2023-06

Prevalence, Associated Risk Factors and Anti-Microbial Susceptibility Profile of Staphylococcus Aureus in Different Dairy Production System, Chagni District, Northwest Ethiopia

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MSc. Thesis

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SUBMITTED TO THE DEPARTMENT OF VETERINARY SCIENCE IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN VETERINARY MICROBIOLOGY

JUNE, 2023

BAHIR DAR, ETHIOPIA

THESIS APPROVAL SHEET

As member of the Board of Examiners of the Master of Sciences (MSc.) thesis open defence Examination, certifying that we have read and evaluated the thesis prepared by **Ahmed Wodaje** entitled **"Prevalence, Associated Risk Factors and Anti-Microbial Susceptibility Profile of *Staphylococcus aureus* in Different Dairy Production System, Chagni District, Northwest Ethiopia".** We here by certify that the thesis be accepted for fulfilling the thesis requirement for the award of the degree of the **Master of Sciences in Veterinary Microbiology.**

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DECLARATION

This is to certify that this thesis entitled **" Prevalence, Associated Risk Factors and Anti-Microbial Susceptibility Profile of *Staphylococcus aureus* in Different Dairy Production System, Chagni District, Northwest Ethiopia"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science in Veterinary Microbiology** to the Graduate program of college of Agriculture and Environmental Sciences, Bahir Dar University by **Ahmed Wodaje** (ID, BDU1100475) is an authentic work carried out by him under our guidance. The matter embodied in this thesis work has not been submitted earlier for award of any degree or diploma to the best of our knowledge and belief.

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ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my advisor Dr. Tiliksew Bialfew for his guidance, supervision, invaluable suggestions and critical review of my thesis. Similarly, I had a great gratitude to my co- advisor Dr. Habtamu Tassew for his comment and advice for the achievement of this thesis.

I would like to thank Bahir Dar University Department of Biology that allowed me to undertake laboratory analysis. Especial thanks also go to Mr. Zemen biotechnology laboratory institute assistant for his unreserved chemical regents and material support.

I am grateful to Amhara Regional Veterinary Laboratory that gave me regents. I also acknowledge Chagni Cattle Breeding and Improvement Ranch manager and farm workers that helped me by giving appropriate samples. Finally, my deepest gratitude is extended to my family, especially my mother and father for their moral for the achievement of this thesis

ABSTRACT

A cross-sectional study was carried out from October 2022 to June 2023 at Chagni cattle breeding and improvement Ranch, and small holder-dairy farms to estimate the prevalence and the anti-microbial susceptibility profile of Staphylococcus aureus isolated from infected cows. The study was conducted on 318 lactating cows selected by simple random sampling techniques in the study areas. The data was analysed using statistical tools. Based on the CMT result and clinical examination, of the 318 cows examined, 44.1%(140/318) were positive for mastitis. Out of this, 35.9% and 8.2% had sub-clinical and clinical mastitis at cow level, respectively. From 1272 quarters examined 55(4.3%) were blind, and 20% (243/1272) were positive for mastitis. In the multivariable logistic regression model, factors significantly associated ($p < 0.05$) with the occurrence of mastitis were age, breed, management system, lactation stage, parity, floor type, and tick on the teat at Chagni cattle breeding and improvement Ranch. In addition, age, lactation stage, and tick on teat were independently associated with mastitis occurrenc in small-holder dairy farms ($p<0.05$). Staphylococcus aureus isolates were detected in 45.7% (64/140) of the samples, of which 9 (34.6%) and 55 (48.2%) isolates from clinical and subclinical mastitis cases, respectively. All (n = 64) isolates of S. aureus were tested for their susceptibility to eight selected antimicrobials. The isolates were highly susceptible to sulfamethoxazole (87.5%) and Gentamycin (79.7 %) followed by Tetracycline (75%), Erythromycin (72%), and Azithromycin (71.8%). However, they were highly resistant to Cefoxitin (65.6%), followed by Tetracycline and ciprofloxacin (25%). The high prevalence of masitis in the study area, more importantly the sub-clinical one. And isolates of S. aureus were resistant to a number of drugs. Hence, implementing hygienic conditions, creating awareness on the control and prevention of subclinical mastitis in dairy farms, and rational use of drugs are recommended.

Key words*: *Antimicrobials, Bovine mastitis, Chagni, Staphylococcus aureus,

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LIST OF ABBREVIATIONS

AST	Antimicrobial Susceptibility Test
BAP	Blood Agar Plate
BC	Bacteriological Culturing
CFU	Colony Forming Unit
CI	Confidence Interval
CLSI	Clinical Laboratory Standard Institute
CMT	California Mastitis Test
CSA	Central Statistical Agency
DNA	Deoxyribonucleic Acid
FAO	Food and Agricultural Organization
H_2O_2	Hydrogen per oxide
HF	Holstein Friesian
IMI	Inflammatory Infections
IU	International Unit
LF	Left Front
LR	Left Rear
MASL	Meters Above Sea Level
MIC	Minimal Inhibitory Concentration
MRSA	Methicillin Resistant *S. aureus*
MSA	Mannitol Salt Agar
NAAS	National Academy Agricultural Sciences,
NMC	National Mastitis Council
PCR	Polymerase Chain Reaction
RF	Right Front
RR	Right Rear
SCC	Somatic Cell Count
SFD	Staphylococcal Food-Borne Disease

CHAPTER 1. INTRODUCTION

1.1. Background and Justification

In Ethiopia, livestock represents a major national resource and form an integral part of the agricultural production system. The total cattle population for the country is estimated to be about 70 million, out of which about 56% are females (CSA, 2020). Cattle contribute between 85 and 89% of total national milk production. Milk is a very nutritious food that is rich in carbohydrates, proteins, fats, and vitamins, and it is one of the most important foods for human beings (Belay Geleta *et al*., 2019). Thus, in Ethiopia dairy industry could play a significant role in reducing the country's poverty and malnutrition for the majority of rural people as well as a significant number of urban and peri-urban people (Mohamed Ahmed *et al.,* 2004).

According to FAO reports, Ethiopia's total annual national milk production is around 3.8 billion liters of raw milk per year and per capita consumption is approximately 19 kg of milk. Furthermore, due to population growth and a change in living standard in the country, it is expected that the demand for milk was estimated to increase by 263% between 2012 and 2050 (FAO, 2019). Nevertheless, this amount is by far below the national demand for milk and milk products in the country. Different factors are thought to be responsible for the insufficiency of dairy products to satisfy the country’s requirements. Of these factors, disease of the mammary gland known as mastitis is the major contributing factor in the reduction of milk production. Mastitis is also associated with number of zoonotic diseases in which milk acts as a vehicle of infection (Demelash Biffa *et al*., 2005).

Bovine mastitis is an inflammation of the mammary gland caused by a variety of microorganisms, mostly bacteria, that gain access to the interior of the udder through the teat canal. It is characterized by physical, chemical, and usually bacterial and pathological changes in milk and glandular tissue (Quinn *et al.,* 2002). Among a wide variety of mastitis-causing microorganisms, bacteria are the most frequent pathogens like *S. aureus*, *S.*

dysgalactiae, S. Agalactiae, S. uberis and Enterobacteriaceae such as *E. coli* (Bradley, 2002; Alebachew Tilahun and Alemu Aylate, 2015; Gashaw Getaneh, 2016).

Mastitis is classified into two types. The first is clinical mastitis, which shows symptoms in the milk or on the animal. The other is subclinical mastitis, which has no visible signs of altering the milk or udder but causes a decrease in milk production, and changes to the composition of the secretion due to the presence of bacteria (Eriskine, 1996). Most frequently, sub-clinical mastitis can be diagnosed by the california mastitis test, somatic cell counting (SCC), and bacteriological culturing (BC) of milk (Zadoks and Schukken, 2006). Despite many years of research, sub-clinical mastitis remains the most prevalent form of mastitis, economically damaging and zoonotic potential disease for the dairy industry and consumers worldwide, irrespective of species of animal (Ojo *et al*., 2009).

Staphylococcus species are common causes of bovine mastitis in dairy herds. Pathogenic strains are usually coagulase-positive (Gorden *et al*., 2008), and they have been found to cause disease in a wide range of hosts, including humans, worldwide. The pathogenic strain of *S. aureus* is particularly the most important pathogen among staphylococci species related to intra-mammary infections in dairy cows, leading to severe economic losses in the dairy industry worldwide (Godden *et al*., 2002). Likewise, these groups of bacteria are also known for widespread transmission to humans through contaminated milk from mastitic cows (Gentilini *et al*., 2000).

Staphylococcus aureus infections can be treated with a wide range of antimicrobial medications. However, the widespread use of antibiotics for remedial purposes or their administration to animals raised for food as growth promoters has been linked to the expansion of resistance in bacterial pathogens that affect both humans and animals (Barber *et al*., 2003). Isolates of *S. aureus* constitute a global problem for the efficient treatment and management of these infections. Oxacillin, methicillin, and other beta-lactam antibiotics, which are commonly used penicillin-related antibiotics, are no longer effective against *S. aureus* (Boyce *et al*., 2005; Peacock and Paterson, 2015).

Mastitis results financial loss, which is caused by a permanent loss of production due to the replacement of secretory tissue with fibrous tissue, discarded milk after antibiotic therapy, early cow culling, veterinary costs, drug costs, increased labor, deaths from acute cases, replacement costs, and a high leukocyte count that result in a loss of income (Sharif and Muhammed, 2009).

The prevalence of *Staphylococcus aureus* in bovine mastitis can vary depending on several factors, including geographical location, management practices, and herd health status. In some regions, it has been reported to be a leading cause of mastitis, while in others, its prevalence may be lower.The prevalence of bovine mastitis in different parts of Ethiopia has been studied and found to be 39.2% in Dire-Dawa (Jafer Kedir *et al.*, 2016), 36.1% in and around Wukro in the Tigray region (Tesfanesh Fentahune *et al*., 2018), 27.2% in and around Dessie (Mulunesh Yenew and Habtamu Addis, 2020), 61.5% in Lome woreda-Oromia (Getachew Nigussie, 2020), and 56.88% in and around Mottta town (Denekew Wubetu, 2021).

1.2. Statement of the Problem

Mastitis, as a disease, has received little attention in Ethiopia, especially the subclinical form, which is mainly caused by *S. aureus* (Hunderra Sori *et al*., 2005; Mekonnen *et al*., 2005). Efforts have been concentrated on the treatment of clinical cases, coupled with the absence of clinical signs to distinguish the disease, and dairy farmers contribute a lot to the economically devastating nature of the disease (Nibret Moges *et al*., 2011; Sefinew Alemu *et al*., 2013).

Generally, the presence of the pathogen on the teat skin end, poor management and poor nutrition, blind treatment of the disease, poor house hygiene, poor milking process, and antibiotic resistance rate are some of the factors that can be pointed out as causes of cow mastitis and resulting economic loss. However, to date, the prevalence and antimicrobial susceptibility profile of *S. aureus* isolated from bovine mastitis have not been investigated in the study area. Therefore, studies on the prevalence of mastitis and the occurrence of *S.*

aureus in mastitic cows as well as antimicrobial susceptibility testing is, crucial for effective management and the selection of appropriate treatment options in the study area.

1.3. Objectives of the Study

1.3.1. General Objective

To estimate the prevalence, assess the potential risk factors, and to isolate and evaluate the antimicrobial susceptibility of *S. aureus* from mastitic milk sample from dairy farms in Chagni cattle breeding and improvement Ranch, and small holder dairy farms, Awi zone.

1.3.2. Specific Objectives

- To estimate the prevalence of mastitis at cow and quarter level
- To assess the potential risk factors associated with prevalence of mastitis in dairy cows.
- To isolating and identifying *S. aureus* from mastitic cow milk in the study area
- To evaluate the anti-microbial susceptibility profile of *S. aureus* from selected antimicrobial discs.

1.4. Research Questions

- What would be the association between *S. aureus* and mastitic cow risk factors?
- How *S. aureus* was isolated and identified from mastitic cow milk?
- What seems the antimicrobial resistance patterns of *S. aureus* to the selected antibiotic discs?

CHAPTER 2. LITERATURE REVIEW

2.1. General Characteristics of *Staphylococcus aureus*

The word Staphylococci were derived from two Greek words *staphyle* which means "bunch of grapes" and *coccus* which means "spherical bacteria" while *aureus* is a Latin word that stands for "gold" and was given to these bacteria because of yellow to yellowish-white colony appearance on enriched medium. *Staphylococcus aureus* is a non-motile, non-spore forming, facultative anaerobe, and pathogenic member of the genus Staphylococci measuring approximately 1μM in size (Qunin *et al*., 2004; Plata *et al*., 2009; Maliha Gulzar and Asima Zehra, 2018; Mahendra *et al*., 2020). It forms golden colonies on rich medium and β-hemolysin on blood agar containing 5% sheep and horse blood due to production of carotenoids and on Gram staining it appears as bluish grape-like colonies because cell division occurs at different planes (Plata *et al*., 2009). *Staphylococcus aureus* is catalase-positive, which distinguishes it from *Streptococcus* spp. This bacterium can grow at temperatures ranging from 5 to 45^{o}C and pH levels ranging from 4 to 10 (Ercolini *et al*., 2006).

2.2. Epidemiology of *Staphylococcus aureus*

Staphylococcus aureus is found as normal flora of humans and animals, and it is an opportunistic pathogen that is found all over the world (Tong *et al*., 2015; Dini *et al*., 2019; Mahendra *et al*., 2020). It can be found on humans and animals of the skin, mucosa of the upper respiratory system, lower urinary tract, and genital tract, as well as transients in the digestive tract. They are environmentally stable and have a selective affinity for particular species (Quinn *et al*., 2011). However, it is well known as an invasive human pathogen that causes significant morbidity and mortality. It has also frequently been isolated from animals and animal-derived food (Tong *et al*., 2015; Wang *et al*., 2018). Evidence of adaptation of mammary associated isolates to host species comes from the acquisition of genes that encode proteins that target cattle-specific (small ruminant specific) molecules, and loss or decay of genes that encode proteins adapted to human targets and adaptation of *S. aureus* to its

environment. Phenotypic diversity can result from the regulation of gene expression (Rainard *et al*., 2018).

Staphylococcus aureus is most commonly found in infected udders, teat canals, and teat lesions, and also the bacteria has been found on teat skin, muzzles, and nostrils. Teat cup liners, milker's hands, washcloths, and flies spread *S. aureus* to uninfected quarters. It does not survive on healthy teat skin but colonizes damaged skin and teat lesions readily. The organisms multiply in infected lesions, increasing the likelihood of teat canal colonization and udder infection (Petersson *et al*., 2010; Rainard *et al*., 2018).

Animals with subclinical types of inflammatory infections (IMI) are a reservoir of *S. aureus* that serves as a source of infection in the dairy environment and represents the most common cause of raw milk contamination. Other sources of *S. aureus* include contaminated milking equipment, bedding, and personnel. Purchased animals that are *S. aureus* infected, and chronically infected are a major source of new *Staphylococcus* infections within a farm (Mahendra *et al*., 2020). Farms and cheese-making plants can serve as a reservoir of *S. aureus* and can spread the microorganism into the environment (Stwart, 2003). Based on epidemiological studies, results of mastitis control programs and molecular data, *S. aureus* is classified as a contagious pathogen (Rainard *et al*., 2018).

Staphylococcus aureus can be transmitted from animal to animal, person to person, as well as from animals to humans and vice-versa. Usually transmission occurs by direct contact, often via the hands with colonized or infected animals or people and contaminated equipment and surfaces (Mahendra *et al*., 2020). The most common transmission pathways include transfer from infected mammary gland to uninfected gland via fomites, such as milking equipment, and the milker's hand. *S. aureus* present in the nose and on the skin, is shed into the environment by infected or colonized people and animals, indicating the possibility of airborne transmission as a possible route for infection. Vectors such as the housefly (Musca domestica) have also been implicated in the transmission of *S. aureus* (Cuny *et al*., 2010; Maliha Gulzar and Asima Zehra, 2018). The ability of a microorganism

to cause disease depends on factors such as the host's susceptibility, immune response, internal and external microbial environment (normal flora), previous infection, and predisposing conditions such as accident/trauma, and the age of animals (Kandi, 2018; Kumari *et al*., 2018).

The prevalence of mastitis is highest in pure exotic breed cattle, followed by crosses and indigenous zebu. The reason for that, exotic breeds give us a higher milk yield than zebus, and High milk yield cows are more susceptible to mastitis than low milk yield cows because they produce more milk, which puts more stress on their udders (Radostits *et al*., 2007; Ataro Abera, 2020). Another factor in the occurrence of mastitis is the age of cows. It has been shown that the manifestation of mastitis in infected quarter's increases with the advancement of age in cows. This may be due to more dilated teat canals in older age and permanent udder tissue damage resulting from the primary infection or due to an increased cellular response to an intra-mammary infection after parturition (Sharma *et al*., 2011; Awale *et al*., 2012).

Cows with the most pendulous quarters appear to be the most susceptible to mammary infections. The pendulous udder exposes the teat and udder to injury, so pathogens easily adhere to the teat and gain access to the gland tissue (Hunderra Sori *et al*., 2005). The cow's environment influences the number and types of bacteria exposed to their ability to resist those organisms. The design of housing system, hygiene, and size of milking cow herd, milking practice, and the climate interact to influence the degree of exposure of a cow to mastitis pathogens (Ataro Abera, 2020).

2.3. Pathogenesis of *Staphylococcus aureus*

Mastitis develops as a result of pathogenic *S. aureus* passing through a mammary quarter's teat duct, multiplying in the teat and gland cisterns, and progressing dorsally to the milk-producing tissues (Akers and Nickerson, 2011). After the bacteria multiply, then produce toxins which cause inflammation of the udder or the corresponding teat. The body produces leucocytes as a result of inflammation, lowering the quality of the milk. The milk becomes watery or curdled, and depending on the severity of the infection, blood streaks may appear.

Infection of the udder usually occurs directly through the teat canal. However, organisms can enter the mammary tissues via the blood. In general, two stages have been described: invasive and infective. During the invasive stage, the organism gains access from the exterior to the teat canal. The infectious stage is the stage of bacterial multiplication and resulting damage to mammary tissues (Maarit, 2008).

Major changes in the composition of the milk secreted from the tissue cells are caused by a series of events following the colonization of the bovine mammary gland by pathogenic agents. Pathogenic bacteria levels initially rise, but this is swiftly followed by a notable increase in the total amount of somatic cells. Somatic cells are of two types, sloughed epithelial cells from udder and leukocytes like lymphocytes, neutrophils, monocytes, eosinophils and basophils. During mammary gland inflammation, epithelial cells decrease from 70% to 35%, whereas leukocytes may increase up to 80% or more in milk. High leukocyte count in milk means either the udder is injured or it is infected (Singh *et al*., 2006).

2.4. Clinical Features of *Staphylococcus aureus* in Cattle

In cattle *S. aureus* causes from simple abscesses to severe mastitis and toxic shock syndrome. It is the most common pathogen in dairy farms, causing both clinical and subclinical mastitis, but the majority of infection is being sub-clinical (Pal, 2018). Dermatitis, gastroenteritis, osteomyelitis, meningitis, pneumonia, endocarditis, and wound infections are all caused by *S. aureus* (Mahendra *et al.,* 2020*).* Staphylococcal per-acute and gangrenous infections cause severe systemic reactions that can be fatal. In gangrenous mastitis, the affected tissue, which becomes cold and bluish black, eventually sloughs-off. Tissue necrosis is attributed to the alpha-toxin which causes contraction and necrosis of smooth muscle in blood vessel walls, impeding blood flow in the affected regions. In addition, this toxin releases lysosomal enzymes from leukocytes (Quinn *et al*., 2004).

2.5. Diagnosis

It is impossible to monitor udder health performance without reliable and affordable diagnostic procedures. There are numerous diagnostic procedures for mastitis with various

principles of action, some of which are based on the detection of udder and milk abnormalities, as well as inflammatory markers. These procedures include physical and clinical examinations of the udder, SCC, and CMT. The other type of diagnosis is more specific and based on mainly on isolation and identification of the causative pathogen of mastitis or the immune response such as bacterial cultures (BC) of milk, biochemical tests, and Polymerase Chain Reaction (PCR). Each diagnostic technique has its own advantages and disadvantages and its performance is dependent on many factors some of which are related to the procedures of sample collection, type, preservation and handling in the laboratory, others include the degree of infection and status of infected udder and infected cow, type of causative pathogen and its virulence and finally those related to the experience of the investigator (Yasser Mahmmod, 2013).

2.6. Antimicrobial Susceptibility Test

The performance of antimicrobial susceptibility testing by the clinical microbiology laboratory is important to confirm susceptibility to chose empirical antimicrobial agents, or to detect resistance in individual bacterial isolates. There are different antimicrobial susceptibility testing methods from this Disk diffusion and Broth dilution tests are commonly used (Murray, 2007). The disk diffusion susceptibility method is simple and practical and has been well-standardized. The test is performed by applying a bacterial inoculum of approximately $1–2\times10^{8}$cfu/ml to the surface of a Mueller-Hinton agar plate. Commercially-prepared, fixed concentration, paper antibiotic disks are placed on the inoculated agar surface. Plates are incubated for 16–24 hours at 35°C prior to determination of results. The zones of growth inhibition around each of the antibiotic disks are measured to the nearest millimeter. The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium. The diameter of zone inhibition for each drug is interpreted using the criteria published by the Clinical and Laboratory Standards Institute (CLSI, 2022). The results of the disk diffusion test are "qualitative," in that a category of susceptibility (susceptible, intermediate, or resistant) with reference of 0.5 McFarland standards on Muller Hinton agar plats.

Broth dilution test is one of the earliest antimicrobial susceptibility testing methods which can be micro-broth or tube-dilution method. The antibiotic containing tubes are inoculated with a standardized bacterial suspension of $1–5\times10^5$CFU/ml. Following overnight incubation at 35°C, the tubes are examined for visible bacterial growth as evidenced by turbidity. The lowest concentration of antibiotic that prevented growth represented the minimal inhibitory concentration (MIC) (CLSI, 2022). Based on this test best drug is prescribed by compared with standard.

2.7. Treatment

The widely used antimicrobial agents for prophylaxis and treatment of *S. aureus* infections in humans and animals are penicillin, chloramphenicol, erythromycin, gentamycin, neomycin, vancomycin, streptomycin, trimethoprim, norfloxacin, and tetracycline. However, the common use of antibiotics in hospitals and veterinary clinics created selective pressure for *S. aureus* to become resistant to antibiotics. *Staphylococcus aureus* develops resistance to antimicrobials by different mechanisms. These include limiting the uptake of the drug, modification of the drug target, enzymatic inactivation of the drug, and active efflux of the drug (Smith *et al*., 2002).

2.8. Prevention and Control of Mastitis

The control of mastitis has been successfully achieved through the establishment of effective herd health control programs (Erskine, 2003). Early diagnosis of mastitis with reliable tests facilitates successful treatment and control. The main control principles include sound husbandry practices and sanitation, a post milking teat dip, treatment of mastitis during the non-lactating period, and culling of chronically infected animals (Sharif and Muhammed, 2009). Successful control of contagious mastitis pathogens is focused on reducing the exposure of teats to pathogens found in milk that originated from infected cows. Control of environmental mastitis can be achieved by reducing the number of bacteria to which the teat is exposed, increasing the immune resistance of the cow, and pre-milking teat dipping with a germicidal (NAAS, 2013). Animal environments should be as clean and dry as possible.

Antimicrobials are routinely used for the treatment of dairy cattle affected by clinical and subclinical infections (Aarestrup, 2005).

To reduce the pathogen exposure to the mammary gland, the farm building must have adequate ventilation and sanitation (Ondiek *et al*., 2013). To reduce the likelihood of spreading disease, the milker's hand should be thoroughly washed, dried, and cleaned. Additionally, all milking implements must be dry and clean. There should be dry bedding available. Since the dung and urine are a constant source of infection at the farm, they should be removed right away (Sharif and Muhammad, 2009; Lehtolainen *et al*., 2003). Milking infected animals last and preventing the animals from lying down after milking are two additional general precautions against contagious and environmental mastitis. To achieve this, feed them right away after milking to make sure they stand for at least 30 minutes. This should give the teat orifice enough time to properly close (Tomita and Hart, 2001).

2.9. Status of Mastitis in Ethiopia

Table 2-1. Prevalence of mastitis in different parts of Ethiopia

No	Overall prevalence	Clinical mastitis	Subclinical mastitis	Name of the researcher, year and areas of the research
1	36.1%	9.4%	26.7%	Tesfanesh Fentahune *et al*. (2018), in Tigray
2	65.3%	22%	43.3%	Addisalem Tadesse (2012), in Addis Ababa
3	5.46%	56.2%	61.7%	Amanu Marama *et al.* (2016), in Holeta
4	62.6%	3.4%	59.2%	Rahmeto Abebe *et al*. (2016), in Hawassa
5	28.2%	3%	25.2%	Molalegne Bitew *et al.* (2010), in Bahir Dar
6	39.32%	11.4%	27.8%	Asmelash Tassew *et al.* (2016), in Assosa town
7	66.6%	12.1%	54.5%	Birhanu Abera *et al.* (2013), in Asella (Oromia)
8	43.6%	12.6%	32.2%	Abayeneh Girma and Dessalew Tamir (2022), in Ethiopia
9	39.6%	16.7%	22.7%	Gutu Kitila *et al*. (2021), west Wollega, (Oromia)
10	40.3%	11.9%	28.3%	Melak Tezera and Endris Aman (2021), in and around Assosa town (Benishangul-Gumuz)
11	46.9%	9.7%	37.2%	Sefinew Alemu *et al*., (2013), in and around Gondar Town, Ethiopia.

CHAPTER 3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted at Chagni Cattle Breeding and Improvement Ranch, and small holder dairy farms, which are found in Chagni, Guanga District. The study area is located in Awi Zone, Amhara Region, 170 km away from Bahir Dar city, and 505 km to the Northwest of Addis Ababa. Geographically, the study area is located at 10° 57′ North longitude and -36° 30′ East latitude. The total area of the Ranch is 1367 hectares. Its average elevation is estimated to be 1750.5 masl, the mean annual temperature range is 13.7 to 29.5ºC, and the mean annual rainfall is 1730mm (Tazeb Gessesse *et al*., 2021).

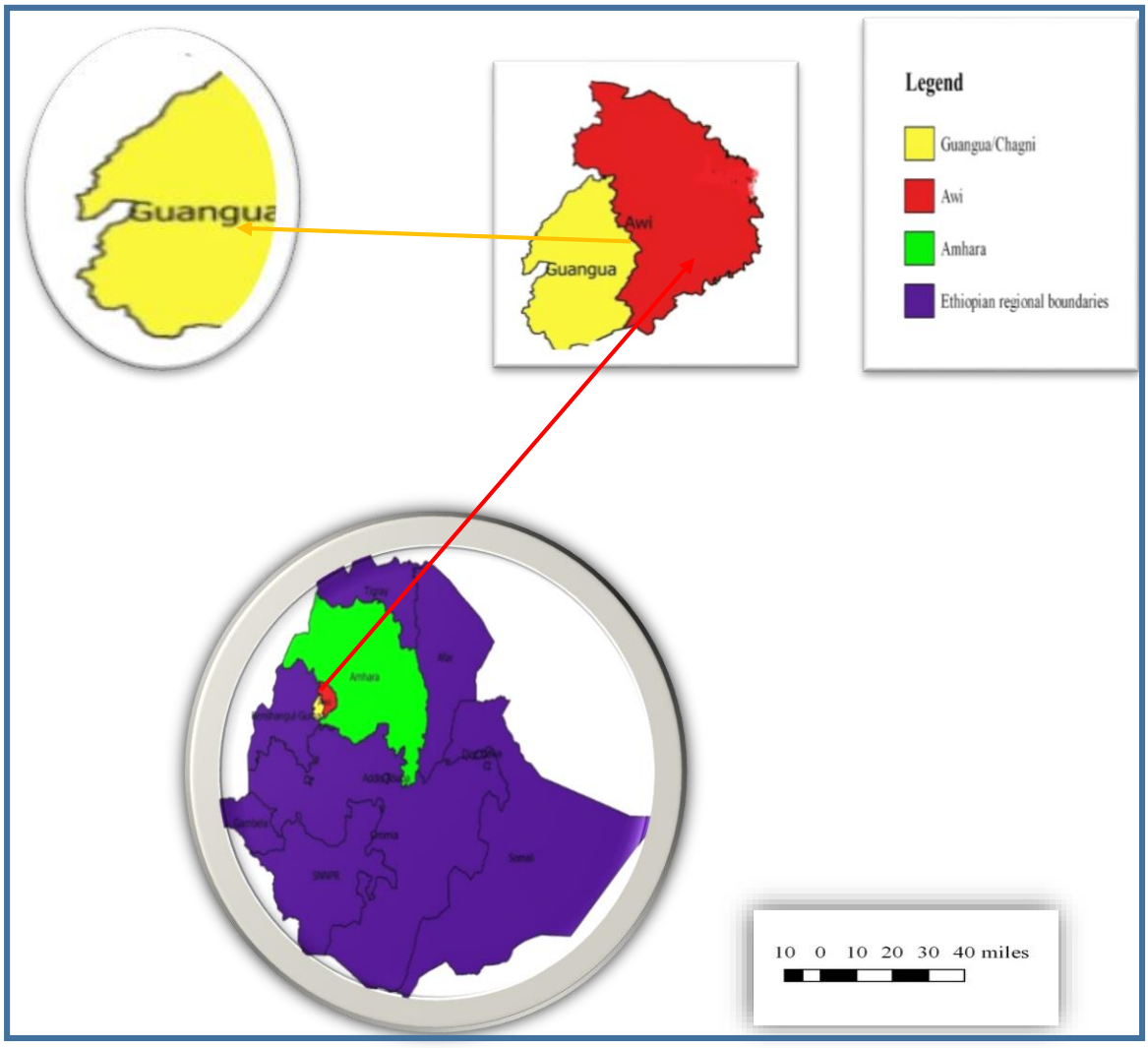


Figure 3-1 Map of the study area

3.2. Study population

The study was conducted in lactating dairy cows from Chagni Cattle Breeding and Improvement Ranch, and small holder dairy farms, irrespective of breed difference. Lactating cows having clinical symptoms of mastitis (the five cardinal signs of inflammation) and apparently healthy were included in the study.

3.3. Study Design, Sample Size Determination and Sampling Strategy

A cross-sectional study was carried out from October 2022 to June 2023 at Changi Cattle Breeding and Improvement Ranch, and small holder dairy farms to estimate the prevalence of mastitis and anti-microbial susceptibility profile of *S. aureus* isolated from bovine mastitic milk.

The sample size was estimated following the method described by Thrusfied (2005).

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

$$n = \frac{1.96^2(0.282)(1 - 0.282)}{0.05^2}$$

$$n = 318$$

Where,

n= sample size, 1.96 = the value of 'Z" at 95% confidence interval

P_{exp} = expected prevalence

d = desired absolute precision

Therefore, **s**ample size was determined at a 95% confidence interval with 5% precision from previous studies in the study area (Molalegne Bitew *et al.*, 2010), with an expected prevalence of 28.2%. Therefore, a total of 318 lactating cows were sampled, 212 from Changi cattle breeding and improvement Ranch out of 462, and 106 from small holder dairy farms out of 231, using a simple random sampling technique. The number of study animals from each production system was selected based on the proportion of the cattle population.

3.4. Questionnaire Survey and Observation

A semi-structured questionnaire was developed, and all information used to evaluate the effect of selected potential risk factors on the occurrence of mastitis was recorded through interviews and personal observation during milk sample collection. Animal-level factors such as age, breed, lactation stage, parity, presence of a tick on the teat, and previous mastitis history were recorded. Farm-level factors such as management system, barn floor type, use of towels, milking sequences, and hygiene were recorded and considered as risk factors. The lactation stage was categorized as early (1-2 months), mid (3-6 months), and late (7 months and above), and parity as few (1–2 calves), moderate (3–5 calves), and many (6 and above calves) (Endrias Zewdu *et al.*, 2022); age as young (3-5 years), adult (6–8 years), and old (9 years and above) (Payne, 1990).

3.5. Physical Examination of the Udder and Milk

The udders of all selected lactating cows were examined by visual inspection and palpation for the presence of any signs of fibrosis, inflammation, damage, tissue atrophy, or enlargement of the supra-mammary lymph nodes. During the inspection and palpation of the gland, the cardinal symptoms of inflammation, uniformity, size, stiffness, blindness, and consistency of the udder quarters were examined. Each teat quarter's milk secretion was analyzed for viscosity and appearance to check for the presence of clots, flakes, blood, and watery discharges. The presence of clots, flakes, blood, and other consistency changes were part of the indicators for clinical mastitis, along with udder and teat morphological changes (Demelash Biffa *et al*., 2005).

3.6. California Mastitis Test (CMT)

The California mastitis test (CMT), which was locally produced and validated with commercial CMT reagents, carried out in the field as a screening test for subclinical mastitis. The somatic cell count of milk was easily determined by the cow's side CMT indicator. It works by rupturing the cell membrane of any cells found in the milk sample, enabling their DNA to react with the test reagent and form a gel (White *et al*., 2005). It was carried out

according to the procedure described by Singh *et al*. (2006). The test cow's udder was cleansed with soap and water, and then dried with a fresh towel. The first few drops of milk were then expelled from each quarter of the udder. After that, around two milliliters of milk samples were placed in each of the four shallow cups on the CMT paddle, and an equal amount of CMT reagent was added to each cup. Each cup was then gently stirred for 15–20 seconds in a circular horizontal plane. Positive samples show gel formation within a few seconds. Cows were classified as positive for CMT when at least one-quarter of the teats were tested positive.

3.7. Milk Sample Collection

Milk samples were collected by standard milk sampling techniques from each of the clinically and subclinically mastitic non-blind quarters of the selected cows for bacterial isolation (NMC, 2004). Aseptic procedures were followed when collecting milk samples in order to prevent contamination with microorganisms present on the skin of the udder and teats, and in the barn environment. Thus, the teat end of each half udder was washed with tap water and soap, and dried. After drying, the teat ends were wiped thoroughly with cotton soaked in 70% ethyl alcohol. Recontamination of teats during scrubbing was avoided by scrubbing carefully, first the teats on the far side of the udder, then those on the near side. A separate pledge of cotton was used for each teat. Teats towards sample collector were sampled first, followed by the far ones. After the first three milking streams (first jets) were discarded, the teat orifice was disinfected again as described by Singh *et al*. (2006). About 10 ml of milk samples from strongly reacting teats from each animal were aseptically placed into sterile test tubes with tight-fitting cups. Consequently, samples were labeled with permanent markers and put in an icebox for transportation to BahirDar Biotechnology Institute laboratory for bacterial isolation, identification, and antimicrobial susceptibility testing. In the laboratory, samples were cultured immediately on standard bacteriological media to isolate and identify *S. aureus* (Sharma and Brinty, 2014).

3.8. Laboratory Examination

3.8.1. Bacterial Isolation and Identification

To comply with the specific objective of the study, bacteriological examination was focused on the isolation and identification of *S. aureus*, which is the most common cause of contagious mastitis in dairy herds worldwide. The isolation of *S. aureus* was carried out according to the standard microbiological procedures described by NMC (2004). In the case of refrigerated milk samples, as bacteria might be concentrated in the cream layer and held in clumps of fat globules, dispersion of fat and bacteria was accomplished by warming the samples at 25 °C for 15 min before plating on mannitol salt agar. Samples were allowed to stand for a while for the foam to disperse before inoculation. The isolation and identification of *S. aureus* was conducted on the basis of colony morphology on mannitol salt agar base, hemolytic properties, Gram-stain, catalase production, and slide and tube coagulase tests (Aycicek *et al.*, 2005).

One standard loop (0.01 ml) of milk sample was streaked on mannitol salt agar (Himedia Labs) using the quadrant streaking method for each cow. After 24-48 hours' incubation, colonies appeared yellow with a change in pH in the media (from red to yellow), regarded as presumptive identification of *S. aureus*. The phenol-red pH indicator detected the acidic metabolic product of mannitol. Fermentation of mannitol by *S. aureus* causes yellow discoloration of the medium. Presumed staphylococcal colonies were then subcultured on nutrient agar plates (NAP) and incubated at 37^{o}C for 24–48 hours to get a pure culture (a clone of cells derived from a single cell). All suspected colonies of *S. aureus* were subjected to Gram's stain and observed under a light microscope for Gram's reaction, size, shape, and cell arrangements. Hence, *S. aureus* is a gram-positive bacterium; they remain stained because of their thick cell wall, which is not permeable to alcohol, and they remain blue or purple. The Gram-stained smears from typical colonies that showed Gram-positive cocci occurring in bunched, grapelike irregular clusters were taken as presumptive *S. aureus* (Quinn *et al*., 2002).

The isolated colonies from nutrient agar were exposed to catalase tests, tube and slide coagulase tests, and hemolytic activity on sheep blood agar. The culture to be tested for catalase was picked using a sterile loop full of nutrient agar plates (NAP) and mixed with a drop of 3% hydrogen peroxide on a clean glass slide, and *S. aureus* (catalase positive) formed bubbles of oxygen liberated within a few seconds. Tube coagulase tests were performed by

adding a pure colony to 0.5 ml lyophilized of rabbit plasma, then mixing and incubating for 4–24 hours at 37°C. The reaction was considered positive (*S. aureus*) if any degree of clotting was visible within the tube when tilted. A slide coagulase test was performed by adding a pure colony to a drop of rabbit plasma and mixing well with a needle; clumping of cocci within 5–10 seconds was taken as positive. The hemolytic activity of *S. aureus* was evaluated after

culturing on a blood agar base enriched with 5% defibrinated sheep blood (Quinn *et al*., 2002). The plates were examined for the presence of Staphylococcus colonies. Isolates supposed to belong to Staphylococcus species on the basis of their morphological aspects (a glistening, smooth, opaque to golden yellow color) and complete hemolytic pattern on the surface of blood agar were collected.

3.8.2. In-vitro Antimicrobial Susceptibility Testing of *Staphylococcus aureus*

The susceptibility of isolates to different anti-microbial agents was determined by the disk diffusion method using commercial disks and interpreted based on CLSI (2022). The disks were impregnated with the following antibiotics: Gentamicin (10µg), Erythromycin (15µg), Tetracycline (30µg), Sulfamethoxazole (25µg), Clindamycin (2µg), Azithromycin (25µg), Ciprofloxacin (5µg), Cefoxitin (30µg). The selection of antibiotics was based on their availability (Table 3.1).

Table 3-1. Inhibition zone diameter (mm) interpretation cut off point (CLSI, 2022)

Antibiotics	**Unit**	**Diameter of zone of inhibition in mm**		
		Resistant ≤	**Intermediate**	**Susceptible ≥**
Clindamycin	2µg	≤14	15-20	≥21
Erythromycin	15µg	≤13	14-22	≥23
Gentamycin	10µg	≤12	13-14	≥15
Sulfamethoxazole	25µg	≤10	11-15	≥16
Tetracycline	30µg	≤14	15-18	≥19
Azithromycin	15µg	≤13	14-17	≥18
Ciprofloxacin	5µg	≤15	16-20	≥21
Cefoxitin	30 µg	≤24	_	≥25

According to CLSI (2022), 24 hours old pure colonies of *S. aureus* cultures were inoculated in saline water (direct colony suspension) to get turbidity equal to 0.5 on the McFarland scale (108 CFU/ml). A sterile cotton swab was dipped into the inoculation, and the excess cultures were removed by pressing the swab to the sides of the tube. The entire Mueller-Hinton agar surface was swabbed. The inoculation was allowed to dry for 15 minutes. Antibiotic discs were applied to the medium using sterile forceps and pressed gently to ensure complete contact with the agar surface. The plates were incubated at 37°C and examined after 24 hours for the presence or absence of zones of inhibition (Kengne *et al*., 2019). The isolates were classified in accordance with the guidelines of the Clinical and Laboratory Standard Institute (CLSI) as susceptible, intermediate, or resistant for each antibiotic tested according to the manufacturer's instructions by measuring the zone of inhibition around the antibiotic disc using a ruler (Huber *et al*.,2011).

3.9. Data Management and Analysis

Collected data was fed to a Microsoft Excel 2016 spread sheet, and the process of data cleaning, handling, validating, and coding was done on this spread sheet. To process the data obtained from the questionnaire, mastitis result, and bacteriological examination, SPSS V23 statistical software was used. A multiple logistic regression analysis was used to identify potential risk factors associated with the prevalence of mastitis. First, univariable logistic regression analysis was performed, and potential risk factors (explanatory variables) with $p < 0.25$ were screened for the multivariable logistic regression. Model fit was examined by post-estimation goodness-of-fit tests, namely the Hosmer-Lemeshow test. The associations were considered statistically significant when $p < 0.05$ at 95% confidence level in multivariable logistic regression analysis. Odds ratios at a 95% confidence interval were used to express the strength of the risk of the diseases associated with the tested factors.

CHAPTER 4. RESULTS

4.1. Prevalence of Mastitis

A total of 318 lactating cows examined for mastitis and 44.1% (140/318) were positive for mastitis and 26 (8.2%) and 114 (35.9%) categorized into clinical and subclinical mastitis, respectively. From 1272 quarters examined 55(4.3%) were blind, and 243 (20%) were positive for mastitis. In quarter prevalence of mastitis in the study site, right rear (RR) showed the highest rate of infection (26.6%) followed by the right front quarter (RF) (21.0%). The detailed prevalence of clinical and sub clinical mastitis at cow, and quarter levels in Chagni Cattle breeding and Improvement Ranch, and in small holder dairy farms are indicated in (Table 4.1).

Table 4-1. Prevalence of clinical and sub clinical mastitis at cow and quarter levels

Observational Level	**Total No of Examined**	**Results**	
		Total No. of Positive	**Prevalence (%)**
Cow level			
Subclinical mastitis	318	114	35.9
Clinical mastitis	318	26	8.2
Overall mastitis	**318**	**140**	**44.1**
Quarters			
Right Front (RF)	305	64	21.0
Left Front (LF)	309	44	14.2
Right Rear (RR)	304	81	26.6
Left Rear (LR)	303	54	17.8
Total Quarter	**1221**	**243**	**20.0**

RF= right front, LF= left front, RR = right rear, LR= left rear

4.2. Logistic Regression Analysis of Risk Factors

4.2.1. Chagni Cattle Breeding and Improvement ranch

Old cows were 5.85 times (OR: 5.85; 95% CI: 2.91-11.78) more likely to have a chance of contracting mastitis than young cows. Cows that given birth >6 and between 3-5 times were

3.89 (OR: 3.89; 95% CI: 1.85-8.1) and 2.13 (OR: 2.13; 95% CI: 1.11-4.07) times more likely to have a chance of contracting mastitis than cows that given birth 1-2 times respectively. Mid and late lactating cows were 96% (OR: 0.04; 95% CI: 0.01-0.10) and 83% (OR: 0.17; 95% CI: 0.08-0.35) less likely to have a chance of contracting mastitis than cows in the early lactation stage respectively. Cross breed cows were 3.55 times (OR: 3.55; 95% CI: 1.19-10.62) more likely to have a chance of contracting mastitis than local breed cows. Intensively managed cows were 3.55 times (OR: 3.55; 95% CI: 1.19-10.62) more likely to have a chance of contracting mastitis than semi-intensively managed cows. Cows that had muddy floor type were 3.55 (OR: 3.55; 95% CI: 1.19-10.62) times more likely to have a chance of contracting mastitis than cows that had concrete floor type and also cows that had tick on teat 2.96 (OR: 4.75: 95% CI: 2.07-10.88) times more likely to have a chance of contracting mastitis than Cows that had not tick on teat. However, mastitis history was not statistically associated with Mastitis prevalence (P>0.05) (Table 4.2).

Table 4-2 Univariable logistic regression analysis of risk factors in Chagni Cattle Breeding and Improvement Ranch

Risk factors	Categories	No. of animals examined	Prevalence (% of positive)	COR (95% CI)	*p*-value
Breed	local	196	75(38.3%)	Ref.	Ref.
	cross	16	11(68.8%)	3.55 (1.19-10.62)	0.023
Age	Young	85	20(23.5%)	Ref.	Ref.
	Adults	57	21(36.8%)	1.9 (0.91-3.96)	0.088
	Old	70	45(64.3%)	5.85 (2.91-11.78)	0.000
Management system	Semi-intensive	196	75(38.3%)	Ref.	Ref.
	Intensive	16	11(68.8%)	3.55 (1.19-10.62)	0.023
Lactation stage	Early	84	61(72.6%)	Ref.	Ref.
	Mid	67	6(9%)	0.04 (0.01-0.10)	0.000
	Late	61	19(31.1%)	0.17 (0.08-0.35)	0.000
Parity	1-2	91	25(27.5%)	Ref.	Ref.
	3-5	74	35(44.6%)	2.13 (1.11-4.07)	0.023
	>6	47	28(59.6%)	3.89 (1.85-8.1)	0.000
Mastitis history	Absent	142	62(43.7%)	Ref.	Ref.
	Present	70	24(34.3%)	0.67 (0.37-1.22)	0.192
Floor type	Concrete	196	75(38.3%)	Ref.	Ref.
	Muddy	16	11(68.8%)	3.55 (1.19-10.62)	0.023
Tick on teat	No	180	63(35%)	Ref.	Ref.
	Yes	32	23(71.9%)	4.75 (2.07-10.88)	0.000
Total		**212**	**86 (40.6%)**		

COR = Crude odd ratio; CI = confident interval

Multivariable logistic regression procedures were used to model the effects of potential risk factors on outcome variables (mastitis). The final multivariable logistic regression model showed that age, breed, management system, lactation stage, parity, floor type and tick on teat were independently associated with mastitis in Chagni Cattle Breeding and Improvement Ranch ($P<0.05$) (Table 4.3).

Table 4-3 Multivariable logistic regression analysis of risk factors in Chagni Cattle Breeding and Improvement Ranch

Risk factors	Categories	No. of animals examined	Prevalence (% of positive)	AOR (95% CI)	*p*-value
Breed	local	196	75(38.3%)	Ref.	Ref.
	cross	16	11(68.8%)	6.09 (1.36-27.28)	0.018
Age	Young	85	20(23.5%)	Ref.	Ref.
	Adults	57	21(36.8%)	2.58 (0.96-6.94)	0.061
	Old	70	45(64.3%)	2.80 (1.10-7.14)	0.031
Management system	Semi-intensive	196	75(38.3%)	Ref.	Ref.
	Intensive	16	11(68.8%)	6.09 (1.36-27.28)	0.018
Lactation stage	Early	84	61(72.6%)	Ref.	Ref.
	Mid	67	6(9%)	0.03 (0.01-0.11)	0.000
	Late	61	19(31.1%)	0.16 (0.07-0.39)	0.000
Parity	1-2	91	25(27.5%)	Ref.	Ref.
	3-5	74	35(44.6%)	2.43 (1.01-5.83)	0.047
	>6	47	28(59.6%)	4.15 (1.49-11.54)	0.006
Mastitis history	Absent	142	62(43.7%)	Ref.	Ref.
	Present	70	24(34.3%)	1.23 (0.47-3.22)	0.670
Floor type	Concrete	196	75(38.3%)	Ref.	Ref.
	Muddy	16	11(68.8%)	6.09 (1.36-27.28)	0.018
Tick on teat	No	180	63(35%)	Ref.	Ref.
	Yes	32	23(71.9%)	2.96 (1.13-7.79)	0.028
Total		**212**	**86 (40.6%)**		

AOR= Adjusted odd ratio; CI = confident interval

4.2.2. Small holder dairy farms

Old cows were 4.08 times (OR: 4.08; 95% CI: 1.47-11.37) more likely to have a chance of contracting mastitis than young cows. Mid and late lactating cows were 97% and 85% less likely to have a chance of contracting mastitis than cows in the early lactation stage respectively, and also the odds of mastitis were 16.43 times higher in cows having tick infestation compared to cows without tick infestation. Cows that given birth >6 and between 3-5 times were 8.36 (OR: 8.36; 95% CI: 2.57-27.17) and 5.98 (OR: 5.98; 95% CI: 2.16-16.61) times more likely to have a chance of contracting mastitis than cows that given birth 1-2 times respectively. Cross breed cows were 6.00 times (OR: 6.00; 95% CI: 2.58-13.97) more likely to have a chance of contracting mastitis than local breed cows. Cows that had muddy floor type were 2.57 times (OR: 3.55; 95% CI: 1.16-5.70) times more likely to have a chance of contracting mastitis than cows that had concrete floor type. Hand prewashing of milkers before milking was 71% less likely to have a chance of contracting mastitis than cows milked without prewashing. However, management system, udder washing and use of towel were not statistically associated with mastitis prevalence (Table 4.4).

Table 4-4 Univariable logistic regression analysis of risk factors in small holder dairy farms

Risk factors	Categories	No. of animals examined	Prevalence (% of positive)	COR (95% CI)	*p*-value
Breed	local	57	18(31.6%)	Ref.	Ref.
	cross	49	36(73.5%)	6.00 (2.58-13.97)	0.000
Age	Young	30	11(36.6%)	Ref.	Ref.
	Adults	39	17(43.6%)	1.34 (0.50-3.54)	0.562
	Old	37	26(70.3%)	4.08 (1.47-11.37)	0.007
Management system	Semi-intensive	63	29(46%)	Ref.	Ref.
	Intensive	43	25(58.1%)	1.63 (0.74-3.56)	0.222
Lactation stage	Early	22	20(90.9%)	Ref.	Ref.
	Mid	46	11(23.9%)	0.03 (0.01-0.16)	0.000
	Late	38	23(60.5%)	0.15 (0.03-0.75)	0.021
Parity	1-2	33	7(21.2%)	Ref.	Ref.
	3-5	47	29(61.7%)	5.98 (2.16-16.61)	0.001
	>6	26	18(69.2%)	8.36 (2.57-27.17)	0.000
Mastitis history	Absent	82	37(45.1%)	Ref.	Ref.
	Present	24	17(70.8%)	2.95 (1.11-7.88)	0.031
Floor type	Concrete	43	16(37.2%)	Ref.	Ref.
	Muddy	63	38(60.3%)	2.57 (1.16-5.70)	0.021
Tick on teat	No	73	32(43.8%)	Ref.	Ref.
	Yes	33	22(66.6%)	2.56 (1.09-6.05)	0.032
Hand prewashing	No	71	43(60.6%)	Ref.	Ref.
	Yes	35	11(31.4%)	0.29 (0.13-0.70)	0.006
Udder washing	No	67	38(56.7%)	Ref.	Ref.
	Yes	39	16(41%)	0.53 (0.24-1.18)	0.121
Use towel	No	90	44(44.4%)	Ref.	Ref.
	Yes	16	10(62.5%)	1.74 (0.58-5.19)	0.319
Milking order	No	77	44(57.1%)	Ref.	Ref.
	Yes	29	10(34.5%)	0.39 (0.16-0.96)	0.040
Total		**106**	**54 (50.9%)**		

COR = Crude odd ratio; CI = confident interval

Multivariable logistic regression procedures were used to model the effects of potential risk factors on outcome variables (mastitis). The final multivariable logistic regression model showed that age, lactation stage and tick on teat were independently associated with mastitis in small holder dairy farms ($P<0.05$) (Table 4.5).

Table 4-5 Multivariable logistic regression analysis of risk factors in small holder dairy farms

Risk factors	Categories	No. of animals examined	Prevalence (% of positive)	AOR (95% CI)	*p*-value
Breed	local	57	18(31.6%)	Ref.	Ref.
	cross	49	36(73.5%)	4.63 (0.68-31.67)	0.118
Age	Young	30	11(36.6%)	Ref.	Ref.
	Adults	39	17(43.6%)	1.63 (0.30-8.89)	0.574
	Old	37	26(70.3%)	6.57 (1.01-42.79)	0.049
Management system	Semi-intensive	63	29(46%)	Ref.	Ref.
	Intensive	43	25(58.1%)	1.36 (0.18-10.56)	0.768
Lactation stage	Early	22	20(90.9%)	Ref.	Ref.
	Mid	46	11(23.9%)	0.01 (0.00-0.078)	0.000
	Late	38	23(60.5%)	0.06 (0.01-0.81)	0.034
Parity	1-2	33	7(21.2%)	Ref.	Ref.
	3-5	47	29(61.7%)	1.47 (0.25-8.50)	0.669
	>6	26	18(69.2%)	4.06 (0.63-26.06)	0.140
Mastitis history	Absent	82	37(45.1%)	Ref.	Ref.
	Present	24	17(70.8%)	1.36 (0.16-11.34)	0.774
Floor type	Concrete	43	16(37.2%)	Ref.	Ref.
	Muddy	63	38(60.3%)	4.67 (0.90-24.28)	0.067
Tick on teat	No	73	32(43.8%)	Ref.	Ref.
	Yes	33	22(66.6%)	16.43 (1.73-155.6)	0.015
Hand prewashing	No	71	43(60.6%)	Ref.	Ref.
	Yes	35	11(31.4%)	0.27 (0.04-2.06)	0.208
Udder washing	No	67	38(56.7%)	Ref.	Ref.
	Yes	39	16(41%)	0.40 (0.09-1.88)	0.248
Milking order	No	77	44(57.1%)	Ref.	Ref.
	Yes	29	10(34.5%)	0.28 (0.03-2.27)	0.233
Total		**106**	**54 (50.9%)**		

AOR = Crude odd ratio; CI = confident interval

4.3. Isolation Rate of *Staphylococcus aureus*

A total of 140 milk samples were collected and subjected to bacterial isolation from 26 clinical and 114 sub-clinical mastitic cows at Chagni Cattle Breeding and Improvement Ranch, and small holder dairy farms. Finally, the isolated *S. aureus* was 45.7% (95% CI: 41.3-49.7) of the total milk samples cultured. The proportion of isolation from milk of clinically and sub-clinically affected cows were 9, (34.6%) and 55, (48.2%), respectively (Table 4.6).

Table 4-6 Frequency of isolation *Staphylococcus aureus* between clinical and sub-clinical mastitis milk samples

Clinical mastitis		**Sub-clinical mastitis**		**Total No. of cultured**	**Total positive (%)**
No sample cultured	No. of positive (%)	No. of sample cultured	No. of Positive (%)		
26	**9(34.6%)**	**114**	**55(48.2%)**	**140**	**64(45.7)**

4.4. Antimicrobial Susceptibility Test Results

All 64 isolates of *S. aureus* from mastitic milk were tested for antimicrobial susceptibility to eight selected antibiotics. The isolates were highly susceptible to sulfamethoxazole (87.5 %) and Gentamycin (79.7 %) followed by Tetracycline (75%), Erythromycin (72%) and Azithromycin (71.8%). However, they were highly resistant to Cefoxitin (65.6%) followed by Tetracycline and ciprofloxacin each (25%) of the isolates (Table 4.7).

Table 4-7Antimicrobial susceptibility profiles of isolated colonies

Antibiotics	**Disc potency**	**Status of antimicrobial against the isolates Isolates (n = 64)**		
		Resistant (%)	**Intermediate (%)**	**Susceptible (%)**
Clindamycin	2µg	12(18.75%)	15(23.4%)	37(57.85%)
Erythromycin	15µg	16(25%)	2(3%)	46(72%)
Gentamycin	10µg	8(12.5%)	5(7.8%)	51(79.7%)
Sulfamethoxazole	25µg	8(12.5%)	0	56(87.5%)
Tetracycline	30µg	16(25%)	0	48(75%)
Azithromycin	15µg	18(28.2%)	0	46(71.8%)
Ciprofloxacin	5µg	16(25%)	8(12.5%)	40(62.5%)
Cefoxitin	30 µg	42(65.6%)	0	22(34.4%)

CHAPTER 5. DISCUSSION

The present study was conducted at Chagni cattle breeding and improvement Ranch and small-holder dairy farms in Awi Zone in order to estimate the prevalence and anti-microbial susceptibility profile of *Staphylococcus aureus* isolated from bovine mastitis and assess the major risk factors associated with mastitis. The result revealed that the overall prevalence of mastitis in dairy cows was 44.1% (95% CI: 39.0-49.4). This result was in agreement with the previous studies conducted by Girma Debele (2010), Elbably *et al.* (2013), Yusuf (2022), and Jafer Kedir *et al.* (2016), who reported a prevalence of 44.1% around Holeta, 42.9% in Egypt, 44.5% in Mogadishu (Somalia), and 39.2% in Dire-Dawa, respectively.

The present finding was comparably higher than the study of Katsande *et al.* (2013), Getahun Kassaye (2006), Tesfanesh Fentahune *et al*. (2018), and Mulunesh Yenew and Habtamu Addis (2020), who reported 21.1% in Zimbabwe, 36.9% in the central highlands of Ethiopia, 36.1% in and around Wukro (Tigray region), and 27.2% around Dessie, respectively. However, the prevalence of the present finding was lower than Mdegela *et al*. (2009), Iraguha *et al.* (2015), Melese Etifu (2012), Addisalem Tadesse and Mersha Chanie (2012), Tariku Sori *et al.* (2011), Tesfaheywet Zeryehun *et al.* (2013), Dabash Hailemeskel *et al.* (2014), Denekew Wubetu (2021), Amanu Marama *et al.* (2016), and Getachew Nigussie (2020), who reported a prevalence of 51.6% in Tanzania, 50% in London, 73% in Alage, 65.3% in Addis Ababa, 75.22% in Jimma town, 74.7% in and around Addis Abeba, 88.9% in North Showa, 56.88% in and around Mottta town, 61.76% in Holeta, and 61.5% in Lome woreda (Oromia), respectively. This prevalence difference could be due to the fact that, mastitis is a complex disease involving interactions of various factors such as management and husbandry, environmental factors, animal risk factors, and causative agents (Demelash Biffa *et al.,* 2005; Radostits *et al.,* 2007).

Comparison of the current findings by study sites indicated that the overall prevalence of clinical mastitis in the study area was 8.2% (95% CI: 5.0–11.9). This result was Comparable to Tesfaye Bekele *et al*. (2019), Elbably *et al.* (2013), and Bedada and Hiko (2011), who

reported a prevalence of 8.3% in Sululta, 9.87% in Egypt, and 10% in Asella, respectively. But lower when compared with the reports of Haben Fesseha *et al.* (2021), Mekibib *et al.* (2010), and Tesfaheywet Zeryehun and Gerema Abera (2017), who reported prevalence of 29.2% in Modjo town, 22.4% in Holeta town, and 12.5% in Eastern Harrarghe. On the other hand, the result of the present finding was higher than the reports of Denekew Wubetu (2021) and Molalegne Bitew *et al.* (2010), who reported 3.67% in and around Motta town, and 3.9% in Bahir Dar, respectively.

The overall prevalence of sub-clinical mastitis in the study area was 35.8% (CI: 30.5–40.9), and this was lower than the prevalence of sub-clinical mastitis reported by Tadesse Teklesilasie *et al.* (2014), 85.4% in Addis Ababa, and Birhanu Abera *et al.* (2013), 54.5% in Asella. The prevalence of the present study was related to that of Elbably *et al.* (2013), Tariku Sori *et al.* (2011), Fufa Abunna *et al.* (2013), Biniam Tadesse *et al.* (2017), Nibret Moges *et al.* (2011), and Shamsaldeen *et al.* (2022), who reported a prevalence of 33.05% in Egypt, 36.67% in Jimma town, 36.86% in Addis Ababa City, 36.18% in and around Walaita Sodo, 30.6% in and around Gondar, and 31.4% in East Coast Malaysia. However, the present finding was higher than Mekibib *et al.* (2010), who reported 25.22% in Holeta.

In most reports, including the present study, clinical mastitis is far less common than subclinical mastitis. This could be due to lack of attention was given to the subclinical mastitis, and also the infected animal does not show any visible symptoms and secretes apparently normal milk. Therefore, farmers not well informed about the invisible loss from subclinical mastitis. In Ethiopia, the subclinical forms of mastitis have received little attention, and efforts have been concentrated on the treatment of clinical cases (Gizat Almaw *et al.*, 2008).

The overall quarter's level prevalence of dairy cow mastitis in the study area was 20.0% (95% CI: 16.9–21.4). These findings were relatively similar to the findings of Kerro and Tareke (2003), who reported 19% in selected areas of southern Ethiopia. The current finding was relatively higher than findings reported by Dimshasha Tolera *et al*. (2021), Sisay Girma

et al. (2012), Molalegne Bitew *et al*. (2010), and Tesfaye Belachew (2016), who reported the prevalence was 8.3% in the west shewa zone (oromia), 10.12% in Doba District (East Hararghe zone), 12.3% in and around Bahir Dar town, and 6% around Bishoftu town, respectively. However, the present finding was lower than Radostits *et al.* (2000), and Edilu and Tola (2017), who reported 25% in London and 44.4% in West Shewa zone, respectively. The difference in the results might be related to the variation in veterinary services, environmental factors, genetic factors, management practices, and hygienic practices at different places. In Ethiopia, a large number of farmers treat their sick animals themselves or get their services from para-veterinarians. Such practices are particularly common in areas where there is limited veterinary service.

In the quarter prevalence of mastitis, the right rear (RR) showed the highest rate of infection, followed by the right front (RF), accounting for 26.6% and 21.0%, respectively, which was in line with the findings of Hunderra Sori *et al*. (2005). The highest prevalence in rear quarters might be due to greater production capacity, the likelihood of fecal and environmental contamination of rear quarters (Yibrah Tekle and Tsega Berihe, 2016), incomplete milking of the rear quarters, and ease of first grasping by the milker's hand in the case of right front quarters (Hunderra Sori *et al*., 2005).

Among the risk factors considered in the current study area, age, breed, management system, stage of lactation, parity, floor type, and tick on the teat were found to be statistically significant ($P<0.05$). Old cows were 2.8 (OR =2.80, 95% CI = 1.10–7.14) and 6.6 (OR =6.57, 95% CI = 1.0–42.79) times more likely to have a chance of contracting mastitis than young cows in Chagni Cattle Breeding and Improvement Ranches and small-holder dairy farms, respectively, by keeping other factors constant. This finding comparable with the findings of Demelash Biffa *et al*. (2005) in Southern Ethiopia, who reported that old cows were 2.6 times more likely to have mastitis than young cows, and Abera *et al*. (2010) in Adama, who reported a more likely occurrence of mastitis in animals aged above 6 years (OR = 3.8, 95% CI = 1.0-15.0). Furthermore, the present finding was in agreement with the findings of Joshi

and Gokale (2006) in India; Mungube *et al*. (2004) in central highland of Ethiopia; Matios Lakew *et al*. (2009) in Asella; Tesfaheywet Zeryehun *et al*. (2013) in around Adiss Ababa; Kerro and Tareke (2003) in Southern Ethiopia; Busato *et al*. (2000) in Switzerland; Hunderra Sori *et al*. (2005) in and around Sebeta; Mekibib *et al*. (2010) in Holeta; Rahman *et al*. (2009) in Bangladesh; Hameed *et al*. (2012) in Pakistan; Sisay Girma *et al*. (2012) in West Harerghe zone; Nibret Moges *et al*. (2012) in Hawassa; Islam *et al*. (2011) in Bangladesh and Jafer Kedir *et al.* (2016) in Dire Dawa City all reported that the less likely chance of mastitis occurrence in younger cows. However, it was different from the findings of Ahmed *et al*. (2015) in Sudan, Sampimon *et al*. (2009) in the Netherlands, and Salvador *et al*. (2012) in the Philippines, which stated that, a higher prevalence of mastitis in heifers. Higher prevalence in older cows in this study might be due to having the largest teats and more relaxed sphincter muscles, which increase the accessibility of infectious agents in the cow's udder, as explained by Radostitis *et al*. (2007), and repeated exposure to milking practices (FAO, 2014).

In the current study, cross-breed cows were 6.09 times more likely to have a chance of contracting mastitis than local-breed cows at Chagni Cattle breeding and improvement Ranch. This finding agreed with the findings of Shittu *et al*. (2012) in Nigeria; Nibret Moges *et al*. (2012) in Hawassa; Joshi and Gokale (2006) in India; Hunderra Sori *et al*. (2005) in and around Sebeta; Matios Lakew *et al*. (2009) in Asella; Rahmeto Abebe *et al*. (2016) in Hawassa; and Iraguha *et al*. (2015) in Rwanda, stating that crossbred dairy cows were generally more susceptible to mastitis than indigenous breeds. It was contrary to the findings of Tesfaheywet Zeryehun and Gerema Abera (2017) in the Eastern Harrarghe Zone and Rahman *et al*. (2009) in Bangladesh, who reported no significant difference between HF crossbreds and zebu. Since the cows in this study were not all under similar conditions, the variability of mastitis occurrence between breeds might be due to other uncontrolled factors, such as management, rather than a true breed difference (Kerro and Tareke, 2003).

The likelihood of mastitis occurrence was 6.09 times higher in cows managed in intensive farming systems than in cows managed in semi-intensive farms at Chagni cattle breeding and improvement Ranch. The current finding was in agreement with the reports of Tesfaye Deme and Biressaw (2015) in Iemu Bilbilo District of Arsi Zone; Jirata Shiferaw and Indalem Telila (2017) in and around Wolayta Sodo; and Hunderra Sori *et al.* (2005) in and around Sebeta, indicating higher prevalence in intensive farms. However, Nibret Moges *et al*. (2011) in Gondar, Rahmeto Abebe *et al*. (2016) at Hawassa milk shed, South Ethiopia, and Amanu Marama *et al*. (2016) in Holeta reported the absence of a significant association ($p > 0.05$) between management systems and the occurrence of mastitis. The variability of reports regarding management systems might be due to variation in the hygienic standards of the dairy environment and milking conditions as well as genetic variation in disease resistance among the breeds maintained in the systems, which overwhelmingly dominate intensive dairy farms in the study area.

Mid- and late-lactation cows were 97% and 84% less likely to have a chance of contracting mastitis than cows in the early lactation stage in Chagni Cattle Breeding and Improvement Ranchi, respectively. However, in small-holder dairy farms, it was 99% in mid-lactation and 94% in late lactation. The current finding was in agreement with Asmelash Tassew *et al.* (2016) in and around Assosa town, and Iraguha *et al*. (2015) in Rwanda. Perhaps this could be linked to the fact that diapedesis of neutrophils into the mammary gland takes longer in recently calved cows (Rostits *et al.* 2007) and increases oxidative stress and reduces antioxidant defense mechanisms during early lactation (Sharma *et al*., 2011). Moreover, the absence of a dry cow therapy regime could possibly be among the major factors contributing to the higher prevalence in early lactation. In contrast to the present finding, Rediet Belayneh *et al*. (2013); Getahun *et al*. (2008); Gizat Almaw *et al.* (2008), who reported higher prevalence of mastitis in late stage of lactation, while Mureithi and Njuguna (2016) in Kenya found a significantly higher prevalence in mid lactation stage.

In the present study, the occurrence of mastitis was significantly influenced by parity number. Cow's having 6 calvings and between 3-5 calvings were, 4.15 and 2.43 times more likely to have a chance of contracting mastitis than cows having 1-2 calvings, respectively. The increased occurrence of mastitis with parity in the current study was in agreement with the previous reports of Mekibib *et al.* (2010) in Holota town, Busato (2000) in Switzerland, and Rgbe Haftu *et al.* (2012) in northern Ethiopia. This difference might be due to the increased opportunity for infection with time and the prolonged duration of infection, especially in a herd without a mastitis control program, and also to the fact that primiparous cows have a more effective defense mechanism than multiparous cows (Radostits *et al*., 2007). In contrast to the present findings, Benta Duro and Habtamu Taddele (2011) in Batu, Ethiopia, and Ahmed *et al*. (2015) in the northern state of Sudan, neither showed a statistically significant association between parity and the occurrence of mastitis.

Cows that had a muddy floor type were 6.09 times more likely to have a chance of contracting mastitis than cows that had a concrete floor type. In this study, the floor system had a significant influence on the occurrence of mastitis. This result was in agreement with the previous reports of Birhanu Abera *et al*. (2013) in Adama town, and Demelash Biffa *et al.* (2005) in southern Ethiopia, which found a high prevalence of mastitis in farms with muddy (soil) floors when compared with concrete (cement) floor types. This is due to associations with poor sanitation and cows that were maintained in dirty and muddy common barns with bedding materials that favor the proliferation and transmission of mastitis pathogens (Asmelash Tassew *et al*., 2016).

Cows that had a tick on their teat were 2.96 times more likely to have a chance of contracting mastitis than cows that did not have a tick infestation. This result was in agreement with the work of Endrias Zewdu *et al*. (2022) in Holeta, Central Ethiopia, who reported that the mammary gland quarters with a tick were 4.31 times more likely to have a chance of contracting mastitis than those without a tick on teat. Moreover, similar results were reported by Matios Lakew *et al*. (2009) in Asella; Tekle Hailemariam and Eyob Eticha (2017) in

Hawassa; Hunderra Sori *et al*. (2005) in Sebeta; Kerro and Tareke (2003) in Southern Ethiopia; and Nibret Moges *et al*. (2012) in Hawassa. This might be due to injuries caused by ticks, which are known to cause direct inflammatory reaction to the mammary gland, necrosis, and abscess formation, which may lead to udder damage and/or exposure to serious secondary infections (Demelash Biffa *et al*., 2005). However, Ahmed *et al*. (2015) in Sudan and Sefinew Alemu *et al*. (2013) in and around Gonder town, Ethiopia, reported that the presence of udder ticks did not show a significant statistical association with mastitis.

Isolation rate of *S. aureus* from milk in the study area was found to be 45.7% (95% CI: 41.3–49.7). This finding was in line with the findings of Tesfanesh Fentahune *et al*. (2018) and Desiye Tesfaye *et al.* (2020), who reported 42.31% in and around Wukro (Tigray) and 42.25% in selected districts of central highland of Ethiopia, respectively. The present finding was comparably higher than the study of Alemu Asefa and Fikadu Kassa (2017), Sema Regasa (2019), Kanenus Dereje *et al.* (2018), *and* Fufa Abunna *et al.* (2013), who reported 39% in Sodo town, 15.3% in Mukaturi and Sululta town (Oromia), 30.9% in Holeta, and 21.13% in Addis Ababa City (Ethiopia), respectively. However, the present study was lower than Zenebe *et al.* (2014) who reported 51.7% in Adigrat, Northern Ethiopia.

The overall isolation rate of *S. aureus* from clinical mastitis was 34.6% (CI: 29.4–37.1). This was in agreement with the report by Kemal Kedir *et al*. (2017) 34.62% in and around Asella town. However, these results were higher than those reported by Gangwal *et al.* (2017) 24%. Additionally, the present prevalence was lower than the 42.85% reported by Saharan *et al* (2022).

The 48.2% (95% CI: 44.5–53.3) overall isolation rate *S. aureus* from sub-clinical mastitis was in agreement with the report by Zenebe *et al*. (2014) in Adigrat, who reported 49.43%. In contrast to the present finding, the higher prevalence was reported by Gianneechini *et al*. (2002) and Rahmeto Abebe *et al*. (2016), who reported 62.8% in Uruguay, and 54.5% in Hawassa, respectively. However, the lowest prevalence of *S. aureus* isolated from subclinical mastitis was reported by Shamsaldeen *et al.* (2022) at 16.5% in East Coast

Malaysia, Workineh *et al*. (2002) at 39.3% in Adiss Ababa, and Bedada and Hiko (2011) at 39.1 in Asella. This difference might be explained by the already documented pieces of evidence that, *S. aureus* species are adapted to survive in the udder (usually establish chronic subclinical infections of long duration) and serving as sources of infection for other healthy cows, and transmitted during the milking process. Transmission among cow's increases whenever there is the absence of dry cow therapy, post-milking teat dipping, and the low culling rate of chronically infected cows (Radostits *et al*., 2007).

All 64 isolates from mastitic milk of *S. aureus* were tested for antimicrobial susceptibility to eight selected antibiotics. The result of this study showed that *S. aurous* isolates were highly susceptible to the tested antibiotics like Sulfamethoxazole (87.5%), Gentamycin (79.7%), Tetracycline (75%), Erythromycin (72%), and Azithromycin (71.8%). These susceptibility profiles were in line with studies by Saikia *et al*. (2009) in Assam (Northeast India), Onwubiko and Sadiq (2011) in Kano (Northwest Nigeria), and Gentilini *et al*. (2000) in Argentina. The possible reason for the high susceptibility of antimicrobials might be, they are not frequently used in the study area of veterinary medicine for treatment of *S. aureus* infections and as growth promoters for animal production. Relatively high resistant isolates were recorded for cefoxitin (65.6%), which is consistent with the studies of Saikia *et al*. (2009) in Assam (Northeast India), and Aetrugh *et al*. (2017) in Libya. The resistance of *Staphylococcus aureus* to certain antibiotic groups in a specific region might be due to their frequent, long-term use of antibiotics and the distribution of the antibiotic-resistant gene among the pathogens all over the world (Sabour *et al*., 2004; Moon *et al*., 2007). 65.6% resistance of isolates to cefoxitin is suggestive that the recovered isolates in our study tend to be methicillin-resistant *Staphylococcus aureus.*

The limitations of this study include failure to perform molecular studies such as genotypic analysis to accurately identify *Staphylococcus aureus*. Therefore, further research should be focused on molecular characterization of *S. aureus* bacteria in mastitis cows in the study area

CHAPTER 6. CONCLUSION AND RECOMMENDATIONS

The results of the present study indicated a relatively high prevalence of bovine mastitis in dairy cows in the study areas. Similarly, the prevalence of subclinical mastitis was higher than that of clinical mastitis. Among the assessed potential risk factors for the prevalence of mastitis, age, breed, management system, lactation stage, parity, floor type, and tick infestation are significantly associated with the occurrence of the mastitis. Mastitis caused by pathogenic Staphylococcus was higher than many previous reports in Ethiopia. *Staphylococcus aureus* isolates from the study sites were found to be relatively resistant to Cefoxitin but showed good susceptibility to Sulfamethoxazole, Gentamycin, Tetracycline, Erythromycin, and Azithromycin. The development of antimicrobial resistance is nearly always a result of repeated therapeutic and/or indiscriminate use of them.

In line with the above conclusion, the following recommendations are indicated*:*

- Regular investigation of mastitis, especially screening of the subclinical form, should be practiced.
- Management, housing, and environmental sanitation, as well as appropriate treatment of cows during the dry and lactation periods, should be practiced.
- Awareness should be created among dairy farm owners and dairy workers about the effect of mastitis on milk quality and the timely treatment of dairy cows.
- Periodic assessment of the antimicrobial sensitivity of drugs and rational use of drugs prior to use should be practiced.

7. REFERENCES

Aarestrup, F. M. (2005). Veterinary drug usage and antimicrobial resistance in bacteria of animal origin. *Journal of basic and clinical pharmacology and toxicology*, *96*(4), 271-281.

Abayeneh Girma and Dessalew Tamir (2022). Prevalence of bovine mastitis and its associated risk factors among dairy cows in Ethiopia during 2005–2022: A systematic review and meta-analysis. *International journal of veterinary science*, *19*.

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

Addisalem Tadesse and Mersha Chanie (2012). Study on the occurrence of bovine mastitis in Addis Ababa dairy farms and associated risk factors, *advances in biological research*, *6* (4), 151-158.

Ahmed, A. G., Elfadil, A. A., and Aldeen, M. T. (2015). Study on bovine mastitis in smallholder dairy cows in Northern state of Sudan. *International journal of veterinary science*, *4*(2), 81-86.

Akers, M. R., and Nickerson, S. C. (2011). Mastitis and its impact on structure and function in the ruminant mammary gland. *Journal mammary gland biology neoplasia*, *16*(4), 275-289.

Alebachew Tilahun and Alemu Aylate (2015). Prevalence of bovine mastitis in lactating cows and its public health implications in selected commercial dairy farms of Addis Ababa, *global journal of medical research*, *15,* 16-24.

Amanu Marama, Gebremeskel Mamu and Tadesse Birhanu (2016). Prevalence and antibiotic resistance of *Staphylococcus aureus* mastitisin in Holeta area, western Ethiopia. *Global veterinaria*, *16*(4), 365-370.

Alemu Asefa and Fikadu Kassa (2017). Incidence of dairy cow mastitis and associated risk factors in Sodo town and its surroundings, Wolaitia Zone, Ethiopia. *Slovak journal of animal science*, *50*(2), 77-89.

Asmelash Tassew, Mesfin Negash, Addisu Demeke, Aklilu Feleke, Biruk Tesfaye and Tesfaye Sisay (2016). Isolation, identification and drug resistance patterns of methicillin resistant *Staphylococcus aureus* from mastitic cow milk from selected dairy farms in and around Kombolcha, Ethiopia. *In journal of veterinary medicine and animal health*, *8*(1), 1–10.

Ataro Abera (2020). Review on prevalence and associated risk factors of bovine mastitis in lactating cows of small holder dairy farms in Ethiopia. *Journal of medical research and health sciences*, *3*(5).

Awale, M. M., Dudhatra, G. B., Avinash, K., Chauhan, B. N., Kamani, D. R., Modi, C. M., Patel, H. B., and Mody, S. K. (2012). Bovine mastitis: a threat to economy. *Open access scientific reports*, *1*(5), 295.

Aycicek, H., Cakiroglu, S. and Stevenson, T. (2005). Incidence of *Staphylococcus aureus* in ready-to-eat meals from military cafeterias in ankara, turkey. *Food control*, *16*(6), 531-534.

Barber, D. A., Miller, G. Y. and Mcnamara, P. E. (2003). Model of antimicrobial resistance and foodborne illness: examining assumptions and practical applications. *Journal of food protection*, *66*(4), 700-709.

Bedada, B., and Hiko, A. (2011). Mastitis and antimicrobial susceptibility test at Asella dairy. Oromia regional state Ethiopia. *Journal of microbiology and antimicrobial*, *3*(9), 228-232.

Belay Geleta, Desta Beyene, Alehegn Wubete and Fufa Abunna (2019). Sub clinical mastitis in dairy farms of Addis Ababa and Sebeta towns, Ethiopia. *Biomedical journal, science and teaching reviews*, *12* (5).

Benta Duro and Habtamu Taddele (2011). Study on prevalence of mastitis and its associated risk factors in lactating dairy cows in Batu and its environs, Ethiopia. *Global veterinarian*, *7(*6), 632-637.

Biniam Tadesse, Biruk Tesfaye, Tesfaye Sisay and Ashenafi Kiros (2017). Isolation and identification of methicilin resistant *Staphylcoccus aureus* (MRSA) from bovine

mastitic milk in and around Wolaita Sodo, southern Ethiopia. *Journal of veterinary science and research, 2*(3), 136.

Birhanu Abera, Diriba Lemma, and Iyob Iticha (2013). Study of bovine mastitis in asella government dairy farm of Oromia regional state, south eastern Ethiopia. *International journal science and research, 1* (2), 134-145.

Boyce, J., Cookson, B., Christiansen, K., Hori, S., Vuopio-Varkila, J., Kocagoz, S., Oztop, Y, Grauls, V., Harbarth, S., and Pittet, D. (2005). Methicillin-resistant *Staphylococcus aureus. Lancet infectious diseases*, *5*(10), 653–663.

Bradley, A. (2002). Bovine mastitis: An evolving disease. *Veterinary journal, (London, England*, *164*(2), 116–128.

Busato, A., Trachsel, P., Schallibaum, M., and Blum, J. (2000). Udder health and risk factors for subclinical mastitis in organic dairy farms in Switzerland. *Preventive veterinary Medicine, 44*(3-4), 205-220.

CLSI (2022). Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; approved guideline. Wayne pennsylvania, pa: *clinical laboratory standards institute*.

CSA (2020). Agricultural Sample Survey 2019/20 (2012 E.C.): Volume II report on livestock and livestock characteristics (private peasant holdings). *Central statistical agency (CSA):* Addis Ababa, Ethiopia.

Cuny, C., Friedrich, A., Kozytska, S., Layer, F., Nübel, U., Ohlsen, K., and Witte, W. (2010). Emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in different animal species. *International journal of medical microbiology*, *300*(2), 109-117.

Dabash Hailemeskel, Petros Admasu and Fekadu Alemu (2014). prevalence and identification of bacterial pathogens causing bovine mastitis from crossbreed of dairy cows in North Showa zone of Ethiopia, *Global veterinarian*, *3*(2), 189-195.

Demelash Biffa, Etana Debela and Fekadu Beyene (2005). Prevalence and risk Factors of mastitis in lactating dairy cows in southern Ethiopia. *International journal of applied research in veterinary medicine, 3*, 189–198.

Denekew wubetu (2021). College of agriculture and environmental science graguate program isolation, identification and antibiotic susceptiblity of *Staphylococcus aureus* from mastitic milk samples from dairy farms in and around motta town, east gojjam zone, Ethiopia.

Desiye Tesfaye, Shimels Tikuye, Bezina Arega, Yohannes Equar, Gebremeskel Mamu, Shelema Kelbessa and Mossisa Dire (2020). Study of prevalence associated risk factors and causative bacteria of bovine mastitis in Ethiopia. *International journal of veterinary science and technology*, *4*(1), 001-006.

Dimshasha Tolera, Bizunesh Mideksa, Petros Admasu, Endrias Zewdu and Lencho Megersa (2021). Prevalence and risk factors of mastitis and isolation, identification and antibiogram of staphylococcus species from mastitis positive zebu cows in toke kutaye, cheliya, and dendi districts, west shewa zone, Oromia, Ethiopia. *In infection and drug resistance*, *14*, 987–998.

Dini, M., Shokoohizadeh, L., Jalilian, F., Moradi, A., and Arabestani, M. (2019). Genotyping and characterization of prophage patterns in clinical isolates of *Staphylococcus aureus*. *BMC research notes*, *12*, 1-6.

Edilu, J. S., and Tola, G. K. (2017). Cross-sectional study on bovine mastitis and its associated risk factors in Ambo district of west Shewa zone, Oromia, Ethiopia. *Veterinary world*, *10* (4), 398–402.

Elbably, M. A., Emeash, H. H., and Asmaa, N. M. (2013). Risk factors associated with mastitis occurrence in dairy herds in Benisuef, Egypt. *World's veterinary journal*, *3*(1), 5-10.

Endrias Zewdu, Addisu Bedashu, Bizunesh Mideksa, Kebede Abdisa, Nega Desalegn, Lencho Megersa and Edilu Jorga (2022). Isolation and identification of *staphylococcus aureus* from milk and milk products, associated factors for contamination, and their antibiogram in Holeta, central Ethiopia. *In veterinary medicine international*.

Ercolini, D., Russo, F., Torrieri, E., Masi, P., and Villani, F. (2006). Changes in the spoilage-related microbiota of beef during refrigerated storage under different packaging conditions. *Applied and environmental microbiology*, *72*(7), 4663-4671.

Eriskine, R. (1996). Intramuscular administration of ceftiofursodiu versus intrammamary infusion of penicillin/novobiocin for treatment of *Streptococcus agalactiae* mastitis in dairy cows. *Journal of American veterinary medicine association, 208*, 258-260.

Erskine, R. J. (2003). Antibacterial therapy of clinical mastitis part I. Drug selection. Part II administration. *North America veterinary conference, Proclamation, 13*, 16.

FAO (2014). Impact of mastitis in small scale dairy production systems. *Animal production and health working Paper*, *13*.

FAO (2019). Livestock, health, livelihoods and the environment in Ethiopia. An integrated analysis. Available at: http://www.fao.org/3/ca3621en/ca3621en.pdff.

Fufa Abunna, Gemechis Fufa, Bekele Megersa and Alemayehu Regassa (2013). Bovine mastitis: prevalence, risk factors and bacterial isolation in small-holder dairy farms in Addis Ababa city, ethiopia. *Global veterinarian, 10* (6), 647-652.

Gangwal, A., Kashyap, S., Katiyar, S., Meena, D., Boyal, P., and Gupta, K. (2017). Isolation and identification of common mastitis causing pathogens from clinical bovine mastitic milk. *Journal of pure and applied microbiology*, *11*(1), 329-333.

Gashaw Getaneh (2016). Prevalence of coagulase negative staphylococcus in mastitis infection in dairy cattle in and around Bahir Dar. *World journal of pharmaceutical and medical research*, *2*(3) 17–23.

Gentilini, E., Denamiel, G., Llorente, P., Godaly, S., Rebuelto, M., and DeGregorio, O. (2000). Antimicrobial susceptibility of *Staphylococcus aureus* isolated from bovine mastitis in Argentina. *Journal of dairy science*, *83*(6), 1224-1227.

Getachew Nigussie (2020). Isolation, Identification and Antimicrobial Susceptibility test of *Staphylococcus aureus* from bovine mastitis in lome woreda of Oromia region, Ethiopia. Msc thesis, Addis Ababa university, college of veterinary medicine and agriculture.

Getahun Kassaye (2006). Bovine mastitis and antibiotic resistance patterns of major pathogens in small holder dairy forms in central high land of Ethiopia. MSC thesis: Debre-Zeit: Factory of veterinary medicine, Addis Ababa university, Ethiopia.

Getahun, K., Kelay, B., Bekana, M., and Lobago, F. (2008). Bovine mastitis and antibiotic resistance patterns in Selalle smallholder dairy farms, central Ethiopia. *Tropical animal health and producton*, *40*(4), 261-268.

Gianneechini, R., Concha, C., Rivero, R., Delucci, I., and Moreno, L. (2002). Occurrence of clinical and sub-clinical mastitis in dairy herds in the west littoral region in Uruguay. *Acta veterinaria scandinavica*, *43*(4), 221–230.

Girma Debele (2010). Study on prevalence of bovine mastitis on Cross breed dairy cow around Holeta areas, west Shoa Zone of Oromia, Ethiopia, *5*(6), 318–323.

Gizat Almaw (2004). A cross-sectional study of bovine mastitis in and around Bahirdar and antibiotic resistance patterns of major pathogens. MSc thesis Addis Ababa university Debre Zeit, Ethiopia.

Gizat Almaw, Ademe Zerihun and Yilkal Asfaw (2008). Bovine mastitis and its association with selected risk factors in smallholder dairy farms in and around Bahirdar, Ethiopia. *Tropical animal health and producton*, *40*, 427-432.

Godden, S. M., Jansen, J. T., Leslie, K. E., Smart, N. L., and Kelton, D. F. (2002). The effect of sampling time and sample handling on the detection of *Staphylococcus aureus* in milk from quarters with subclinical mastitis. *Canadian veterinary journal*, *43*(1), 38-42.

Gordeon, J.R., and Lowy, F.D. (2008). Pathogenesis of methicillin-resistant *Staphylococcus aureus* infection. *Clinical infectious disease*, *46*, 350–359.

Gutu Kitila, Bedaso Kebede and Moti Wakgari (2021). Prevalence, aetiology and risk factors of mastitis of dairy cows kept under extensive management system in west Wollega, western Oromia, Ethiopia. *Veterinary medicine and science*, *7*(5), 1593-1599.

Haben Fesseha, Mesfin Mathewos, Saliman Aliye and Amanuel Wolde (2021). Study on prevalence of bovine mastitis and associated risk factors in dairy farms of Modjo

town and suburbs, central oromia, ethiopia, *veterinary medicine*: *research and reports*, *12*, 271-283,

Hameed, S., Arshad, M., Ashraf, M., Avais, M., and Shahid, M. (2012). Cross-sectional epidemiological studies on mastitis in cattle and buffaloes of Tehsil Burewala, Pakistan. *Journal of animal and plant sciences*, *22*(3), 371–376.

Huber, H., Giezendanner, N., Stephan, R., and Zweifel, C. (2011). Genotypes, antibiotic resistance profiles and microarray-based characterization of methicillin-resistant *Staphylococcus aureus* strains isolated from livestock and veterinarians in Switzerland. *Zoonoses and public health*, *58*(5), 343-349.

Hunderra Sori, Ademe Zerihun and Sintayehu Abdicho (2005). Dairy cattle mastitis in and around sebeta, Ethiopia. *International journal of applied reviews Veterinary medicine, 3*, 332-338.

Iraguha, B., Hamudikuwanda, H., and Mushonga, B. (2015). Bovine mastitis prevalence and associated risk factors in dairy cows in Nyagatare district, Rwanda. *Journal of the south african veterinary association*, *86* (1), 1-6.

Islam, M. A., Islam, M. Z., Rahman, M. S., and Islam, M. T. (2011). Prevalence of subclinical mastitis in dairy cows in selected areas of Bangladesh. *Bangladesh journal of veterinary medicine*, *9*(1), 73-78.

Jafer Kedir, Haimanot Disassa, Hawi Jaleta, Tilahun Zenebe and Girma Kebede (2016). A Study on bovine mastitis, isolation and identification of staphylococcus species in dairy farms of dire dawa city, eastern Ethiopia. *Global veterinaria*, *16*, 222-230.

Jirata Shiferaw and Indalem Telila (2017). Prevalence of bovine mastitis and assessment of risk factors in and around Wolayta Sodo, Ethiopia. *International journal of homeopathy and natural medicines*, *2*(1), 1–7.

Jorgensen, J. H., and Turnidge, J. D. (2015). Susceptibility test methods: dilution and disk diffusion methods. *Manual of clinical microbiology*, 1253-1273.

Joshi, S., and Gokhale, S. (2006). Status of mastitis as an emerging disease in improved and periurban dairy farms in India. *Annals of the new york academy of sciences*, *1081*(1), 74–83.

Kandi, V. (2018). Coral dermatitis or infectious dermatitis: report of a case of *Staphylococcus aureus*. *Infection of skin after scuba diving*. *Cureus*, *10*(2), 2196.

Kanenus Dereje, Abriham Kebede, Nigus Abebe and Yobsan Tamiru (2018). Isolation, identification and antimicrobial susceptibility test of mastitis causing bacteria at Holeta agricultural research center dairy farms. *International journal of animal science and technology*, *2*, 6-13.

Katsande, S., Matope, G., Ndengu, M., and Pfukenyi, D. (2013). Prevalence of mastitis in dairy cows from smallholder farms in Zimbabwe. *Onderstepoort journal of veterinary research*, *80*(1), 1-7.

Kemal Kedir, Tesfaye Sisay, Ashanafi Shiferaw, Muhammad hussien and Aman Fato (2017). Prevalence, risk factors and multidrug resistance profile of *Staphylococcus aureus* isolated from bovine mastitis in selected dairy farms in and around Asella town, Arsi Zone, south eastern Ethiopia. *African journal of microbiology research*, *11* (45), 1632-1642.

Kengne, M., Fotsing, O., Ndomgue, T., and Nwobegahay, J. (2019). Antibiotic susceptibility patterns of *Staphylococcus aureus* strains isolated at the Yaounde central hospital, Cameroon: a retro prospective study. *Pan african medical journal*, *32*(1).

Kerro, D., and Tareke, F. (2003). Bovine mastitis in selected areas of southern Ethiopia. *Tropical Animal Health and Production*, *35*, 197–205.

Kumari, T., Bhakat, C., and Choudhary, R. (2018). Review on Sub Clinical Mastitis in Dairy Cattle, *International journal of pure applied biological science*, *6*(2), 1291-1299.

Lehtolainen, T., Pohjanvirta, T., Pyorala, S., and Pelkonen, S. (2003). Association between virulence factors and clinical course of *Escherichia coli* mastitis. *Acta veterinary scand*, *44*(4), 1-3.

Maarit, H. (2008). *Staphylococcus aureus* in bovine intramammary infection molecular, clinical and epidemiological characteristics; *department of production animal medicine faculty of veterinary medicine university of helsinki helsinki, Finland*, 11-79.

Mahendra Pal, Gemechu Berhanu, Lencho Megersa and Venkataramana Kandi (2020). "Epidemiology, pathogenicity, animal infections, antibiotic resistance, public health significance, and economic impact of *Staphylococcus aureus*: a comprehensive review." *American journal of public health research*, *8* (1), 14-21.

Maliha Gulzar, and Asima Zehra (2018). *Staphylococcus aureus*: A brief review. *International journal of veterinary science review*, *4*(1), 020-022.

Matios Lakew, Tadele Tolosa and Worku Tigre (2009). Prevalence and major bacterial causes of bovine mastitis in Asella, South Eastern Ethiopia. *Tropical animal health and production*, *41*, 1525–1530.

Mdegela, R., Ryoba, R., Karimuribo, E., Phiri, E., Loken, T., Reksen, O., Mtengeti, E., and Urio, N. (2009). Prevalence of clinical and subclinical mastitis and quality of milk on smallholder dairy farms in Tanzania. *Journal of the south african veterinary association*, *80*(3), 163–168.

Mekibib, B., Furgasa, M., Abunna, F., Megersa, B., and Regassa, A. (2010). Bovine mastitis: Prevalence, risk factors and major pathogens in dairy farms of Holeta town, central Ethiopia. *veterinary world*, *3*(9), 397-403.

Mekonnen, H., Workineh, S., Bayleyegne, M., Moges, A., and Tadele, K. (2005). Antimicrobial susceptibility profile of mastitis isolates from cows in three major Ethiopian dairies. *Medical Veterinary*, *176*, 391-394.

Melak Tezera and Endris Aman (2021). Prevalence and associated risk factors of bovine mastitis in dairy cows in and around Assosa town, Benishangul-Gumuz regional state, western Ethiopia. *Veterinary medicine and science*, *7*(4), 1280-1286.

Melese Etifu (2012). Study on prevalence of mastitis and associated risk factors with isolation and antimicrobial susceptibility of major pathogens in Alage state dairy

farm, Ethiopia. Addis Ababa University College of veterinary medicine and agriculture, MSc thesis.

Mohamed Ahmed, Simeon Ehui and Yemesrach Assefa (2004). Dairy development in ethiopia. International food policy research institute, eptd discussion paper, 123. Washington, dc, u.s.a.

Molalegne Bitew, Arega Tafere and Tadele Tolosa (2010). Study on bovine mastitis in dairy farms of Bahirdar and its environs. *Journal of animal and veterinary advances*, *9* (23), 2912-2917.

Moon, J. S., Lee, A. R., Kang, H. M., Lee, E. S., Kim, M. N., Paik, Y. H., and Koo, H. C. (2007). Phenotypic and genetic antibiogram of methicillin-resistant staphylococci isolated from bovine mastitis in Korea. *Journal of dairy science, 90***(**3), 1176-1185.

Mulunesh Yenew and Habtamu Addis (2020). Study on the prevalence and associated risk factors of bovine mastitis in and around Dessie town, south Wollo, north eastern Ethiopia, biomedicine and nursing, *6*(3), 59-76.

Mungube, E., Tenhagen, B., Kassa, T., Regassa, F., Kyule, M., Greiner, M., and Baumann, M. (2004). Risk factors for dairy cow mastitis in the central highlands of Ethiopia. *Tropical animal health and production*, *36*(5), 463-472.

Mureithi, D., and Njuguna, M. (2016). Prevalence of subclinical mastitis and associated risk factors urban and peri-urban areas of Thika sub country, Kenya. *Livestock research for rural development, 28*(2), 456-471.

Murray, P., Baron, E., Jorgensen, J., Landry, M., and Pealler, M. (2007). Manual of clinica l microbiology 9th edition" editorial asm press. *Washington dc, USA.*

NAAS (2013). Mastitis management in dairy animals. Policy paper. *National academy of agricultural sciences, new delhi*, *61*, 12-36.

Nesru Hussein (1999). A cross-sectional and longitudinal study of bovine mastitis in urban and peri urban dairy system in the addis ababa region. Unpublished MSC thesis, free university of berlin and Addis Ababa university.

Nibret Moges, Tekle Hailemariam, Tewodros Fentahun, Mersha Chanie, and Achenef Melaku (2012). Bovine mastitis and associated risk factors in small holder lactating dairy farms in Hawassa, southern Ethiopia. *Global veterinaria*, *9*(4), 441–446.

Nibret Moges, Yilikal Asfaw and Kelay Belihun (2011). A cross sectional study on the prevalence of sub clinical mastitis and associated risk factors in and aronund Gondar, northern Ethiopia. *International journal of animal and veterinary advances*, *3*(6), 455–459.

NMC (2004). Microbiological procedures for the diagnosis of udder infection, 3rd ed., *national mastitis council inc. Arlington*,*4*, 47.

Ojo, O., Oyekunle, M., Ogunleye, A., and Otesile, E. (2009). *Escherichia coli. O157: h7* in food animals in part of south-western Nigeria: prevalence and in vitro antimicrobial susceptibility. *Tropical veterinaria*, *26*, 23-30.

Ondiek, J. O., Ogore, P. B., Shakala, E. K., and Kaburu, G. M., (2013). Prevalence of bovine mastitis, its therapeutics and control in Tatton agriculture park, Egerton university, Njoro district of Kenya. *Basic research journal of agricultural science and review*, *2*(1), 15-20.

Onwubiko, N. E., and Sadiq, N. M. (2011). Antibiotic sensitivity pattern of *staphylococcus aureus* from clinical isolates in a tertiary health institution in Kano, northwestern Nigeria. *Pan african medical journal*, *8*(1).

Pal, M. (2018). Mastitis is a major production disease of dairy animals. *Agriculture world*, *4*, 46-51.

Payne, W. (1990). An Introduction to animal husbandry in the tropics. 4th ed. Longman group: *Harlow, Essex, UK*, 414-415.

Peacock, S. J., and Paterson, G.K. (2015). Mechanisms of methicillin resistance in *Staphylococcus aureus*. *Annual review of biochemistry*, *84*, 577-601.

Petersson, C. S., Mullarky, I. K., and Jones, G. M. (2010). *Staphylococcus aureus* Mastitis: Cause, Detection, and Control, 404-229.

Plata, K., Rosato, A., and Wegrzyn, G. (2009). *Staphylococcus aureus* as an infectious agent: *overview of biochemistry and molecular genetics of its pathogenicity. Acta biochimical polonica, 56*(4), 597-612

Quinn P. J., Carter M. E., Markey B., and Carter, G. R. (1994). *Veterinary clinical microbiology*. *Wolfe publication, London, UK*, 254-258.

Quinn, P. J, Carter, M. E, Markey, B. K., and Carter, G. R. (2004). Mastitis. In: clinical veterinary microbiology, *mosby international limited, London*, 327-344.

Quinn, P. J., Leonard, F. C., Markey, B. K., Carter, W. E., and Donnelly, W. J. (2002). *Clinical veterinary* microbiology. *Wolfe, baltimore*, 465-472.

Quinn, P. J., Markey, B. K., Leonard, F. C., Hartigan, P., Fanning, S., Fitzpatrick, E., and John, W. (2011). *Veterinary microbiology and microbial disease*.

Radostits, O. M., Gay, C. C., Blood, D. C. and Hinchcliff, K. W. (2000). A textbook of the diseases of cattle, sheep, pigs, goats and horses. *Veterinary medicine*, *9*, 603-700

Radostits, O. M., Gay, C. C., Hinchcliff, K. W., and Constable, P. D. (2007). A textbook of the diseases of cattle, horses, sheep, pigs and goats. *Veterinary medicine*, *10*, 2045-2050.

Rahman, M., Bhuiyan, M., Kamal, M., and Shamsuddin, M. (2009). Prevalence and risk factors of mastitis in dairy cows. *Bangladesh veterinarian*, *26*(2), 54-60.

Rahmeto Abebe, Hagere Hatiya, Mesele Abera, Bekele Megersa and Kassahun Asmare (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), 1-11.

Rainard, P., Foucras, G., Fitzgerald, J., Watts, J., Koop, G., and Middleton, J. (2018). Knowledge gaps and research priorities in *Staphylococcus aureus* mastitis control. *Transboundary and emerging diseases, 65*, 149-165.

Rediet Belayneh, Kelay Belihu and Alehegne Wubete (2013). Dairy cow mastitis survey in Adama Town, Ethiopia. *Journal of veterinary medicine animalhHealth*, *5*(10), 281-287.

Rgbe Haftu, Habtamu Taddele, Getachew Gugsa and Shewit Kalayou (2012). Prevalence, bacterial causes, and antimicrobial susceptibility profile of mastitis isolates from cows in large-scale dairy farms of Northern Ethiopia. *Tropical animal health production*, *44*, 1765-1771.

Sabour, P., Gill, J., Lepp, D., Pacan, J., Ahmed, R., Dingwell, R., and Leslie, K. (2004). Molecular typing and distribution of *Staphylococcus aureus* isolates in Eastern Canadian dairy herds. *Journal of clinical microbiology*, *42*(8), 3449-3455.

Saharan, D., Jaidiya, K., Meena, Y., Bhalothia, S., and Choudhary, S. (2022). Isolation and identification of bacterial flora in milk of cattle affected with clinical mastitis. *The Pharma innovation journal*, *11*(7), 2613-2618.

Saikia, L., Nath, R., Choudhury, B., and Sarkar, M. (2009). Prevalence and antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* in Assam. *Indian journal of critical care medicine: peer-reviewed, official publication of Indian society of critical care medicine*, *13*(3), 156-158.

Salvador, R. T., Beltran, J. M., Abes, N. S., Gutierrez, C. A., and Mingala, C. N. (2012). Prevalence and risk factors of subclinical mastitis as determined by the california mastitis test in water buffaloes (bubalis bubalis) in nueva ecija, philippines. *Journal of dairy science*, *95*(3), 1363-1366.

Sampimon, O. C., Barkema, H. W., Berends, I. M., Sol, J. and Lam, T. J. (2009). Prevalence and herd-level risk factors for intra-mammary infection with coagulase-negative staphylococci in Dutch dairy herds. *Veterinary microbiology*, *134* (1), 37-44.

Sefinew Alemu, Firaol Tamiru, Gizat Almaw and Awoke Tsega (2013). Study on bovine mastitis and its effect on chemical composition of milk in and around Gondar town. *Journal of veterinary medicine and animal health, 5*(8), 215–221.

Sema Regasa, Shimelis Mengistu and Ashebr Abraha (2019). Milk safety assessment, isolation, and antimicrobial susceptibility profile of *Staphylococcus aureus* in selected dairy farms of Mukaturi and Sululta town, Oromia region, Ethiopia. *Veterinary medicine international,*

Sharif, A., and Muhammed, G. (2009). Mastitis control in dairy animals. *Pakistan veterinary journal*, *29*(3), 145-148.

Sharma, I., and Brinty, A. (2014). Isolation and identification of *Staphylococcus aureus* from bovine mastitis milk and their drug resistance patterns in Silchar town dairy farms, NE India. *Online international interdisciplinary research journal*, *4*, 256-260.

Sharma, N., Singh, N., and Bhadwal, M. (2011). Relationship of somatic cell count and mastitis: an overview. *Asian-australasian journal of animal sciences*, *24*(3), 429-438.

Shamsaldeen, I., Saeed, K., Anis,a M., Hidayatul, A., Hashimy, S., Khadijah, D., Nordin, F., Nik Azmi, N., Mohd, F., Erkihun, A., Maizan, M., Che Wan, S., and Nor Fadhilah., K. (2022). Prevalence, antimicrobial resistance, and characterization of *staphylococ cus aureus* isolated from subclinical bovine mastitis in east coast Malaysia. Animals, *12*(13).

Shittu, A., Abdullahi, J., Jibril, A., Mohammed, A., and Fasina, F. (2012). Sub-clinical mastitis and associated risk factors on lactating cows in the savannah region of nigeria. *BMC veterinary research*, *8*, 1-6.

Singh, A., Jaiswal, S., Singh, D., and Singh, H. (2006). Bovine mastitis and udder affections. 1st edition, 52-53.

Sisay Girma, Alo Mammo, Ketema Bogele, Teshale Sori, Fanos Tadesse and Tariku Jibat (2012). Study on prevalence of bovine mastitis and its major causative agents in west Harerghe zone, Doba district, Ethiopia. *Journal veterinary medicine animal health*, *4*(8), 116-123.

Smith, D. L., Harris, A. D., Johnson, J. A., Silbergeld, E. K., and Morris, J. G. (2002). Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria. *Proceedings of the national academy of sciences*, *99*(9), 6434-6439.

Stawart, C.M. (2003). *Staphylococcus aureus* and *Staphylococcal enterotoxins*. In: hocking ad (ed) food borne microorganisms of public health significance. 6th ed. *Australian institute of food science and technology (nsw branch), sydney*, 359-380.

Tadesse Teklesilasie, Mulisa Megersa Kebeta and Teka Fayera (2014). Bovine mastitis: prevalence and isolation of major pathogens in dairy farms of selected sites in Addis Ababa, Ethiopia. *Applied journal of hygiene, 3* (3), 31-37.

Tariku Sori, Jemal Hussein and Molalegne Bitew (2011). Prevalence and susceptibility assay of *Staphylococcus aureus* isolated from bovine mastitis in dairy farms of Jimma town, South west Ethiopia. *Journal of animal and veterinary advances,10*(6), 745-749.

Tazeb Gessesse, Yohannes Dagnew, Solomon Abegaz and Assemu Tesfa (2021). Growth performance and survival rate of fogera and their crossbred calves at government ranches in Ethiopia. *Frontiers in animal science*, 2.

Tekle Hailemariam and Eyob Eticha (2017). Bovine mastitis and its selected risk factors in smallholder lactating dairy farms in Hawassa, Ethiopia. *World applied sciences journal*, *35*(5), 703–709.

Tesfaheywet Zeryehun and Gerema Abera (2017). Prevalence and bacterial isolates of mastitis in dairy farms in selected districts of eastern Harrarghe zone, eastern Ehiopia. *Journal of veterinary medicine*, 1-6.

Tesfaheywet Zeryehun, Tilahune Aya and Regassa Bayecha (2013). Study on prevalence, bacterial pathogens and associated risk factors of bovine mastitis in small holder dairy farms in and around Addis Ababa, Ethiopia. *Journal of animal and plant science*, *23* (1), 50-55.

Tesfanesh Fentahune, Habtamu Taddele and Marshet Adugna (2018). Prevalence of mastitis and identification of its bacterial causative agents in small holder dairy farms in and around Wukro of Tigray region, Ethiopia. *International journal of advanced research in biological sciences*, *5*(11), 10–22.

Tesfaye Bekele, Matios Lakew, Getachew Terefe, Tafesse Koran, Abebe Olani, Letebrihan Yimesgen, Mekdes Tamiru and Tilaye Demissie (2019). Study on bovine mastitis with isolation of bacterial and fungal causal agents and assessing antimicrobial resistance patterns of isolated Staphylococcus species in and around Sebeta town, Ethiopia, *african journal of microbiology research*. *13*(1), 23-32.

Tesfaye Belachew (2016). Bovine mastitis: prevalence, isolation of bacterial spe-cies involved and its antimicrobial susceptibility test around Debrezeit, Ethiopia. *Journal* of veterinary scince technology, *7*, 6.

Tesfaye Deme and Biressaw, S. (2015): Prevalence of bovine mastitis and determinant of risk factors in lemu Bilbilo District, Arsi Zone. *Global journal of veterinary medicine and research, 3*, 80–85.

Thrusfield, M. (2005). Veterinary epidemiology (3rdedn) blackwell science. *United Kingdom, 158*.

Tomita, G., and Hart, S. (2001). The mastitis problem. Proc. 16th ann. Goat field day. Langston university, *Langston, Oklahoma*, 6-9.

Tong, S., Davis, J., Eichenberger, E., Holland, T., and Fowler, V. (2015). *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clinical microbiol review 28*(3), 603-660.

Wang, X., Liu, Q., Zhang, H., Li, X., Huang, W., Fu, Q., and Li, M. (2018). Molecular characteristics of community-associated *staphylococcus aureus* isolates from pediatric patients with bloodstream infections between 2012 and 2017 in shanghai, china. *Frontiers in microbiology*, *9*, 1211.

White, D., Walmsley, M., Liew, A., Claycomb, R., and Mein, G. (2005). Chemical and rheological aspects of gel formation in the california mastitis test. *Journal of dairy research*, *72*(1), 115–121.

Workineh, S., Bayleyegn, M., Mekonnen, H., and Potgieter, L. (2002). Prevalence and aetiology of mastitis in cows from two major Ethiopian dairies. *Tropical animal health and production*, *34*(1), 19–25.

Yasser Mahmmod (2013). The future of PCR technologies in diagnosis of bovine mastitis pathogens. *Advances in Dairy Research*, *2*, 106.

Yibrah Tekle and Tsega Berihe (2016). Bovine mastitis: prevalence, risk factors and major pathogens in the Sidamo Zone SNNPRS, Ethiopia. *European journal of biology and medical science research*, *4,* 27–43.

Yusuf-Isleged, M. A. (2022). Prevalence and associated risk factors of bovine mastitis on dairy cattle in Mogadishu Somalia. *Animal and veterinary sciences, 10*(2), 21-27.

Zadoks, R. N., and Schukken, Y. H. (2006). Use of molecular epidemiology in veterinary practice. Veterinary clinics: *food animal practice*, *22*(1), 229-261.

Zenebe, N., Habtamu, T., and Endale, B. (2014). Study on bovine mastitis and associated risk factors in Adigrat, northern Ethiopia. *Africa journal microbiology research, 8*(4), 327–331.

8. APPENDICES

Appendix 1. Questionnaire format

Owners Name: _____Address_____ ID/Animal name

Date of sample Collection_____

1. History of cow:

Age: Young_____ Adults_____Old_____

Breed: Local (Fogera)_____ Cross (Fogera X HF) _____

Management system: Intensive___Semi-intensive___

Parity: 1-2_____3-5_____>6_____

Stage of lactation: Early (1-2 mo) ____Mid(3-6mo) ___ Late(>7mo) ____

Types of mastitis: Clinical_____Sub-Clinical_____

Mastitits History: Present _____Absent_____

Tick on teat/Udder: present _____ Absent_____

CMT result: RR _____ RF _____ LF _____ LR _____

2. **Milking practice**:

Do you wash hand before milking? yes_____ no_____

Do you wash Udder before milking? Yes_____ No_____

Do you use towel? Yes____ No_____

Do you practice milking mastitic cows at last: yes_____ no___

3. **Floor Type:** Concrete (cement)_____Muddy(soil)_____

Appendix 2: Record sheet for risk factors of *Staphylococcus aureus* mastitis

No	Ear Tag/Animal Name	Sample code	Breed	Age	Parity	Lactation stage	Management	Floor type	Udder - washing	Tick on udder	Use towel	Wash hand mikers	Mastitis history	Mastitis type	*Mastitic posetive*

Appendix 3. **Record sheet for laboratory investigation of *Staphylococcus aureus***

No	Ear Tag/Animal name	Sample code	Manitol fermentation	Haemolysis	Gram satin	Catalase test	Coagulase test	Antimicrobial sensitivity test	+ve result (*S. aureus*)

Appendix 4. Age determination of cattle by dentition (Payne, 1990)

Eruption time of teeth	Time of wear
I1 = 1.5-2 years	5 years all incisors in wear
I2= 2-2.2 years	6 years – I1 is leveled and neck is visible
I3 = 3 years	7 years –I2 is leveled and neck is visible
C = 3.5- 4 years	8 years –I3 is leveled and neck is visible
	9 years – C is leveled and neck is visible

I=incisor teeth, C = canine teeth

Appendix 5. Procedure of milk sample collection (NMC, 2004)

1. Label tubes prior to sampling (date, ear tag and sample number).
2. The udder and teat were examined for any clinical signs of mastitis and history of mastitis.
3. The udder, especially the teats were cleaned and dried before milk sample collection.

4. Dust, particles or other filth was removed by brushing the surface of the teats and udder with a dry towel. The teats were washed with tap water and dried.
5. Then the teats were disinfected with cotton soaked in 70% ethyl alcohol.
6. After the first three milking streams were discarded then the teat orifice was disinfected again. A single cotton ball or alcohol swab should not be used on more than one teat.
7. Approximately 2-3 ml of milk was dropped starting from the closest teat and move to teats on the far side of the udder aseptically from each teat in each hole of CMT paddle then equal amount of CMT reagent was added in each sample.
8. Based on the thickness of the gel formed by CMT reagent and milk mixture the sample recorded as positive and negative to sub clinical mastitis.
9. Finally, 10 ml milk was taken from CMT positive sample which strongly form gel. The CMT positive milk samples were held in an icebox for transportation to Biotechnology laboratory institute for bacterial isolation and identification
10. In the laboratory, samples were cultured immediately on a standard bacteriological media.

Appendix 6. California mastitis test (CMT) (NMC, 2004)

❖ **Principle**: -

✓ The test is based on the fact that milk from an infected mammary gland contains an increased number of somatic cells, mainly leukocytes (white blood cells). The CMT detects the presence of these cells by mixing a small amount of milk with a CMT reagent. The reagent lyses the cell membranes, releasing the intracellular contents of the DNA, forming a gel-like mass that indicates the presence of somatic cells.

❖ **Procedures: -**

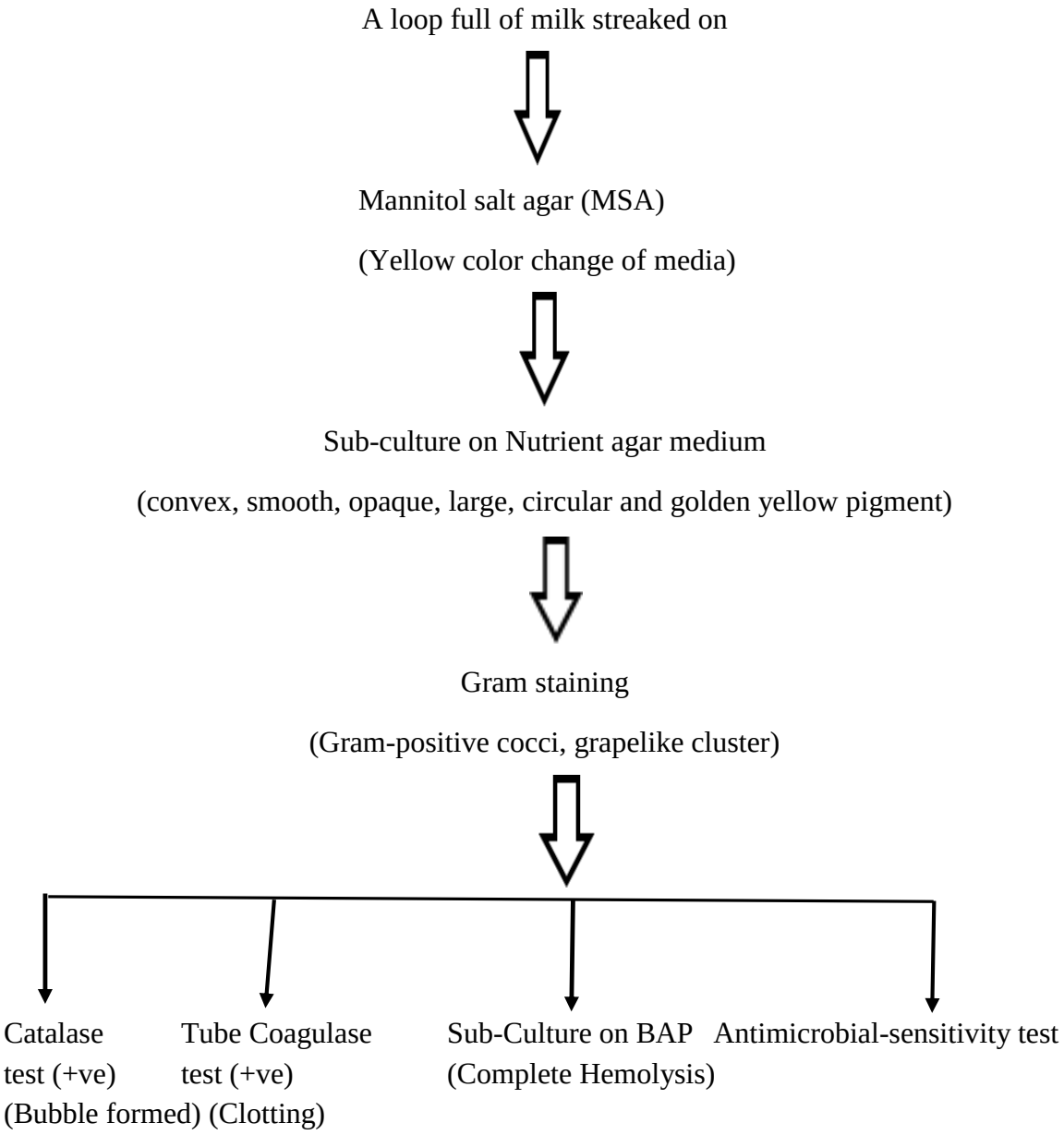
✓ Take about 2-3 ml of milk from each quarter. This is the amount of milk that would be left in the cups if the CMT Paddle were held nearly vertical.

✓ Add an equal amount of CMT solution to each cup in the paddle.

✓ Rotate the CMT Paddle in a circular horizontal motion to thoroughly mix the contents.

✓ Read the test quickly. Visible reaction disintegrates after about 15-20 seconds. The reaction is visually scored. The more gel formation, the higher the score.

Appendix 7. Flow chart of the laboratory analysis protocol of *S. aureus* (Quinin *et al*., 2002)



Appendix 8. Media ingredients and its preparation (most of them were from Biomark and Himedia Laboratories pvt. Ltd)

No	**Media**	**Composition Ingredients**	**Quantity(grm/L)**	**Preparation (direction) Powder ×Distilled Water**
1	Blood agar base	Sodium chloride	5.00	Suspend 40.0 grams in × 1L of distilled water. Bring to the boil to dissolve comp letely. Sterilize by autoclaving at 121^0c f or 15 minutes. Cool to 45 ^{0}C and add 5% v/v sterile def ibrinated blood. Mix well and pour in to sterile petri plate
		Meat infusion	10.00	
		Tryptose	10.00	
		Sodium chloride	5.00	
		Agar	15.00	
		Final pH (at 25^0c)	7.3±0.2	
2	Nutrient agar	Yeast extract	2.0	28gram × 1L of distilled water. Bring to the boil for 1minute to dissolve completely. Sterilize by autoclaving at 121^{O}C for 15 minutes.
		Beef extract	1.00	
		Pepttic digest of animal tissue	5.0	
		Sodium chloride	5.0	
		Agar	15.0	
		Final pH (at 25^0c)	7.4 ± 0.2	
3	Mannitol salt agar	Proteose peptone	10.00	111.02 gram × 1L of distilled water and bring to the boil to dissolve completely. Sterilize by autoclaving at 121^{O}C for15 minutes. Cool to 45-50 ^{0}C
		Meat extract B	1.00	
		Sodium chloride	75.00	
		D-Mannitol	10.00	
		Phenol red	0.025	
		Agar	15.00	
		Final pH (at 25^0c)	7.4±0.2	
4	Nutrient Broth	Peptic digest of animal tissue	5.00	Susped 13 gram × 1L of distilled water and bring to the boil to dissolve completely. Sterilize by autoclaving at 121^{O}C for15 minutes. Cool to 50 ^{0}C
		Beef extract	1.50	
		Yeast Extract	1.50	
		Sodium chloride	5.00	
		Final pH (at 25^0c)	7.4±0.2	
5		Beef infusion	300.00	

	Mueller-Hinton Agar	Acid hydrolysis of casein	17.00	Susped 39 gram × 1L of distilled water and bring to the boil to dissolve completely. Sterilize by autoclaving at 121^{O}C for15 minutes.
		Agar	17.00	
		Starch	1.50	

Appendix 9. Primary and secondary identification tests of *Staphylococcus aureus*

1. Mannitol salt agar and Mannitol fermentation test (Quinn *et al*., 2004).

❖ **Principle: -**

- ✓ The principle of the test is based on the ability of *S. aureus* to ferment mannitol. The MSA contains 7.5% NaCl, which inhibits the growth of most bacteria except for halophilic organisms such as Staphylococci. The medium also contains mannitol as the carbohydrate source and phenol red as a pH indicator. When *S. aureus* ferments mannitol, it produces acid, which lowers the pH of the medium, causing the phenol red to turn yellow. Non-fermenting bacteria will not produce acid and the medium will remain pink.

❖ **Procedure**

- ✓ Culture CMT positive milk sample on mannitol salt agar and incubated at 37^{0}C and examined after 24-48 hours for growth and change in the colour of the medium.
- ✓ The presence of growth and change of pH in the media (red to yellow colour) were regarded as confirmative identification of Staphylococci.
- ✓ Phenol red pH indicator detected the acidic metabolic product of mannitol.
- ✓ Fermentation of mannitol by *S. aureus* causes yellow discolouration of the medium.
- ✓ Colonies that develop weak or delayed yellow colour after 24 hours of incubation were taken as *S. intermedius* and colonies that failed to produce any change on the medium were considered as *S. hyicus*.

2. **Gram's staining** (Quinn *et al*., 2004)

❖ **Principle: -**

- ✓ Differentiate bacterial species into two groups of gram positive and gram negative based on the physical and chemical properties of their cell walls. Gram-positive bacteria retain the crystal violet stain and appear purple under the microscope, while gram-negative bacteria lose the crystal violet stain and appear pink or red after the counterstain is applied.

❖ **Procedure**

- ✓ Make a thin bacterial colony smear and allow it to dry on the air.
- ✓ Fix the dried smear by passing through the Bunsen flame two to three times taking care not to overheat the smear.
- ✓ Flood the fixed smear with Gram's crystal violet (primary stain). Let stand for 60 seconds.
- ✓ Pour off the stain and gently wash with tape water.
- ✓ Flood with Gram's iodine (mordant) solution. Allow it to remain for 60 seconds.
- ✓ Pour off the iodine solution and gently wash with tape water.
- ✓ Decolorize with Gram's decolorizer solution (95% Ethanol alcohol) for 15-20 seconds until the blue dye no longer flows from the smear and gently wash the smear with tape water.
- ✓ Counter stain with Gram's safranin solution (counter stain) for 60 seconds.
- ✓ Wash off the red safranin solution with water. Blot with bibulous paper to remove the excess water. Alternatively, the slide may shake to remove most of the water and air-dried.
- ✓ Examine the finished slide under a microscope (oil immersion objective).
- ✓ Interpretation: Bluish purple colour indicate Gram positive and pinkish colour indicate Gram negative bacteria.

3. Catalase test (Quinn *et al*., 2004)

❖ **Principle: -**

✓ The principle of the catalase test is to detect the presence of the enzyme catalase in bacterial cells. Catalase is an enzyme that breaks down hydrogen peroxide into water and oxygen. Bacteria that produce catalase can rapidly break down hydrogen peroxide into water and oxygen, resulting in the formation of bubbles, while those that do not produce catalase cannot. This test is commonly used to differentiate between Staphylococcus species, which are catalase-positive, and Streptococcus species, which are catalase-negative.

❖ **Procedure: -**

✓ Transfer a small amount of bacterial colony to a surface of clean, dry glass slide using a loop or sterile wooden stick.

✓ Place a drop of 3% H_2O_2 on to the slide and mix.

✓ A positive result is the rapid formations of oxygen (within 5-10 sec.) as evidenced by bubbling.

✓ A negative result is no bubbles or only a few scattered bubbles.

4. Coagulase test (Quinn *et al.*, 2002)

Principle: -

✓ The test is based on the ability of the bacteria to produce the enzyme coagulase, which can convert fibrinogen to fibrin, thereby causing clotting of blood plasma. The coagulase test is used to differentiate between *Staphylococcus aureus*, which produces the enzyme coagulase, and other Staphylococcus species that do not produce coagulase.

❖ **Procedure: -**

▪ **Tube coagulase test**

✓ Three test tubes are taken and labeled “test”, “negative control” and “positive control”.

✓ Each tube is filled with 1 ml of 1 in 10 diluted rabbit plasma.

✓ To the tube labeled test, 0.2 ml of overnight broth culture of test bacteria are added.

- ✓ To the tube labeled positive control, 0.2 ml of overnight broth culture of known *S. aureus* is added.
- ✓ To the tube labeled negative control, 0.2ml of sterile broth is added.
- ✓ All the tubes are incubated at 37^{o}C and observe the suspensions at half hourly intervals for a period of four hours.
- ✓ Positive result is indicated by gelling of the plasma, which remains in place even after inverting the tube.
- ✓ If the test remains negative until four hours at 37^{o}C, the tube is kept at room temperature for overnight incubation.

▪ **Slide coagulase test**

- ✓ Divide the slide into two sections with pencil and should be labeled as test and control.
- ✓ Place a small drop of distilled water on each area.
- ✓ Emulsify one or two colonies of Staphylococcus
- ✓ The test suspension is treated with a drop of citrated plasma and mixed well with a needle.
- ✓ Do not put anything in the other drop that serves as control. The control suspension serves to rule out false positivity due to auto agglutination.
- ✓ Clumping of cocci within 5-10 seconds is taken as positive.

5. **Disc-diffusion Antibiotic Sensitivity Test** (Quinn *et al*., 2004).

❖ **Principle: -**

- ✓ The disc-diffusion antibiotic sensitivity test, also known as the Kirby-Bauer test, is a laboratory method used to determine the effectiveness of antibiotics against bacteria. The size of the zone of inhibition indicates the susceptibility of the bacterial strain to the antibiotic. A larger zone of inhibition indicates greater susceptibility, while a smaller zone indicates resistance. The results are reported as susceptible, intermediate, or resistant, based on established breakpoints for each antibiotic.

❖ **Procedure: -**

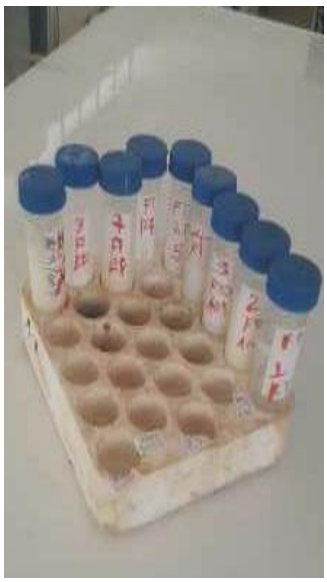
- ✓ At least 4-5 well isolated colonies of the same morphological type are selected from nonselective agar plate and just the top of the colonies are touched.
- ✓ Then a suspension was made in a saline or broth without pre incubation.
- ✓ The turbidity of both suspensions is adjusted by comparison with a 0.5 McFarland turbidity standard.
- ✓ The standard and the test suspension are placed in similar 4-6 ml, thin glass tube.
- ✓ The turbidity of the test suspension is adjusted with broth or saline and compared with the turbidity standard, against a white back ground with contrasting black lines, until the turbidity of the test suspension equates to that of the turbidity standard.
- ✓ Inoculation of bacterial suspension.
- ✓ A sterile, non-toxic swab on an application stick is dipped in to the standardized suspension of bacteria and excess fluid is expressed and rotating the swab firmly against the inside of the tube above the fluid level.
- ✓ The swab is streaked in three directions and continuously brushed over the Muller Hinton or by rotating the plate for complete cover of the agar surface.
- ✓ The inoculated plates are allowed to stand for 3-5 minutes but no longer than 15 minutes and the discs are placed on the agar surface using sterile forceps or an antibiotic dispenser.
- ✓ Each disc is gently pressed with the point of a sterile forceps to ensure complete contact with the agar surface. The disc should be placed no closer together than 24 mm (center to center).
- ✓ This is equivalent to 6 discs per standard 90 mm Petri plate.
- ✓ After incubation, the diameter of the zones of inhibition are measured to the nearest mm using a ruler or caliper.
- ✓ The diameters are read from the back of the plate, when the test is on the comparatively clear Muller-Hinton medium.
- ✓ The diameter of the zones should be read across the center of the discs.
- ✓ An interpretation of the size of the zones of inhibition is made with reference.

Appendix 1: **Photo taken during physical examination, CMT testing and sample collection at the field**

Milk smaple taking from dairy cow

Photo taken during CMT testing

Appendix 2: Photo taken during media preparation and culturing

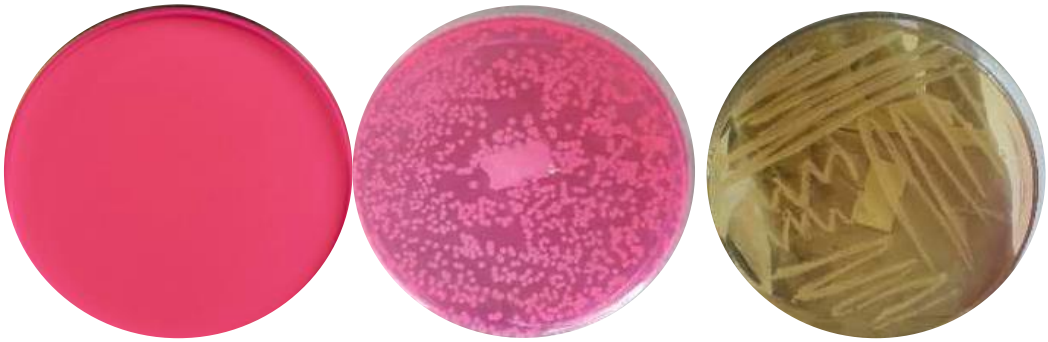


Collected milk smaple

media preparation

Culturing on MSA

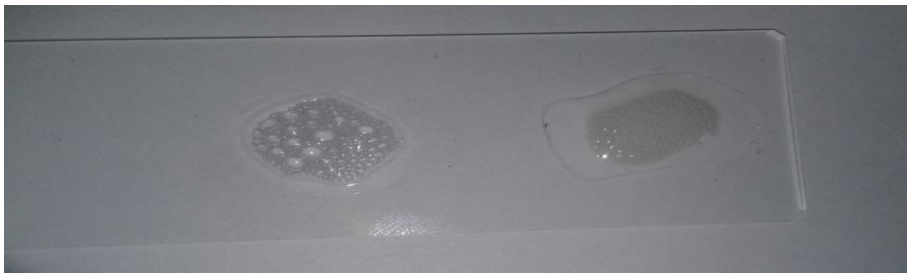
Appendix 3: **Photo taken in culture of agar media**



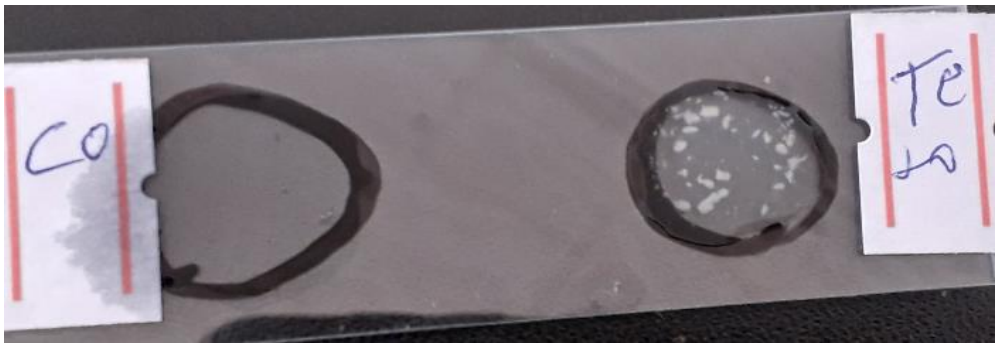
Uninoculated MSA Growth of other than *S. aureus* Growth of *S. aureus* on MSA

Pure colonies subcultured on nutrient agar

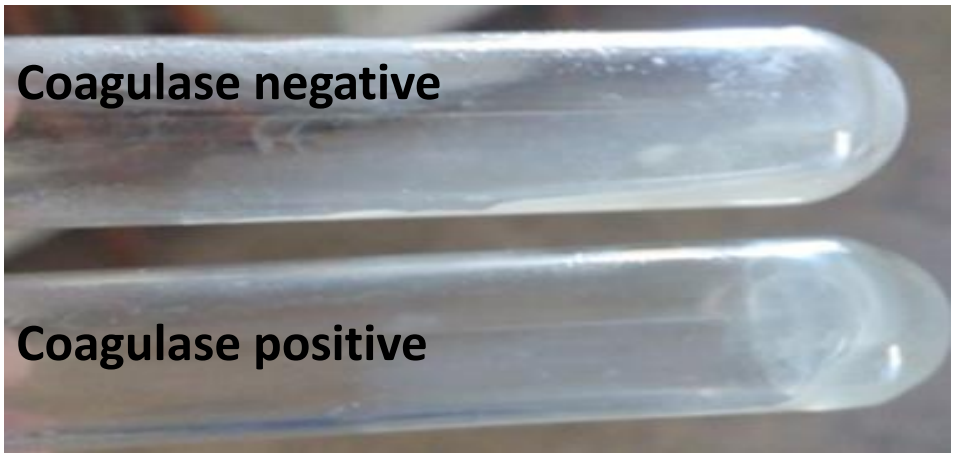
Appendix 4: **Photo of results of biochemical test**

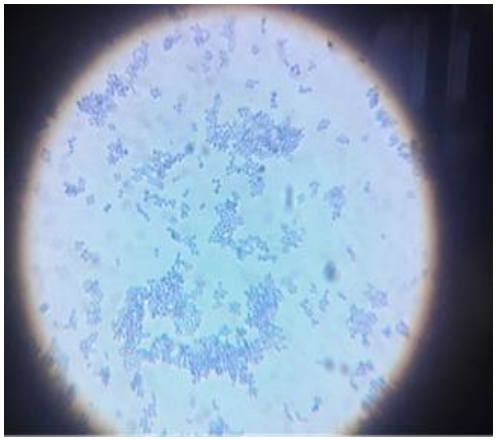


Catalase-Posetive Catalase-negative



Slide-Coagulase- negative Slide- Coagulase-Positive

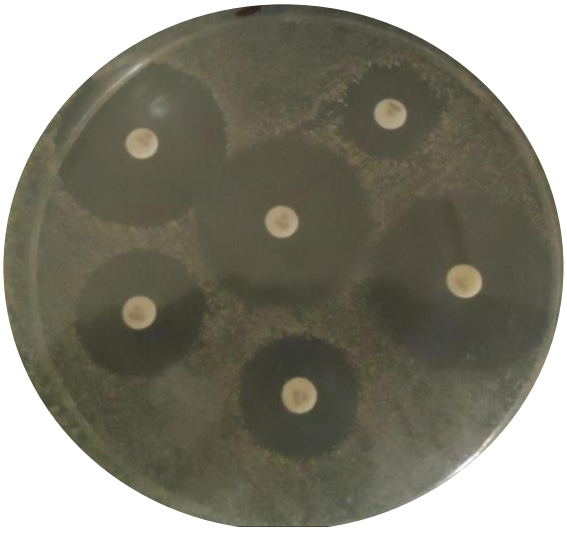




Gram positive Cocci *S. aureus*

Appendix 5: Photo of results of antimicrobial sensitivity test

Standardization inoculum suspension with 0.5 McFarland standard



Antimicrobial-sensitivity test of *S. aureus*