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Clinical and Subclinical Bovine Mastitis: *Staphylococcus aureus* Isolation and Identification from Dairy Farms Located in and Around Hawassa Town, Southern Ethiopia

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Abstract

Background: Mastitis is an expensive disease of dairy cattle in most countries, including Ethiopia. The most commonly recovered bacterial pathogen from mastitis milk is *Staphylococcus aureus* in dairy herds worldwide, with huge economic losses in the dairy industry.

Objective: To isolate and identify *Staphylococcus aureus* from bovine mastitis milk. Additionally, risk factors associated with the occurrence of mastitis were determined.

Methods: A cross-sectional study was conducted from March 2021 to August 2021 on dairy farms in and around Hawassa town. A total of 250 lactating cows were examined for clinical and subclinical mastitis from randomly selected dairy farms. Clinical signs and the California Mastitis Test (CMT) were used to identify clinical and subclinical mastitis, respectively.

Results: During the study period, 50.8% of cows had mastitis, of which 4.8% and 46% showed clinical and subclinical mastitis, respectively. The quarter-level prevalence was 27.4%; of which the clinical form was 2.9%, while the subclinical mastitis was 24.5%. Logistic regression analysis showed a significant association among cows of different age groups, lactation stages, and farm hygienic status with the occurrence of mastitis ($p < 0.05$). A bacteriological study targeting *S. aureus* was conducted with all ($n=127$) milk samples collected from clinical and subclinical mastitis cows. Bacterial identification targeting *S. aureus* was done, and this agent was identified in 60 (47.2%) milk samples. This pathogen was found to be higher (47.8%) in subclinical than in clinical (41.6%) mastitis.

Conclusion: This study showed that mastitis was prevalent in dairy cattle of the study area, with a higher case of *S. aureus* in subclinical mastitis. However, the recovery of *S. aureus* in nearly half of the mastitis milk indicated the possible presence of other pathogens. Therefore, further study to recover other potential pathogens commonly causing mastitis can be a good approach.

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Introduction

Ethiopia has one of the largest livestock resources in Africa, with a national herd estimated at 60.39 million cattle and 64 million sheep and goats (CSA, 2018). Dairy cows represent the largest population of cattle production in the country, where about 42% of the total cattle are milking cows. However, per capita consumption of milk in Ethiopia is as low as 17 kg per head, while the average figure for Africa is 26 kg per head (Abera et al., 2011).

Mastitis is known for its damage to the udder tissue, which is reported in numerous species, mainly in domestic dairy animals. This pathology is the most frequent disease of dairy cattle and can be potentially fatal (Gutierrez-Chavez et al., 2019). Bovine mastitis has been reported as the most important disease on dairy farms because of the reduction of farm profitability, decreased milk production, discarded milk, treatment costs, and culling (Julian, 2016). Mastitis can be manifested by a wide range of clinical and subclinical conditions. Clinical mastitis is characterized by sudden onset, alterations of milk composition and appearance, decreased milk production, and the presence of the cardinal signs of inflammation in infected mammary quarters. It is readily superficial and visually detected. It occurs when the inflammatory response is strong enough to cause visible changes in the milk (clots, flakes), the udder (swelling), or the cow (inappetence or fever).

Subclinical mastitis (SCM) refers to inflammation of the mammary gland in the absence of visible gross lesions in the udder or its secretions, with the presence of pathogenic microorganisms and an increased number of somatic cells in the milk (Smith, 1996; Radostits et al., 2007). Even if there is a great loss related to both conditions, clinical mastitis continues to be a problem in many dairy herds (Gezehagn et al., 2020). Moreover, mastitis has serious zoonotic potential associated with the shedding of bacteria and their toxins in the milk. Mastitis is caused by a wide spectrum of pathogens and is epidemiologically categorized into contagious and environmental mastitis. Contagious pathogens are those for which the udders of infected cows serve as the major reservoir. They spread from cow to cow, primarily during milking, and tend to result in chronic subclinical infections with flare-ups of clinical episodes (Abebe et al., 2016). Environmental bacteria live in the surrounding environment of the cows and are considered opportunistic, causing clinical infections with short duration (Blowey and Edmondson, 2010).

Milk contamination sources include the internal and external sources of the udder. External sources include skin, milking equipment, milkers, contaminated water, and milk transportation tankers. Increasing different bacterial populations will also change milk components and result in an unfavorable odor and flavor, increased rate of spoilage, and a decrease in its maintenance. It also increases the risk of transmission of zoonotic diseases (Mekuria et al., 2014).

Microbes that are commonly isolated from milk include *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Mycobacterium*, *Campylobacter*, *Leptospira*, *Clostridium*, *Pseudomonas aeruginosa*, and *Proteus* species (Angulo et al., 2009; Abeer et al., 2012). Among bacteria causing mastitis, only *Streptococcus agalactiae*, *Staphylococcus aureus*, *Mycoplasma* species, and *Corynebacterium bovis* are considered fully contagious. Among these, *S. aureus* is currently the most frequently isolated contagious pathogen in subclinical and chronic bovine mastitis worldwide (Zecconi, 2010).

Staphylococcus aureus is one of the most important pathogens in humans and animals (Douaa et al., 2016). The organism is well adapted to survive in the udder and usually establishes a mild subclinical infection of long duration. Bacteria are shed into milk from infected quarters (Abera et al., 2010). The prevalence of mastitis, in most countries, reaches up to 50% in cows and 25% in quarters (Radostitis et al., 2000). *S. aureus* is considered the third most important cause of disease in the world among the reported foodborne illnesses (Peles et al., 2007). The bacteria persist in mammary glands, teat canals, and teat lesions of infected cows and are contagious (Wolfe et al., 2010).

Infected cows and quarters are normally identified through bacteriological examination of milk samples (Artursson et al., 2010). *S. aureus* is transmitted from an infected to an uninfected mammary gland during the milking process. Shared equipment, udder cloths, and even milkers' hands can transmit *S. aureus* between cows if good hygienic practices are not followed. Environmental factors, such as bedding, housing, and feedstuffs, can also be contaminated and play a role in spreading *S. aureus* infection. Transmission occurs mainly at milking time through contaminated milking machines, clothes, and hands of milkers or machine operators (Abera et al., 2010).

More attention has been given to the diagnosis of subclinical mastitis using indirect tests. The indirect tests depend on the cellular interaction between the reagent and certain protein factors in mastitis milk. The most common methods are somatic cell count (SCC) and the California mastitis test (CMT) (Rafik et al., 2014). During SCC, the adherence of bacteria stimulates macrophage migration and the migration of neutrophils from blood into the milk, which will lead to a high somatic cell number (SCC), swelling of the mammary gland, damage to the host defense system, and epithelial cells (Douaa et al., 2016). When milk and CMT reagent are mixed in equal amounts, the CMT reagent dissolves or disrupts the outer cell wall and the nuclear cell wall of any leukocyte, which are primarily fat (detergent dissolves fat). DNA is now released from the nuclei. DNA will string or gel together to form a stringy mass. As the number of leukocytes increases in a quarter, the amount of gel formation will increase in a linear fashion (Melleneger, 2001).

Several bacterial pathogens are implicated in bovine mastitis. From an epidemiological and pathophysiological standpoint, the pathogens are regarded as contagious, teat skin opportunistic, or environmental (Radostits et al., 2007).

Staphylococcus aureus is the etiological agent more commonly associated with the disease and is normally related to

both subclinical and chronic infection, leading to severe economic loss to dairy farms (Kubota et al., 2007). According to a livestock agriculture office report, the incidence of mastitis has become popular, and some professionals also recommend digging in detail about the area, as such the study was designed to figure the prevalence value and to identify the factors associated with mastitis infection. In addition, although many studies have been conducted by different researchers on the isolation and identification of *S. aureus* from bovine mastitis milk in Ethiopia, including the present study area, it is necessary to update the information to find out if there was any change in the epidemiology of the bacteria. Accordingly, the objectives of this study were to isolate and identify *S. aureus* from bovine mastitis milk and estimate the prevalence of the pathogen and associated risk factors of bovine mastitis in Hawassa town.

Materials and Methods

Study Area

The study was conducted in Hawassa town and its surroundings. Hawassa is the capital city of the Sidama Region, which is located 275 km south of Addis Ababa with a total human population of 157,879. Geographically, it lies between 7°03'1.35"N latitude and 38°29'43.81"E longitude at an altitude of 1750 meters above sea level. The area annually receives an average of 800 - 1000 mm of rainfall, of which 67% falls in the long rainy season, which extends from June to September, with an average annual temperature of 22°C and 51.8% mean relative humidity. The area is mainly covered by dry savanna and bush-type vegetation. The total livestock population of the Sidama region (including Hawassa town) is estimated to constitute 2,413,482 cattle, 308,903 goats, 467,858 sheep, 34,709 horses, 16,376 donkeys, 1,824,841 poultry, and 44,364 beehives (CSA, 2020).

Study Animals

The study was conducted on lactating cows of both exotic (Holstein-Friesian and Jersey) and crossbred (Holstein Friesian-Zebu crosses). The cows were randomly selected from small, medium, and large dairy farms kept under intensive and semi-intensive management systems. For simplicity and categorization, Megersa et al. (2011) number of cattle categories was used: small (with fewer than 10 cattle), medium (10 to 50 animals), and large (≥ 50 animals).

Study Design

A cross-sectional study was conducted from March 2021 to August 2021 on dairy farms in and around Hawassa to isolate and identify *Staphylococcus aureus* from clinical and subclinical mastitis cows.

Sample Size Determination

The sample size was calculated according to the formula given by Thrusfield (2005). The expected prevalence of 81.1% by Duguma et al. (2014) was used, together with a 95% confidence interval and a significance level of 5%. The minimum

number of cattle needed in the study was calculated to be 236. However, we included 250 lactating cows in this study.

Study Methodology

Each selected lactating cow was screened for mastitis based on clinical examinations and the California Mastitis Test (CMT). Cows with mastitis were subjected to bacteriological examination to identify *Staphylococcus aureus*. Furthermore, information regarding potential risk factors for both clinical and subclinical mastitis, such as husbandry systems, the status of farm hygiene, and previous history of mastitis, was collected from interviews with farm owners. Additionally, animal identification, including breed, age, body condition score (BCS), parity, lactation stage, and average milk yield per day, were recorded on the sheet designed for sample collection. Age and BCS were determined with dentition (Johnson, 1998) and body condition (Sharad et al., 2016) observation, respectively.

Clinical Inspection of the Udder

The udders of the study cows were examined visually and by palpation for the presence of any abnormalities, such as hard and swollen quarters, pain (kicking upon touching the udder), heat, and abnormal secretion in the mammary gland (the presence of clots or flakes in milk or watery consistency, and blood-tinged secretions).

California Mastitis Test (CMT)

Cases of subclinical mastitis were diagnosed based on CMT results (i.e., observation of the nature of gel formation), which show the presence and severity of the infection. From each quarter of the udder, a squirt of milk sample was dropped into each of the strip cups on the CMT paddle, and an equal amount of CMT reagent was added to each cup and mixed gently. The test result was interpreted based on the thickness of the gel formed by the CMT reagent and milk mixture and scored as 0 (negative), T (trace), 1 (weak positive), 2 (distinct positive), and 3 (strong positive). Finally, quarters with a CMT score of 1 or above were judged as positive for subclinical mastitis; otherwise, they were considered negative (Quinn et al., 2002).

Milk Sample Collection

Milk samples were collected from both positive cases of clinical and subclinical mastitis. The udder was washed first with tap water, and then the teats were dried with a clean cloth. Approximately 10 ml of milk was taken from each quarter after discarding the first three milking streams aseptically into sterile bottles for bacteriological investigation and labeled. The sample was placed in an icebox containing ice packs and transported immediately to the microbiology room of Hawassa University's veterinary medicine (HU-FVM) laboratory.

Udder Cleaning

Udder cleaning was made with clean water, soap, and dried with a clean towel, followed by teat disinfection with 70% alcohol before milk sampling. 10 ml of milk was taken after discarding the forestrip milk from each dairy cow.

Transportation was done to the HU-FVM lab by keeping an icebox containing ice packs. Upon arrival, the collected samples were immediately stored at 4 °C for a maximum of 24 hours until culturing the next day.

Isolation and Identification

Udder cleaning was performed with clean water, soap, and dried with a clean towel, followed by teat disinfection with 70% alcohol before milk sampling. Ten milliliters of milk was taken after discarding the forestrip milk from each dairy cow. Transportation was done to the HU-FVM lab by keeping an icebox containing ice packs. Upon arrival, the collected samples were immediately stored at 4 °C for a maximum of 24 h until culturing the next day. The bacteriological culture was performed following the standard microbiological technique (Quinn et al., 2002). A loopful of milk sample using an inoculating needle was streaked on sterile 5% sheep blood agar (Oxoid, UK), and swab samples were streaked on blood agar media using a cotton applicator, and the plates were incubated aerobically at 37 °C and examined after 24–48 h of incubation. The colonies were identified based on morphological characteristics, hemolytic pattern, and Gram's staining reaction. The representative colonies that were positive for Gram's staining and had a typical grape-like structure under a microscope were further sub-cultured on nutrient agar plates (Oxoid, UK) and incubated at 37 °C for 24 hrs. Pure colonies were preserved and maintained on nutrient slants for further characterization of the isolates. Eventually, identification of the agent was done based on biochemical tests such as catalase, coagulase, mannitol salt agar, and purple agar base tests.

A catalase test using 3% hydrogen peroxide (H₂O₂) was performed to identify catalase-positive and catalase-negative bacteria. The colonies that were identified by Gram staining and catalase tests were subcultured on Mannitol Salt Agar (MSA) plates and incubated at 37°C, examined after 24–48 hrs for growth and change in the color of the medium. The presence of growth and change of pH in the media (red to yellow color) were regarded as confirmative identification of the salt-tolerant staphylococci. The fermentation of mannitol by *S. aureus* causes yellow discoloration of the medium (Quinn et al., 2002).

The tube coagulase test was conducted according to the method of Robertson et al. (1999). Accordingly, 0.1 ml of fresh cultures of suspected staphylococci grown on Nutrient Broth for 18-24 hours was added to 0.5 ml of 1/10 diluted sterile rabbit plasma (Sigma) in the test tube. The tube was incubated at 37°C and examined every 4-24 hours to see the presence of clotting. The reaction was considered coagulase-positive if any degree of clotting was visible (Tallent et al., 2001). The suspected culture was also inoculated on a purple agar base with 1% maltose media and incubated at 37°C for 24 hrs. Samples were considered positive for *S. aureus* when the suspected isolates were catalase-positive, coagulase-positive, and showed rapid fermentation of maltose on PAB (Quinn et al., 2002).

Data Collection and Analysis

The collected data about the risk factors, including breed, age, parity, lactation stage, housing, and previous history of mastitis, along with the laboratory results, were entered into a Microsoft Excel spreadsheet and coded before statistical analysis. The prevalence of mastitis was calculated by dividing the number of mastitis-positive cows (clinical and

subclinical) by the total number of animals examined. The degree of association between risk factors and the prevalence of mastitis was analyzed using the odds ratio (OR). Furthermore, logistic regression was used to examine the association of the potential risk factors with the occurrence of mastitis using STATA Corp. (version 12.0 statistical software). In all analyses, a 95% confidence level and a p-value < 0.05 were used to determine statistical significance.

Results

Prevalence of Mastitis

In this cross-sectional study, from a total of 250 lactating cows examined, 127 (50.8%) were found to be affected with clinical and subclinical mastitis infection. Of these, 4.8% (12/250) and 46% (115/250) showed clinical and subclinical mastitis, respectively. The quarter-level overall prevalence of mastitis was 27.4% (274/1000), while quarter-level clinical and subclinical mastitis were 2.9% (29/1000) and 24.5% (245/1000), respectively. There was a higher prevalence of subclinical mastitis than clinical mastitis, both at the cow and quarter levels (Table 1).

Table 1. Prevalence of mastitis at cow and quarter level

Forms of mastitis	Cow level (N= 250)	Quarter level (N= 1000)
	No. positive (%)	No. positive (%)
Clinical	12 (4.8%)	29 (2.9%)
Subclinical	115 (46%)	245 (24.5%)
Total	127 (50.8%)	274 (27.4%)

Bacterial Isolation

A total of 127 exotic and crossbred lactating cows with either clinical or subclinical (CMT positive) mastitis were examined for the isolation of *S. aureus*. *S. aureus* was isolated from 41.6% (5/12) of the clinical cases and 47.8% (55/115) of the subclinical cases, respectively. The overall prevalence of *S. aureus* was 47.2% (60/127), as indicated in Table 2.

Table 2. Prevalence of *S. aureus* in clinical and subclinical mastitis

Form of mastitis	No. of cow examined	No. of isolated <i>S. aureus</i> Cases (%)
Clinical	12	5 (41.6)
Subclinical	115	55 (47.8)
Total	127	60 (47.2)

Risk Factors Associated with Mastitis

Multivariate logistic regression analyses were conducted to determine the presence of an association between the risk

factors and the outcome variable (mastitis). Accordingly, the analysis showed that the odds of being infected with mastitis for exotic breed types were 1.88 times greater compared to cross-bred types (OR=1.88; 95% CI=0.96, 3.66; $p < 0.064$). Regarding one of the significant variables, which is age, the risk of having mastitis for cows aged 4-5 years was 3.7 times greater than for those aged <3 years (OR=3.7; 95% CI=1.63, 8.43; $p < 0.002$). Likewise, the odds of having mastitis for the >6 years age category were 17.61 times greater compared to that of the reference age group (OR=17.61; 95% CI=5.3, 58.44; $p < 0.001$). From the age variable, the study concludes that as age increases, the risk of living with or being infected with mastitis also increases. Another significant variable, the lactation stage, showed us that >7 months was a significant category for mastitis compared with <3 months; in other terms, the possibility of being infected with mastitis in the >7 month group was twice as great as in the <3 month group (OR=2.1; 95% CI=1.0, 4.32; $p < 0.049$). Lastly, the most responsible variable for mastitis was farm hygienic status per day. In such cases, the risk of mastitis infection for farms with hygienic status maintained three times per day was 2.94 times greater than for those with hygienic status maintained four times per day (OR=2.94; 95% CI=1.58, 5.81; $p < 0.002$). Similarly, for farms with hygienic status maintained twice per day, the risk was 5 times greater for the occurrence of mastitis compared to those with hygienic status maintained more than four times per day (OR=5.1; 95% CI=1.85, 13.7; $p < 0.002$) (Table 3).

Table 3. Logistic regression analysis of potential risk factors for the occurrence of mastitis in the study area

Risk factors	Categories	No. of cows		Univariable COR (95% CI)	Multivariable AOR (95% CI)	p-value
		Examined	Positive (proportion)			
Breed	Cross	86	32 (37.2)	1	1	0.064
	Exotic	164	83 (50.6)	1.72 (1.0, 2.9)	1.88 (0.96, 3.66)	
Age (years)	<3	93	21 (22.6)	1	1	0.002
	4-5	98	47 (47.9)	3.16 (1.68, 5.92)	3.7 (1.63, 8.43)	
	>6	59	47 (79.7)	13.43 (6.04, 29.85)	17.61 (5.3, 58.44)	
Parity	<2	134	43 (32.1)	1	1	0.512
	>3	116	72 (62.1)	3.46 (2.06, 5.83)	1.29 (0.59, 2.79)	
Lactation stage (Months)	<3	85	27 (31.7)	1	1	0.094
	4-6	89	43 (48.3)	2.01 (1.08, 3.72)	1.81 (0.9, 3.62)	
	>7	76	45 (59.2)	3.11 (1.63, 5.95)	2.1 (1.0, 4.32)	
Milk yield per day (lit.)	<10	86	45 (52.3)	1	1	0.531
	11-15	82	34 (41.5)	1.56 (0.84, 2.80)	1.26 (0.61, 2.63)	
	>16	82	36 (43.9)	1.41 (0.76, 2.58)	1.39 (0.66, 2.94)	
Husbandry system	Intensive	233	102 (43.7)	1	1	0.213
	Semi-intensive	27	13 (48.1)	1.10 (0.49, 2.45)	1.97 (0.68, 5.88)	
Farm hygienic status per day	> 4 times	36	10 (27.7)	1	1	0.002
	3	130	53 (40.7)	2.36 (1.37, 4.15)	2.94 (1.58, 5.81)	
	2	84	52 (61.9)	4.24 (1.80, 9.90)	5.1 (1.85, 13.7)	

Discussion

In the present study, the overall prevalence of mastitis in dairy cows was 50.8% in cows and 24.7% at the quarter level. This result was in line with a prevalence of 46.7% in cows and 29% at the quarter level reported by Abera et al. (2010) in Adama town and 52.8% in cows reported by Hundera et al. (2005) around Sebeta. This report is relatively similar to the assertion by Radostits et al. (2000). In most countries, irrespective of the cause, the prevalence of mastitis is about 50% in cows and 25% in quarters. However, the present findings are lower than the previous reports of Abebe et al. (2016), Zeryehun and Abera (2017), Elemo et al. (2017), and Tegegne et al. (2020) who reported in Hawassa, South Ethiopia (62.6%); Eastern Harrarghe Zone, Eastern Ethiopia (64.3%); in Asella, Southern Eastern Ethiopia (65.36%); Addis Ababa,

central Ethiopia (70%), respectively. However, the finding was higher than the previous reports of Workineh et al. (2002) (38.2% in Adami-Tulu, central Ethiopia), Mungube et al. (2004) (39.8% in and around Addis Ababa), and Kerro and Tareke (2003) (40% in Southern Ethiopia). This variability in the prevalence of mastitis, irrespective of the cause, between different reports could be attributed to differences in breeds, farm management practices, or environmental conditions in different parts of the country (Radostits et al., 2007).

The occurrence of clinical mastitis in the present study was 4.8%, and that of subclinical mastitis was 46% at the cow level. This also provides further support for other studies in different regions of the country which have concluded that subclinical mastitis is more prevalent than clinical mastitis. In support of this claim, Kerro and Tareke (2003) (62.9% versus 37.0%), Abebe et al. (2016) (59.2% versus 3.4%) in southern Ethiopia, Mekbib et al. (2010) (48.6% versus 22.4%) in Holeta, Abera et al. (2010) (36.7% versus 10.0%) in Adama, and Tassew et al. (2017) (27.86% versus 11.45%) in and around Assosa town reported the higher prevalence of subclinical than clinical mastitis. This variation in prevalence between subclinical and clinical mastitis may be attributed to the difficulty of detecting subclinical mastitis by the owners compared to the easily detectable clinical cases, which prompt owners to seek treatment for their animals (Radostits et al., 2007).

The prevalence of mastitis in the current study was significantly higher during the late stage of lactation (59.2%). This is in agreement with the reports of Almaw et al. (2008), Getahun et al. (2008), and Abera et al. (2012). However, another report from Kerro and Tareke (2003) disagreed with our current study, with its higher prevalence of mastitis during the early stage of lactation. The variations in the effect of stages of lactation among different studies could be related probably to disparities in age, parity, and breed of the sampled animals (Isae and Kurtu, 2018).

The present study revealed that the prevalence of mastitis was higher in older cows (>6 years of age) (79.7%) than in younger (<3 years) cows (22.6%). This contradicts the findings by Kerro and Tareke (2003), and Busato et al. (2000), who found that the risk of clinical and subclinical mastitis increases significantly with the advancing age of the cow. It has been well documented that older cows have larger teats and more relaxed sphincter muscles, which increase the accessibility of infectious agents in the cows' udder (Radostits et al., 2007). The increased prevalence of mastitis with parity reported in the current study is in line with previous reports (Zeryehun et al., 2013; Abunna et al., 2013; Belayneh et al., 2014; Dabele et al., 2021). This could be because primiparous cows have a more effective defense mechanism than multiparous cows (Erskine, 2001). The latter type of cows increases the chance of infection over time and the prolonged duration of infection in multiparous cows (Radostits et al., 2007). The higher prevalence of mastitis (61.9%) was reported in cows kept in houses cleaned twice per day (less frequently cleaned) than in cows kept in houses cleaned four times or more per day (more frequently cleaned). Husbandry system and previous history of mastitis were not significantly affecting the prevalence of mastitis in this study.

According to the microbiological findings of this study, the overall prevalence of *S. aureus* isolates was 47.2% from both clinical and subclinical mastitis cows. This finding was in line with the previous findings of Mekibib et al. (2010) (47%) and Legesse et al. (2015) (48.3%) from Holeta town and Addis Ababa city, respectively. However, the present finding was higher than other studies (Workineh et al., 2002; Tesfaye et al., 2013; Zeryehun et al., 2013; Yohannis and Molla, 2013).

On the contrary, a higher isolation rate than the present result was reported by another group of studies in the country (Abebe et al., 2016; and Zenebe et al., 2014). The variability in the occurrence of *S. aureus* in mastitic cows among different reports may be attributed to differences in farm management practices and environmental inconsistency.

In this study, *Staphylococcus aureus* was more frequently identified in subclinical mastitis than in clinical cases. This is almost similar to previous studies that proved *Staphylococcus aureus* is the principal causative agent of subclinical mastitis (Tassew et al., 2017). This difference might be explained by the already documented evidence that *Staphylococcus* species are adapted to survive in the udder and usually establish chronic subclinical infection of long duration, from which it is shed through milk, serving as a source of infection for other healthy cows and transmitted during the milking process (Radostits et al., 2007).

Conclusion and Recommendations

Mastitis is one of the most important infectious diseases of dairy cows. The subclinical form is the most prevalent type of mastitis in the study farms, which might indicate that dairy farm owners give more attention to clinical mastitis than to subclinical mastitis, which receives very little attention regarding the status of subclinical mastitis. The prevalence of mastitis was found to be significant. Increasing age, increased number of parities, late stage of lactation, and poor farm hygienic status were associated factors with the occurrence of *S. aureus* implicated bovine mastitis in the present study. Based on the conclusions, the researchers recommended regular screening for early detection and treatment of subclinical mastitis and to raise awareness in the community of the risk of *S. aureus* as a public health concern.

Statements and Declarations

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Availability of Data and Materials

The data from the current study are available.

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