Microbial Quality and Safety of Raw Cow Milk in Girar Jarso District of Oromia Regional State, Ethiopia

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ABSTRACT

The study aimed to evaluate the quality and safety of raw cow milk collected from dairy producers and collection centers in urban and peri urban areas of Girar Jarso district. A total of 60 milk samples (40 from milk producers and 20 from milk collectors) were collected for physico-chemical, and microbial quality and safety analysis. The analyses were made at Dairy Technology and Microbiology Laboratory of Holetta Agricultural Research Center. There were significant differences (P<0.05) in temperature (29.75±0.52 and 22.35±0.52°C), pH (6.69±0.02 and 6.55±0.02), specific gravity (1.026±0.002 and 1.023±0.002) and fat content (4.02±0.10 and 3.75±0.14%) between producers and collection centers milk samples, respectively. The mean total aerobic mesophilic bacterial count (TAMBC), coliform count (CC) and spore forming bacterial count (SFBC) for milk samples collected from producers were 6.42±0.07, 4.49±0.09 and 2.59±0.05 log10 cfu/ml, respectively. However, significantly higher (P<0.05) bacterial counts were observed for milk samples taken from milk collectors (7.49 log10 cfu/ml) than that of producer milk samples (6.42 log10 cfu/ml). Out of a total milk samples collected from producers, 57, 7.5 and 15% were positive for Staphylococcus aureus, Salmonella spp. and Listeria monocytogenes, respectively. The microbial quality of milk produced and marketed in the study areas was found to be substandard and could cause public health risks for raw milk consumers. This calls for establishment and enforcement of quality and safety control systems for both milk producers and collectors in order to improve the quality and safety of milk.

Keyword: raw milk, microbial quality, physico-chemical, safety.

INTRODUCTION

Milk and milk products are ideal media for multiplication of various microorganisms if it is not properly handled (Soomro *et al*., 2002). Most of the bacteria in fresh milk from a healthy animal are either harmless or beneficial. The health statuses of an animal or the milk handler, or contaminants from polluted water, dirt, manure vermin, cuts and wound can make raw milk potentially dangerous (Zelalem Yilma, 2012). The major determinant that influences the quality and safety of dairy products is the quality of raw milk. Therefore, milk should have normal composition, be free from adulteration and must be produced under hygienic conditions (Chamberlain, 1990).

The microbial contamination of milk not only reduces its nutritional quality but also may threaten the health of the consumers. Microorganisms may contaminate milk at various stages including production, procurement, processing and distribution. It is assumed that differences in feeding and housing strategies of cows may influence the microbial quality of milk (Coorevits *et al*., 2008). In addition to health of animal, cleanness of animal, milking practices, milk handling, and equipments used may affect quality and safety of milk. Bacteria in raw milk can affect the quality, safety, and consumer acceptance of dairy products. Several human microbial pathogens could contaminate milk and render it unsafe for public consumption. These include pathogenic *Escherichia coli*, *Staphylococcus aureus*,

Salmonella spp., *Listeria monocytogenes, Brucella abortus, Mycobacterium* spp., *Campylobacter* spp., *Leptospira* spp., *Clostridium* spp., *Pseudomonas aeruginosa* and *Proteus* spp. (Jayarao *et al*., 2006).

There is a steady challenge to those involved in milk production to prevent or minimize the entry and subsequent growth of microorganisms in milk (O'Connor, 1995). Therefore, an understanding of the microbial load of raw milk is needed to measure its hygienic quality. High microbial load and presence of harmful pathogenic microorganisms in the milk samples are evidences of unhygienic milk production conditions (Abrahamsen *et al*., 2007).

Although milk and milk products represent an important place in the nutrition of consumers as well as nutrition and income of producers, there is limited work so far undertaken regarding physico-chemical and microbiological quality of raw milk in Girar Jarso district. Moreover, there is no formal quality control system in place to monitor and control the quality and safety of milk produced and sold in the district. Therefore, studying the quality and safety of cow milk in the study area has paramount importance. The results generated will be used to design appropriate intervention strategies for the policy makers to improve the production, quality and safety of cow milk. This is mainly important for producing milk of good hygienic quality, which is necessary to produce milk products with superior quality and prolonged shelf-life and thereby provide safe and wholesome food for the consumers. Therefore, the objective of this study was to evaluate the physico-chemical properties, microbiological quality and safety of raw cow milk produced and marketed in Girar Jarso District.

MATERIALS AND METHODS

Description of the Study Area

The study was conducted in urban and peri urban areas of Girar Jarso District that is found in North Shewa Zone of Oromia Regional State. The district is located at a distance of 112 km from Addis Ababa. Geographically it lies at 09°45’121’’N latitude and 038°46’728’’E longitude and at an altitude of 2677 meters above sea level. The area receives mean maximum and minimum temperature of 22.13°C and 10.26°C, respectively and average long-term annual rainfall of 1000 mm (WFEDO, 2010). The livestock sub-sector is an integral component in the farming practice of the study area. This sub-sector contributes to the livelihood of the population in terms of milk and milk products. High proportion of households in the district (80%) is selling raw milk (WFEDO, 2010).

Milk Sampling Techniques

Milk samples were collected from milk producers and collectors. One of the lots was placed in a separate sterile container for microbiological analysis while the second one was used for determination of the physico-chemical properties. All laboratory analyses were conducted in duplicates.

A total of 60 samples of morning raw cow milk (250mL) were randomly collected from dairy farmers milk containers at farm gate and milk collection centers from bulk milk. Milk samples were collected aseptically using sterile bottles and immediately kept in an ice box and transported to Holetta Agricultural Research Center, Dairy Technology and Microbiology Laboratory for analysis. The milk samples were kept in refrigerator at 4°C upon arrival.

Physico-Chemical Analysis of Raw Milk

Physicochemical parameters such as Temperature, pH, Titratable acidity, Total solids (TS), Ash, Solids not fat (SNF) and protein contents were determined following the procedures described by O'Connor (1995). Temperature of the milk sample was determined by a thermometer and pH was measured by digital pH meter after calibration using buffer solutions (4 and 7). While titratable acidity of the milk samples was determined by measuring the amount of lactic acid produced per ml of NaOH. Ten ml of milk sample was pipetted into a beaker and 3 to 5 drops of 0.5% phenolphthalein indicator was added. Then milk sample was titrated by 0.1N NaOH until a pink color persisted. The titratable acidity of the milk was expressed as percent of lactic acid (O'Connor, 1995).

$$\%lactic\ acid = \frac{ml\ NaOH(0.009)}{ml\ milk\ sample\ used} x100$$

For determination of specific gravity, fresh milk sample was filled sufficiently into a glass cylinder (100ml capacity) then a Lactometer was gently inserted into the milk. The lactometer was allowed to freely float until it reached equilibrium. Then the readings were recorded at the lower meniscus. The following formula was used to calculate the specific gravity of the milk.

$$Specificgravity = \frac{L}{1000} + 1$$

Where: L = corrected lactometer reading at a given temperature.
If the temperature of the milk is between 17 and 24°C, the following correction factors were used to determine L:

Temperature °C	17	18	19	20	21	22	23	24
Correction	–0.7	–0.5	–0.3	0	+0.3	+0.5	+0.8	+1.1

To determine total solid (TS) of milk three grams of milk sample was placed into pre–dried and weighed crucible. The sample was dried at 100 $\pm$ 2°C in a hot air oven (PBSO42, England) for two hours. Finally, the dried milk samples were taken out from the oven and placed in desiccators to cool at ambient temperature. Then it was weighed again and TS content was expressed as a percentage (O'Connor, 1995).

$$Total\ solids\ (\%) = \frac{weight\ of\ dried\ sample}{weight\ of\ sample} X\ 100$$

The ash content of the cow milk sample was determined gravimetrically. The dried milk sample used for the determination of total solids content was ignited in a muffle furnace at 540 -550°C for five hours. The milk sample was burnt until black color disappeared or the ash residue become white. Finally, the ash content was calculated. The Gerber method was used to determine milk fat content. Ten ml of sulfuric acid was dispensed into a Gerber butyrometer. Then, 11ml of milk sample and one ml of amyl alcohol were added into the butyrometer having sulfuric acid, respectively. Then the content was shaken and the butyrometer was placed in a water bath at 65ºC for five minutes. The sample was centrifuged for five minutes at 1100rpm. Finally, the sample was placed in water bath with a temperature of about 65ºC for 5 minutes and fat percentage was recorded from the butyrometer reading (O' Connor, 1995). Solids-not-fat (SNF %) content were determined by subtracting the percentage of fat from the total solids (O' Connor, 1995).

% SNF = % TS – % Fat

Total protein content of the milk samples was determined by the formaldehyde (formal) titration (O' Connor, 1995). Saturated aqueous potassium oxalate (0.4ml) was dispensed and 0.5ml of 0.5% phenolphthalein solution was added to 10ml of milk sample. The sample was allowed to stand for two minutes and titrated with N/9 NaOH until a pink color was obtained. Two ml of neutral 40% formalin which was discharge the pink color added and continue the titration with N/9 NaOH until a pink color was obtained. The number of ml of the NaOH used after the addition of the formalin multiplied by 1.74 gives the percentage protein in the milk.

Microbiological Analysis

The microbiological analysis was done through enumeration of total coliform count (TCC), total aerobic mesophilic bacterial count (TAMBC) and spore-forming bacterial count (SFBC) and isolation and identification of microbial pathogens such as *Salmonella spp., staphylococcus aureus* and *Listeria monocytogenes.*

For TAMBC determination, 1ml of milk sample was diluted in 9ml sterile peptone water (Oxoid, CM0009) and serial dilutions (tenfold) were made in sterile peptone water diluents until the expected level of count of 30-300 was obtained. One ml of the milk sample from a chosen dilution was placed on the sterile plate. Then, plate count agar media (Oxoid, CM0325) of 15-20ml was poured on to the plate and thoroughly mixed with the sample and allowed to solidify for 15 minutes. Then the plates were incubated for 48 $\pm$2hrs at 35°C in an inverted position. Finally, colonies were counted manually using colony counter (FDA, 2003).

Total colony count was determined using sterile violet red bile agar (VRBA) (Oxoid, CM0107). One mL of raw milk sample was added into a sterile test tube containing 9 mL of sterile peptone water (Oxoid, CM0009). After thoroughly mixing, the sample was serially diluted up to 10^{-9} and duplicate samples (each with 1mL) were pour plated using sterile 15-20mL VRBA. After gently mixing, the resulting plates were allowed to solidify and then incubated at 32 ± 1^{o}C for 24hrs (Murphy, 1996). Following incubation, typical dark red or purplish red or pink colonies appearing on the plates, measuring 0.5mm or more in diameter on un-crowded plates and with bile precipitation around them were counted as coliforms (FDA, 2003). For SFBC determination, milk samples were first heat treated in a water bath (Chifton, UK) at 80^{o}C for 10 minutes. Appropriate dilutions of the milk samples (1ml) were plated on duplicate solid plate count agar (Oxoid, CM0325) media. Then, colonies were counted after 3 days of incubation at 30^{o}C (Roberts and Greenwood, 2003).

Isolation and identification of selected pathogenic bacteria

Three pathogenic bacterial species were isolated to measure safety of milk samples in the study area. Isolation and identification of *Salmonella* spp involved three steps based on ISO-6579, 2000. First, 25ml of milk sample was pre-enriched with 225ml of buffered peptone water (BPW) (Oxoid, CM509) and incubated for 24hrs at 37°C. A portion (0.1ml) of the pre-enriched cultured was transferred to10 ml of Rappaport Vassilidis (RVs) broth (Oxoid, CM0866) and incubated at 42°C for 24hrs. Finally, a loop full of a culture broth was streaked on the surface of a dry Xylose Lysine Deoxycholate (XLD) (HIMEDIA, M031) agar plate. Then the plates were incubated at 37°C for 24hrs and the incubation was prolonged to 48 hrs for those that did not show any growth during the 24hrs of incubation. Characteristic *Salmonella* spp. colonies, having a slightly transparent zone of reddish color and a black center, were sub-cultured on nutrient agar (Oxoid, CM0003). To confirm the presence of

Salmonella spp. further tests were made including gram staining and biochemical tests such as Triple Sugar Iron (TSI), Voges-Proskauer (VP), Methyl red, glucose fermentation and Citrate utilization tests (ISO, 2003).

For isolation and characterization of *Staphylococcus aureus*, 1ml of milk sample was transferred to a test tube containing 9ml of normal peptone water. This was again serially diluted until an appropriate dilution was obtained. After properly shaken the homogenate, 0.1 ml of the appropriate dilution was spread-plated on the surface of a dry Baird Parker Agar (Oxoid, CM0275) plate using a sterile bent glass rod and incubated at 37°C for 24 - 36hrs. White yellow colonies surrounded by clear haloes were considered as colonies belonging to *Staphylococcus aureus*. Further confirmation of *Staphylococcus aureus* colonies was made using gram staining and biochemical tests such as methyl red test, catalase test, coagulase test, mannitol fermentation, oxidase test and VP tests (FDA, 2003).

For isolation and characterization of *Listeria monocytogenes*, a portion of 25ml of milk sample was pre-enriched in 225ml of the BPW (Oxoid, CM 0509) at 32°C for 24hrs. Then, 0.1ml of pre-enriched sample was inoculated into 10ml Listeria enrichment broth part A (HIMEDIA, M569) and Listeria enrichment broth part B (HIMEDIA, M569) and incubated at 37°C for 24hrs. A loop full of the enriched culture broth was taken and streaked on PALCAM agar. (HIMEDIA, M1064) and incubated for 48hrs at 37°C. Colonies that were gray-green with black precipitate were considered as *Listeria monocytogenes*. Further confirmation of the identity of suspected colonies of *Listeria monocytogenes* was made using gram staining and biochemical tests such as TSI, VP, Methyl red and Citrate tests (FDA, 2003).

Data Analysis

Physicochemical and microbial analyses data were analyzed using analysis of variance (ANOVA), SAS procedure, version 9.0 (SAS, 2009). Tukey's Studentized Range (HSD) test was employed to detect mean differences between sample sources. The numbers of microorganisms (colony forming units) per milliliter of milk samples were expressed using the following mathematical formula (FDA, 2003):

$$N = \frac{\Sigma C}{(n1*2)+(0.1*n2)} *_d$$

Where,

N = Number of colony-forming units per ml of milk

$\sum C$ = Sum of all colonies counted on plates

n_1 = Number of plates in the first dilution counted

n_2 = Number of plates in the second dilution counted

d = Dilution factor of lowest dilution used

Microbial count data were first transformed into logarithmic values ($\log_{10}$) before statistical analysis. The $\log_{10}$ transformed values were analyzed using the General Linear Model (GLM) of SAS software.

$Y_{ij} = \mu + L_{i+} C_j + e_{ij}$

Where

Y_{ij} = the dependent variables

μ =overall mean,

L_i = location effect (peri-urban and urban) and

C_j= collection site effect (producers and collection center)

e_{ij} = random error

RESULTS

Physicochemical Properties of Raw Milk

The temperature of milk samples collected from urban producers (29.5^0C ± 0.61) was significantly (P<0.0001) higher than urban collectors (19.9^0C ± 0.86) (Table 1). The temperature of milk from peri urban producers (30^0C ± 0.86) were also significantly higher (P<0.0001) than peri urban collectors (24.8 ±0.6^0C). The mean value of temperature for milk sampled from producers and collectors of the study area were also significantly different.

The overall mean pH value of milk collected from the producer and collector in the study areas was 6.69±0.02 and 6.55±0.02, respectively (Table 1). The pH of milk from peri-urban producer was significantly (P<0.01) higher than that of the urban milk producer. Similarly, the pH of milk sample collected from urban collector was significantly (P< 0.05) lower than the milk sample collected from urban producer. The titratable acidity of milk samples in the current study was found to be significantly (P<0.05) lower for milk samples collected from peri urban producers (Table 1). The average titratable acidity/percent of lactic acid of milk from producer and collector falls almost in the range of fresh milk pH that is 0.14 to 0.16%. The specific gravity of milk samples in the current study were significantly (P<0.001) different among the two locations (Table 1). The specific gravity of milk samples collected from the two sites did not lie within the normal range (1.027-1.035) of the specific gravity of milk.

Table 1: Physical properties (means ± SE) of raw cow milk produced and marketed in the study area

Parameters	Milk sources	Location		Overall mean
		Urban	Peri urban	
Temp (^{0}C)	Producers	29.50 ±0.61[a]	30.00 ±0.86[a]	30.00 ±0.86
	Collection center	19.90 ±0.86[c]	24.80 ±0.61[b]	22.35±0.52
pH	Producers	6.62±0.03 [b]	6.76±0.03 [a]	6.69±0.02
	Collection	6.49±0.04 [b]	6.62±0.04 [b]	6.55±0.02
Acidity	Producers	0.17±0.004[a]	0.15±0.004[b]	0.16±0.003
	Collection center	0.17±0.006 [a]	0.18±0.006[a]	0.175±0.003
Specific gravity.	Producers	1.024±0.002 [b]	1.026±0.002 [a]	1.026±0.002
	Collection center	1.023±0.003[b]	1.026±0.003[a]	1.023±0.002

Means in all columns and rows bearing different superscripts for the same parameter are significantly different (P<0.05). Temp. = Temperature.

Chemical composition of raw cow milk in the study areas

The mean value of fat content of milk samples from the urban producer and urban collector 3.69±0.14 and 3.57±0.20%, respectively showed significant deference (P<0.001). The overall mean fat content of milk samples from urban producer was significantly (P<0.0001) higher than milk sample from peri urban producer of the study area (Table 2). The total solids (TS) content of milk samples obtained in the current study showed highly significant differences (P<0.001) among locations (Table 2). Likewise, the TS content of milk samples collected from peri-urban and urban producers were significantly different. The mean value of Protein, SNF and Ash content of milk samples obtained in the current study did not show significant differences (P<0.05) between locations and collection centers.

Table 2: Chemical composition (mean ± SE) of raw cow milk produced and sold in study area

Parameters (%)	Milk sources	Location		Overall mean
		Urban	Peri urban	
Fat	Producers	3.69 ±0.14^{c}	4.34 ±0.14^{a}	4.02±0.10
	Collection center	3.57 ±0.20^{d}	3.94 ±0.20^{b}	3.75±0.14
Protein	Producers	3.44±0.10	3.51±0.11	3.47±0.07
	Collection center	3.25±0.76	3.26 ±0.76	3.26±0.05
TS	Producers	11.73±0.39^{b}	13.51±0.39^{a}	12.62±0.28
	Collection center	10.96±0.56^{b}	12.25±0.56^{b}	11.60±0.39
SNF	Producers	8.04±0.33	9.16±0.33	8.60±0.23
	Collection center	7.49±0.48	8.31±0.48	7.90±0.33
Ash	Producers	0.66±0.02	0.67±0.02	0.66±0.01
	Collection center	0.72±0.03	0.67±0.03	0.69±0.02

Means in all columns and rows bearing with different superscripts for the same parameter are significantly different (P<0.05). TS= Total Solid SNF= Solid Not fat.

Microbial Quality of Raw Cow Milk in the Study area

The mean value of aerobic mesophilic bacterial count (AMBC) of raw milk samples collected at producers was significantly (P<0.05) lower than that of the milk collectors (Table 3). However, significantly (P<0.05) lower bacterial counts of raw milk in both sampling sources were observed in urban areas of the district. The difference in the overall mean AMBC observed in the study area might be associated with the difference in hygienic practices during milking, milk storage condition, and the cleanliness of milk utensils.

In the current study, the TCC of raw milk sampled from collection site (7.05±0.10) were significantly (P<0.001) higher than the TCC of milk sampled from producers. The spore forming bacteria count (SFBC) from milk samples of peri urban collector was significantly (P<0.0001) higher than that of urban collectors with the mean values of 4.13±0.10 log10cfu/ml and 3.27±0.10 log10cfu/ml, respectively (Table 3). Similarly, the SFBC in peri urban producer was significantly (P<0.05) higher than the urban producer with 2.77±0.10 log cfu/ml and 2.42±0.74 log10 cfu/ml, respectively.

Table 3: Total microbial counts (log cfu/ml) in raw cow milk produced and marketed in the study area

Parameters	Milk Sampling sources	Location		Overall mean
		Urban	Peri urban	
AMBC	Producers	6.22 ±0.10^{c}	6.62 ±0.13^{b}	6.42±0.07
	Collection center	6.99 ±0.15^{b}	7.99 ±0.15^{a}	7.49±0.10
TCC	Producers	3.87 ±0.13^{d}	5.10±0.13^{c}	4.49±0.09
	Collection center	6.96±0.18 ^{b}	7.13±0.18 ^{a}	7.05±0.10
SFBC	Producers	2.42±0.74^{d}	2.77±0.10^{c}	2.95±0.05
	Collection center	3.27±0.10^{b}	4.13±0.10^{a}	3.70±0.07

Means in all columns and rows bearing with different superscripts for the same parameter are significantly different at P<0.05. AMBC = Aerobic Mesophilic Bacteria Count; TCC= Total Coliform Count; SFBC = Spore Forming Bacteria Count.

Safety of raw cow milk in the study area

Staphylococcus aureus is one of the major pathogens that affect the quality and safety of raw milk in the study area. It is a major causative pathogen of clinical and subclinical mastitis. The results of the current

study showed that 55 - 70% of the milk samples taken from producers and milk collectors were contaminated by *Staphylococcus aureus*. The mean *Staphylococcus aureus* count of the milk samples sampled from producers was 3.44±0.18 log10cfu/ml, while 5.64±0.26 log_{10} cfu/ml was found for milk collectors (Table 4).

Table 4. **Staphylococcus aureus** ***count (mean ± SE) (log CFU/ml) in raw cow milk produced and marketed in the study areas***

Parameters	**Location**	**Milk Sampling sources**	
		Producers	**Collection center**
Staphylococcus aureus count	Urban	2.79 ±0.26^{c}	5.48±0.36^{b}
	Peri-urban	5.48±0.26^{b}	5.79 ±0.36^{a}
	Overall mean	3.44±0.18	5.64±0.26

Means in all columns and rows bearing with different superscripts for the same parameter are significantly different at P<0.05.

As observed in the present study, the proportion of positive *Salmonella* spp. was higher for collection centers than the producers, with the minimum value observed at urban producers (5%) and the maximum was recorded for peri urban milk collectors (50%). The higher prevalence of *Salmonella* spp. in the current study might be related to poor hygienic handling practices of raw milk (Table 4). Prevalence of *Listeria monocytogenes* was high in all milk sampling sites. Out of the total 60 milk samples considered from different sources, about 25% were found to be contaminated with *Listeria Monocytogenes*. The highest prevalence rate of about 50% was recorded for peri urban milk collection site of the study districts. In the present study the frequency of detection of *Salmonella* spp., *Listeria monocytogenes* and *staphylococcus aureus* were higher in milk collection centers.

Table 5: Prevalence of **Staphylococcus aureus*****, Salmonella spp. and*** **Listeria monocytogenes** ***in raw cow milk produced and marketed in the study areas***

Parameters	Sampling sources	Location		
		Urban	Peri urban	Overall means
Staphylococcus aureus (%)	Producers	55	60	57.5
	Collection center	60	70	65
Salmonella spp. (%)	Producers	5	10	7.5
	Collection center	30	40	35
Listeria monocytogenes (%)	Producers	10	20	15
	Collection center	40	50	45

DISCUSSION

Physicochemical Properties of Raw Milk

The mean value of temperature for milk sampled from producers and collectors of the study area were significantly different that might be because of temperature difference of the environment, equipment

used and time elapsed since production. This result is in agreement with the finding of Almaz Kehase (2014) who reported milk temperature of 30.61±0.52 and 21.05± 0.42^0C from dairy farms and milk vendor, respectively in Mekelle.

The pH of milk from peri-urban producer and collectors was significantly different from that of the urban milk producer but were in the range of pH of cow's fresh milk which varies between 6.6 and 6.8 (Van den Berg 1988). This value is in agreement with the value recorded by Srairi *et al*. (2005) in Morocco (6.74 ± 0.14). However, Abebe Bereda *et al*. (2012) reported a lower pH value of 6.15 for milk in Ezha district of the Gurage zone. The low pH of milk in the collection site as compared to the pH values of milk from farms gate might be due to the proliferation of acid producing bacteria during transportation of milk samples.

The average titratable acidity of milk from producer and collector (0.16±0.003 and 0.17±0.003 %, respectively) were almost in the range of fresh milk acidity. Asaminew and Eyassu Seifu (2011) reported higher titratable acidity for milk samples collected from individual farmers with (0.23 ±0.01%) and dairy cooperatives (0.28±0.01% lactic acid) in Bahir Dar District. Normal fresh milk should have an apparent acidity of 0.14 to 0.16 % and the percentage of acid present in milk is a rough indication of its age or freshness and the manner in which it has been handled (O'Connor, 1995). The specific gravity of milk samples collected from the four sites was slightly below the normal range (1.027-1.035) of the specific gravity of milk suggesting that these milk samples might have been adulterated with water.

Chemical composition of raw cow milk in the study areas

The overall mean fat content of milk samples from urban producer was significantly higher than that of milk sample from peri urban producer of the study area which could be attributed to variation in breeds of animal, feeds, stage of lactation and health, physiological status of animal. The average TS content in this study is lower than the average result of 13.4% TS obtained from similar study conducted in Shashemene on milk from dairy cooperative collection centers, small scale milk producers, kiosks and hotels (Teshome Gemechu *et al.*, 2015).

Microbial Quality of Raw Cow Milk in the Study area

There were significantly ($P<0.05$) lower AMBC of raw milk in both urban and peri urban producers sampling sources. The difference in the overall mean AMBC observed in the study area might be associated with the difference in hygienic practices during milking, milk storage condition, and the cleanliness of milk utensils. According to Ethiopian standard (ES, 2009), the overall AMBC recorded in the present study was higher than 6 log10 cfu/ml and put the microbial quality of milk as bad microbial quality due to high bacterial load.

Earlier study by Alganesh Tola (2016), showed similar total AMBC of 6.97±0.35, 7.11±0.33 7.92±0.35log10 cfu/ml from Selale, Debre Birhan and Ejere milk producers, respectively. Similarly, Haile Welearegay et al. (2012) reported an average AMB count of 7.28 log10cfu/ml for milk samples collected from different farm sizes in Hawassa, southern Ethiopia. The result of the present study is not in agreement with the findings of Zelalem Yilma (2010), Haile Welearegay et al. (2012) and Teklemichael Tesfaye (2012) who reported a total bacterial count of 9.10 log10 cfu/ml for milk samples collected from different parts of Ethiopia; 10.28 log10 cfu/ml from distribution containers (at selling point) and 9.137 log10cfu/ml from vendors, respectively. According to ES (2009), good quality milk should not contain a

total bacterial count of more than 5 log10 cfu/ml and the result of the current study revealed higher bacterial counts which call for measures to reduce bacterial load to meet standard set by Quality and Standards Authority of Ethiopia (QSAE).

The overall mean total coliform count (TCC) observed in this study at producers (4.49±0.09) was lower than the result of Teshome Gemechu *et al*. (2014) who reported an average TCC of 4.99±0.081log cfu/ml for milk marketed in Shashemene town. The current result is also lower than that of Amistu Kuma *et al* (2015) and Asaminew Tassew and Eyassu Seifu (2011) who reported TCC of 5.42±1.735 to 5.78±0.985 log10cfu/ml and 4.84 log10cfu/ml in milk samples of special zone of Oromia and Bahir Dar milk shed, respectively. However, the current study showed higher TCC than the finding of Abebe Bereda *et al.* (2012) who report 4.18 ± 0.01 log10 cfu/ml for raw milk samples in the Ezha districts of the Gurage Zone. In the current study, the TCC of raw milk sampled from collection site (7.05±0.10) were significantly (P<0.001) higher than the TCC of milk sampled from producers. According to Ethiopian standard (2009) the TCC of good quality raw milk should not exceed 3 log10 cfu/ml. The presence of high TCC in milk could be attributed to unsanitary conditions of milk production, processing and storage conditions in the study area. Moreover, their presence in large number in dairy products is also an indicator of potential hazard to the consumer's health due to possible presence of other enteric pathogens (Godefay Bekele and Molla Bayileyegn, 2000).

The spore forming bacteria count (SFBC) from milk samples of peri urban producers and collector was significantly higher than that of urban producers and collectors These results are higher than the finding of Mulugojjam Adugna *et al.* (2013) (2.1 log 10cfu/ml) for camel milk in Eastern Ethiopia and lower than the finding of Teshome Gemechu *et al.* (2014) who reported SFBC of 4.703 ± 0.069 log10 cfu/ml in milk samples collected from Shashemene town. The ubiquitous nature of aerobic spore-forming bacteria leads to numerous points of potential entry into raw milk. Soiling of the udder and teats is considered as one of the most important factors in the contamination of raw milk by spores. High levels of spores in feed may also lead to large quantities of spores in the feces, which in turn can contaminate the udder and teats of lactating cows (Almaz Kehase, 2014).

Safety of raw cow milk in the study area

Staphylococcus aureus is one of the major pathogens that affect the quality and safety of raw milk in the study area. It is a major causative pathogen of clinical and subclinical mastitis. The results of the current study showed that 55 - 70% of the milk samples taken from producers and milk collectors were contaminated by *Staphylococcus aureus*. The mean *Staphylococcus aureus* count of the milk samples collected from producers was 3.44±0.18 log10cfu/ml, while 5.64±0.26 $\log_{10}$ cfu/ml was found in milk samples taken from milk collectors (Table 4). Similarly, Almaz Kehase (2014) found about 46 - 60% *Staphylococcus aureu*s positive milk samples in Mekele dairy farms and vendors. The same author also stated that *Staphylococcus aureus* is often found in raw milk and dairy products due to contamination caused by poor hygienic conditions or from mastitic cows. *Staphylococcus aureus* is a common cause of mastitis in dairy cattle and can enter the milk supply from sores on the teats of cows or from the hands and nasal discharges of dairy farmers and workers (Aberra Aseffa, 2010).

As observed in the present study, the proportion of positive *Salmonella* spp. was higher for collection centers than the producers, with the minimum value observed in samples from urban producers (5%) and the maximum was recorded for peri urban milk collectors (50%). The higher prevalence of *Salmonella* spp. in the current study might be related to poor hygienic handling practices of raw milk.

The consumption of raw cow milk produced and marketed in the study areas may pose public health hazards unless strict control measures taken. Hailemariam Mekonnen *et al.* (2006) and Mulugojjam Adugna *et al.* (2013) reported the high prevalence of *Salmonella* (33 - 83%) in milk samples collected from small, medium and large-scale dairy farms in Ethiopia and 79.17% prevalence in camel milk samples from eastern Ethiopia. The water which was used for cleaning of milking equipment could be one possible source of pathogenic bacteria and responsible for the contamination of milk and its products. The difference in prevalence of *Salmonella* may be due to differences in hygienic practices employed by the producers and collectors (Almaz Kehase, 2014).

Listeria monocytogenes showed high prevalence in the current study areas with prevalence rate of 25% and the highest prevalence rate was about 50% for peri urban milk collectors. Eyasu Tigabu et al. (2015) found 18.9% prevalence rate of *L. monocytogenes* in raw milk and milk products produced in urban and peri-urban areas of central Ethiopia. The current high prevalence rate might be due to the feeding of *Listeria* contaminated feed during grazing which may have direct contact to the soil, due to contaminated udders, and milking equipment, and animals with listeria mastitis. Since these pathogenic bacteria are present in raw milk, it is a major public health concern, especially for those individuals who frequently drink raw milk (Lang Halter et al., 2013).

In the present study the frequency of detection of *Salmonella* spp., *Listeria monocytogenes* and *staphylococcus aureus* were higher in milk collection centers. This could be attributed to poor post-harvest hygienic handling practices of milk by the collectors. Matofari *et al*. (2007) suggested that contamination of camel milk by pathogen was influenced by post-harvest handling of the milk rather than camel infection by the pathogen.

CONCLUSIONS

The microbial quality of milk produced and marketed in the study area was found to be substandard which could be attributed to poor hygienic milk handling practices and absence of cooling system along the milk market chain. In addition, the presences of pathogenic microorganisms mainly *Staphylococcus aureus, Listeria monocytogenes* and Salmonella species are potential cause of public health hazards. Therefore, awareness needs to be created through trainings among dairy cow owners and workers on the importance of hygienic milking techniques, and use of clean dairy equipment, washing of utensils and milkers hands using properly treated water plus detergents to improve the milk hygienic quality. Moreover, milk collection centers should be equipped with cold chain and the necessary milk handling equipment to avoid/minimize milk contamination. It is also necessary to establish and enforce quality and safety control systems in order to produce and market wholesome milk and dairy products.

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