

Full Length Research Paper

***Staphylococcus aureus* and other *Staphylococcus* species in milk and milk products from Tigray region, Northern Ethiopia**

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***Staphylococcus aureus* is an important pathogen that can cause Staphylococcal Food Poisoning (SFP). Milk and dairy products are frequently contaminated by this bacterium. In this study, 310 samples (168 bovine raw milk and 142 dairy products) were collected in the Tigray region of Northern Ethiopia, with the objective of detection and enumeration of *S. aureus* and other *Staphylococcus* species. Baird-Parker agar for isolation and 16S rRNA gene sequencing for species identification were employed. *S. aureus* was found in 38.7% of samples with mean count of 4.35 +/- 0.97 log₁₀ CFU ml⁻¹. The prevalence of *S. aureus* was significantly greater in raw milk samples (47%) than in dairy products (28.8%). Of all *S. aureus* positive samples, 34.2% contained ≥5 log₁₀ CFU ml⁻¹. Samples from cafeterias and restaurants showed the greatest prevalence of *S. aureus* (P<0.05) compared to other sampling points. Samples from small-scale dairies were twice more likely to be contaminated by *S. aureus* than traditionally managed dairies (P<0.05, OR=2.0). Coagulase-negative staphylococci (CNS) were found in 51.6% of the samples, with mean count of 6.0±1.21 log₁₀ CFU ml⁻¹. Ten species of CNS were identified and *S. epidermidis* (36.13%) was the most frequent. The frequency of isolation found in this study indicates that *S. aureus* and other *Staphylococcus* spp. may impose public health hazard in dairy products. Therefore, further studies on the enterotoxigenic potential of the isolates, and molecular epidemiology to trace the sources of the contamination are recommended.**

Key words: Coagulase negative staphylococcus (CNS), dairy products, Ethiopia, *Staphylococcus aureus*, raw milk.

INTRODUCTION

Staphylococcal Food Poisoning (SFP) is among the most prevalent causes of gastroenteritis worldwide. In the

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United States, the 2006 Centre for Disease Control (CDC) annual report showed that *Staphylococcus aureus* enterotoxigenesis was ranked third among bacterial food-borne outbreaks (CDC, 2009), while it was ranked as fourth in Europe (European Food Safety Authority, 2010). In China, a retrospective study (1994 to 2005) revealed that *S. aureus* was the second most common food-borne agent in homes (Wang et al., 2007). In developing countries like Ethiopia, there is little available data on the incidence of SFP. However, considering the poor hygiene conditions during production & processing of foods, as well as the shortage of cooling facilities, a high occurrence of SFP is likely. *S. aureus* grows at a wide temperature range between 6 to 48°C with optimum of 35 to 41°C. It tolerates a pH between 4 to 10 with optimum of 6 to 7, a salt concentration of 0 to 20%, and a water activity (a_w) level of 0.83 to 0.99 with optimum at 0.99 (Cretenet et al., 2011).

These growth characteristics enable the bacterium to grow in a wide range of foodstuffs including milk and dairy products (Loir et al., 2003; Meyrand et al., 1998). Although, *S. aureus* is ubiquitous in nature, humans and animals are the primary reservoirs. Around 50% of healthy individuals harbour the bacteria in their nasal passage, throat and skin (Bergdoll and Wong, 2006; Hennekinne et al., 2011) whereas the mastitic cow is a common source of *S. aureus* in raw milk (Kadariya et al., 2014; Loir et al., 2003). *S. aureus* has many potential virulence factors and staphylococcus enterotoxin (SE) is one of among several responsible for food poisoning. Ingestion of less than 1.0 µg enterotoxin causes SFP (Seo and Bohach, 2007). To date, 21 SE and SEI (Staphylococcus Enterotoxin Like: SE that lack emetic properties) have been identified and designated as SEA-SEE (classical), SEG-SEI (new), SEIJ-SEIV (new) (Argudin et al., 2010; Bennett and Hait, 2011; Hennekinne et al., 2011; Ortega et al., 2010). Recent research reported that among *S. aureus* food isolates, 57% up to 72.8% harbour the classical and/or new SE genes (Akineden et al., 2001; Rosec and Gigaud, 2002).

The milk production systems in Ethiopia are classified into rural/traditional, peri-urban and urban production systems (Redda, 2001). The traditional/rural dairy represents the milk production in the mixed and pastoral/agro-pastoral farming systems and accounts for 97% of the total national production. The majority of the milking cows here are the indigenous *zebu* breed. The urban and peri-urban production systems include small and large scale dairy farms with a commercially oriented purpose and use exotic cross breed animals (Ahmed et al., 2003). Like all parts of Africa, traditional milk processing is a common practice in Ethiopia. The naturally sour/fermented milk (Rego) and buttermilk (Auso) are made from raw bulked milk. Butter and Ethiopian cottage cheese (Ajibo) are made by churning of soured milk and heating of sour buttermilk, respectively. The traditional milk preparation activities have been

shown to be unhygienic and consequently expose to microbial contamination (Yilma et al., 2007).

Due to favourable agricultural policy and gradual improvement in living standards of the population, there is an on-going expansion of small-scale dairy farmers around the major cities of Ethiopia. The country showed 3 % increase in annual milk production in the past decade compared to 1.63-1.66 % of the previous two decades. This trend is also predicted to continue as there is great livestock potential and a suitable climatic environment for dairying (Ahmed et al., 2004).

However, if milk safety and quality standards are not in place, the high nutritional composition and neutral pH of milk may convey many food-borne pathogens and thereby constitute a public health challenge to consumers (Angulo et al., 2009). In the country clinical and subclinical mastitis mainly caused by *S. aureus* are a major challenge in the majority of Ethiopian dairy farms (Abera et al., 2010; 2009; Sori, 2011), moreover, a recent survey conducted in central Ethiopia also reported that 31.8% of farmers consume raw milk (Makita et al., 2012) that increased the risk of milk borne contaminations.

Therefore, in order to protect consumers from microbial hazards it is of paramount importance to study and monitor the type and level of pathogenic microbes such as *S. aureus* in the milk value chain. Such surveillance data may provide a basis for risk assessment study as well as give a foundation for the establishment of national milk quality standards that currently do not exist in Ethiopia. In light of this, there is a knowledge gap concerning the occurrence and distribution of *S. aureus* and other *Staphylococcus* species in the milk value chain in the Northern part of Ethiopia. The present study has therefore been designed to fill this gap.

MATERIALS AND METHODS

Project area and origin of samples

The study was conducted in Tigray region, Northern Ethiopia. Seven sampling areas (Mekelle, Shireendasselase, Hageselame, Adigidome, Wukro, Adigrate and Maichew) were selected purposely in order to cover the major cities (with relatively large number of milk and milk product shop or cooperatives), geographical location (south, north, west and east) and agro climatic zones (highland medium and lowland). The spatial distribution of the sampling areas in the region and their metrological data are presented in Figure 1 and Table 1, respectively.

Study design and sampling points

A cross sectional study was conducted from August, 2012 to January, 2013. Samples were collected randomly from small-scale dairy farms, traditionally managed dairies at household level, milk collection centres/cooperatives, milk and milk product shops, market places and cafeteria and restaurants. A free informed consent was obtained from the milk and milk product owners, after explaining the research purpose, potential benefits, risks if any, and

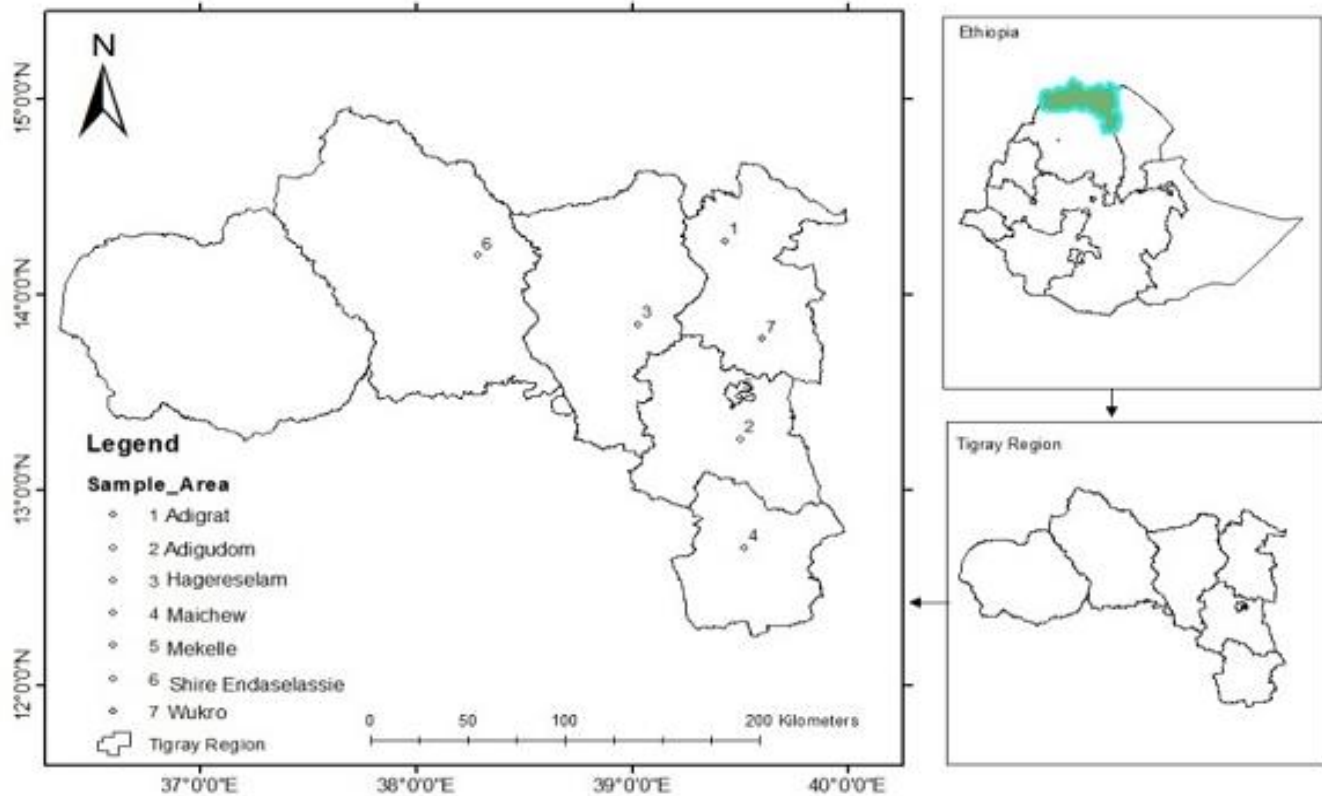


Figure 1. Map of the project area: Spatial distribution of the sampling sites.

Table 1. Metrological data of the sampling areas.

Code	Name	Elevation (m)	Mean annual temperature (°C)
1	Adigrat	2509	15.51
2	Adigudom	2107	-
3	Hagereselam	2663	16.75
4	Maichew	2402	17.11
5	Mekelle	2221	18.32
6	Shire Endasselassie	1732	21.57
7	Wukro	1783	19.6

Source: Ethiopian meteorology agency, 2013.

confidentiality of the research project.

Sampled milk and products

A total of 310 samples (168 bovine raw bulk milk and 142 milk product) were collected. These were: raw bovine bulk milk (n= 168), naturally soured/fermented raw milk (n=51), butter milk (n=44), butter (n= 32), Ethiopian cottage cheese (n=7), Cheese (n= 4) and Cake made from milk (n=4).

Sampling methodology

From each sampling unit, 100 ml of mixed raw bulk milk or 100 g of

milk product was collected in a sterile container and maintained at 4°C.

Microbial analysis

Milk and milk products

From the raw milk samples, tenfold serial dilutions were made. While for the milk products, 11 g of the product was mixed with 99 ml peptone water (Sigma-Aldrich, Switzerland) and blended for 2 min in a stomacher (Stomacher® 400, UK) before further dilutions. The isolation and enumeration of *S. aureus* were performed as per Ahmed and Carolyn (2003) with some modifications. Briefly, 100 µl of each tenfold dilution of the samples was spread with a sterile

bent glass rod into duplicate Baird-Parker agar (Oxoid, England) supplemented with egg yolk tellurite (Merck, Germany). Typical and non-typical colonies were differentially counted after incubation of the plates at 37°C for 48 h. Circular colonies of 2 to 3 mm diameter, jet-black to gray-black in colour and surrounded by an opaque halo and clear zone were considered as a typical *S. aureus* colonies while gray colonies without halos or clear zones were considered as non-typical.

Both typical and non typical colonies were gram-stained and tested for catalase activity (3% H₂O₂, VWR/International). Moreover, to ensure the purity of the colony before further biochemical tests, 3 to 4 colonies from each type of colony were sub-cultured into Brain Heart Infusion (BHI) broth (Oxoid, England) and incubated overnight at 37°C. From the overnight culture, the following tests were performed as per Bennet and Lancette (2001) recommendations: tube coagulase test on rabbit plasma with EDTA (Remel, Lenexa, KS, USA); DNase test on agar with toluidine blue (Sigma-Aldrich, USA), and anaerobic fermentation of 1% mannitol (Sigma-Aldrich, USA).

Pure cultures were preserved with 85% glycerol in cryovials (Sarstedt, Nümbrecht, Germany) and stored at -20°C until they were transported to Norwegian University of Life Sciences (NMBU), Norway for further molecular work. At NMBU, they were stored at -80 until analysis.

Genetic identification

The isolates of *Staphylococcus* species were cultured on Baird Parker agar, supplemented with egg yolk tellurite, followed by sub-culturing of a single colony in BHI to obtain a pure culture.

DNA extraction

From the over-night BHI culture, DNA was extracted by GenElute™ Bacterial Genomic DNA Kit (Sigma-Aldrich, USA) as per the manufacturer's instructions with some minor modifications. In summary, 1 ml of the broth was centrifuged at 16 363 x g for 2 min. The pellet was washed twice with 0.9% aqueous NaCl solution, homogenized with 200 µl of lysis solution and incubated at 37°C for 90 min. The suspension was treated with 20 µl each of RNase and proteinase K, followed by 200 µl of lysis solution C, and incubated at 55°C for 60 min. The lysate was treated with 200 µl of ethanol and transferred into the new binding column and centrifuged at 3968 x g for 1 min. The content was washed twice with washing solution 1 and concentrated wash solution and the eluate was discarded. The column was put into a new 2 ml tube and the DNA was collected with elution buffer solution. The concentration (ng/ µl) and quality (260/280 and 260/230 ratio) of the collected DNA was determined by NanoDrop® - 2000 spectrophotometer (Thermo Fischer Scientific Inc. Waltham MA, USA). The genomic DNA was stored at -20°C until use.

16S rRNA gene sequencing

According to the recommendation by Clarridge III (2004), the final identification of the isolates to species level was performed by sequencing the 16S rRNA gene. The oligonucleotide sequences: Forward: 5'-TCCTACGGGAGGCAGCAG-3' Reverse: 5'-CGGTTACCTTGTTACGACTT-3' primers described by Vebø et al. (2011) were used to amplify the 16S rRNA gene with expected 1200 bp product. The amplification was conducted with a final PCR reaction volume of 50 µl. It contained, 5 µl of 10x Thermopol™ reaction buffer (BioLabs, New England, USA), 0.25 µl Taq Polymerase of 5,000 µ/ml (BioLabs), 1 µl of each primer of 10 pMol con, 1 µl of 10 Mm deoxynucleotide triphosphate mixture (Sigma-

Aldrich, USA) 2 µl of genomic DNA and finally adjusted to final volume of 50 µl with milliQwater. The amplification was carried out in C1000™ Thermal cycler (BIO RAD laboratories) programmed to initial denaturation of 95°C for 1 min, 30 cycles of 95°C for 30 sec., 55°C for 30 sec. 68°C for 80 s and a final extension period at 68°C for 5 min.

After amplification, the expected PCR product (1200 bp) was verified by gel-electrophoresis (Figure 2) and purified by QIAquick® PCR purification kit (QIAGEN®, Oslo, Norway) as per the company's procedures. The purified PCR products were mixed with the same primer and sent to GATC Biotech AG (European Genome and Diagnostic Centre, Konstanz, Germany) for sequencing as per the company's instructions. The sequence results were bioedited (BioEdit version 7.0.0) and compared in GenBank using the nucleotide BLAST algorithm (<http://www.ncbi.nlm.gov/blast>). Maximum identification at species level was considered with result scores of 99 to 100% that appear in the first row.

Statistical analysis

Descriptive statistics (mean, max., min. SD, median) and log-transformed counts was calculated using Microsoft Excel (Windows version 8.1 version). Epi info™ version 7.1.3.10 (CDC, Atlanta, USA) was utilized to analyse rates, confidence intervals (CI) and significant associations between explanatory variables (type of samples, different localities, sampling points) and the contaminations. Difference with P-values <0.05 was considered as statistically significant.

RESULTS

S. aureus in milk and milk products

The overall point prevalence of *S. aureus* in both raw milk and milk products was 38.7% (95% CI 33.4 to 44.2%) with a mean count of $4.35 \pm 0.97 \log_{10}$ CFU ml⁻¹. A significant difference (P< 0.05) in the occurrence of *S. aureus* was observed between raw bulk milk, 47.0%, (95% CI 39.63 - 54.5%) and milk products, 28.9%, (95% CI 22 - 36.8%) (Table 2).

S. aureus in dairy products in different localities

The prevalence of *S. aureus* in products in the different geographical localities of the project area is shown in Figure 3. The highest prevalence (56.38%, 95% CI 46.3 to 65.9%) was observed in Shire area and the lowest (8.69 and 95% CI 2.41 - 26.7%) in Maichew area (P<0.05).

S. aureus at different sampling points in the milk chain

Analysis of the prevalence of *S. aureus* in relation to sampling points in the milk supply chain showed that the prevalence was significantly (P<0.05) higher in samples from cafeterias and restaurants than in samples from other sampling points (Table 3). Odds ratio analysis of two dairy production systems indicated that milk samples



Figure 2. Gel electrophoresis of PCR product of 16 S RNA gene amplified with universal primer (lane 1= 100 bp DNA ladder, lane 2, 4, 5, 6, 7, 8= Amplified PCR product (1200 bp) positive samples, lane 3=negative control).

Table 2. Prevalence and mean count of *S. aureus* in raw bulk milk and milk products.

Type of sample	n	Number of positives	% prevalence (95% CI)	Mean count \pm SD (log ₁₀ CFU ml ⁻¹)
Milk				
Raw bulk milk	168	79	47 (39.6-54.6)	4.34 \pm 0.98
Milk products				
Naturally soured/fermented raw milk	51	13	25.4 (15.6-38.9)	4.02 \pm 1.06
Butter milk	44	14	31.8 (20-46.6)	4.47 \pm 0.94
Butter	32	8	25 (13.3-42.1)	4.89 \pm 0.87
Ethiopian cottage cheese	7	2	28.6 (8.22-64.1))	3.31 \pm 0.43
Cheese	4	2	50 (15-85)	4.46 \pm 0.91
Cakes made from milk	4	2	50 (15-85)	4.32 \pm 1.58
Sub total	142	41	28.9 (22-36.8)	4.34 \pm 0.99
Total	310	120	38.7 (33-44.2)	4.35 \pm 0.97

from small- scale dairies (46.42%) were twice more likely to be contaminated by *S. aureus* than samples from traditional dairies (29.49%) ($P < 0.05$, OR = 2.07).

Classification of *S. aureus* counts

Out of the total 120 *S. aureus* positive samples 41 (34.2%) contained ≥ 5 log₁₀ CFU ml⁻¹ *S. aureus*. Classification

of samples based on their *S. aureus* count is shown Table 4.

Identification of coagulase-negative staphylococcus (CNS) and other microorganisms

Based on the microbiological and 16 S r RNA gene sequence results, out of the total 310 milk and milk product samples, 276 (89%) were found contaminated

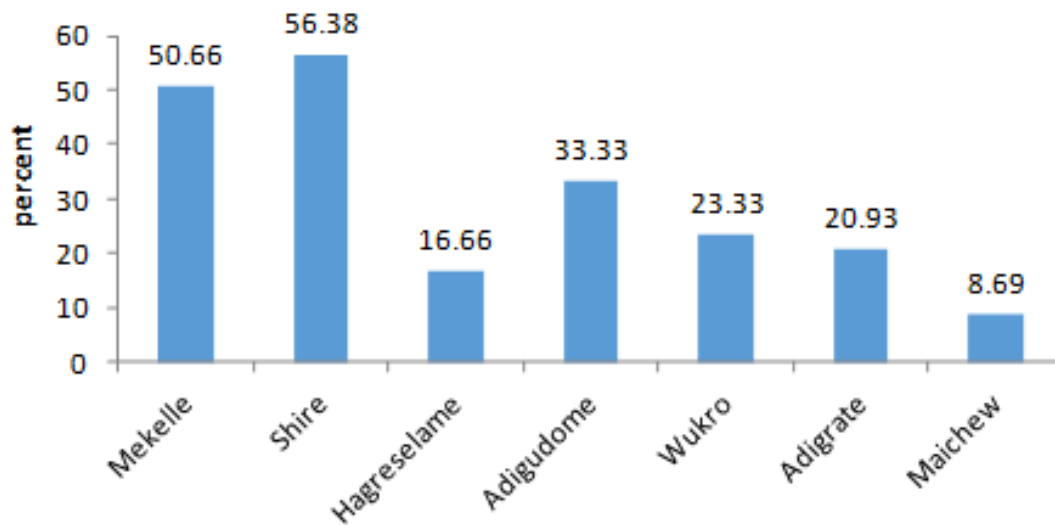


Figure 3. Point prevalence (%) of *S. aureus* in different localities of the project area.

Table 3. Distribution of *S. aureus* at different sampling points in the milk supply chain.

Sampling site	Total sampled	Positive for <i>S. aureus</i>	% (95 % CI)
Small scale dairy farms	56	26	46.4 (34-59.3)
Traditional dairies	139	41	29.5 (22.5-37.6)
Milk and milk product shops, milk collection center/Cooperatives	32	13	40.6 (25.5-57.7)
Cafeterias and Restaurants	83	40	48.2 (37.8 -58.8)
Total	310	120	-

Table 4. Classification of *S. aureus* positive samples based on *S. aureus* count (\log_{10} CFU ml⁻¹).

Sample type	$\leq 3 \log_{10}$ CFU ml ⁻¹	$> 3 - < 5 \log_{10}$ CFU ml ⁻¹	$5 \log_{10}$ CFU ml ⁻¹
Raw milk	-	50	29
Naturally fermented milk	1	9	3
Buttermilk	-	11	3
Butter	-	4	4
Cottage cheese	1	1	-
Cheese	-	1	1
Cakes made from milk	-	1	1
Total	2 (1.67 %)	77 (64.2%)	41 (34.2%)

with one or two types of either *Staphylococcus* species or other microorganisms (*Enterococcus* and *Macroccoccus* species). From the 276 positive samples, 318 isolates were identified. Coagulase-negative staphylococci (CNS) were found in 51.61% of the samples with a mean count of $6.07 \pm 1.21 \log_{10}$ CFU ml⁻¹. Ten different species of CNS were identified and *S. epidermidis* was the dominant species (36.13%). The type of identified isolates and their

respective microbial load is summarized in Table 5.

DISCUSSION

S. aureus is one of the most important food-borne microorganisms, responsible for SFP. Consumption of 100 ng of *Staphylococcus* enterotoxin (SE) produced by

Table 5. Prevalence and microbial count of *Staphylococcus* species and others microorganisms from milk and milk products.

Identified species	Isolates (n)	%	Microbial count Log ₁₀ CFU ml ⁻¹			
			Mean	Max	Min	SD
Coagulase positive staphylococcus (CPS)						
Species						
<i>Staphylococcus aureus</i>	120	38.7	4.35	5.95	3	0.97
Coagulase negative staphylococcus (CNS)						
Species						
<i>Staphylococcus epidermidis</i>	112	36.13	6	8.81	3	1.26
<i>Staphylococcus cohnii</i>	16	5.16	5.6	7.8	4.07	1.01
<i>Staphylococcus haemolyticus</i>	10	3.22	6.78	8.61	5.72	0.85
<i>Staphylococcus sciuri</i>	6	1.94	6.99	7.81	6.07	0.82
<i>Staphylococcus warneri</i>	5	1.61	6.28	7.81	5.07	0.94
<i>Staphylococcus hominis</i>	5	1.61	6.27	7.81	5	1.1
<i>Staphylococcus succinus</i>	2	0.65	5.48	5.65	5.32	0.17
<i>Staphylococcus carnosus</i>	2	0.32	6.17	6.81	5.54	0.63
<i>Staphylococcus caprae</i>	1	0.32	7.81	-	-	-
<i>Staphylococcus devriesei</i>	1	0.32	6.81	-	-	-
Sub-total	160	51.61	6.07	8.81	3	1.21
Other microorganisms						
<i>Enterococcus faecalis</i> and other <i>Enterococcus</i> species	36	11.61	6.0	7.81	4.5	0.99
<i>Macrocococcus caseolticus</i>	2	0.65	6.49	6.81	6.17	0.32
Sub-total	38	12.26	6.04	7.81	4.57	0.98
Total isolates	318	-	5.41	8.81	3	1.38

enterotoxigenic strains causes SFP (Hennekinne et al., 2011; Seo and Bohach, 2007). This amount of toxin is produced when the enterotoxigenic *S. aureus* population is greater than 5 log₁₀ CFU ml⁻¹ (Food and Drug Administration, 2012; IESR, 2001). In the present study, 34.4% of the positive samples contained >5 log₁₀ CFU ml⁻¹ of *S. aureus* which implies that there was high probability for production of SE. Once SE is produced, it will remain structurally stable and biologically active as it is thermo-stable, and also resistant to low pH, freezing, and to the action of different enzymes. Normal pasteurization does not denature the toxin (Bergdoll and Wong, 2006; Hennekinne et al., 2011; Loir et al., 2003).

S. aureus could multiply in wide range of growth limiting determinants such as temperature, aw, NaCl and gaseous atmosphere (Cretenet et al., 2011; Kadariya et al., 2014) and hence samples that contained < 5 log₁₀ CFU ml⁻¹ of *S. aureus* could also impose a public health hazard. An increase in time or temperature before consumption could lead to further proliferation of the pathogen and the production of toxins by enterotoxigenic strains if any.

The point prevalence of *S. aureus* in the Shire area is statistically higher (P < 0.5) than the Maichew area. This

difference may be attributed to differences in the milk hygiene situation during production, transportation, milk processing or storage. In addition to this situation, the relatively higher annual mean temperature of Shire (21.6°C) may encourage greater growth of the pathogens than Maichew, where the temperature is cooler (17.3°C). In agreement with this suggestion, Luca et al. (1997) reported that *S. aureus* is more prevalent during the warmest months of the year suggesting the impact of temperature on the prevalence of the pathogen.

This study shows that *S. aureus* was statistically more prevalent (P < 0.05) in raw bulk milk than in the milk products. This may be related to the fact that during the traditional milk product preparation such as natural sour milk, butter milk and Ethiopian cottage cheese, the milk usually undergoes a natural spontaneous fermentation and/or is exposed to heat treatment, usually in the range of 50°C to 100°C for 50 to 70 min (Yilma et al., 2007), which may kill the *S. aureus*. Fermentation process may also reduce the growth of *S. aureus* as it competes poorly with other microbes like lactic acid bacteria. Whenever there are competing microorganisms in the milk, the growth, and SE production of *S. aureus* will be decreased as a result of low pH and presence of H₂O₂ and possibly

other antimicrobial substances (Hennekinne et al., 2011; Loir et al., 2003).

The prevalence of *S. aureus* was significantly higher in cafeteria and restaurant samples than from other sampling points. This situation may occur because the initial *S. aureus* load from the farm may multiply during transportation, as cold chain facilities are not available in all sampling points, or more *S. aureus*, from human sources, may contaminate the milk because of poor personal and/or equipment hygiene during the value chain. Although a molecular epidemiological study is required to verify the source, the obtained results indicates that there is need to improving food handlers and equipment hygiene as well as application of cold chain facilities in the milk value chain in order to protect the consumer from milk-borne hazards.

The higher prevalence of *S. aureus* in small-scale dairy farms compared to the traditionally managed dairy (P<0.05) may be associated with the type of milking cows available in the respective farms. In the traditional dairy, the local indigenous *zebu* breed is usual, whereas in small-scale dairy farms, cross-bred animals are the dominant milking cows. *S. aureus* is the major causative agent of sub-clinical mastitis in dairy cows (Akineden et al., 2011; Petersson-Wolfe et al., 2010), and consequently a major source of raw milk contamination (Kadariya et al., 2014). However, the susceptibility of the dairy cows to mastitis varies between breeds. Pure dairy cows are 6.3 times and crossbred cows are 3.1 times more likely to be exposed to mastitis than the local *zebu* breed (Katsande et al., 2013). Therefore, this genetic difference may be a further reason for the difference in the occurrence of *S. aureus* in the two dairying systems.

S. aureus is one of the most important food-borne pathogens in Africa as well as in other parts of world. In Zimbabwe, a prevalence of 75.8% with mean count of 5.4 log₁₀ CFU ml⁻¹ in raw milk and 40% with mean count of 4.7 log₁₀ CFU ml⁻¹ in processed milk was reported (Mhone et al., 2011), which is higher than this study. Poor hygienic practices during production and /or processing may give rise to this situation. In Nigeria, *S. aureus* was reported in the range of 25 to 45% in locally produced fermented milk, *Nono* (Nnadi, 2006) while in Pretoria, South Africa, 40% of the milk samples collected from milk shops were found to be contaminated with *S. aureus* (O'Ferrall-Berndt, 2003). Milk collected from large and small-scale dairy farms in Kenya for a multidrug resistance study reported a 30.6% prevalence of *S. aureus* (Shitandi and Sternesj, 2004). The aforementioned similar prevalence of *S. aureus* in milk and milk products indicates that *S. aureus* is one the most important public health concerns and warrants further attention in the improvement of food safety in Africa.

In Europe, Asia and USA, *S. aureus* has become one of the most important milk-borne pathogens. *S. aureus* was reported at a prevalence of 62% at dairy farms in

Minnesota, USA, (Haran et al., 2011) and at prevalence of 26% from milk-producing herds in Ireland (Murphy et al., 2010). In Italy, 43% of raw milk intended for Caprino cheese making (Foschino et al., 2002) and 68% of raw milk from the Reconcavo area, Brazil (Oliverira et al., 2011) was positive for *S. aureus*. In India, a prevalence ranging from 61.7 to 65.6% was reported in raw cow's milk (Lingathurai and Vellathurai, 2010; Singh et al., 2010). Similar to this study, all the above surveys showed the importance of *S. aureus* in the milk supply chain of the respective areas.

In this study, the presence of 51.61% CNS with a mean count of 6.07±1.21 log₁₀ CFU ml⁻¹ in the samples indicated that the hygienic condition of the milk and milk products, during production, transportation, processing and storage was poor and needs attention for improvements. In a similar research conducted in Sudan by Suliman and Mohamed (2010), out of 644 raw milk samples, 44.7% were positive for CNS, which is lower than that of this study, however, similar to this study finding, the dominant species was *S. epidermidis*. In addition to the above hygiene-related implications, the finding of high percentage of CNS in the milk and milk product may have some risk associated with Staphylococcal Food Poisoning. Recent research outputs reported the enterotoxinogenic potential of CNS (Guimarães et al., 2013; Loir et al., 2003; Madhusoodanan et al., 2011; Park et al., 2011; Vera et al., 2010) and the involvement of CNS in SFP. Podkowik et al. (2013), confirmed the presence of localized elements in the genome of some CNS which are involved in the coding and production of SE. A food-borne disease outbreak caused by *S. epidermidis* was also reported in USA (Breckinridge and Bergdoll, 1971). These facts necessitate further screening of the CNS for enterotoxinogenic potential.

Conclusions

The study showed that *Staphylococcus* spp. in general, and *S. aureus* in particular, are common in milk and milk products in the study area and may impose a public health hazard. From a food safety point of view, food handlers at different points in the milk value chain should be educated on how to reduce contamination of milk and milk products from staphylococci species and other pathogens through personal and equipment hygiene as well as through provision of cold-chain equipment in the milk supply chain. On the other hand, farmers should also be educated on methods of reducing raw milk contamination from the environment and from the cow (mastitis) itself. Governmental regulatory bodies should also propose a standard for microbial limits for milk and milk products that reach the consumer and the milk processing plants. Further study on the enterotoxinogenic potential of *S. aureus* and the other coagulase-negative

staphylococcus (CNS) to assess the risk of SFP should be undertaken. Molecular epidemiological studies aimed to trace the source of *S. aureus* in milk and milk products will be of paramount importance in the control strategy.

Conflict of Interests

The authors have not declared any conflict of interests.

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