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Isolation and Identification of *Staphylococcus aureus* from Bovine Subclinical Mastitis in Dairy Farms of Assosa Town

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Abstract: A cross sectional study was carried out from March, 2019 to June, 2019 to estimate the prevalence of *Staphylococcus aureus* in subclinical mastitis and to assess its associated risk factors in and around Assosa town. From the study area a total of 149 lactating cows were tested for mastitis using the California Mastitis Test (CMT). Out of 149 lactating cows, a total of 58 milk samples were found to be CMT positive (Subclinical mastitis). Samples were collected from all subclinical mastitic cows under aseptic precautions and processed by using standard bacteriological techniques. Mannitol salt agar was used as selective medium for isolation and identification of *Staphylococcus aureus*. It was identified on the basis of their morphological, cultural and biochemical characteristics. From 58 CMT positive milk samples *Staphylococcus aureus* was isolated only from 20 samples with overall prevalence of 34.5%. Even though it was not statistical, *Staphylococcus aureus* prevalence showed significant variation among cows of different age groups, production systems, lactation stages, body condition scores, party level and breed. There was statistically significant association ($P < 0.05$) in prevalence of *Staphylococcus aureus* between hygiene score isolated from subclinical mastitic milk samples. The prevalence of this pathogen between hygiene score was found to be 9.3% and 21.2% in good and poor, respectively. In conclusion, this study confirms the importance of *Staphylococcus aureus* as mastitis causing bacterium and identifying the association between the risk factors and this pathogen as cause of subclinical mastitis play a paramount importance.
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1. Introduction

Bovine mastitis is the most prevalent and costly diseases in dairy cows or some livestock milk industries with losses lead to reducing of milk production, changing in milk composition and reducing the quality of milk, increasing veterinary services, and increasing labor costs (Hillerton and Berry 2005). Bovine mastitis is inflammation of the mammary gland can be divided into two types that manifested by the appearance of inflammation at the udder of dairy cow; asymptomatic (subclinical mastitis) and symptomatic mastitis (clinical mastitis), whereas subclinical mastitis can be occurred up to 40 times more common than clinical cases (Islam *et al*., 2011).

Subclinical mastitis seems to be an important type of mastitis in dairy cow because it is a hidden mastitis or invisible problem in the herd (Yadav *et al*., 2014). Staphylococcus species are one of the main etiological agent for bovine mastitis especially *Staphylococcus aureus*, which is considered as a contagious pathogen that often cause bovine subclinical mastitis (NMC, 1996). This bacterium can spread from infected cow to another cow in a herd through many routes with contaminated milking equipment or through hands of farmers. *S. aureus* infection in subclinical bovine mastitis becomes an important problem in dairy farm industry (Plozza *et al*., 2011).

Staphylococcus aureus is a gram positive, aerobic and facultative anaerobic, oxidase-negative, catalase positive, non-motile and non-spore forming cocci. Upon culturing, this bacterium produces smooth, circular, convex and lustrous colony with size ranges from 0.5-1.5 µm in diameter (Jahan *et al*., 2015). After gram staining under microscope, the *S. aureus* organism appears like irregular three dimensional bunches of grapes like cluster of cells. The colony pigmentation may vary from

grey, grey-white, grey-white with yellowish to orange shades and in blood agar typical β-hemolysis may be produced; depending on the growth condition (Deresse *et al*., 2012).

However, *Staphylococcus aureus* is also present in a variety of locations in the dairy farms, in many occasions it was isolated from swabs taken from the cows head, skin swabs, legs and nasal mucosa and also on the milkers' hands. Even though, an infected udder quarter remains the main reservoir of the bacteria, which transmitted mostly during the milking time (Green and Bradley, 2004). Mastitis in cattle caused by *S. aureus* can either be subclinical or clinical. Subclinical mastitis is the most common type of global importance in the dairy industry and has great economic importance to dairy farmers (Abera *et al*., 2010*).*

From a food safety point of view, the staphylococcal enterotoxin production is the most crucial problem, which leads to the staphylococcal food poisoning outbreaks in humans linked to milk and milk products including cheese, are the third most common food intoxication in the world. The ability to produce one of the enterotoxins was observed in more than 20% of the *S. aureus* strains; even more than 50% of the strains were found (Boynukara *et al*., 2008). Furthermore, 65% up to 84 % of all of these strains were of human origin (Normanno *et al.,* 2007).

Few studies have been carried out in some areas of Ethiopia to assess the status of *Staphylococcus aureus* in bovine subclinical mastitis. However, the present study was done to assess the status of *S. aureus* in bovine subclinical mastitis in and around Assosa town Benishangul-Gumuz regional state, Western Ethiopia. Even though, the current study is not first of its kind, there was no sufficient manuscript pertaining to the prevalence of *Staphylococcus aureus* from subclinical mastitic cows. Moreover, the study on isolation and identification of this pathogen in the study area and screening of milk for such pathogenic organisms will play a vital role in restricting its effect on causing bovine mastitis. Therefore, the objectives of the present study were:

- To isolate and identify *Staphylococcus aureus* from subclinical bovine mastitis in and around Assosa town from dairy farms.
- To determine risk factors associated with *Staphylococcus aureus* in subclinical bovine mastitis.

2. Literature Review

Staphylococcus aureus is one of the most common causes of mastitis in cows. The main source for the organism is from milk handlers since most humans carry the organisms in their nostrils. This is the most common type of subclinical mastitis and has great economic importance to dairy farmers (Abera *et al*., 2010).

2.1. Etiology

Large numbers of microorganisms have been involved in causing subclinical mastitis (Upadhayay, 2005). However*, S. aureus* is the predominant cause of subclinical mastitis in many countries. The important source of infection is infected udder, teat ducts or teat lesion. Extra mammary source do exist notably the vagina and tonsil but don't appear important in the pathogenesis of infection. Most commonly *staphylococcus aureus* udder infection is chronic, acute mastitis being less common than with other bacteria. However, acute gangrenous *staphylococcus aureus* infection can arise in which uncontrolled growth of the organisms occurs, elaborating large quantities alpha toxin. Such infections are probably not due to strains of increased virulence but rather to a failure by the host to mount an effective defense. Workers in California showed the critical role of the PMN (polymorphonucleocytes) in these defense since subclinically infected cows made neutrogenic, rapidly developed acute gangrenous *Staphylococcus aureus* mastitis (Abera *et al*., 2010).

2.2. Epidemiology

In most countries surveys the incidence of subclinical mastitis irrespective of cause show comparable of about 40% morbidity rate amongst dairy cows and greater infection rate as measured by an indirect test about 25%, (Upadhayay, 2005). Historically, S*taphylococcus aureus* was one of the most common causes of bovine subclinical mastitis in dairy cattle worldwide. In the last 25 years, the prevalence of infection and the occurrence of clinical mastitis due to *S. aureus* has decreased in herds using effective mastitis control measures. However, surveys indicate that 50%-100% herds may be infected. In low somatic cell count (SCC) herds, the prevalence infection in cow's ranges from 1-10%. In other hands, especially those with high SCC, up to 50% cows may be infected with *S. aureus*, with quarter infection rate ranging from 10%-25%.

The prevalence of infection of *Staphylococcus aureus* in heifers at parturition can range from 5-15%. The majority of intra-mammary infections due *S. aureus* are subclinical. The incidence subclinical mastitis due *to S. aureus* depends on its prevalence infection in the herd (Deresse *et al*., 2012).

2.2.1. Source of infection and method of transmission

Staphylococcus aureus is ubiquitous in the environment of dairy cattle. The major reservoirs of *S. aureus* are infected udders, teat canals, and teat lesions, but these bacteria also have been found on teat skin, muzzles, and nostrils. The bacteria are spread to uninfected quarters by teat cup liners, milkers' hands,

washcloths, and flies. Staphylococci do not persist on healthy teat skin but readily colonize damaged skin and teat lesions. The organisms multiply in infected lesions and result in increased chance of teat canal colonization and subsequent udder infection (Boss *et al.,* 2016).

2.2.2. Risk factors

Animal, age and parity: Several animal risk factors influence the prevalence of infection and occurrence of subclinical mastitis due to *S. aureus* .The prevalence of infected cow increase with age, peaking at seven years. Surveys show the prevalence of intra-mammary infection in dairy heifers a few days before their first parturition reveals that 45% are infected and the quarter infection rate may be 15%. Some studies found intra-mammary infection in 97% of heifers and 74% quarters (Esron *et al*., 2005).

Breed and level of inherited resistance: The incidence of mastitis is greater in Holstein Friesians than Jerseys, but this may reflect difference in management rather than true genetic difference. Level of inherited resistance is possibly related to teat shape and anatomy of the teat canal. The presences of skin lesion on teats act as a predisposing factor for mastitic organisms (Miller *et al*., 1996).

Environmental and management risk factors: Several herd level management risk factors are important for the spread of *S. aureus.* Poor treatment and udder cleaning can allow spread of the organism among quarters of the same cows, and can allow contamination of milking unit, which are commonly transferred among cows without washing rising. The use of high line parlors is risk, this may be due to the greater fluctuation in vacuum especially when units are removed, and leading to a greater occurrence of teat and impacts in with bacteria in the milking unit may inter the teat canal to establish a new udder infection (Lemma and Kassa, 2001).

Other disease: Retained placenta is often associated with high prevalence of mastitis. The general phenomena are that quarters which are already infected with a minor or a major infection are less likely to acquire a new infection than an infected quarter (Radiostitis *et al*., 2000).

2.3. Pathogenesis

The disease can be reproduced experimentally by the injection of *Staphylococcus aureus* organism in to the udder of cattle but, there is considerable variation in the types of mastitis produced. This does not seem to be due to difference in virulence of the strains used, although strain variation do occur, but may be related to the size of inoculums used or, more probably, to the lactation status of the udder at the time of infection. It is possible to induce *S. aureus* infection in the bovine teats cistern, the teat tissues are able to mount a marked local inflammatory response but in spite large numbers of neutrophil that invade the teat, they are unable to control the infection, except when the numbers of bacteria are low. Infection during early lactation may result in per acute form of mastitis, with gangrene of the udder. During the later stage of lactation or during dry period new infection are not usually accompanied by a systemic reaction but result in the chronic or acute forms. Chronic *S. aureus* mastitis in cow has been converted to the gangrenous form by the experimental production of a systemic neutropenia (Carter and Wise, 2004).

2.3. Clinical Signs

Although, 50% cattle in herd may be affected only a few animals will have abnormalities recognizable by the milkers. Many cases are characterized by slowly developing in duration and atrophy with the occasional appearance of clots in the milk or wateriness of the first streams. The SCC of the milks increased, as well as CMT results of infected quarters, but the disease may go unnoticed until much of the functional capacity the gland is lost. The infection can persist and the disease may progress slowly over a period of many months (Sangvik *et al.,* 2011).

2.4. Diagnosis

Bacteriological culture of milk is the best method for identifying cows with *Staphylococcus aureus* intra-mammary infection. A problem in the laboratory identification of *S. aureus* is that are shed cyclically from infected quarters, so that a serious samples are necessary to increase overall test sensitivity. The sensitivity of a single sample may as low as 75%. In an attempt to decrease to the cost of sampling all quarters for culture, an alternative strategy to use somatic cell count as a screening test in order to identify which cows selected to culture for *S. aureus* (Radostits *et al.*, 2007).

2.5. Treatment

Antimicrobial agents are used extensively for the treatment and control of bovine mastitis. In mammary antibiotic preparation are available in farmers in many countries and this easy access are likely to result in excessive antimicrobial chemotherapy, (Suriyasathaporn *et al.,* 2006). Antibiotic used in the treatment of subclinical mastitis can be administered by parental or intra-mammary roots. Cephalosporin, cloxacilin, erythromycin, penicillin, penicillin combined with novobicocin, tetracycline and trypsin are antimicrobial agents used for the treatment of *S. aureus* pathogen. Dry cow therapy is important. Chronic or subclinical mastitis best treated at drying off with long acting intra-mammary antimicrobial infusion that is beta lactamase resistant (D'Amico *et al*., 2009).

2.6. Control and Prevention

To reduce the source of *S. aureus* organism, a program of early identification, culling and segregation of mastitic cow in dairy herd is important. Although, successful

implementation of all these aspects is challenging satisfactory control of *S. aureus* mastitis has historically been difficult, however, at the present time infection rate can be rapidly and profitably reduced from average level 30% to 10% or less.

Hygienic practice, washing and drying of the udders before milking, regular milking machine maintenance, teat dipping after milking are the major control programs of *S. aureus*. Teat dipping in 10% iodine or 0.5% chlorhedine is completely effective against *S. aureus* mastitis. Elimination of existing infections by dry cow therapy and immunization with vaccine may reduce future impact.

2. Materials and Methods

3.1. Study Area

The study was conducted in and around Assosa, which is the capital city of the Benishangul-Gumuz Regional State and composed of 74 administrative peasant associations, which is located at 8°30'and 40°27' N latitude and 34°21' and 39°1' E longitude 687kms Northwest of Addis Ababa (CSA, 2015). The altitude of Asossa ranges from 580 to over 1560 meter above sea level. The area is characterized by low land plane agroecology which has 'kola' micro climate with land covering 2317km^2 area according to National Meteorological Service Agency with average annual rainfall of 850-1316mm with unimodal type of rainfall that occurs between April and October. Its mean annual temperature ranges between 16.75 °C and 30 °C. Asossa zone has 35.6% of the livestock population of the region constituting 61, 234 cattle (CSA, 2015).

Figure 1: Map of the study area (**Source:** www.google.com)

3.2. Study Population

The study animals were apparently healthy lactating cows in dairy farms in and around Assosa town. Both cross (Holstein Friesian-zebu crosses) and indigenous (local zebu) breed were included in the study from different peasant associations of the study area. The inclusion criteria of the study were based on the availability of subclinical mastitis after screening with California Mastitis Test and willingness of the farm owners.

3.3. Study Design

A cross sectional study was carried out from March, 2019 to June, 2019 to determine the prevalence and to isolate and identify *Staphylococcus aureus* from bovine with subclinical mastitis in dairy farms in and around Asossa town.

3.4. Sampling Methods and Sample Collection

Purposive sampling technique will be employed as sampling strategies to select the dairy farms. This sampling approach was to find people who are willing to provide sample. Lactating cows were selected by simple random sampling technique for CMT screening from each selected dairy farms.

All the collected data about age, parity, lactation stage, body condition scores and previous history of mastitis and the housing system were recorded. Depending on CMT results, cases were categorized as either positive or negative. The age of the study animals was determined from birth records and categorized as young adults (≥ 3 - 5 years), adults (> 6 - ≥ 9 years), and old (> 9 years). Parity was also categorized as few (with 1 - 2 calves), moderate (3 - 4 calves) and many (> 4 calves). Lactation stage was classified as early (< 3 m), medium (3 - 6 m) and late (> 6 m); and the housing were categorized as good (house with concrete floor) and poor (house with mud floor).

Accordingly, in the present study a total of 149 milk samples were checked for the presence of

subclinical mastitis. After milking out and discarding the first two drops, about 2ml of milk were tested on CMT paddle from each quarter and about 20 ml of milk were aseptically collected from each mastitis positive quarter using sterile universal bottle. Out of 149 samples only 58 are found to be positive for subclinical mastitis. Therefore a total of 58 milk samples were collected from subclinical mastitis. Purposively all CMT positive milk samples were subjected to bacteriological analysis for isolation and identification of *S. aureus*, which was the focus of the current study.

The universal bottle will be labeled with permanent marker before sampling. Aseptic procedure was applied during collecting milk samples in order to prevent contamination with microorganisms present on the skin udder and teats, hands of samplers and barn environment by keeping in icebox containing ice packs. The samples were collected from March 2011 to May 2011 and investigation was carried out following collection. The laboratory works was accomplished in the laboratories of the Department of Veterinary Science in Assosa University. Upon arrival, the collected samples were immediately store at 4 °C for a maximum of 24hours until culturing the next day for microbiological examination. Milk samples were cultured and different biochemical tests were employed for isolation and identification of *S. aureus* from subclinical mastitic cow after CMT test. Only CMT positives results were subjected to different primary and secondary biochemical test for isolation and identification.

3.5. Sample Size Determination

Sample size was calculated with an expected prevalence of 40.39% from a previously published research work of Tassew *et al.* (2017) on prevalence of *S. aureus* in subclinical mastitis infection in and around Assosa, Benishangul-Gumuz Regional State, Ethiopia The desired sample size for the study was determined by using the formula described by Thrusfield (2005).

$$n = \frac{1.96^2 P_{exp}(1 - P_{exp})}{d^2}$$

$$n = \frac{(1.96)^2 * 0.4039 * (1-0.4039)}{(0.05)^2} = 370$$

Where; n is the sample size, Z(1.96) is the statistic corresponding to level of confidence 95%, *P* is the expected prevalence and d is precision which was taken as 5%. Therefore, a total of 370 samples were needed but due to limitation of CMT reagent for screening subclinical mastitis and shortage of time for bacterial isolation, only 149 samples were checked with CMT and all CMT positive results were subjected to bacteriological analysis.

3.6. Culturing, Isolation and Biochemical Test

Bacteriological culture was performed following the standard microbiological technique (Fujikawa *et al*., 2006). Culturing and biochemical tests for isolation and identification of *S. aureus* was conducted by direct streaking of loopful of milk onto 7% sheep blood agar and incubating aerobically at 37 °C for 24-48hours. The plates were examined for the presence of *S. aureus* colonies. After growth of presumptive colonies were identified by using conventional bacteriological techniques on the basis of colony characteristics, pigment production and hemolysis. Presumed *S. aureus* colonies were then sub-cultured on nutrient agar plates (NAP) and mannitol salt agar (MSA) medium and incubated at 37 °C for 24-48 hours to get a pure culture (clone of cells derived from a single cell). MSA is a selective media for Staphylococcus species. The final identification of the *S. aureus* was done based on Gram staining, catalase test, oxidase test, and coagulase test after culturing on nutrient agar plates.

3.7. Data Analysis

All the necessary data were collected and registered precisely. The data obtained at the time of study were classified, entered, coded and filtered using Microsoft Excel® 2010 spreadsheet. Before subjected to statistical analysis, the data were thoroughly screened for errors and improper coding. Then the data subjected to Chi-square test ($\chi 2$) in order to assess the association between the different risk factors and occurrence of *S. aureus* in dairy cattle by making use of SPSS version 20 for appropriate statistical analysis.

4. Results

A total of 149 milk samples were screened by CMT reagent and 58 milk samples had been showed subclinical mastitis. Out of 58 subclinical mastitic milk collected, *S. aureus* was isolated only in 20 samples (34.5%). The total percentage of CMT positive accounts 38.9%.

Table 1: Total prevalence of subclinical mastitis and *S. aureus*

Criteria	Total sample examined	No of positive	Prevalence (%)
CMT	149	58	38.9%%
S. aureus	58	20	34.5%

The prevalence of subclinical mastitis between ages was found to be 29.2%, 38.9% and 48.9% in young, adult and old, respectively. The highest prevalence was observed in old as compared to young and adult. The

prevalence of subclinical mastitis between parity was found to be 28.8%, 42.9% and 46.3% in few, moderate and many, respectively. The prevalence of subclinical mastitis between production systems was found to be 51.1% and 30.0% in intensive and extensive, respectively. The highest prevalence was observed in intensive as compared to extensive production systems.

The prevalence of subclinical mastitis between mastitis histories was found to 34.8% and 52.9% without and with mastitis history, respectively. The prevalence of subclinical mastitis between body condition score was found to be 35.2% and 47.7% in poor and good, respectively. The prevalence of subclinical mastitis between breed was found to be 27.6% and 60.8% in local poor and cross, respectively. The prevalence of subclinical mastitis between hygiene score was found to be 53.8% and 30.9% in poor and good, respectively. The prevalence of subclinical mastitis between lactation stages was found to be 62.5%, 15.8% and 46.2% in early, middle and late, respectively.

There was no statistically significant association ($P>0.05$) in the prevalence of subclinical mastitis between age, party, mastitis history and body condition score. There was statistically significant association ($P<0.05$) in the prevalence of subclinical mastitis between production systems, breed, hygiene score and lactation stage (Table 2).

Table 2: The association of age, parity, production system, mastitis history, body condition score, breed, hygiene score and lactation stage with CMT test

Risk factors	Total sample examined	Number of CMT positive	Prevalence (%)	χ2	*p*-value
Age					
Young	48	14	29.2%		
Adult	54	21	38.9%	3.904	0.142
Old	47	23	48.9%		
Parity					
Few	52	15	28.8%		
Moderate	56	24	42.9%	3.535	0.171
Many	41	19	46.3%		
Production system					
Intensive	49	28	51.1%		
Extensive	100	30	30.0%	10.191	0.001
Mastitis history					
No	115	40	34.8%		
Yes	34	18	52.9%	5.640	0.056
Body condition					
Poor	105	37	35.2%		
Good	44	21	47.7%	2.034	0.154
Breed type					
Local	98	27	27.6%		
Cross	51	31	60.8%	15.583	0.000
Hygiene score					
Poor	52	28	53.8%		
Good	97	30	30.9%	7.479	0.006
Lactation stage					
Early	40	25	62.5%		
Middle	57	9	15.8%	23.327	0.000
Late	52	24	46.2%		

The prevalence of *S. aureus* between ages was found to be 12.5%, 14.8% and 12.8% in young, adult and old, respectively. The highest prevalence was observed in adult as compared to young and old. The prevalence of *S. aureus* between parity was found to be 11.5%, 14.3% and 12.8% in few, moderate and many, respectively. The highest prevalence was observed in moderate as compared to few and many. The prevalence of *S. aureus* between production systems was found to be 12.0% and 16.3% in extensive and intensive production system, respectively. The highest prevalence was observed in intensive as compared to extensive production system. The prevalence of *S. aureus* among mastitis history was found to be 13.9% in cows without history of mastitis

and 11.8% with history of mastitis. The highest prevalence was observed in cows without history of mastitis as compared to with history of mastitis.

The prevalence of *S. aureus* among body condition score of animals was found to be 16.2% in poor and 6.8% in good. The highest prevalence was observed in poor body condition as compared to good. The prevalence of *S. aureus* between breed types was found to be 12.2% and 15.7% in local and cross breeds, respectively. The highest prevalence was observed in cross breeds as compared to local breeds. The prevalence of *S. aureus* between hygiene score was found to be 21.2% and 9.3% in poor and good, respectively. The highest prevalence was in poor hygiene score as compared to good. The prevalence of *S. aureus* between lactation stages was found to be 25.0%, 5.3%, and 13.5% in early, middle and late stage, respectively. The highest prevalence was observed in middle lactation stage as compared to early and late.

There was no statistically significant association ($P>0.05$) in the prevalence of *S. aureus* between ages, party, production system, mastitis history, body condition score, breed and lactation stages isolated from subclinical mastitic milk samples. There was statistically significant association ($P<0.05$) in prevalence of *S. aureus* between hygiene score isolated from subclinical mastitic milk samples (Table 3).

Table 3: The association of age, parity, production system, mastitis history, body condition score, breed, hygiene score and lactation stage with *S. aureus*

Risk factors	Total sample examined	Number of positive	Prevalence (%)	χ2	p-value
Age					
Young	48	6	12.5%		
Adult	54	8	14.8%	0.143	0.931
Old	47	6	12.8%		
Parity					
Few	52	6	11.5%		
Moderate	56	8	14.3%	0.247	0.884
Many	47	6	12.8%		
Production system					
Intensive	49	8	16.3%		
Extensive	100	12	12.0%	0.530	0.467
Mastitis history					
No	115	16	13.9%		
Yes	34	4	11.8%	0.104	0.747
Body condition					
Poor	105	17	16.2%		
Good	44	3	6.8%	2.344	0.126
Breed type					
Local	98	12	12.2%		
Cross	51	8	15.7%	0.342	0.559
Hygiene score					
Poor	52	11	21.2%		
Good	97	9	9.3%	4.108	0.043
Lactation stage					
Early	40	10	25.0%		
Middle	57	3	5.3%	7.879	0.19
Late	52	7	13.5%		

5. Discussion

Out of 58 CMT positive samples processed, 20 (34.5%) samples yielded the growth of *S. aureus*, identified based on cultural and biochemical characteristics (gram-stained smears of the pure cultures exhibited clusters of gram-positive *S. aureus*, catalase positive, coagulase positive and oxidase negative test results were obtained). This study described the isolation and identification of *S. aureus* from subclinical mastitic cow milk obtained from in and around Assosa town dairy farm.

In the present study, the prevalence of *S. aureus* in subclinical mastitis was 34.5%. This is due to *S. aureus* is adapted to survive in the udder and usually establishes chronic subclinical infection of long duration from which it is shaded through milk serving as sources

of infection for other healthy cows and transmitted during the milking process (Radostits *et al.,* 2000).

The present study with regard to the bacteriological analysis of milk sample was higher than the report of Thaker *et al* (2013) which was 6.25% in Gujarat region of India, the report of Fagundes *et al* (2010) (10.8%) from Sao Paulo state, Brazil, and Ayano *et al* (2013) (13.8%) from Holleta.

However, the present finding was inconsistent with the earlier findings in Holleta agricultural research center reported as 43.3% by Duguma *et al* (2013), 48.75% in Hawassa by Daka *et al* (2012) and 47.1% in Holleta town by Mekibib *et al* (2010). Similarly, this result was also disagree with previous findings of Kerro and Tareke (2003) who reported 40.3%, 39.1% and 39.2% of *S. aureus* isolates at Assela, Addis Ababa and Southern Ethiopia, respectively. It was also smaller comparable with findings of Lakew *et al* (2009) who reported 41.1% and 43.3% in dairy cows, respectively.

In addition to this, the report of Anueyiagu and Isiyaku (2015) (43.75%) in Plateau State, Nigeria and Tassew *et al* (2017) who reported 27.86% prevalence of *S. aureus* from subclinical mastitis in and around Assosa town from subclinical mastitis also larger than current report. From all these study results of above mentioned indicates prevalence of *S. aureus* is varied from place to place and regions to regions around the world and it highlights that hygienic practice of milking and production system variation across the world.

According to the work of Anueyiagu, and Isiyaku (2015) lactation stage was found to have statistically significant association with *S. aureus* from subclinical mastitis, which disagree from our result. Similar to the report of Tassew *et al* (2017) the current study finding showed that age and parity have no statistically significant association with the prevalence of *S. aureus* from sub-clinical mastitis.

6. Conclusion and Recommendations

The prevalence of *S. aureus* can most likely be attributed to the wide distribution of the organism inside mammary glands and on the skin of teats and udders. From the present study out of 58 CMT positive milk samples *Staphylococcus aureus* was isolated only from 20 samples with overall prevalence of 34.5%. Even though it was not statistical *Staphylococcus aureus* prevalence showed significant variation among cows of different age groups, production systems, lactation stages, body condition, party and breed level. There was statistically significant association in the prevalence of *Staphylococcus aureus* between hygiene score isolated from subclinical mastitic milk samples. The prevalence of this pathogen between hygiene score was found to be 21.2% and 9.3% in poor and good, respectively. In this study confirms the importance of this pathogen as a mastitis causing bacterium and identifies risk factors associated with the disease in Ethiopia. *S. aureus* adapts very well in the udder and establishes chronic and subclinical infections. From there it is shed into the milk, which serves as a source of infection for healthy cows during the milking process. Therefore, based on the above conclusion the following recommendations were forwarded:

- There must be adequate sanitary measure in dairy farms.
- There should be regular dry cow therapy or intra-mammary infusion.
- Milkers' should wash and disinfect their hands before milking cows.
- Avoid the use of common towel for more than one animal.
- Infected animal should be immediately treated before transmitting to others.
- There must be segregation of *S. aureus* infected cows into one group and milk them at last.
- Cull *S. aureus* infected cows from the dairy farm instead of keeping them in breeding list.

7. References

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