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Handling Practices, Evaluation of Adulteration and Microbial Quality of Raw Cow Milk around Bahir Dar, Ethiopia

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Abstract
Background: Despite milk is a highly nutritious food, it can easily be contaminated with physical, chemical and microbiological hazards.**Objective:** The study was conducted to assess handling practices, evaluation of adulteration and microbial quality of raw cow milk collected from farmers and dairy cooperatives at Andassa, Sebatamit and Tis Abay kebeles around Bahir Dar city, Ethiopia. **Method:** The study involved both cross-sectional survey aimed at assessing the handling practices and a laboratory- based investigation aimed to determine adulteration and microbial quality of the milk. A total of 94 respondents were selected using simple random sampling technique and interviewed using a semi structured questionnaires. A total of 39 samples of raw cow milk were collected from farmers and dairy cooperatives.**Result:** The mean fat content of raw milk obtained from farmers and dairy cooperatives were 4.23 % and 4.31% respectively and the mean specific gravity of raw milk obtained from farmers and dairy cooperatives were 1.029. The overall mean total bacterial count, coliform count, isolated E.coli and Staphylococcus count of raw milk samples obtained from farmers at Sebatamit (7.22±0.56, 4.70±0.79, 3.15±0.65 and 4.97±0.52 $\log_{10}$ cfu/ml, respectively), Andassa (6.91±0.68, 5.02±0.59, 3.42±0.78 and 4.95±0.47 $\log_{10}$ cfu/ml, respectively) and Tis Abay kebeles (6.83±0.68, 5.05±0.63, 2.98±0.55 and 4.95±0.55 $\log_{10}$ cfu/ml, respectively) and dairy cooperatives at Sebatamit (6.42 ± 0.42, 4.41 ± 0.85, 2.91 ± 0.59 and 4.83 ± 0.37 $\log_{10}$ cfu/ml, respectively), Andassa (7.38 ± 0.48, 4.87 ± 0.67, 3.38 ± 0.61 and 4.64 ± 0.32 $\log_{10}$ cfu/ml, respectively) and Tis Abay kebeles(6.82 ± 0.34, 4.09 ± 0.69, 3.07 ± 0.65 and 4.75 ± 0.70 $\log_{10}$ cfu/ml, respectively). The result of this study indicated that, about 72.3% of the farmers at the study Kebeles use common towels to dry the udder and teats of each cow. Overall, about 66.2%, 21.1% and 12.6% of the farmers respectively used warm water, cold water and both warm and cold water alternatively for washing udder.**Conclusion:** The findings from this study suggest that the milk obtained from most farmers at the study area do not adulterate milk and some of the farmers had adulterated milk according to the East Africa raw cow milk standard. The results showed that the microbial quality of raw milk obtained from farmers and dairy cooperative were not satisfactory. Therefore, these findings highlight the need to implement improved hygiene practices to apply effective monitoring at all levels of dairy chain.
Keywords: Raw Cow Milk, Hygienic Practices, Adulteration, Microbial Quality, Farmers, Dairy Cooperative.

BACKGROUND

Milk is a highly nutritious food, ideal for microbial growth. Chemically, milk is a complex mixture of fat, protein, carbohydrate, minerals, vitamins and other miscellaneous constituents dispersed in water, make it a complete diet (Haug et al., 2007). The major components of milk are water (87.4%), milk solids (12.60%), solids-not-fat (9.0%), fat (3.60%), protein (3.40%), milk sugar or lactose (4.90%) and ash or minerals (0.70%) (Ramesh, 2006).The adulteration of milk is done to derive undue profit by adding water or extraction of fat. Besides this, milk is also adulterated with urea, detergent and vegetable fat. Addition of adulterants like water, starch, salt, pulverized soap, detergents, urea, skim milk powder and preservatives like formalin and hydrogen peroxide in milk will deteriorate its overall quality (Chagas et al, 2007). Adulteration of milk can cause the deterioration of dairy products, therefore milk quality requires the necessity and greater emphasis on regulatory aspects with advanced methods of analysis and monitoring milk production and processing (Fox and McSweeney, 1995).

The presence of food- borne pathogens in milk is due to direct contact with contaminated sources in the dairy farm environment and to excretion from the udder of an infected animal (El Zubeir et al. (2005). According to Asaminew (2007), the overall milking hygienic practice followed by the farmers in Bahir Dar Zuria and Mecha Woreda is poor. But, provision of milk and milk products of good hygienic quality, quantity and good composition is desirable from consumer health point of view (Giangiacomo, 2000). Nutritionally enriched milk and its products with enhanced biological potential and without health risks are generally demanded (Imran et al., 2008). Hygienic control of milk and milk products in Ethiopia is not usually conducted on regular bases. Apart from this, door-to-door raw milk delivery in the urban and peri-urban areas is commonly practiced with virtually no quality control at all levels (Godefay and Molla, 2000). Thus, the purpose of this research was to assess handling practices, evaluation of adulteration and microbial quality of raw cow milk collected around Bahir Dar, North-West Ethiopia.

MATERIALS AND METHODS

Study design: The study was carried out using both cross-sectional survey aimed to assess handling practices and a laboratory- based investigation aimed to determine adulteration and microbial quality of raw cow's milk collected at Sebatamit, Andassa and Tis Abay kebeles. A total of 94 respondents were selected using simple random sampling technique and interviewed using a semi structured questionnaires. All the samples were collected using proportional random sampling method.

Interview Questionnaires

Semi-structured questionnaires were prepared for conducting face-to-face interviews with the selected farmers and dairy cooperatives. Semi-structured questionnaire was used to gather information on the handling practices of milk in the studied kebeles.

Milk Sample Collection and Transport: Raw milk samples were collected from farmers and dairy cooperatives in the selected kebeles around Bahir Dar city.

A total of 39 samples of raw cow's milk were collected in the morning from farmers and dairy cooperatives from Andassa, Sebatamit and Tis kebeles who took part in the interview. Thirty raw cow milk samples from farmers and nine raw milk samples from dairy cooperatives were collected. The raw cow's milk sample was collected aseptically in sterilized plastic bottles, kept in an icebox and transported to the Food Microbiology laboratory, Bahir Dar University, Institute of Technology. Then the milk was analyzed for its chemical and microbiological qualities. All the analysis was conducted in triplicate.

Evaluation of milk adulteration

The adulteration of the collected raw milk samples were evaluated through determination of specific gravity and fat content.

Determination of Specific Gravity: Density (g/ml) was determined by using a lactometer. Adulteration with water was tested by specific gravity (SG) using a lactometer at a standardized milk temperature. The lactometer was allowed to float freely in a cylinder, containing sufficient milk sample, until it reached equilibrium and readings taken below the meniscus (O'Connor, 1995). Accordingly, the following formula was used to calculate the specific gravity of the milk.

$$\textbf{Specific gravity} = L/1000 + 1$$

Where, L= corrected lactometer reading at a given temperature, i.e., for every degree above 15.6 oC, 0.2 was added to the lactometer reading but for every degree below 15.6 oC, 0.2 was subtracted from the lactometer reading.

Determination of Fat Content

The Gerber method using butyrometer was used to determine the milk fat content. Ten ml of sulfuric acid was dispensed into a butyrometer. Then, 11 ml of milk and one ml of amyl alcohol were added into a butyrometer having the sulfuric acid. The butyrometer was closed with rubber cork and the sample was shaken and inverted several times until all the milk was digested by the acid. Then the butyrometer was placed in a water bath at 65ºC for five minutes. The sample was centrifuged for five minutes at 1100 rpm. Finally, the sample was returned back to the water bath and kept for 5 minutes at 65ºC and fat percentage was read from the butyrometer scale (O'Connor, 1995).

Determination of Microbial Quality of Raw Cow

MilkMicrobiological analysis was done using appropriate media designed for cultivation, enumeration and identification of the different microbial groups.

Total bacterial count: One ml of milk sample was added into sterile test tube containing nine ml peptone water up to serial dilution of 10-6 and mixed thoroughly. Appropriate decimal dilution of milk samples were pour-plated on 15-20 ml Standard Plate Count Agar (SPCA) solution and mixed thoroughly. The plated sample was allowed to solidify and then incubated at 32 °C for 48 h (Richardson, 1985). Colony counts were made using colony counter.

Coliform Count: Appropriate decimal dilutions were surface plated and incubated at 32°C for 24 hours on Violet Red Bile Agar and typical dark red colonies on uncrowned plates was considered as coliforms and counted. This was followed by a confirmatory test by transferring and incubating four to five typical colonies from each plate transferred into tubes containing 2% Brilliant Green Lactose Bile Broth. Gas production within 48 hours of incubation at 35°C was considered as sufficient evidence for the presence of coliforms (Richardson, 1985).

Finally, the plate counts were calculated as; N, the number of colony forming units of coliforms per ml of milk sample using the formula

$$\text{"}\mathbf{N} = \text{"}\ (\sum\text{"}\mathbf{C}\text{"})/\text{"}\mathbf{n1} + \mathbf{n2}\text{"}$$

Where, N=Number of colonies per ml of milk sample; ∑C=Sum of all colonies on plates counted; n1=Number of plates used in lowest dilution counted; n2=Number of plates used in highest dilution counted; d=dilution factor of the lowest dilution used

Isolation of Escherichia Coli: The test was done by plating one ml sample onto MacConkey agar media. The

plates were incubated at 35°C for 48. Plates showing positive coliform were subjected to the confirmatory test using Brilliant green bile lactose broth in test tubes with inverted Durham tubes and incubated at 44ºC for 48 h. Each positive tube was sub-cultured into Escherichia coli broth medium and then incubated at 44.5°C for 24 h. For the isolation and identification of E. coli, the enriched sample was cultured on selective medium Levine Eosin Methylene Blue (EMB) Agar and incubated at 37°C for 24 h (Harrigan, 1998). Morphologically typical colonies (at least 4/plate) producing metallic sheen were taken into nutrient broth for further identification. Biochemical tests were performed to confirm Escherichia coli using Gram staining, Catalase test, Indole, Methyl red, Voges- Proskauer test.

Staphylococcus count

One ml sterile pipettes were used to place 0.1 ml aliquots from each dilution into two properly labeled mannitol salt agar (MSA) plates. The plates was spread and incubated at 37°C for 45± 2 Hrs. The number of yellow colonies in un-crowded plates was counted. For confirmation, four to five of typical colonies per MSA plate were streaked on Mannitol salt agar which was followed by Gram stain, coagulase test, catalase test and mannitol fermentation (ISO, 1999; Yousef and Carlstrom, 2003).

Data analysis: Descriptive statistics such as frequency distributions and percentages were analyzed using statistical program for social sciences, version 20 (IBM SPSS Inc., Chicago, USA), Data obtained from a laboratory analyses were analyzed performing analysis of variance (ANOVA) using SAS software (version 9.1.3) to compare the mean values of the treatment using LSD at significant level of ($p<0.05$).

RESULT AND DISCUSSION

Milk Handling Practices: Table 1 shows the handling practices of farmers in the study Kebeles. About 72.3% of the farmers at the study Kebeles use common towels to dry the udder and teats of each cow. While about 1.1% reported that they do not practice udder washing and drying. Using common towels to dry the udder and teats of each cow may favor contamination of milk from the udder and teats of infected cows Gran et al. (2002). About 66.2%, 21.1% and 12.6% of the farmers used warm water, cold water and both warm and cold water alternatively for washing udder respectively. Pre-milking, udder preparation and teat sanitation play important role in the microbial load of milk infection with mastitis, and environmental contamination of raw milk during milking (Depiazzi and Bell, 2002). Accordingly, about 51%, 11.7%, 20% and 17.3% of the respondents clean the barn daily, once a week, two times per week and three times per week respectively. In agreement to this study, about 87% of the respondents cleaned their barn on daily basis, while few (9%) of them cleaned only once or twice a week in the Ethiopian highlands (Zelalem, 2010). However, proper and clean housing environment is a pre-requisite to produce milk and milk products of acceptable quality (Asaminew, 2007).

All the farmers milk their cows by using hand milking either washing cow teats or letting calf to suckle its dam for minutes to stimulate milk let-down. About 58.5% of farmers milk their cows using hand milking by washing teats without calf suckling while 41.5% of farmers milk their cows by hand after calf suckling and they believe that during calf suckling for milk letdown, the teats get washed by the saliva of calf and therefore it is not as such important to wash the teats before milking. Calf suckling attributes to contamination of the milk from infected calf while milking. As a result, washing teat after calf suckling was counted as removing contaminant from the teat as well as delaying the contamination of milk occurred from the saliva of the calf. Restricted suckling before and after milking is used in most dual purpose cattle production systems of Latin America, partly as a consequence of difficulties in milking cows with Bos indicus genes without the presence of the calf (Merbis et al., 2001).

The result of this study indicated that about 83.2% of the farmers clean their containers before and after milking. Proper cleaning of equipment used for storage, processing and further handling of milk and milk products are essential to keep microbial contamination of the products to a minimum. Among the factors that affect the quality of dairy products, adequately performing milking procedures and cleanness of the milking utensils is commonly mentioned (Almaz et al., 2001). Thus, cleaning and disinfection of equipment after each milking is important to reduce contamination of milk by microorganisms from the equipment and with rinsing, about 10% of the number of bacteria found in milk can be reduced (Murphy, 1996). As indicated in Table 1, the entire farmers milk their cows twice a day (morning and evening). About 80.7% farmers did not have separate place for milking. The milking area must minimize the risk of contamination from any source, including dust, flies, birds or other animals (Food Hygiene Regulations, 2006). The study showed that about 66.7% dairy cooperatives used both Jerry can and Aluminium can and about 33.3 % of the respondents use only jerry-cans for milk collection. This study is in agreement with the findings of (Yitaye et al., 2009) and Teklemichael, 2012). All respondents of dairy cooperatives washed milk containers with hot water and soap. All of the respondents from dairy cooperatives testing quality of milk by using, lactometer, alcohol test and boiling at collection centers.

The survey data showed that, the entire dairy cooperative did not use cooling systems for storing milk before selling. They were either keeping it at room temperature until it was sold or transporting it at ambient temperature to selling points. Nevertheless, due to the absence of appropriate cooling systems at milk collection

centers, milk in the present study area was usually transported at ambient temperatures to selling points. This may leads to increased microorganism in the milk and cause health problem among consumers. These results are in agreement with those of Ali et al., (2010) and Hussain (2001). Therefore, it would be beneficial to have an access to cooling facilities for retarding bacterial growth in raw milk during collection and transportation to the selling points.

Table 1 Handling practices of farmers at Sebatamit, Andassa and Tis Abay Kebeles.

Variables	Study Kebeles			
	SB (N=30)	AN(N=28)	TA(N=36)	Mean (N=94)
Use of towel for drying udder (%)				
Common towel	63.3	78.6	75	72.3
Individual towel for each cow	33.3	21.4	25	26.6
No washing and drying	3.3	-	-	1.1
Type of water used for udder washing (%)				
Cold	23.3	17.9	22.2	21.1
Warm	63.3	71.4	63.9	66.2
Both alternatively	13.3	10.7	13.9	12.6
Barn hygiene/cleaning (%)				
Daily	46.7	53.6	52.8	51.0
Once a week	13.3	10.7	11.1	11.7
Twice a week	20	17.9	22.2	20.0
Three times per week	20	17.9	13.9	17.3
Presence of separate worker for milk (%)				
Yes	83.3	75	63.9	74.1
No	16.7	25	36.1	25.9
Practice of washing the udder and teats before milking (%)				
Yes	76.7	85.7	83.3	81.9
No	23.3	14.3	16.7	18.1
Practice of washing hands with soap before milking (%)				
Yes	16.7	14.3	25	18.7
No	83.3	85.7	75	81.3
Frequency of milking (%)				
Once a day	-	-	-	-
Twice a day	100	100	100	100.0
Techniques of milking (%)				
Washing teat	60	57.1	58.3	58.5
Calf sucking	40	42.9	41.7	41.5

N=Number of respondents, SB: Sebatamit, AN: Andassa, TA: Tis Abay

Adulteration of raw cow milk: Adulteration results with respect to specific gravity and fat content of raw cow milk collected from farmers (Table2) and those of dairy cooperatives across study Kebeles are shown in Table 3.

Specific gravity: The study shows that the mean specific gravity of raw milk obtained from farmers and dairy cooperatives were (1.029). There was no significant difference (P>0.05) in specific gravity of raw cow milk among study Kebeles (Table 2). Similarly, there was no significant difference (P>0.05) in specific gravity of raw cow milk among the dairy cooperatives in the study Kebeles (Table 3). For normal whole cow milk specific gravity ranges from 1.028 g/ml – 1.036 g/ml based on the East African Community (EAC) (2006) standard. Having specific gravity below recommended level implies that there was adulteration of milk with water which contributes to production of poor quality milk Ali et al., (2010).

The findings from this study suggest that about 23.3% milk samples collected from farmers at the study Kebeles were adulterated milk. The adulteration of milk with water was found to be a common practice by farmers from the study Kebeles. This practice has been reported not only to decrease the quality of the milk but also causes major economic losses for the processing industry; as it introduces chemical and microbial health hazards (Hussain, 2001).

Fat content: The mean fat content of raw milk obtained from farmers and dairy cooperatives were (4.23 % and 4.31% respectively). There was no significant difference (P>0.05) in fat content of raw milk collected from dairy cooperatives among the study Kebeles (Table 3). The result of present finding was comparable with the result of Zelalem et al. (2009) in terms of fat content, indicating 5.43%. The current study also was comparable with the earlier findings of Rehrahie and Andinet (2007) who reported 6.01% for Borana cows, Asaminew (2007)

reported 4.71 % fat for local cows' milk in Bahir Dar milk shed. The fat content of milk varies from animal to animal, and is influenced by a number of factors: genetic breed, the ration feed, season, and age of the cow, stage of lactation and adulteration of the milk (Raff, 2011). The United State public health service (USPHS) Milk Ordinance and Code recommended a minimum of 3.25% butterfat in farm milk (FAO, 2007). Ethiopian standard (2009) for the fat content of whole milk is 3.50%. The overall mean value of the fat content of milk samples obtained from farmers and dairy cooperatives in the study area fall within the acceptable value set by Ethiopian standard and USPHS.

Table 2 Adulteration of raw cow milk collected from farmers between study Kebeles.

Variables	Sebatamit(n=10)	Andassa(n=10)	Tis Abay(n=10)	Overall mean(n=30)
Fat content	4.31±0.50a	4.05±0.66a	4.34±0.57a	4.23
S. gravity	1.029±0.00a	1.029±0.00a	1.029±0.00a	1.029

Different letters with the same row shows significantly different from each other at ($p<0.05$).

Table 3 Adulteration of raw cow milk collected from cooperatives among study Kebeles.

Variables	Sebatamit (n=3)	Andassa (n=3)	Tis Abay (n=3)	Overall mean (n=9)
Fat content	4.17 ± 0.21b	4.54 ± 0.16a	4.20 ± 0.39 b	4.31
S. gravity	1.029 ± 0.00 a	1.030 ± 0.00 a	1.030 ± 0.00 a	1.029

Different letters with the same row shows significantly different from each other at ($p<0.05$).

Microbial Quality of Raw Cow Milk

Total Bacterial Count: The mean total aerobic bacterial count of raw milk collected from farmers and dairy cooperatives were 6.99 log10 CFU/ml and 6.87 log10 CFU/ml respectively. There was no significant difference ($P>0.05$) in total bacterial count of raw milk samples collected from dairy cooperatives at Sebatamit and Tis Abay Kebeles (Table 5). Similarly, there was no significant difference ($P>0.05$) in TBC of raw milk collected from farmers at Andassa and Tis Abay Kebeles (Table 4). In the present study, total bacterial count of raw milk collected from farmers in the study area were lower than that reported by Francesconi (2006), who found high total bacterial count of 108cfu/ml from raw milk sample collected in dairy cooperatives operating in Ethiopia. Alganesh et al. (2007) also reported higher total bacterial count of cows' milk produced in Bila Sayo and Guto Wayu districts of Eastern Wollega which were 7.4 x 107 and 2.0 x 107 cfu/ml, respectively. Furthermore, higher total bacterial count (7.58log10 cfu/ml) was reported by Asaminew and Eyassu (2011) for milk samples obtained from farmers in Bahir Dar Zuria district. According to standards indicated by East African community of raw cow milk (2007) a good quality raw cow milk should have TBC of less than 5.3 log10 cfu/ml. In the present study, total bacterial count of raw milk collected from farmers (Table 4) and dairy cooperatives (Table 5) in the study Kebeles were exceeding East African community standards of raw cow milk. Higher TBC of milk samples obtained from farmers could be attributed to improper cleaning of the udder and milking containers before and after milking, failure to use separate towel for each cow, lack of knowledge about clean milk production, improper cooling system and milk contamination from the hands of handlers. Higher microbial loads observed in dairy cooperatives may be use of plastic containers for collecting and keeping milk, further contamination of the milk during transportations, absence of cooling systems at milk selling points. El Zubeir et al., (2007) found that the milk collected from Khartoum North and Omdurman at Sudan has relatively high viable bacterial count and concluded that unsanitary conditions in the farms associated with mishandling of milk and lack of cooling during transportation could be the reason for this high bacterial load. In general, using plastic buckets for milk collection and keeping raw milk at room temperature until sold out in dairy cooperative may lead to high number of total bacterial count in the study Kebeles.

Coliform Count

The mean total aerobic bacterial count of raw milk collected from farmers and dairy cooperatives were 4.92 log10 CFU/ml and 4.46 log10 CFU/ml respectively. The analysis of variance (ANOVA) suggested that there was a significant difference ($P<0.05$) in coliform count of raw milk collected from farmers (Table 4) and dairy cooperatives (Table 5) among the study Kebeles. In the current study, the coliform count of raw milk collected from farmers in the study area was higher than that reported by Asaminew and Eyassu (2011) who found coliform count of (4.94 ± 0.23 log10 cfu/ml) in milk samples collected from Bahir Dar Zuria district). However, Teklemichael (2012) reported lower mean values of coliform counts of (4.130 ± 0.757 log10 cfu/ml) from milk samples collected from Dire Dawa town dairy farms. Correspondingly, the coliform count of raw cow milk collected from farmers in the three Kebeles were lower than Gemechu et al (2014) and Derese (2008) who reported the coliform bacteria count (4.999log10 cfu/ml) from shashemane town and coliform count 4.84 log cfu/mL in milk samples collected in the Bahir Dar milkshed respectively. According to Zelalem and Faye (2006) investigation higher coliform count of 6.57log10 cfu/ml for raw cow's milk collected from different producers in the central highland of Ethiopia. Meanwhile according to East African community standards for

coliform count of raw milk (2007) good quality raw cow milk should not exceed coliform count of 3 (log10 cfu/ml). This implied that all the milk samples from farmers and dairy cooperatives analyzed for coliform count were above the recommended EAC levels for coliform count.

In reference to this limit, the presence of high numbers of coliforms in milk indicates that the milk has been contaminated with feacal materials, unclean udder and teats of cow's, inefficient cleaning of the milking containers, poor hygiene of the milking environment, contaminated water and cows with subclinical or clinical coliform mastitis (Jayarao et al., 2004).

Escherichia coli count

The mean Escherichia coli count of raw milk collected from farmers and dairy cooperatives were 3.18 log10 CFU/ml and 3.12 log10 CFU/ml respectively. The presence of Escherichia coli (one of the member of coliform bacteria) in milk is a common indicator of feacal contamination. The status of milk quality based on bacterial contamination with E.coli showed that there was no statistical significant difference (P>0.05) in the counts of E.coli of raw milk collected from farmers among study Kebeles (Table 4). The value of E. coli of raw milk collected from farmers and all dairy cooperatives in the study Kebeles was lower than the reported value for E.coli (3.93 ± 0.01cfu/ml) by Ali and Abdelgadir (2011) at Sudan from raw milk samples. The overall values of E. coli count observed in the current study were much higher when compared with the recommended values given by the microbiological standards of raw milk for EU and US (0 cfu/ml) (FAO WHO, Codex Standard (2000). The presence of E. coli was used as an indication of faecal contamination which indicates possible presence of enteropathogenic bacteria in milk Abeer et al. (2012). Contamination of milk with E.coli may be attributed to unhygienic milking environment or other sources of faecal contamination including unclean udder of the cow during milking. Again the presence of Coliforms like E. coli is an indication of poor level of hygiene of the milkers' utensils, water and the milking environment. This agreed with Najib (2003) who indicated that the source of E. coli found in raw milk include soil, manure, unsanitary equipment and human faeces.

Staphylococcus count

The mean Staphylococcus counts of raw milk collected from farmers and dairy cooperatives were 4.96 log10 CFU/ml and 4.74 log10 CFU/ml respectively. There was no significant difference (p>0.05) in staphylococcus bacteria count of raw milk samples collected from dairy cooperatives (Table 5) in the study Kebeles. According to Teklemichael et al. (2013) the percentage of detection of Staphylococcus count in milk samples obtained from dairy farms and vendors was 25% and 50%, respectively. According to European law, Council Directive 92/46/EEC of 16 June 1992, raw cow's milk intended for direct consumption without any heat treatment or raw milk for manufacturing of products without heat treatment has a threshold value of 5.0x102 cfu/mL, which is considered as satisfactory. The maximum value of 2.0x103cfu/mL is considered unsatisfactory if reached. The Staphylococcus counts in dairy cooperatives (Table 5) in the study Kebeles were higher than European law. All raw cow milk samples in this study Kebeles were not within an acceptable level. This situation may occur because the initial Staphylococcus load from the farm may multiply during transportation, as cold chain facilities are not available in all sampling points, may contaminate the milk because of poor personal and/or equipment hygiene during the value chain. Staphylococcus is the major causative agent of sub-clinical mastitis in dairy cows Akineden et al., (2011) and consequently a major source of raw milk contamination (Kadariya et al., 2014). Andreoletti et al (2009) mentioned that Staphylococcus count is carried by approximately half the human population; due to absence of cooling during transportation is another factor that might increase S. aureus in milk. Contamination of raw milk after handling under non hygienic conditions and mastitis considered as another source of Staphylococcus (Fook et al., 2004).

Table 4 microbial load (log10 cfu/ml) of raw cow milk collected from farmers among study Kebeles.

Variables	Study Kebeles			
	Sebatamit (n=10)	Andassa (n=10)	Tis Abay (n=10)	Overall mean (n=30)
TBC	7.22±0.56a	6.91±0.68 b	6.83±0.68b	6.99
CC	4.70±0.79b	5.02±0.59ba	5.05±0.63a	4.92
E. coli	3.15±0.65b	3.42±0.78 a	2.98±0.55b	3.18
Staphylococcus	4.97±0.52a	4.95±0.47a	4.95±0.55a	4.96

Different letters with the same row shows significantly different from each other at (p<0.05). Ms: milk sample, TBC: total bacterial count, CC: Coliform count.

Table 5 microbial load (log10 cfu/ml) of raw cow milk collected from dairy cooperatives between study Kebeles.

Variables	Study Kebeles			
	Andassa (n=1)	Sebatamit (n=1)	Tis Abay (n=1)	Overall mean (n=3)
TBC	7.38 ± 0.48 a	6.42 ± 0.42 b	6.82 ± 0.34 b	6.87
CC	4.87 ± 0.67a	4.41 ± 0.85ba	4.09 ± 0.69 b	4.46
E. coli	3.38 ± 0.61a	2.91 ± 0.59 a	3.07 ± 0.65 a	3.12
Staphylococcus	4.64 ± 0.32 a	4.83 ± 0.37 a	4.75 ± 0.70 a	4.74

Different letters with the same row shows significantly different at ($p<0.05$).TBC: total bacterial count, CC: Coliform count.

CONCLUSIONS

The statistical analysis showed that about 76.7 % of milk samples collected from farmers in the study Kebeles was free from adulterants according to the East Africa raw cow milk standard. In conclusion, high bacterial loads found in this study show that there is a failure to prevent bacterial growth in milk during transportation from farm to consumer, absence of cooling system, poor sanitary condition of the milk containers, poor udder and teats cleaning practice, failure to use separate towel for each cow and the poor personal hygiene of the milkers. and results in raw milk that is beyond the limits for safe consumption. Therefore, these findings highlight the need to implement improved hygiene practices and to apply effective monitoring at all levels of dairy chain.

RECOMMENDATION

Based on the findings, the following recommendations are given.

i. Hygienic measures such as washing the milking containers, use of separate towel for udder washing and drying of udder, use stainless steel containers instead of plastic containers for transporting, collection and storage of milk should be applied.
ii. Adequate awareness on hygienic production, handling and the importance of raw milk quality control and safety should be given for farmers, dairy cooperative and individual collectors and realistic standards.
iii. Milk testing facilities should be put in place at milk collection centers need to be Strengthened in order to avoid adulteration.

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