)(B) | 6817<br>(Standard) | Proteus mirabilis | 1.89 | Proteus mirabilis | 1.88 |
| B7<br>(+)(B) | 6818<br>(Standard) | Enterobacter hormaechei | 1.83 | Enterobacter hormaechei | 1.76 |
| B8<br>(+++)(A) | 6819<br>(Standard) | Staphylococcus aureus | 🟩 | Staphylococcus aureus | 🟩 |
| B9<br>(+)(B) | 6820<br>(Standard) | Staphylococcus sciuri | 1.85 | **No Organism Identification Possible** | [illegible] |
| B10<br>(-)(C) | 6821<br>(Standard) | **No Organism Identification Possible** | [illegible] | **No Organism Identification Possible** | [illegible] |
| B11<br>(+++)(A) | 6822<br>(Standard) | Staphylococcus sciuri | 🟩 | Staphylococcus sciuri | 1.75 |

*Result overview table—continued on next page*

Report created at [illegible]

Research Use Only

Page 7 of 104

MALDI TOF OUTPUT

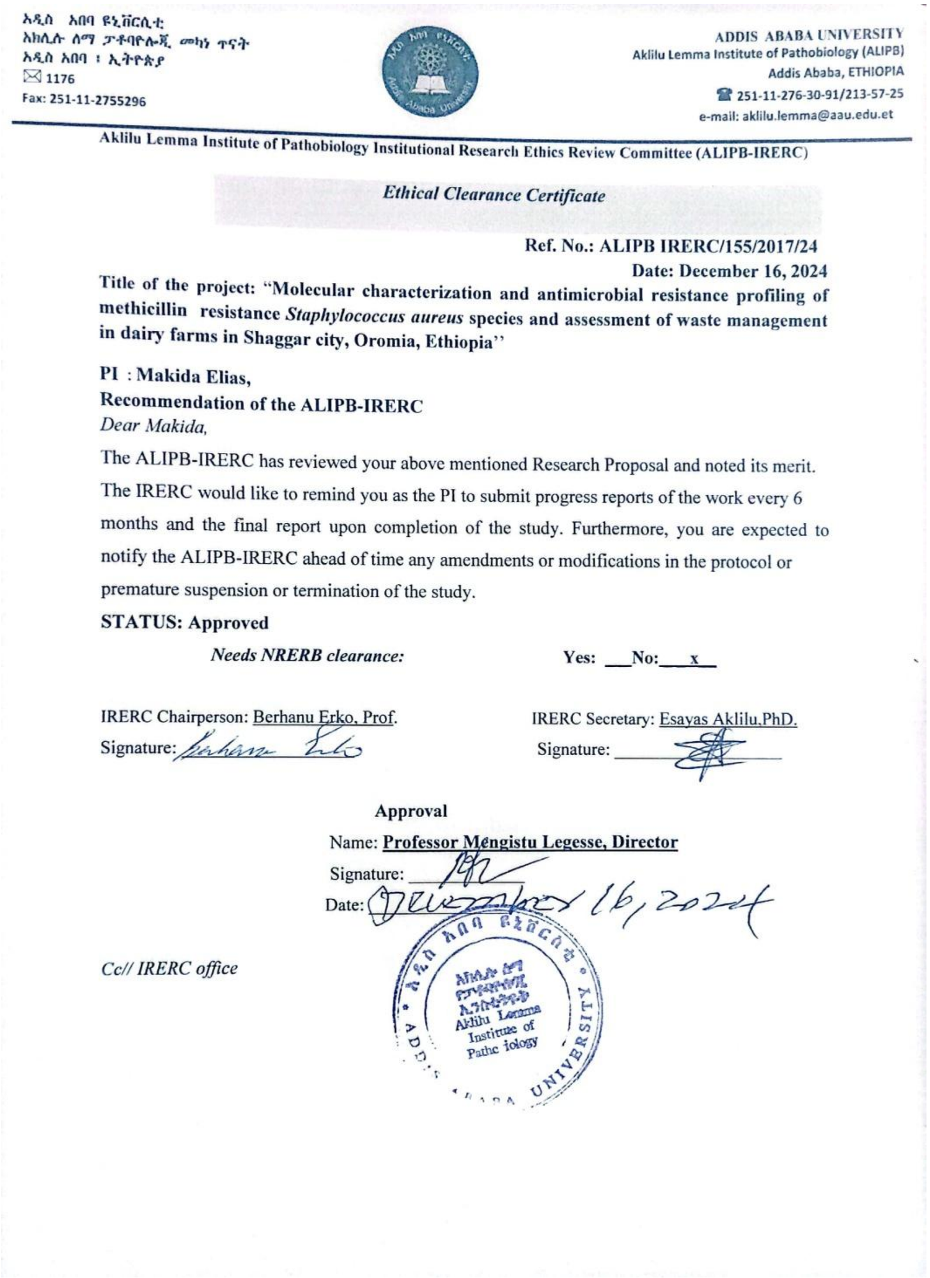
**characteristics of *S. aureus* on mannitol salt agar media with negative control**

McFarland Densitometer

Antimicrobial susceptibility test results

**Annex 6:** Ethical clearance

1.Human ethical clearance



አዲስ አበባ ዩኒቨርሲቲ
አክሊሉ ለማ ፓቶባዮሎጂ መካነ ጥናት
አዲስ አበባ ፡ ኢትዮጵያ
✉ 1176
Fax: 251-11-2755296

ADDIS ABABA UNIVERSITY
Aklilu Lemma Institute of Pathobiology (ALIPB)
Addis Ababa, ETHIOPIA
☎ 251-11-276-30-91/213-57-25
e-mail: aklilu.lemma@aau.edu.et

**Aklilu Lemma Institute of Pathobiology Institutional Research Ethics Review Committee (ALIPB-IRERC)**

***Ethical Clearance Certificate***

**Ref. No.: ALIPB IRERC/155/2017/24**
**Date: December 16, 2024**

**Title of the project: "Molecular characterization and antimicrobial resistance profiling of methicillin resistance *Staphylococcus aureus* species and assessment of waste management in dairy farms in Shaggar city, Oromia, Ethiopia''**

**PI : Makida Elias,**
**Recommendation of the ALIPB-IRERC**
*Dear Makida,*

The ALIPB-IRERC has reviewed your above mentioned Research Proposal and noted its merit. The IRERC would like to remind you as the PI to submit progress reports of the work every 6 months and the final report upon completion of the study. Furthermore, you are expected to notify the ALIPB-IRERC ahead of time any amendments or modifications in the protocol or premature suspension or termination of the study.

**STATUS: Approved**

***Needs NRERB clearance:*** **Yes: ___No:__x__**

IRERC Chairperson: Berhanu Erko, Prof.
Signature: Berhanu Erko

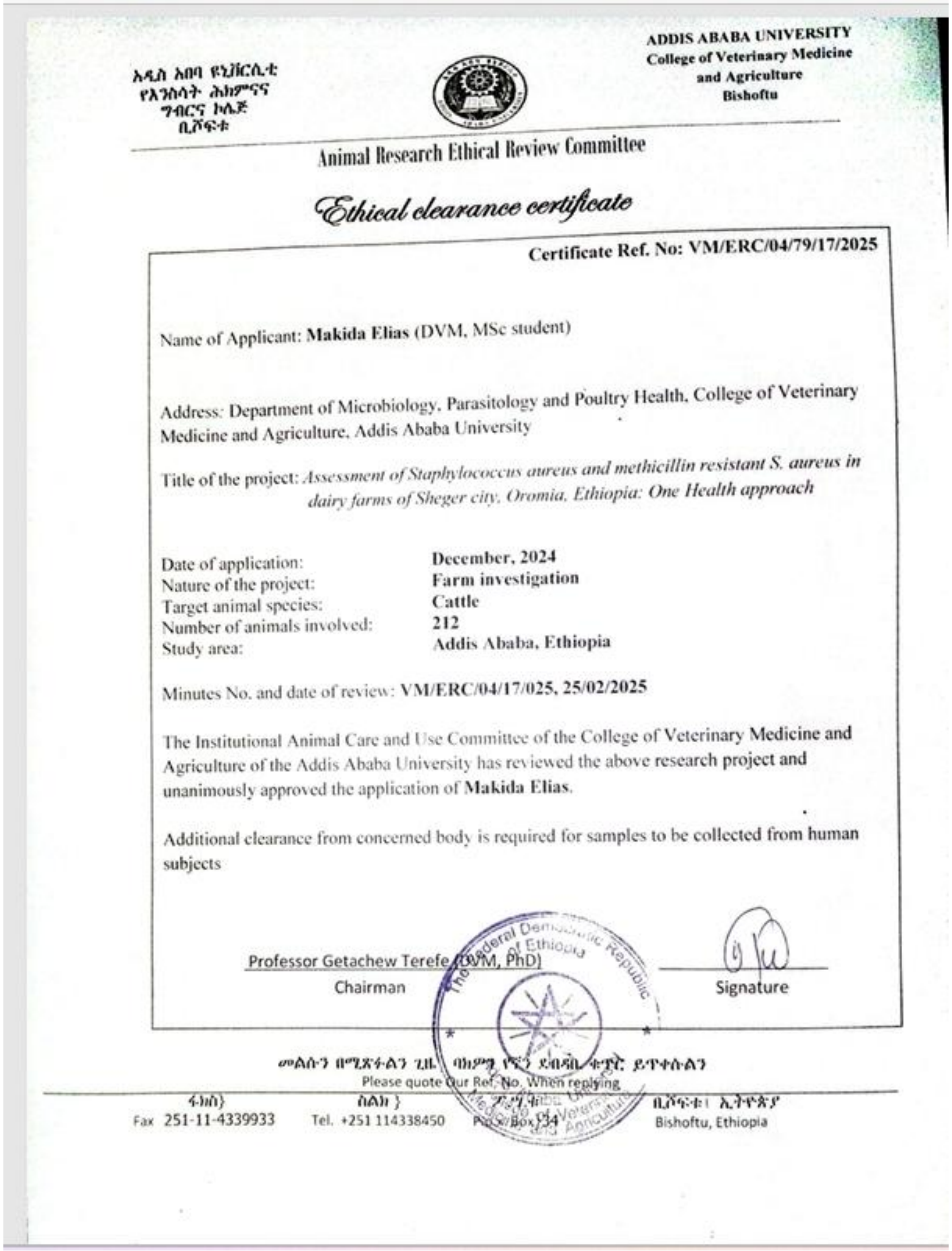
IRERC Secretary: Esayas Aklilu,PhD.
Signature:

**Approval**

Name: **Professor Mengistu Legesse, Director**
Signature:
Date: December 16, 2024

*Cc// IRERC office*

2. Animal ethical clearance



አዲስ አበባ ዩኒቨርሲቲ
የእንስሳት ሕክምናና
ግብርና ኮሌጅ
ቢሾፍቱ

**ADDIS ABABA UNIVERSITY**
**College of Veterinary Medicine**
**and Agriculture**
**Bishoftu**

## Animal Research Ethical Review Committee

## *Ethical clearance certificate*

**Certificate Ref. No: VM/ERC/04/79/17/2025**

Name of Applicant: **Makida Elias** (DVM, MSc student)

Address: Department of Microbiology, Parasitology and Poultry Health, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *Assessment of Staphylococcus aureus and methicillin resistant S. aureus in dairy farms of Sheger city, Oromia, Ethiopia: One Health approach*

| | |
|---|---|
| Date of application: | **December, 2024** |
| Nature of the project: | **Farm investigation** |
| Target animal species: | **Cattle** |
| Number of animals involved: | **212** |
| Study area: | **Addis Ababa, Ethiopia** |

Minutes No. and date of review: **VM/ERC/04/17/025, 25/02/2025**

The Institutional Animal Care and Use Committee of the College of Veterinary Medicine and Agriculture of the Addis Ababa University has reviewed the above research project and unanimously approved the application of **Makida Elias**.

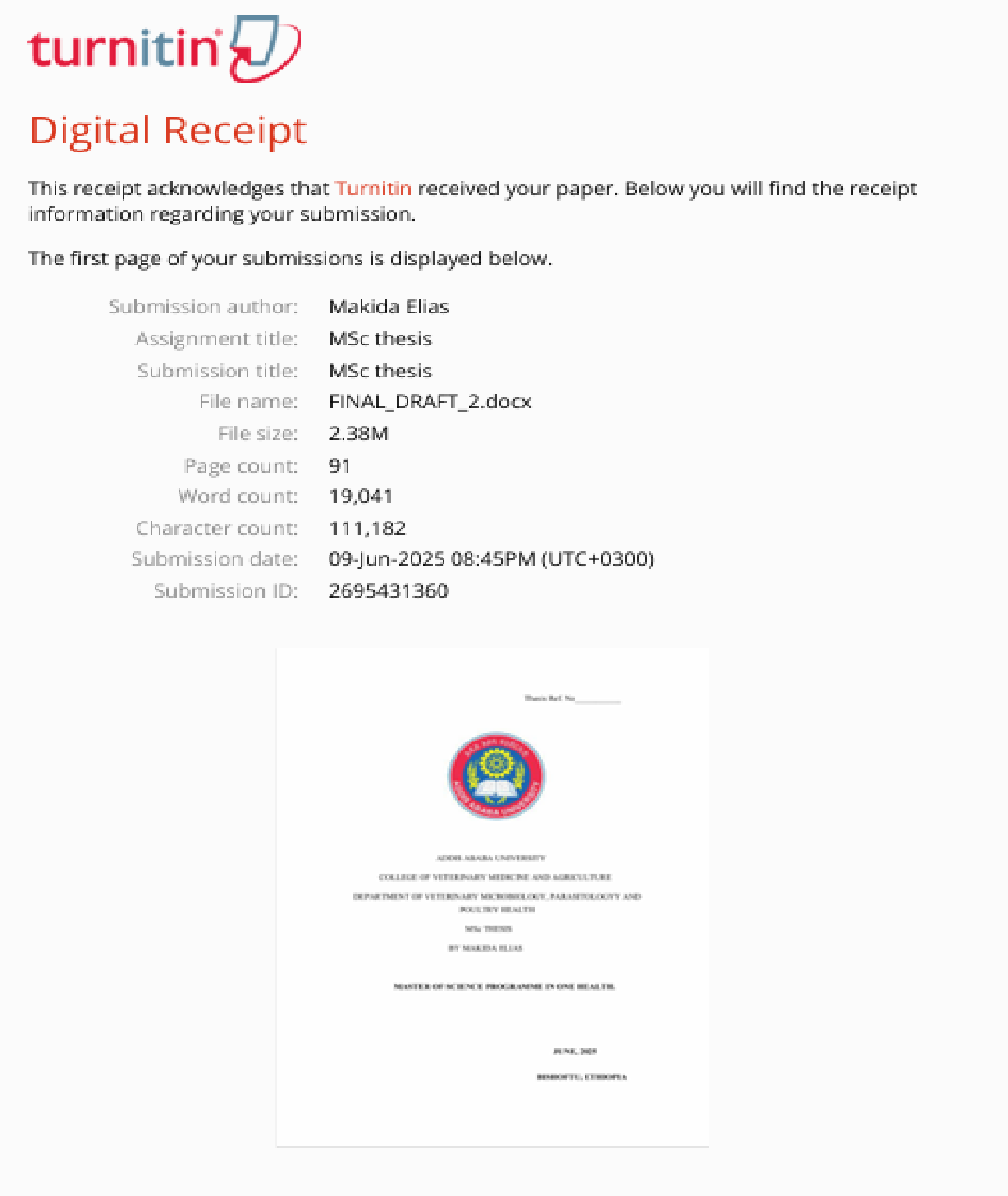
Additional clearance from concerned body is required for samples to be collected from human subjects

Professor Getachew Terefe (DVM, PhD)
Chairman

Signature

መልሱን በሚጽፉልን ጊዜ ባክዎ የኛን ደብዳቤ ቁጥር ይጥቀሱልን
Please quote Our Ref. No. When replying

ፋክስ Fax 251-11-4339933
ስልክ Tel. +251 114338450
ፖ.ሣ.ቁ P.O.Box 34
ቢሾፍቱ፣ ኢትዮጵያ Bishoftu, Ethiopia



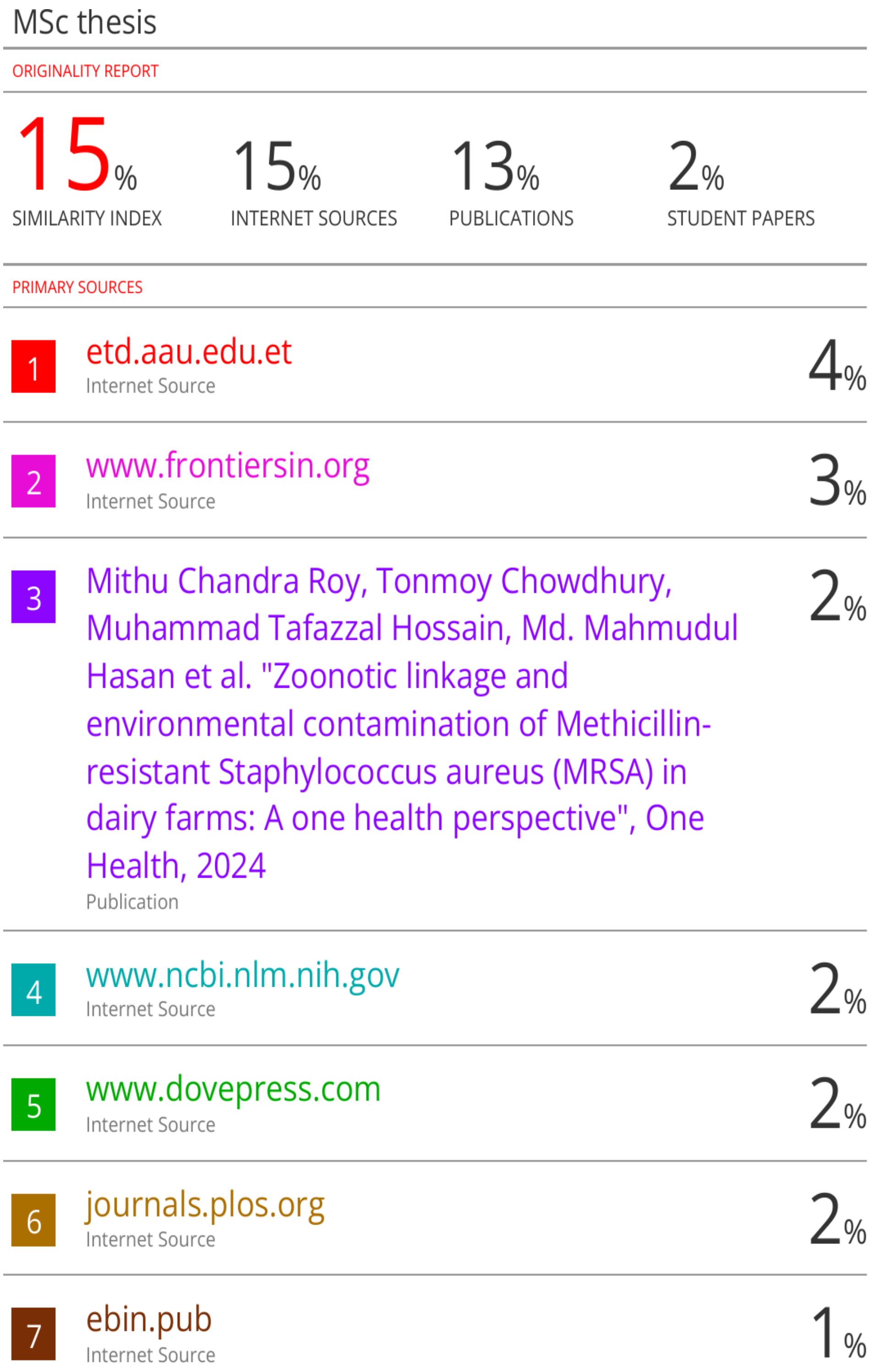
turnitin

# Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

| | |
|---|---|
| Submission author: | Makida Elias |
| Assignment title: | MSc thesis |
| Submission title: | MSc thesis |
| File name: | FINAL_DRAFT_2.docx |
| File size: | 2.38M |
| Page count: | 91 |
| Word count: | 19,041 |
| Character count: | 111,182 |
| Submission date: | 09-Jun-2025 08:45PM (UTC+0300) |
| Submission ID: | 2695431360 |



MSc thesis

ORIGINALITY REPORT

| 15% | 15% | 13% | 2% |
|---|---|---|---|
| SIMILARITY INDEX | INTERNET SOURCES | PUBLICATIONS | STUDENT PAPERS |

PRIMARY SOURCES

| | | |
|---|---|---|
| 1 | etd.aau.edu.et<br>Internet Source | 4% |
| 2 | www.frontiersin.org<br>Internet Source | 3% |
| 3 | Mithu Chandra Roy, Tonmoy Chowdhury, Muhammad Tafazzal Hossain, Md. Mahmudul Hasan et al. "Zoonotic linkage and environmental contamination of Methicillin-resistant Staphylococcus aureus (MRSA) in dairy farms: A one health perspective", One Health, 2024<br>Publication | 2% |
| 4 | www.ncbi.nlm.nih.gov<br>Internet Source | 2% |
| 5 | www.dovepress.com<br>Internet Source | 2% |
| 6 | journals.plos.org<br>Internet Source | 2% |
| 7 | ebin.pub<br>Internet Source | 1% |