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Microbiological quality and safety of milk production and marketing in Hawassa district, Ethiopia

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The microbiological quality and safety of milk samples from different sources in Hawassa distinct from southern nations, nationalities and people regional state was evaluated. A total of 63 raw milk samples were obtained from three selected dairy farms, urban and rural households. Twenty-seven pasteurized milk samples were obtained from three retail brands from various supermarkets in Hawassa city. Each milk sample was collected in triplicate monthly over three months. Total bacterial count (TBC), coliform counts (CC), total staphylococci counts (TSC), yeast and mould counts (YMC) were isolated and identified by morphological and biochemical tests following the standard methods. Household milk samples had a higher TBC (7.32 log CFU/ml) than dairy farm milk samples (6.83 log CFU/ml) and pauperized milk samples (6.75 log CFU/ml). Similarly, household milk samples had significantly higher Coliform load compared to dairy farms and pasteurized milk samples. Total staphylococci counts (TSC) and YMC significantly vary between sources. Household milk samples had the highest TSC and YMC count while pasteurized milk samples had the least TSC and YMC count. Twelve bacterial genera were identified from each milk sample from all sources. However, the degree of occurrences of each genus varies between milk sources. While the isolation rate of *Enterobacter*, *Escherchia,* and *Shigella* species of raw milk samples from the households was significantly higher than in milk samples from dairy farms, the percentage of positive milk samples for *Proteus* species, coagulase negative *Staphylococcus* and coagulase postive *Staphylococcus* was higher in dairy farm milk samples than in milk sample from households. The present study has shown that the quality of milk produced in the area and the retail brands of pasteurized milk sold in various supermarkets in the area had poor microbiological quality and are unsafe for consumption. Hence, adequate sanitary measures should be taken at all stages from production to consumption to keep the safety of the consumers particularly children.

Key words: Coliform count, dairy farms, milk, total bacteria count, total staphylococci count, yeast, mould count.

INTRODUCTION

Milk is a single most completed food produced naturally. It is a complex mixture of fat, protein, carbohydrates, minerals, vitamins, and other miscellaneous constituents dispersed in water, making it a complete diet (Haug et al., 2007). The nature of milk and its chemical composition renders it one of the ideal culture media for microbial

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growth and multiplication (Woldemariam and Asres, 2017). The safety of dairy products with respect to food-borne diseases is a great concern around the world. This is especially true in developing countries where production of milk and various dairy products take place under rather unsanitary conditions and poor production practices (Abebe et al., 2014).

With more than 54 million heads, Ethiopia has the largest livestock population in Africa and holds great potential for dairy development (Leta and Mesele, 2014). On the other hand, the traditional milk production system, which is dominated by indigenous breeds of low genetic potential for milk production, accounts for about 98% of the country's total annual milk production which concerns the microbiological quality and safety of the product (Hiwot et al., 2016).

Due to the highly perishable nature of milk and mishandling, the amount produced is subjected to high post-harvest losses. Losses of up to 40% have been reported in Ethiopia for milk and dairy products from milking to consumption. Microbial spoilage is found to be one of the causes that offer the losses (Felleke, 2003; Amentie et al., 2016). Such losses are mainly attributed to the employment of hygienic practices at the time of milking, long storage time at high ambient temperature, adulteration of milk, transportation equipment, and distribution systems, which lead to losses in spoilage due to bacterial contamination and due to its intrinsic factors (Felleke, 2003).

The microbial load of milk is a significant factor in determining its quality and safety. It indicates the hygienic level exercised during milking including cleanliness of the milking utensils, storage condition, and transportation as well as the cleanliness of the udder of the individual animal (Yilma and Faye, 2006). Milk may contain few microorganisms when it leaves the udder but it may be contaminated at various stages from the cow, milker (manual as well as automated), extraneous contaminants or use of unclean water for cleaning the under as well as the milking equipment (Hayes et al., 2001). Milk produced under hygienic conditions from healthy animals should not contain more than 5 Log CFU/mL (Prejit et al., 2007).

In Ethiopia, in most cases, milk is produced and marketed without quality control measures (Yilma and Faye, 2006). Information on the microbial and chemical properties of marketed raw and pasteurized milk is not available though it is essential to understand the overall quality of the products being marketed and consumed. Therefore, the objectives of this study were to assess the bacteriological quality and safety of milk from production to marketing in the study area.

MATERIALS AND METHODS

Study site and sample size

The study was conducted in and around Hawassa, the capital city of the Southern Nations Nationalities and Peoples Regional State (SNNPRS) of Ethiopia. Three dairy farms were selected from 12 dairy farms randomly as suggested by Ike (2002). For household milk sampling, milk producing households were selected from 4 urban kebeles and from 2 surrounding rural kebeles. Three different pasteurized milk brands commonly available in Hawassa city supermarkets and dairy shops were selected. Sample size of ninety was collected from all groups: 27 milk samples from dairy farms, 36 samples from households and 27 samples from retail brands. Each milk sample was collected in triplicates every month for a total of 3 months.

Milk sample collection

About 200 ml triplicate samples of raw whole morning milk were collected from each group every month for three months for microbiological quality analysis following the standard methods as described by Marth and Steele (2001). Whole milk was sampled within 1 h after milking from the farmer's milking containers and the bulk milk of dairy farms at the farm gate. A total of 300 ml plastic packed samples of branded pasteurized milk samples were also purchased in triplicate from supermarkets and dairy shops. All samples were kept in an icebox and transported to Hawassa University, Dairy Science Laboratory.

Microbial analysis method

Microbial analyses were carried out to investigate the microbial quality and safety of milk samples from different sources in the study area using standard methods. Microbial enumeration, isolation, and identification were performed for total bacterial count (TBC), coliform count (CC), total staphylococci counts (TSC) and yeast and mold count (YMC). To determine TBC and CC, peptone water and total plate count agar (both from Oxoid) were sterilized by autoclaving at 121°C for 15 min. The violet red bile agar (VRBA: Oxoid) used for determination of CC was sterilized by boiling (Ruegg and Reinemann, 2002).

Total bacterial count (TBC)

One milliliter of milk from each sample was mixed with 9 mL of 0.1% peptone water (a standard maximum recovery diluent, Oxoid) and homogenized by shaking. Then each sample was serially diluted up to $1:10^{-7}$ in duplicates. One milliliter of each duplicate was pour plated using 15 to 20 ml standard plate count agar (Oxoid, UK) and mixed thoroughly. The plated sample was allowed to solidify and then incubated at 30 ± 2°C for 48 h (Laird et al., 2004). Dilutions with the total number of colony count between 30 and 300 per plate were selected and colonies were counted using digital colony counter (Marth and Steele, 2001). Then TBC was expressed as the number of the organism of colony forming units per ml (CFU/mL) of samples according to ISO (1999).

CC and *Escherichia coli* identification

One milliliter of milk from each sample was mixed with 9 mL of 0.1% peptone water (a standard maximum recovery diluent, Oxoid) and homogenized by shaking. Each sample was serially diluted up to $1: 10^{-5}$ and duplicate samples (1 ml) were pour-plated using 15 to 20 ml Violet Red Bile Agar (VRBA) at room temperature (Pharma, US). After thoroughly mixing, the plated sample was allowed to solidify and then incubated at 30°C for 24 to 48 h. Colonies were counted as previously described. Typical dark red colonies on uncrowned plates were considered as coliform colonies. For the

confirmatory test, four to five typical colonies from each plate were transferred into tubes containing 2% Brilliant Green Lactose Bile Broth (BGLB; Oxoid, UK). Gas production within 48 h of incubation at 35°C was considered as sufficient evidence for the presence of coliforms (Marth and Steele, 2001). Samples which gave positive results for coliforms test, further identification was done using an Indole, Methyl red, Voges Proskauer, and Citrate (IMVIC; Oxoid, England). Catalase and sugar tests as described for isolation and identification of *E. coli* (Salman and Hamad, 2011).

Total staphylococci counts

For direct plate count analysis of raw milk samples, 1 ml aliquots from each group was plated onto mannitol salt agar (MSA; Oxoid, UK) plates with appropriate dilutions. Each plate was spread by bent glass rod and incubated at 37°C for 24 ± 2 h. Following the incubation period, the positions of typical colonies were marked on the bottom of the plates. Plates were re-incubated for further 24 ± 2 h at 37°C, new typical colonies and atypical colonies were marked. Plates containing a maximum of 300 colonies with 150 typical and/or atypical colonies at three successive dilutions were taken for enumeration. One of the plates should contain at least 15 typical colonies characterized by golden yellow, smooth, circular, convex, and moist to be considered as total staphylococci colonies. Colonies were counted as previously described. One to two typical and atypical suspect colonies were transferred from each MSA (Oxoid, England) plate into nutrient broth (NB; CDH, India) tubes and incubated at 35°C for 48 h for the isolation and identification of catalase positive and negative staphylococci. Following the incubation period, a loop full of NB were streaked on the nutrient agar (Oxoid, England) plates and incubated at 35°C for 48 h. The pure isolate colonies were subjected to Gram staining and catalase test for confirmation (Altuntas, 2015). Biochemical and sugar test was carried out following the standard of manufacturing instructions.

Isolation and identification of *Salmonella*

A portion of 1 ml of milk was pre-enriched in 9 ml of lactose broth at 37°C for 24 h. Then, 1 ml of the pre-enrichment sample was inoculated into 10 ml cystine selenite broth (Oxoid, England) and incubated at 37°C for 24 h. A loop full of selective enrichments were streaked on Xylose-lysine decarboxylate (XLD; HiMedia, India) and *Salmonella-Shigella* agar (SSA; HiMedia, India) and incubated at 37°C for 24 h. All suspected non-lactose fermenting *Salmonella* colonies were picked from all plate agars and streaked onto a nutrient agar plate and then incubated at 37°C for 24 h. From each agar plate pure isolate single colonies were picked and inoculated into biochemical tubes for biochemical tests which include triple sugar iron (TSI) agar (Oxoid, England), ornithine decarboxylate broth (CDH, India), Simmon's citrate agar (Oxoid, England), H_2S, indole and motility (SIM test; CDH, India), and urea broth (Oxoid, England). Then the tubes were kept in an incubator for 24 or 48 h at 37°C followed by identification of typical test for *Salmonella* as previously described (Richter et al., 2000).

Yeast and mould count (YMC)

Milk samples were diluted following similar methods as for TBC but dilutions were spread plated on yeast and malt extract chloramphenicol agar which consists of 5 g yeast extract, 20 g glucose, 0.1 g chloramphenicol, 0.01 g bromophenol blue, and 15 g agar per liter of distilled water at a pH of 6.0 and potato dextrose agar (PDA). The dried plates were then incubated at 25°C for 3 to 5 days. Colonies with a blue-green color were counted as yeasts and moulds (Lavoie et al., 2012).

Statistical analysis

The number of microorganisms (colony forming units) per ml of milk was calculated using the following formula (International Dairy Federation, 1991).

$$\text{Count} = S_k/n_1 + 0.1n_2 \text{ x } d$$

Where, S_k = sum of all colonies counted (between 30 and 300), n_1 = number of the plate from the lowest dilution used for computing the count, n_2 = number of plates in the next dilution factor used for computing the count, d = reciprocal of the dilution factor of the lowest dilution used for computing the A count corresponding to n_1.

Recorded laboratory result and data were entered into Excel spreadsheet. TBC, CC, TSC and YMC were logarithmically transformed, and the results were analyzed using General Linear Model (GLM) procedure of SAS (2008) for least square means. Tukey's Studentized Range (HSD) tests method was used for test significant difference between sources of samples. For nonparametric data, the median was used for significance test. A statistical significance level of 0.05 was used to test the difference between groups.

RESULTS

Bacteriological quality of milk from dairy farms

TBC was statistically significantly different between the dairy farms ($p<0.05$). The mean TBC for SOS, Hawassa University and Beteseb dairy farms was 6.22 ± 0.16, 6.82 ± 0.16 and 7.46 ± 0.16 log CFU/mL, respectively (Table 1). SOS dairy farm had the lowest coliform count (5.54 ± 0.19 log CFU/mL) as compared to Hawassa University (7.10 ± 0.19 log CFU/mL) and Beteseb (7.23 ± 0.19 log CFU/mL) dairy farms ($p<0.05$). On the other hand, SOS and Hawassa University dairy farms had similar mean TSC as well as YMC, while the TSC and YMC from Beteseb dairy farms where significantly higher ($p<0.05$) than SOS and Hawassa university dairy farms (Table 1).

Milk samples from urban and rural households had similar bacteriological quality

Although there are some variations within households, milk samples collected from urban and rural households had similar bacterial counts (Table 2). Milk samples from urban households had 7.11 ± 0.19, 7.07 ± 0.23, 7.51 ± 0.14 and 7.01 ± 0. 21 log CFU/mL for TBC, CC, TSC and YMC respectively. Similarly, milk samples collected from rural households were found to contain 7.52 ± 0.19, 7.40 ± 0.23, 7.45 ± 0.14, 7.42 ± 0.21 Log CFU/mL for TBC, CC, TSC and YMC, respectively (Table 2). The overall mean of TBC, CC, TSC and YMC was 7.32 ± 0.19, 7.24 ± 0.23, 7.48 ± 0.14 and 7.21 ± 0.21 Log CFU/mL, respectively for samples from both urban and rural

Table 1. Microbial count of dairy farms in the study area.

Sample source	N	Quality indicator parameter, Mean ± SE (Log CFU/mL)			
		TBC	CC	TSC	YMC
SOS dairy farm	9	6.22 ± 0.16^{a}	5.54 ± 0.19^{b}	6.76 ± 0.28^{b}	6.64 ± 0.32^{c}
Hawassa University dairy farm	9	6.82 ± 0.16^{b}	7.10 ± 0.19^{a}	6.94 ± 0.28^{b}	6.93 ± 0.32^{c}
Beteseb dairy farm	9	7.46 ± 0.16^{c}	7.23 ± 0.19^{a}	7.66 ± 0.28^{a}	7.33 ± 0.32^{b}
Total	27	6.83 ± 0.16	6.63 ± 0.19	7.12 ± 0.28	6.96 ± 0.32

Column mean value with different superscript letters for each milk quality parameters are significantly different (p<0.05). SE: Standard error of mean; N: number of observation; TBC: total bacteria count; CC: coliform count; TSC: ttal staphylococci count; YMC: yeast and mould count.

Table 2. Microbial count of milk samples from urban and rural households in the study area.

Household	Milk sample Source Kebeles	N	Quality indicator parameter, Mean ± SE (Log CFU/mL)			
			TBC	CC	TSC	YMC
Urban	Hyk Dar kebele	9	6.98 ± 0.19^{a}	7.31 ± 0.23^{a}	7.66 ± 0.14^{b}	7.29 ± 0. 21^{a}
	Daka kebele	9	7.24 ± 0.19^{ab}	6.84 ± 0.23^{b}	7.36 ± 0.14^{b}	6.73 ± 0.21^{b}
	Sub-total	18	7.11 ± 0.19*	7.07 ± 0.23*	7.51 ± 0.14*	7.01 ± 0. 21*
Rural	Bushulo Kebele	9	7.54 ±0.19^{a}	7.43 ± 0.23^{a}	7.48 ± 0.14^{a}	7.46 ± 0.21^{a}
	Odahe kebele	9	7.51 ± 0.19^{a}	7.38 ± 0.23^{a}	7.42 ± 0.14^{a}	7.38 ± 0.21^{b}
	Sub-total	18	7.52 ± 0.19*	7.40 ± 0.23*	7.45 ± 0.14*	7.42 ± 0.21*
Total		36	7.32 ± 0.19	7.24 ± 0.23	7.48 ± 0.14	7.21 ± 0.21

Column mean value with different superscript letters or symbols for each milk quality parameters are significantly different (p<0.05). SE: Standard error of mean; N: number of observation; TBC: total bacteria count; CC: coliform count; TSC: total staphylococci count, YMC: yeast and mould count.

Table 3. Microbial count of retail Brands from the supermarkets in the study area.

Source of pasteurized milk	N	Quality indicator parameter, Mean ± SE (Log CFU/mL)			
		TBC	CC	TSC	YMC
Almi	9	7.50 ± 0.23^{a}	6.20 ± 0.19^{b}	7.21 ± 0.27^{a}	7.13 ± 0.24^{a}
Mama	9	7.24 ± 0.23^{ab}	6.05 ± 0.19^{ab}	7.15 ± 0.27^{a}	6.81 ± 0.24^{ab}
Shola	9	6.53 ± 0.23^{b}	5.38 ± 0.19^{a}	6.31 ± 0.27^{b}	6.10 ± 0.24^{a}
mean	27	6.75 ± 0.23	5.87 ± 0.19	6.89 ± 0.27	6.68 ± 0.24

Column mean value with different superscript letters for each milk quality parameters are significantly different (p<0.05). SE: Standard error of mean; N: number of observation; TBC: total bacteria count; CC: coliform count; TSC: total staphylococci count, YMC: yeast and mould count.

kebeles (households) of the study area (Table 2).

Almi pasteurized milk had higher bacterial counts

The bacteriological quality of three pasteurized milk retail brands (*Almi, Shola,* and *Mama*) was compared in the study area. *Almi* pasteurized milk had considerably higher TBC counts (7.50 ± 0.23 Log CFU/mL) as compared to milk samples from *Mama* (7.24 ± 0.23 log CFU/mL) and *Shola* (6.53 ± 0.23 log CFU/mL) and the difference was statistically significantly different (P < 0.05). *Almi* pasteurized milk had higher CC count (6.20 ± 0.19 log CFU/mL) than *Mama* (6.05 ± 0.19 log CFU/mL) and *Shola* (5.38 ± 0.19 log CFU/mL) pasteurized milk samples (P < 0.05). similarly, *Almi* pasteurized milk samples had higher TSC and YMC counts as compared to pasteurized milk samples from *Mama* and *Shola* brands (P < 0.05) (Table 3).

Microbial load of milk samples from different sources

The microbial load of milk samples obtained from

Table 4. Bacterial quality of milk samples from different sources.

Source of milk	N	Quality indicator parameter, Mean ± SE (Log CFU/mL)			
		TBC	CC	TSC	YMC
Retail brands	27	6.75 ± 0.23[a]	5.87 ± 0.19 [a]	6.89 ± 0.27 [a]	6.68 ± 0.24 [a]
Dairy farms	27	6.83 ± 0.16 [a]	6.63 ± 0.19 [a]	7.12 ± 0.28[ab]	6.96 ± 0.32[ab]
Households	36	7.32 ± 0.19[b]	7.24 ± 0.23[b]	7.48 ± 0.14[b]	7.21 ± 0.21[b]

Column mean value with different superscript letters for each milk quality parameters are significantly different (p<0.05). SE: Standard error of mean; N: number of observation; TBC: total bacteria count; CC: coliform count; TSC: total staphylococci count, YMC: yeast and mould count.

Table 5. Common bacterial isolates from different sources of milk samples in the study area.

Isolated bacterial species	Sources of samples		
	Dairy farms (N=27) n (%)	Households (N=36) n (%)	Supermarket (N=27) n (%)
Citrobacter genera	4 (14.8)[a]	5 (13.9)[a]	2 (7.4)[b]
Enterobacter genera	6 (22.2)[a]	9 (25.0)[a]	10 (37.0)[b]
Escherichia coli	6 (22.2)[a]	11 (30.6)[b]	5 (18.5)[a]
Klebsiella genera	5 (18.5)[a]	7 (19.4)[a]	4 (14.8)[a]
Proteus genera	6 (22.2)[b]	4 (11.1)[a]	3 (8.3)[a]
Pseudomonas aeroginosa	4 (14.8)[b]	3 (8.3)[a]	2 (7.4)[a]
Salmonella genera	6 (22.2)[a]	7 (19.4)[a]	3 (8.3)[b]
Shigella genera	6 (22.2)[a]	9 (25.0)[a]	9 (33.3)[b]
Staphylococcus (cogulase (-))	5 (18.5)[b]	4 (11.1)[a]	4 (14.8)[a]
Staphylococcus (cogulase (+))	6 (22.2)[a]	7 (19.4)[a]	6 (22.2)[a]
Listeria monocytogens	2 (7.4)[a]	2 (5.6)[a]	1 (2.8)[b]
Yersina enterocolitica	2 (7.4)[a]	4 (11.1)[b]	2 (7.4)[a]

Raw percentage value with different superscript letters for each milk source is significantly different (p<0.05). Statistical test: nonparametric test.

different sources (farms, households and retail brands) were compared. Milk samples from households had the highest TBC counts (7.32 ± 0.19 log CFU/mL) as compared to milk samples from dairy farms and retail brands. Similarly, household milk samples contained the highest CC and TSC counts as compared to milk samples from other sources. Milk samples from retail brands and dairy farms had similar TBC, CC and TSC count. On the other hand, the YMC was significantly different between all groups and the lowest count was obtained in milk samples from retail brands (Table 4).

Total bacterial load of pasteurized milk on its shelf-life

The bacterial growth we investigated during the shelf-life of the milk which is supposed to be 21 days. It was found that in all retail milk brands, total bacterial count significantly increased from day 7 to day 27 (Figure 1). The bacterial growth significantly increased from day 7 to day 21 in milk samples from Almi retail brand sd compared to the other two.

Bacterial isolates from milk samples

Twelve bacterial genera were identified from all milk sources by biochemical tests. The percentage of positive samples for *E. coli* and *Yersina enterocolitica* test was higher in household milk samples than milk samples from dairy farms and retail brands. On the other hand, milk samples from dairy farms had a higher percentage of *Proteus genera, Staphylococcus* (coagulase negative) and *Pseudomonas aeroginosa* than milk samples from retail brands and households. Surprisingly, milk samples from retail brands had a significantly higher percentage of *Shigella* and *Enterobacter* genera than the other two milk sample sources. However, retail brand milk samples had a lower percentage of *Salmonella* and *Listeria monocytogens* as compared to the other two sources (Table 5).

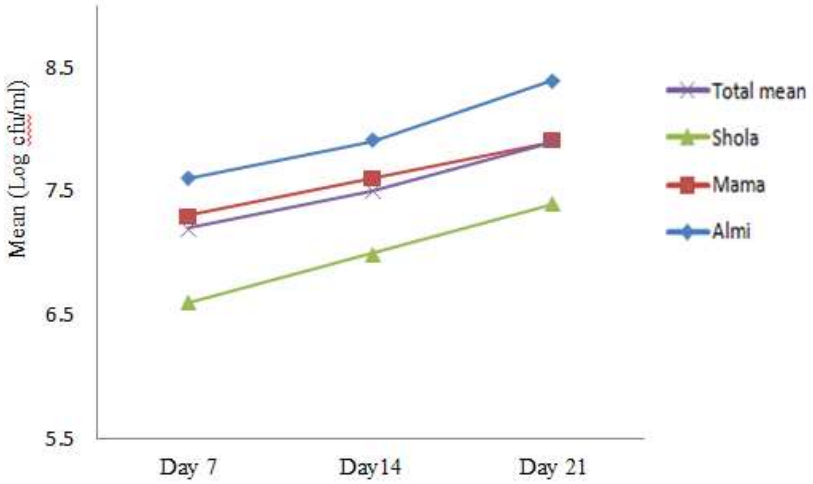

Figure 1. Total bacterial load of pasteurized milk in day 7, 14 and 21.

DISCUSSION

In the present study, the overall mean value of total bacterial count was 6.83 log10 CFU/mL milk samples. TBC count obtained in the present study in all sources are generally higher than the acceptable limit (5 log CFU/mL) (Revelli et al., 2004). However, the total bacterial count obtained in the present study is lower than previous reports of Tola et al. (2007) (7.6 log CFU/mL) in East Wollega, Ethiopia and Yilma and Faye (2006) (8.38 log CFU/mL) in central highlands of Ethiopia. On the other hand, the present TBC count is higher than the reports made (Godefay and Molla, 2000; Mogessie and Fekadu, 1993). Mogessie and Fekadu (1993) reported 5.5 Log CFU/mL of TBC in milk samples obtained from Awassa College of Agriculture dairy farm, while Godefay and Molla (2000) reported 6.0 Log CFU/mL TBC in milk samples collected from selected dairy farms in Addis Ababa.

The mean coliform count in milk samples in this study was 6.63 Log CFU/mL which is higher than the previous report by Fekadu (1994) who reported a mean of 3.8 Log CFU/mL of CC in Southern Ethiopia. The higher coliform count observed in this study may be due to the initial contamination of the milk samples either from the cows, the milker, milk containers or the milking environment.

None of the three retail brands met the minimum quality standard of coliform counts (<100 cells/ml pasteurized milk) which indicates that in all retail brands either pasteurization is inadequate or cross-contamination during and after pasteurization (de Oliveira et al., 2015). This finding highlights the need for strict quality control by regulatory bodies in such retail milk brand which could cause serious health problem particularly in children who frequently consume milk.

Among the pasteurized milk samples, the highest mean TBC (7.50 log CFU/mL) was found in samples belonging to *Almi* brand and the lowest from *Shola* brand (6.53 log CFU/mL) (Table 3) which shows differences in hygienic practice within the processing plants. The overall mean TBC for the retail brands (6.75 log CFU/mL) was higher than the report of Nanu and Latha (2007) for packaged milk samples (4.76 log CFU/mL) and Mahari and Gashe (1990) for pasteurized milk count 7 Log CFU/mL as it left the pasteurizing unit. However, the population increased 2 to 4 fold as a result of subsequent contamination which may be attributed to post pasteurization contamination which includes: improperly cleaned pasteurizer equipment, storage tank, packaging units, package material and working personnel

The high bacterial load could also be associated with the original heavy load of bacteria in raw milk before pasteurization. Raw milk ready for pasteurization must be within the count rate of 1×10^5 to 3×10^5 (Jayarao et al., 2004). Also, bacterial cells can recover after thermal injury under the favorable tropical temperatures that prevail during transportation or at retail outlets that do not have chilling facilities and electric power cuts (Omore et al., 2001).

The total mean for TBC of the current study was 7.2, 7.5 and 7.9 Log CFU/mL for days 7, 14 and 21, respectively. This shows the storage time increases the quality and safety of pasteurized milk decreases due to increased total bacterial load. Previous studies in different areas had reported similar findings (Angelidis et al., 2016).

In this study, 12 bacterial genera were isolated from all milk samples. However, the degree of occurrences of each genus varies between milk sources. While the isolation rate of *Enterobacter*, *Escherichia*, and *Shigella* spp. of raw milk samples from the households was significantly higher than in milk samples from dairy farms,

the reverse was true for *Proteus* spp., *coagulase-negative Staphylococcus* and *coagulase positive Staphylococcus. Enterobacter*, *Escherichia,* and *Shigella* spp. are related to personal hygiene and the higher percentage of these genera in household milk samples could be associated with the poor personal hygiene of the households. The finding of a higher percentage of *Proteus* spp. in dairy farms may be associated with the milking environment hygiene as proteus bacilli are widely distributed as saprophytes being found in decomposing animal matter, manure, soil and mammalian intestine.

Although *E. coli, Klebsiella, Enterobacter, Citrobacter, Proteus, Pseudomonas, Salmonella, Shigella* and *Yersinia* species were both fecal and nonfecal organisms isolated from all sample sources; the existence of fecal coliform bacteria may not necessarily indicate direct fecal contamination of milk but it is a precise indicator of poor sanitary practices during milking and further handling processes. The presence of *E. coli* implies a high concern for safety that other enteric pathogens may be present in the sample (Hayes et al., 2001). The incidence of fecal coliforms in raw milk has received considerable attention, partly due to their association with contamination of fecal origin and the consequent risk of more pathogenic fecal organisms being present, partly because of the spoilage that can result from their growth in milk at ambient temperatures. Sporadic high coliform counts may also be a consequence of unrecognized coliform mastitis, mostly caused by *E. coli* (Suojala et al., 2013).

During the present study, the hygienic condition of the environment where cows are kept and where milking takes place was assessed. It was found that animals are kept in open muddy barn, and regular hygienic conditions of the cows were poor. The provisions of adequate facilities for the cleaning, disinfection, and storage of utensils and milking equipment and the refrigeration of milk to a temperature of 3.3°C are essentials. The milking areas must be clean and should be free from harmful microorganisms (de Oliveira et al., 2015).

Psychrotrophic bacteria are important, because although mostly not thermoduric, many of them produce extracellular thermostable proteolytic and lipolytic enzymes which can survive pasteurization and thus affect the shelf life and quality of the dairy product (de Oliveira et al., 2015). In this study, psychrotrophic bacterial isolates (*Pseudomonas* spp.) were isolated from milk samples from all sources including the pasteurized retail brands which call for attention to the way milk is produced in the country particularly in pasteurized retail milk brands as the society directly consumes these milk brands. Furthermore, the health status of each milking cow should not be ignored as it may contribute to the poor quality of milk.

Lack of knowledge on clean milk production, use of contaminated milking equipment coupled with lack of potable water for cleaning purpose might have contributed to the poor hygienic quality of milk. The use of insufficient and poor quality water for cleaning of milk handling equipment can result in milk residues on equipment surfaces that provide nutrients for the growth and multiplication of bacteria that can then contaminate the milk. Differences in microbial qualities of milk produced by the different dairy farms presumed to be the result of variations in production, processing and preservation practices followed at various stages. There is no as such a standard practice in the method of processing and handling of the dairy products in these farms. The existence of such variation suggests the need for intervention aimed at developing a standard code of practice for milk production and marketing system in the country in general and in the study area in particular.

Conclusion

The present study has shown that the quality of milk produced in the study area was poor and below the standard. This was evident from the high TBC, CC, YMC and TSC in the milk. Hence, adequate sanitary measures should be taken at all stages from production to consumption. These measures include proper handling of the cow, personal hygiene, use of hygienic milking and processing equipment and improving milk and milk handling environment. The poor bacteriological quality observed in the present study requires further investigation of the health status of the animals, and the significance of the effect of containers to ascertain their contribution on microbial quality. Provision of continuous training to all stakeholders who involved in milk production chain could be one of the key intervention areas to improve the quality and safety of the milk consumed in the area. At the same time, it was suggested that milk production and marketing regulatory mechanism should be in place to protect the public health and safety.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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