EJSIT
European Journal of Science, Innovation and Technology
ISSN: 2786-4936
www.ejsit-journal.com
Volume 4 | Number 4 | 2024

Isolation and Identification of *Staphylococcus* Species from Raw Milk, Swab of Towels, Tank (Bucket) and Milkers' Hand in Smallholder and Dairy Farms in and around Asalla Town, Oromia, South East Ethiopia

Dimshasha Tolera & Derartu Ketama
Asalla Regional Veterinary Laboratory, Ethiopia

ABSTRACT

A cross-sectional study was conducted in and around Asalla town from November 2019 to April 2020, to estimate prevalence and risk factors associated with *Staphylococcus* species and to isolate and identify of *Staphylococcus* species from milk, swab of towel, swab of bucket and swab of milkers' hand sample in and around Asalla town dairy farms and smallholder farms. A total of 215 samples were collected from smallholder and dairy farms. These samples contained 162 raw milk, 29 swabs towel, 13 milkers' hands swab and 11 swabs of bucket. Primary and secondary biochemical tests were used to identify the *Staphylococcus* species, and risk factors were assessed through interview. *Staphylococcus* species identified from all samples were categorized into *Staphylococcus aureus*, *Staphylococcus intermedius* and *Staphylococcus hyicus*. The prevalence of *Staphylococcus aureus*, *Staphylococcus hyicus* and *Staphylococcus intermedius* found from milk in the study area was 14.89%, 3.7% and 3.72%, respectively. The prevalence of *Staphylococcus aureus*, *Staphylococcus hyicus* and *Staphylococcus intermedius* found from towels was 2.79%, 0.47% and 2.32%, respectively. Using the chi-square test, the association between risk factors and isolation of *Staphylococcus* species from the collected samples was analyzed. The result indicated that the prevalence of *Staphylococcus* species in milk and swab had a statistically significant association based on type of farm (P= 0.047), age (P=0.003), management system (P=0.037) and drainage condition (P= 0.010). The current study revealed that the prevalence of *Staphylococcus* species in raw milk was high as compared to other samples. Therefore, raw milk should be pasteurized and handled hygienically. In addition, further research should be done on other risk factors responsible for milk contamination in the study area.

Keywords: dairy farm, prevalence, smallholder farm, species, *Staphylococcus*

INTRODUCTION

Milk can harbor a variety of microorganisms and can be an important source of foodborne pathogens. The presence of foodborne pathogens in milk is due to direct contact with contaminated sources (Roberts & Greenwood, 2003) in the dairy farm environment, contaminated milking equipment, the hands of the milkers (Cullor, 2004) and excretion from the udder of an infected animal (Oliver *et al*., 2005). The contamination of milk with pathogenic bacteria has been known to occur mainly due to unhygienic way of handling and processing (Singh & Prakash, 2010).

Staphylococci are normal inhabitants of the skin and mucous membranes of animals and humans. They are also wide spread in nature and have been isolated sporadically from a wide range of environmental sources such as air, water, soil and plant surfaces, meat, poultry and dairy products (Singh & Prakash, 2010). Pathogenic strains are usually coagulase-positive and have been found to cause disease in their hosts throughout the world (Larsen *et al*., 2000). They are capable of causing mild to life threatening diseases, which also includes food borne illnesses. Several species in this genus are having capability to produce a wide range of heat stable enterotoxins (Fagundes *et al*., 2010).

Milk is considered vehicles of *S. aureus* for infection in humans (Zecconi & Hahn, 2000). Milk has been known to be contaminated by *S. aureus* when there is infection of the mammary gland or by bad hygiene habits, such as coughing or sneezing and not washing hands when handling milk storage equipment, during or after milking, and in this case, human activity is responsible for the contamination, as these bacteria colonizes the nasal pathways in human beings (De Oliveira *et al*., 2011). Humans and animals have been known as the primary reservoirs (Argaw, 2015). The presence of *S. aureus* in food has been known to cause food poisoning by releasing enterotoxins into the food and also cause Toxic Shock Syndrome by release of super antigens into the blood stream (Todar, 2005). Pathogenicity of *S. aureus* is due to the membrane active substances such as cytolytic toxins, consisting of four haemilysins and a leukocidin. This genus may have alpha, beta, gamma and delta haemolysin and the pathogenic members of species aureus display beta haemolysis (Presscott *et al*., 2002).

Statement of the Problem

In Ethiopia, there is no standard hygienic condition followed by producers during milk production. The hygienic condition has been known to vary according to the production system, adapted practices, level of awareness, and availability of resources. In most of the cases under smallholder condition, the common hygienic measures taken during milk production especially during milking are limited to letting the calf to suckle for few minutes and/or washing the udder before milking. The quality of the water used for cleaning purpose (to wash the udder, milk equipment, hands), however, is not secured (Yilma, 2003).

Studies conducted in the country showed that *Staphylococcus* species is distributed at different parts of Ethiopia and sometimes with higher prevalence. Thus, Fikru (2014) reported 17.2% from farm and abattoir samples at Addis Ababa; Lencho (2015) reported 13.9% from farm samples (milk, udder, hand, and utensil swab) at Ambo and Guder town, Ayele (2017) reported 19.6% at farm level and 80% at milk collection center at Sebeta and Abuna *et al*. (2016) reported 49.2% from Farm, Abattors and personnel at Asalla town. The prevalence of *Staphylococcus* species and risk factor contributing to contamination of milk of dairy farm is limited in the study area. There is a need for study on the status of *Staphylococcus* species and associated risk factor to forward the possible management options for *Staphylococci* species.

General Objective

To estimate prevalence and risk factors associated with *Staphylococci* and to isolate and identify of *Staphylococcus* species from milk, swab of towel, swab of Bucket and swab of milkers' hand sample in and around Asalla town dairy and smallholder farms.

Specific Objectives

- ✓ To estimate prevalence *Staphylococcus* species in and around Asalla town dairy farm and smallholder farm;
- ✓ To assess risk factors associated with *Staphylococcus* species;
- ✓ To isolate and identify *Staphylococcus* species from milk, swab of towel, swab of Bucket and swab of milkers' hand sample.

MATERIALS AND METHODS

Study Area

The study was conducted in and around Asalla town dairy farm and smallholder farms from November to June 2020. Asalla town is located in Oromia region. The town, which is the capital of Arsi zone, is located at about 175 km Southeast of Addis Ababa at 6°59' to 8°49' N latitudes and 38°41' to 40°44' E longitudes with an altitude of the area ranges from 2500 to 3000 meter above sea level. Agricultural production system of the study area is of mixed crop and livestock production. Dairy farming using improved breeds is a common practice in urban

and peri-urban areas. The area is characterized by mid subtropical temperature ranging from 5°C-28°C and with relative humidity ranging from 43 to 60%. The annual average rainfall is 1200 mm and mostly with clay type of soil and in rare case black soil. The area has a bimodal rainfall occurring from March to April (short rainy season) and July to October (long rainy season). The area covers 23674.72 km square and topographically has highland escapement and lowland areas. The area is densely populated, with livestock population of 85,893 cattle, 57,118 sheep, 10,725 goats, 7841 horses, 15,642 donkeys, 517 mules and 35,489 poultry. The farmers in the area practice mixed crop-livestock farming system. The high land areas are found centrally and the low lands dominate the periphery of the area (APEDO, 2007).

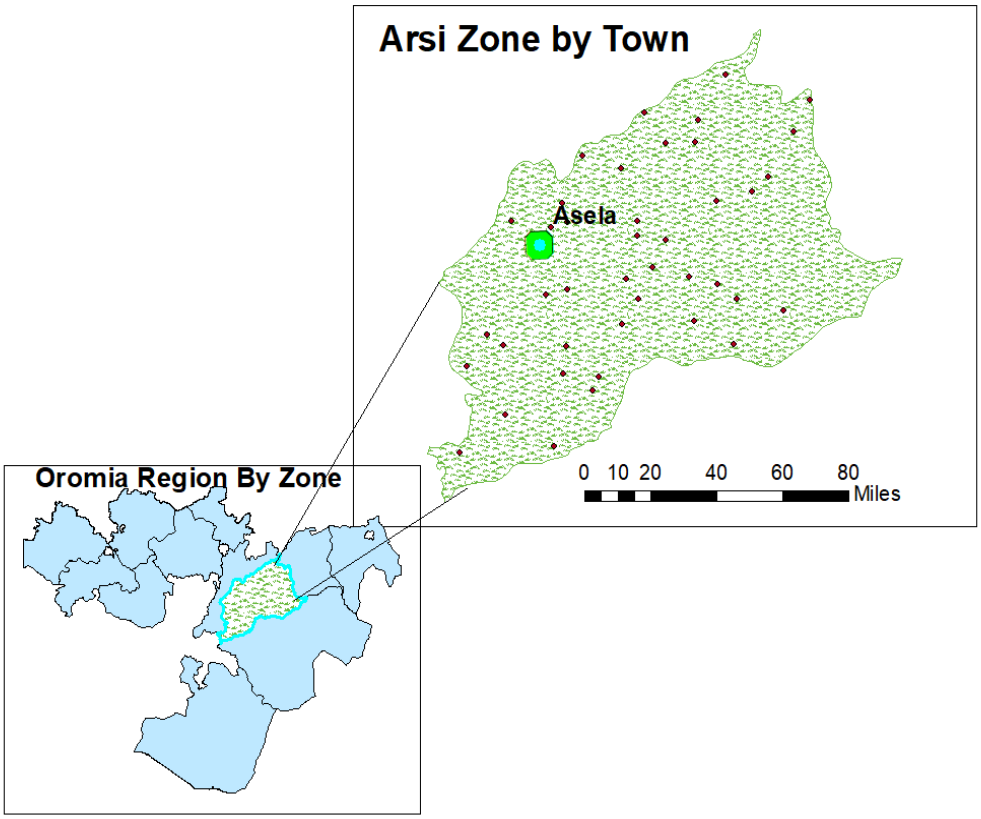

Figure 1: Map of Study Area

Study Population

The study population was apparently healthy lactating cows in selected dairy farm and smallholder farm which are kept under intensive and semi-intensive management system.

Study Design and Sample Type

A cross-sectional study was conducted in the selected dairy farms and smallholder farm from November, 2019, to April, 2020, to conduct different sample from milk, swab of towel, swab of bucket and swab of milkers' hand, then to isolation of *Staphylococcus* species.

Sample Size Determination and Sampling Strategy

The sample size was calculated using the formula described by Thru field (2005) at 5% precision and expected prevalence of 16.8% which was reported by Mokennen *et al*. (2011) from Bishoftu, which has similar features with the current study area. A total of 215 samples were collected by simple random sampling techniques from lactating cows in purposively selected dairy farms based on the availability of one or more lactating animals and willingness

of the dairy farm owners to be part of the study. Ten lactating cows from the selected farms were selected using simple random sampling techniques after assigning of identification tags for each lactating animal. In addition, 162 milk, 29 towels swab, 13 milkers hand swabs and 11 swabs bucket were sampled (Table 1). The milker population serves in the smallholder and dairy farms are few in numbers; as the result all of them were included. Therefore, a total of 215 samples were considered for the present study.

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

Where n = required sample size, 1.96 = the value of Z at 95% confidence interval, P_{exp} = expected prevalence, and d = desired absolute precision.

Table 1: Number and type of samples considered for the study

Type of samples	Total sample collected
Milk	162
Towel swab	29
Milkers' hand swab	13
Bucket swab	11
Total sample collected	**215**

Study Methodology

Sample Collection and Transport

5milliliter volume of raw milk sample was collected aseptically from each apparently healthy lactating cow using sterile universal bottles. The swab samples from milkers' hands, milking buckets, and drying towels are taken using sterile swabs and kept in sample bottles containing sterile physiological saline solution to prevent desiccation. All samples are immediately transported using a box containing an ice to bacteriology laboratory at Asalla Regional veterinary Laboratory and the samples are kept at 4^0 C for isolation of the target bacteria within 24 hrs of collection.

Questionnaire Survey

Questionnaire interview was used to collect information on possible risk factors for *Staphylococcus* species in the current study was age of the study dairy cows are determined from information of cattle birth records kept, transferred with the cattle as they move from one operation to another or from owner and categorized according to Abera *et al*. (2013) as young (≥ 3 - 5 years), adults (> 6 - ≥ 9 years), and old (> 9 years). Parity will be also categorized as few (with 1 - 2 calves), moderate (3 - 4 calves), and many (> 4 calves). Also, lactation stage will be classified as early (< 3 months), medium (3 - 6 months), and late (> 6 months). Animal's body condition score will be categorized as poor, moderate and good based on vertebrae at middle of the back, fat deposit behind shoulder and in brisket area, rear view of the hook bone (cross-section), side view of the line between hook and pin bones, and cavity between tail head and pin bone Sharad *et al*. (2016). Drainage conditions of the milking areas are categorized as poor and good from view of accumulated dirty sewage and muddy or properly cleaned area. Milkers who served in dairy farms at selected area are part of the study. In addition to animals, milkers' hands, milking bucket, and drying towels was a part of the study

Cultural procedure

A loop full of the pre-enriched milk sample was streaked (seeded) aseptically onto sterile blood agar plates (BAP) enriched with 7% heparinized sheep blood and incubated at 37^0C for 24-48 hours under aerobic culture conditions. The plates were examined for the presence of *Staphylococcus*. Colonies of *Staphylococcus* species were identified on the basis of their morphological aspects (creamy, greyish, white or yellow colonies) and haemolytic pattern on

the surface of BAP. Presumed staphylococcal colonies were sub-cultured on nutrient agar plates (NAP) and incubated at 37^0C for 24-48 hours to get a pure culture (clone of cells derived from a single cell). The pure isolates from NAP were preserved and maintained for biochemical differentiation tests. Pure culture of a single colony type from the NAP were inoculated into nutrient slants and incubated at 37^0C for 24-48 hours under aerobic culture conditions; the pure isolates in the nutrient slant were preserved and maintained at 4^0C for further analysis (Radostits *et al*., 2007).

Isolation and identification of Staphylococcus species

The samples were directly inoculated on blood agar plate containing 7% sterile sheep blood. It was incubated at 37 ^{0}C for 24- 48 hrs. Then the colonies characteristics were seen and recorded. The colonies grown on blood agar plates were taken and smeared. Gram's staining was employed for identifying cell morphology, color and shape. Suspected colonies were sub-cultured on nutrient agar plates (Quinn *et al.,* 2005) from which further biochemical tests such as catalase test, coagulase test and growth characteristics on mannitol salt agar were performed.

Gram's staining

All suspected cultures of *Staphylococcus* species colonies were picked and smeared on labeled clean glass slide. The smeared slides were stained using Gram stain. Once stained, the smear was examined using the oil immersion (100X magnification) lens. *Staphylococcal* organism on microscopic examination appeared in pairs, or grape like clusters (Quinn *et al*., 2002).

Catalase test

Suspected colonies from cultured (18-24 hrs) samples were picked and placed on a clean glass slide. Then one drop of 3% H_20_2 was placed over the organism and liberation of gas was observed immediately. If it produces gas bubbles it is recorded as catalase-positive organism whereas; non-gas producing organism is recorded as catalase-negative (Quinn *et al*., 2002).

Growth of Staphylococcus on mannitol salt agar plate

The colonies that were identified by Gram staining and catalase test were sub-cultured on Mannitol Salt Agar (MSA) plates and incubated at 37^OC examined after 24-48hrs 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*. Phenol red pH indicator detected the acidic metabolic product of mannitol. Fermentation of mannitol by *S. aureus* causes yellow discoloration of the medium (Quinn *et al*., 2005). Colonies that develop weak or delayed yellow color after 24 hrs of incubation were taken as *S. intermedius* and colonies that failed to produce any change on the medium were considered as *S. hyicus* and CNS (Quinn *et al*., 2005).

Tube coagulase test

Colonies that were grown on the MSA plate were sub-cultured on nutrient medium broth incubated at 37^{o}C for 24 hrs. The 0.5 ml of rabbit plasma and a drop of young colonies taken from Nutrient Broth (NB) were mixed and incubated for 4-24hrs at 37^{o}C. The clotting of suspension was evaluated at 30 minutes intervals for the first 4hr of the test and then after 24hrs incubation. The reaction was considered as positive, if any degree of clotting was visible (Bennett & Lancette, 2001).

Data Management and Analysis

The collected data are entered and analyzed using STATA version 11.0 computer software. Descriptive statistics are applied to compute prevalence of *Staphylococcus* species and proportions of questioner data. Chi-square test will be used to check the presence of association between risk factors and isolation of *Staphylococcus* species. The significance level will be adjusted at $P \leq 0.05$.

RESULTS

Isolation and Identification of *Staphylococcus* Species

Out of 215 samples collected from smallholder and dairy farms, 70 (32.55%) *Staphylococcus* species isolates were identified. The prevalence of *Staphylococcus* species in dairy and smallholder farms was 41(19.06%) and 29(13.49%), respectively, as indicated in Table 2 and Figure 2.

Table 2: Prevalence of *Staphylococcus* species in dairy and smallholder farms

Type of farm	Total no. of sample examined	Number of *Staphylococcus* species isolated			
		S. aureus (%)	*S. intermedius (%)*	*S. hyicus*	Total prevalence (%)
Dairy farm	105	21(9.77)	8(3.72)	12(5.58)	41(19.06)
Smallholder farm	110	22(10.23)	4(1.86)	3(1.39)	29(13.49)
Total isolated	**215**	**43(20)**	**12(5.58)**	**15(6.97)**	**70(32.55)**

Note: X^2= 3.9361, p-value= 0.047

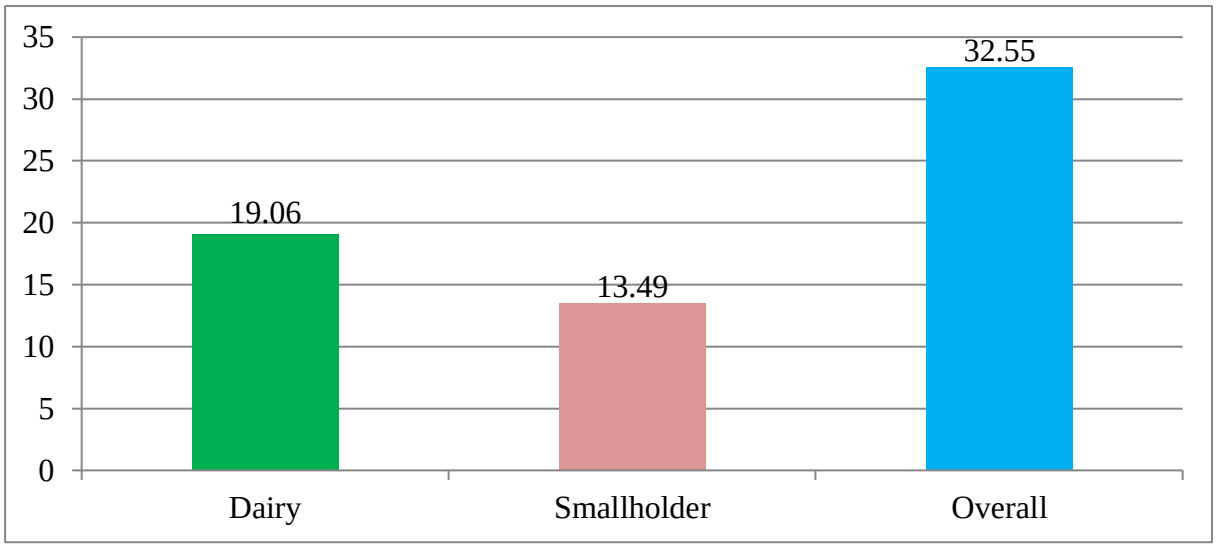

Figure 2: Overall prevalence of *Staphylococcus* species between dairy farms and smallholder farms

Each of samples was examined with primary and secondary biochemical tests and species of *Staphylococcus* distributed in all samples were categorized into four. They are *S. aureus* with a total of 43 isolates (20%); the second, third and fourth were represented respectively by *S. intermedius* with 12 isolates (5.58%), *S. hyicus* with 15 isolates (6.98%). There was no statistical association between species of *Staphylococcus* identified and type of samples (Table 3).

In the present study, the prevalence of *S. aureus, S. intermedius* and *S. hyicus* in fresh raw milk was 14.89%, 3.7% and 3.72% respectively. The percentage of *S. aureus* found to be contaminated the milk, swab of towel, swab of Bucket and milkers' hands were 14.89%, 2.79%, 0.46% and 1.86% respectively. The number of *S. intermedius* isolate identified contaminated the milk, swab of towel, swab of Bucket and milkers' hands were 3.7%, 0.47%, 0.47% and 0.94% respectively. In addition, 3.72%, 2.32%, 0.47%, 0.47% isolates of *S. hyicus* were identified from contaminated the milk, Swab of towel, swab of Bucket and milkers' hands respectively. The percentage of *S. aureus* identified was high when compared to other *Staphylococci* (Table 3 and Figure 3).

Table 3: Prevalence of *Staphylococcus* species isolated from various sample

Sample type	No. of identified *Staphylococcus* species			
	S. aureus (%)	*S. intermedius (%)*	*S. hyicus (%)*	Total isolates (%)
Milk	32(14.89)	8(3.7)	8(3.72)	48(22.31)
Swab of towel	6(2.79)	1(0.47)	5(2.32)	12(5.57)
Swab of milkers' hand	4(1.86)	2(0.94)	1(0.47)	3(3.27)
Swab of bucket	1(0.46)	1(0.47)	1(0.47)	7(1.4)
Total	**43(20)**	**12(5.58)**	**15(6.98)**	**70(32.55)**

Note: X^2= 4.4834, p-value= 0.214

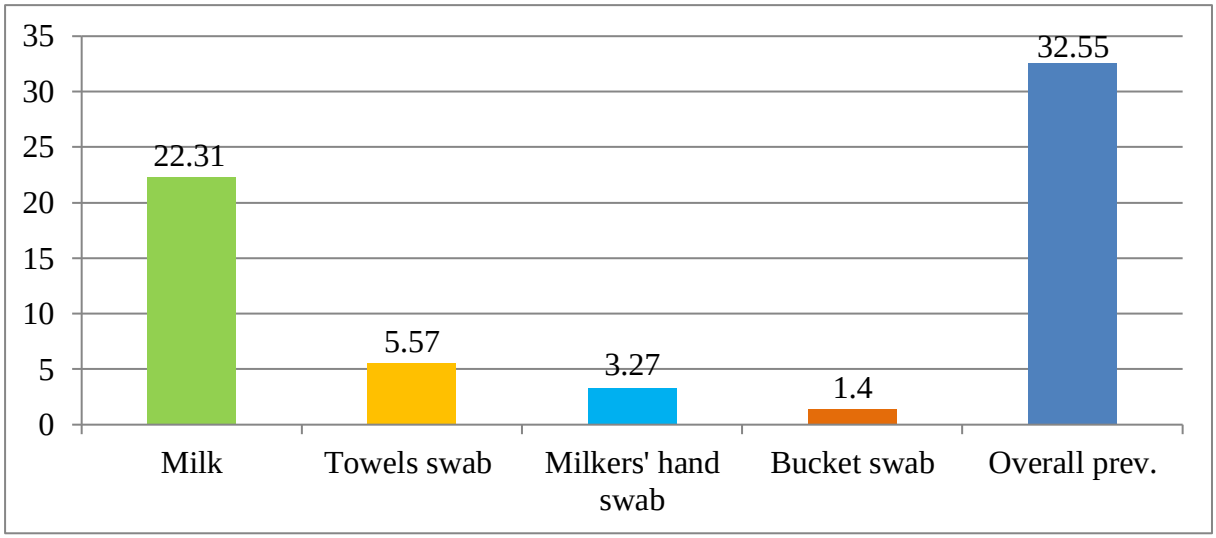

Figure 2: Overall prevalence of *Staphylococcus* species derived from milk, swab towel, swab milkers' hand and bucket swab

Association between Risk Factors and *Staphylococcus* Species Isolation

The chi-square test showed that three variables were found to be insignificantly ($P \geq 0.05$) associated with the isolation of *Staphylococcus* species, those are (lactation, parity and body conditions) but, the prevalence of *Staphylococcus* species were statistically significant variation ($p \leq 0.05$) with respect to type of farm (P= 0.047), age (P=0.003), management system (0.037) and drainage condition (P= 0.010).

Table 4: Association between risk factors and *Staphylococcus* species isolated

Risk factor	Category	Total sample examined	Positive isolate	Prevalence (%)	Chi-square(x^2)	P-value
Type of Farm	Dairy farm	105	41	19.06	3.9361	0.047
	Smallholder farm	110	29	13.48		
Age	Young	32	4	1.86	11.4142	0.003
	Adult	88	25	11.62		
	Old	95	41	19.06		
Parity	Few	109	31	14.41	1.8568	0.395
	Medium	71	27	12.55		
	Many	35	23	10.69		
Lactation	Early	146	45	20.93	5.0638	0.080
	Medium	44	12	5.58		
	Late	25	13	6.05		
Body condition	Poor	41	12	5.58	1.7890	0.409
	Medium	130	40	18.60		
	Good	44	18	8.37		

Management system	Intensive	108	28	13.02	4.3472	0.037
	Semi-intensive	107	42	19.53		
Drainage condition	Poor	26	15	6.97	9.2888	0.010
	Medium	187	55	25.58		
	Good	2	0	0		

DISCUSSION

Overall Prevalence of *Staphylococcus* Species

Cow's milk may be contaminated from different sources and at different processes. Milk can be contaminated by microorganisms directly from the milk handlers who have direct or indirect contact with the milk especially if these persons are in the process of shedding pathogenic organisms and the cow itself, from air/ dust, unclean milk containers and the milk handlers. Pathogens and other organisms can gain access to milk as a result of the milk handlers' activities such as coughing, sneezing, scratching and from body surfaces in contact with milk, particularly the fingers (Getahun & Gebre-Selassie, 2003)

The present study showed that 70*Staphylococci* isolates were detected out of 215 samples collected; 48 (22.31%) originated from raw fresh cows' milk, 12(5.58%) towels swab, 7 (1.4%) swabs of bucket and 3 (3.27%) swabs of milkers' hands. Lencho (2015) reported a prevalence of 30 (12.4%) towels swab and 18 (7.3%) swabs of milkers' hands that agree with the current study and 40.8% *Staphylococcus* from milk, that disagrees with the present study. The variation in prevalence of *Staphylococcus* reported from milking utensils may be usage of disinfectant when cleaning utensils, size of the sample and study methods designed.

The prevalence of *S. aureus* in milk was 14.89%. This finding is similar with the study done by Lencho (2015) who reported 13.9%, Mekuria *et al.* (2013) who reported 15.5 %, 17.2% reported by Gizaw (2014) and Abunna *et al*. (2016) who reported 14.9% *S. aureus* isolates from milk at Asalla town. However, the present findings were lower than that of Bendahou *et al*. (2008) who reported 40% *S. aureus* isolates from milk and milk products at North Morocco. This finding is also in contrast with findings of Jahan *et al*. (2015) who reported 25.5% *S. aureus* from raw milk. In the current study, milk samples were collected directly from cows' udder before contacting milking utensils that might decrease the prevalence of *S. aureus.*

The study revealed that 5.58% *S. intermedius* and 11.7% *S. hyicus* isolates were identified from all samples (out of 215). This result agrees with the study done by Lencho (2015) who reported 6.2% of *S. intermedius* and 11.7% of *S. hyicus* and Gizaw (2014), who reported 7.4% of *S. intermedius* and 8.2% *S. hyicus* from milk and human origin samples. The current finding is also disagreed with study of Bendahou *et al*. (2008) who reported 4 % *S. hyicus* in dairy cows and Mokennen *et al*. (2011) study, who reported the prevalence of 4% *S. hyicus*.

Out of 40 swabs of milking utensils (towels swab and bucket swab) of a dairy farm and smallholder farm, 7 (3.25%) isolates were found to be *S. aureus*. The study conducted by Lencho (2015) reported 9% *S. aureus* isolated from milking equipment (towels swab and bucket swab) was similar with the current study and in similar with Pramar *et al.* (2014), reported high prevalence 18.8% of *S. aureus* which were isolated from swabs of milking equipment of a dairy farm. The dissimilarity of prevalence of *S, aureus* isolation probable the hygienic status of equipment sampled of present the study was better than the previous study.

A total of 4 swabs of from milker hands were collected and 1.86% (4/215) was found to *be S. aureus*. This finding is similar with Pramar *et al*. (2014), who reported 2.55% prevalence of *S. aureus* from swabs of milker's hands and lower than with Lencho (2015) who reported 20% prevalence of *S. aureus* from swabs of milkers' hands. This variation might be due to small number of samples in the current study.

Association between Risk Factors and *Staphylococcus* Species Isolation

When prevalence of *Staphylococcus* species in smallholder and dairy farms is compared there was higher prevalence of the *Staphylococcus* species in dairy farms than in smallholder farms. Even though, there is statistically significant ($p \leq 0.05$) association in smallholder and dairy farms. The probable reason for this could be free movement out of door is not common in dairy farms, kept always in house and failure to use separate towel for individual cows; there could be high chance of contamination of the udder and milk with pathogenic micro-organisms compared to smallholder farms which might have increase the prevalence of *Staphylococcus* species.

The study also revealed that statistically significant association was observed among age categories ($p \leq 0.05$). High prevalence of *Staphylococcus* species recorded in old. This could be due to the fact that old cows are more susceptible to infection than young and adult cows because of weak immune system and they lose their electrolyte by giving large amount of milk production for long time. The present study showed that the prevalence of *Staphylococcus* species showed in lactation stage has no statistically significant variation ($p \geq 0.05$). Additionally, body condition of dairy cow has no statistical association ($p \geq 0.05$). This is due to the fact that *Staphylococcus* species is ubiquitous in nature, with human s and animals as the primary reservoirs. They are present in the nasal passages and throat, in the hair, and on the skin of healthy individuals (Mekonnen, 2015). The present study showed significantly ($p \leq 0.05$) high prevalence of *Staphylococcus* species in semi-intensive than intensive management system. This is due to association with cows which were maintained in dirty and muddy common barns with bedding materials and failure to use separate towel for individual cows; there could be high chance of contamination of the udder and milk with pathogenic microorganisms The present study showed significantly associated with *Staphylococcus* species ($p \leq 0.05$)) with high prevalence in poor than in good drainage condition. This is due to association with poor hygiene of milking area; cows which were milking in dirty, muddy, and sewage full drainage are increase milk contamination and favor the proliferation and transmission of *Staphylococcus* species to udder of cow.

CONCLUSIONS AND RECOMMENDATIONS

The present study showed a higher prevalence of *Staphylococcus* species in dairy farms than in smallholder farms. The result of fresh milk sample, swab samples from the towels, milkers' hands and storage container showed higher prevalence of Staphylococcus species respectively. This indicated possible contamination of milk with *Staphylococcus* species from milkers' hands, towels and bucket. The presence of pathogenic *S. aureus* poses a public health hazard and rise concerns about the safety of these food product.

Based on the above findings of the present study the following recommendations are forwarded.

- Antiseptics and disinfectant should be encouraged after washing hand and cleaning milking utensils.
- The site of the udder placement enables to harbor the microorganisms from the floor and environment, thus cleaning the udder with antiseptics and drying using clean and separate towels for each individual's cow should be preferred.
- Raw milk intended for human consumption must be subjected to pasteurization or heat treatment at least equivalent to pasteurization temperature.
- Further research should be done broadly on smallholder farms and other risk factors responsible for milk contamination in the study area.

REFERENCES

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

Abunna, F., Abriham, T., Gizaw, F., Beyene, T., Feyisa, A., Ayana, D., ... & Duguma, R. (2016). Staphylococcus: isolation, identification and antimicrobial resistance in dairy cattle farms, municipal abattoir and personnel in and around Asella, Ethiopia. *Journal of Veterinary Science & Technology*, *7*(6), 383.

Argaw, S. (2015). A Review on *Staphylococcal* Food Poisoning. *Food Science and Quality Management*, *40*, 58-59.

Ayele, Y., Gutema, F. D., Edao, B. M., Girma, R., Tufa, T. B., Beyene, T. J., ... & Beyi, A. F. (2017). Assessment of Staphylococcus aureus along milk value chain and its public health importance in Sebeta, central Oromia, Ethiopia. *BMC microbiology*, *17*, 141.

Bendahou, A., Lebbadi, M., Ennanei, L., Essadqui, F.Z., & Abid, M. (2008). Characterization of *Staphylococcus* species isolated from raw milk and milk products in North Morocco. *J Infect Developing Count*., *2*, 218-225.

Bennett, R. & Lancette, G. (2001). Bacteriological Analytical Manual, *S. aureus.* http://www.fda.gov/food/foodscienceresearch/laboratorymethods.

Cullor, J. (2004). Risks and prevention of contamination of dairy products. *Rev. Sci. Tech. Off. Int. Epiz*., *16*, 472-481.

De Oliveira, L., Barros, L., Silva, V. & Cirqueira, M. (2011). Study of *S. aureus* in raw and pasteurized milk consumed in the Reconcavo area of the State of Bahia, Brazil. *J Food Process Technol*., *2*, 1-5.

Fagundes, H., Barchesi, L., Nader Filho, A., Ferreira, L. M., & Oliveira, C. A. F. (2010). Occurrence of Staphylococcus aureus in raw milk produced in dairy farms in São Paulo state, Brazil. *Brazilian Journal of Microbiology*, *41*, 376-380.

Fikru, G. (2014). *Epidemiology and Its Drug Resistance in Cattle, Food Chains and Human in Central Ethiopia*. Addis Ababa University, Addis Ababa, Ethiopia.

Getahun, T. & Gebre-Selassie, S. (2003). Assessment of the Bacteriological Quality of Milk at Dairy Farms and Individual Breeders in Jimma Town, South West Ethiopia. *Ethiop J Health Sci.*, *13*, 21-29.

Gizaw, F. (2014). *Staphylococcus: Epidemiology and Its Drug Resistance in Cattle, Food Chains and Human in Central Ethiopia*. Thesis. Addis Ababa University, Ethiopia.

Jahan, M., Rahman, M., Parvej, S., Chowdhury, S. Z. H., Haque, E., Talukder, A. K. & Ahmed, S. (2015). Isolation and characterization of *S. aureus* from raw cow milk in Bangladesh. *J. Adv. Vet. Anim. Res*., *2*, 49-55.

Larsen, H., Sloth, K. & Elsberg, C. (2000). The dynamics of *S. aureus* intramammary infections in nine Danish dairy herds. *Vet Microbiol.*, *71*, 89–101.

Lencho, M. (2015). *Identification and Antimicrobial Susceptibility Profiles of Staphylococcus Species Isolated from Raw Milk, Swabs of Udders, Milking Utensils and Milkers Hands in Small Holder and Dairy Farms in Ambo and Guder town*. Addis Ababa University, Addis Ababa, Ethiopia.

Mekonnen, A. (2015). A Review on *Staphylococcal* Food Poisoning. *Food Science and Quality Management*, ***40***, 1-14.

Mekuria, A., Asrat, D., Woldeamanuel, Y. & Tefera, G. (2013). Identification and antimicrobial susceptibility of S. aureus isolated from milk samples of dairy cows and nasal swabs of farm workers in selected dairy farms around Addis Ababa, Ethiopia. *Afr. J. Microbiol. Res*., *7*, 3501-3510.

Mokennen, M., Pal, M. & Kyule, M. (2011): Isolation and Identification of *Staphylococcus* species from Raw Bovine Milk in DebreZeit, Ethiopia. *Vet. Res*., *4*, 45-49.

Oliver, S.P., Jayarao, B.M. & Almeida, R.A. (2005). Review Foodborne Pathogens in Milk and the Dairy Farm Environment: Food Safety and Public Health Implications. *Foodborne Pathogens and Dis.*, *2*, 115-119.

Pramar, B.C., Dhami, M.N. & Nayak, J.B. (2014), Isolation, Characterization and Antibiotics Sensitivity Pattern of Staphylococcus aureus from Man, Animal and Environment. *Sch J Agric Vet Sci*., *1*, 173-179.

Presscott, L. Harley, J.P. & Klein, D.A. (2002). *Text book of Microbiology* (5th ed.). Brown Publishers, pp. 441–42.

Quinn, P., Carter, M, Markey, B. & Carter, GR. (2002). *Veterinary Microbiology Microbial Diseases- Bacterial causes of Bovine Mastitis* (8th ed.). Mosby international Limited, London, pp. 465-475.

Quinn, P., Markey, B., Donnelly, W., Leonard, F. & Maghire, D. (2005). *Veterinary Microbiology and Microbial Disease*. Blackwell Science, Ltd., London, pp. 468-470

Radostits, O., Gay, C., Hinchcliff, K. & Constable, P. (2007). *Mastitis in Veterinary medicine: Text book of the disease of cattle, horse, sheep, pigs and goats* (10th ed.). Elsevier London, pp. 674-762.

Roberts, D. & Greenwood, M. (2003). *Practical Food Microbiology* (3rd ed.). *Blackwell Publishing*, USA, p. 173.

Sharad, M. Kiran, K. & Ashutosh, D. (2016). Body condition scoring dairy cattle: a review. *Research Reviews: Journal of Veterinary Sciences*, *2*, 1–8.

Thrusfield, M. (2007). *Veterinary Epidemiology* (3rd ed.). Blackwell Science, p. 233.

Zadoks, R. N., Allore, H. G., Barkema, H. W., Sampimon, O. C., Wellenberg, G. J., Gröhn, Y. T., & Schukken, Y. H. (2001). Cow-and quarter-level risk factors for *Streptococcus uberis* and *Staphylococcus aureus* mastitis. *Journal of Dairy Science*, *84*(12), 2649-2663.