Hygienic Practices, Microbial Quality and Safety of Raw Cow's Milk and Traditional Fermented Milk (*Irgo*) in Selected Areas of Ethiopian Central Highlands

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Abstract: A semi-structured questionnaire was used to interview a total of 320 smallholder farmers to assess the hygienic practices and quality of milk and traditional fermented milk. Eighty samples each for raw and fermented milk were collected for microbial analysis using standard procedures. The majority (96.3%) of the milkers washed their hands during milking and 90.7% of the milkers washed udder before milking. However, only 3.5% of the respondents used individual clean towel to dry hands and 19.6% of the respondents to clean udder prior to milking. Plastic containers were the most frequently used milk utensils for various purposes. About 53.8% of the respondents' clean milk utensils with cold water and detergents; while 46.2% used warm water and soap. Tap, river, spring and bore-well were the common sources of water used to clean udder, hands and milk containers. The majority of the respondent's stored milk at room temperature until sold. The average aerobic mesophilic bacterial, coliforms, lactic acid bacteria, and yeast and mould counts in raw milk was 6.8, 3.5, 2.9, and 5.06 log cfu/mL, respectively. Significantly low aerobic mesophilic (5.3 log cfu/mL) and coliform counts (2.5 log cfu/mL) were recorded for fermented milk samples. About 7.5% of raw milk and 2.5% of the fermented milk samples were positive for *Listeria monocytogenes*. However, none of the samples were positive for *Salmonella*. Results revealed that the microbial quality and safety of raw and fermented milk produced in the central highlands of Ethiopia are not as per the standard set by European Union and may be considered as less quality product. Therefore, implementing hygienic handling practices of milk and milk products throughout the dairy value chain is essential to ensure the safety and suitability of these food products for consumers.

Keywords: *Fermented milk, Microbial quality, Raw milk, Safety*

Introduction

Though milk and its derivatives are nutritious food sources for human being, it also serves as ideal media for the multiplication of various microorganisms if not handled in hygienic manner (Parekh and Subhash, 2008). Some of the microorganisms can multiply to high counts and produce toxins, which lead to food poisoning and cause economic loses. In Ethiopia, previous studies revealed that the microbial quality of milk and milk products are frequently substandard (Zelalem, 2010; Abebe *et al.,* 2012; Haile *et al.,* 2012). Presence of pathogenic microorganisms such as *Listeria monocytogenes, Staphylococcus aureus, Escherichia coli* O157:H7 and *Salmonella* spp. were also confirmed in milk in Ethiopia (Fanta *et al.,* 2012; Haile *et al.,* 2012).

Several approaches had been devised to minimize the possibilities of microbial contamination of milk and milk products. These include improvement of animal health, hygiene of the milk handler, cleanliness of containers, proper transportation facilities, and cooling and pasteurization of raw milk and various dairy products (Lore *et al.,* 2006). Complying with all strategies may be challenging in developing countries where there is generally insufficient dairy infrastructure including clean water and cooling facilities. Moreover, consumption of unpasteurized milk is still common in most parts of Ethiopia predisposing consumers to food-borne diseases.

In Ethiopia, milk and milk products are important for family consumption and as a source of income to purchase other household necessities. Consequently, production of high quality milk should be of priority in order to manufacture good quality end products of long shelf life and market value added products. The evaluation of hygiene and safety is conducted by measuring various indicators, for instance bacterial counts such as total bacteria, coliforms, yeast and mould and lactic acid bacteria and detecting pathogenic microorganisms such as *Salmonella* spp. and *Listeria monocytogenes*. This study was conducted to evaluate the hygienic conditions practiced during handling of raw milk and traditional fermented milk termed locally '*Irgo'* and to determine the microbial quality and safety of the products in the study areas.

Materials and Methods

Description of the Study Areas

The study was conducted in eight selected dairy potential areas of the Ethiopian central highlands namely Debre Berhan, Sheno, Sendafa, Chancho, Fiche, Degem, Debre Zeit, and Asella, which are located within a radius of 175 km from the capital city, Addis Ababa in an altitude range of 1600 to 3000 meters above sea level. The mean annual rainfall varies from 860 to 1200 mm. Among the sites, the highest (28^{o}C) average annual temperature is reported for

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Debre Zeit while the lowest (2.4°C) is reported for Debre Berhan.

Methods of Data Collection and Sampling

Data were collected simultaneously in all the study areas using a semi-structured questionnaire. The questionnaire focused on hygienic practices during handling of milk and milk products. Four dairy potential *kebeles* (the smallest administrative unit in Ethiopia) were identified from each of the study sites and 40 households having at least one milking cow were purposively selected. Among the 320 households interviewed, 80 were randomly taken and 80 samples each of raw and *Irgo* were collected. The samples were aseptically collected into a labeled sample bottles, securely capped, kept in ice box, transported to Holetta Agricultural Research Center, Dairy Microbiology Laboratory, placed in a refrigerator at 4°C and analyzed within 24h.

Microbial Analysis

One milliliter of homogenized sample was added into sterile test tube having 9 mL sterile peptone water and mixed thoroughly by using vortex mixer. One milliliter of the sample was taken from the chosen dilution to obtain an expected count of 30 to 300 for Aerobic Mesophilic Bacterial Count (AMBC), 15 to 150 for Coliform count (CC), and 10 to 200 for Yeast and Mould count (YMC) (Richardson, 1985). The media and sample dilutions were gently mixed clockwise, anti-clockwise, and back and forth thrice and allowed to set. All counts were made with duplicate plates. Aerobic mesophilic bacteria were counted on pour plates of Plate Count Agar (PCA) incubated in an inverted position at 30°C for 48h. Lactic acid bacteria were enumerated on pour plates of de Man Rogosa and Sharpe (MRS) agar incubated in an inverted position at 32°C for 48h anaerobically in anaerobic jar. Yeast and mould counts were made on pour plates of Potato Dextrose Agar (PDA) with addition of streptomycin and chloramphenicol and followed by incubation at 25°C for 3 - 5 days, while CC were enumerated on pour plates of Violet Red Bile Agar (VRBA), incubated in an inverted position at 37°C for 24h.

For detection of *Listeria monocytogenes,* well mixed testing samples (25 mL) were homogenized in 225 mL of Listeria Enrichment Broth A and B and incubated for 24h at 37°C (Yousef and Carlstrom, 2003). A loop full of the enrichment culture broth was streaked in duplicate onto Polymyxin-Acriflavin-Lithium Chloride-Ceftazidime-Aesculin-Mannitol (PALCAM) agar and incubated for 48h at 37°C. Suspected *Listeria monocytogenes* colonies were further characterized using gram staining and catalase test.

For *Salmonella* species identification, the sample (25ml) was pre-enriched with 225 mL of Buffered Peptone Water (BPW) and incubated for 24h at 37°C. A portion (0.1 mL) of the pre-enriched culture was transferred to 10 mL Rappaport-Vassiliadis (RV) broth and incubated at 42°C for 24h. A loopful of the enrichment broth culture was then transferred to Xylose Lysine Deoxycholate (XLD) agar and incubated at 37°C for 24h. Characteristic *Salmonella* colonies having a slightly transparent zone of reddish color and black center were sub-cultured on nutrient agar and confirmed biochemically using Triple Sugar Iron (TSI) and Simon citrate agar.

Data Analysis

Descriptive statistics was used to compute the variability of different parameters involved in the evaluation of the milk hygienic quality using SPSS software (ver.16). Microbiological counts were first transformed into logarithmic values (log_{10}cfu/mL) and analyzed using the General Linear Model. The difference was declared as significant when P-value was less than 0.05. The model used for this study was $Y_{ij}=\mu+\beta_i+e_{ij}$, where, Y_{ij} = microbial count, μ = overall mean, β_i = product type and e_{ij} = random error.

Results

Hygienic Practices during Milking

The majority (96.3%) of the sample respondents washed their hands before milking (Table 1), which is important in minimizing potential contamination of milk from milkers' hands. About 54.5% of the respondents had access to tap water for hand, udder and milk utensils washing. However, river in Asella, bore-well in Sendafa, and spring in Degem were the major sources of water used.

Most (69.2%) of the respondents used cold water and soap, while the rest (30.9%) washed their hands with cold water but did not use any detergent. Higher proportion of the households (52 - 80%) in Chancho, Asella and Degem wash hands without detergents. Moreover, no hand drying was practiced before milking by significant proportion (43%) of the respondents and only 3.5% practice hand drying using clean towel. The remaining 53.6% dried their hands with a piece of any cloth they grab including that they dressed. Hair cover and wearing gown were not practiced during milking and subsequent handling of raw and fermented milk.

Most of the respondents (90.7%) said that they wash the udder of milking cows prior to milking (Table 2). Forty-two percent of the respondents used the same towel to dry the udder of all lactating cows; while 38% used their hands to wipe the water from the udder. Only 19.6% use individual towel for each lactating cow. About 72.5% use warm water for cleaning udder. None of the respondents practice discarding of the foremilk showing limitations of sanitary procedures during milking.

Table 1. Source of water and hygienic practices of milkers in the study areas (% respondent)

Variables	Study sites (N=320)								Overall mean
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	
Hygienic practice during milking									
Hand wash	97.2	96.8	97.7	96.3	94.4	94.3	95.9	97.8	96.3
Hair cover	2.8	3.2	2.3	1.8	5.6	3.7	2.0	1.2	2.8
Dress gown	0.0	0.0	0.0	1.9	0.0	2.0	2.1	1.0	0.9
Water sources									
Tap	77.0	72.0	0.0	82.0	95.0	0.0	100	9.8	54.5
River	18.0	23.0	0.0	12.5	5.0	12.5	0.0	90.2	20.2
Spring	5.0	5.0	0.0	5.5	0.0	87.5	0.0	0.0	12.9
Bore-well	0.0	0.0	100	0.0	0.0	0.0	0.0	0.0	12.5
Water type and detergent use for cleaning									
Cold water	0.0	20.0	17.5	52.5	0.0	80.0	0.0	77.5	30.9
Cold water and soap	100	80.0	82.5	47.5	100	20.0	100	22.5	69.1
Practice of hand drying									
Piece of cloth	56.0	48.0	47.2	51.0	55.0	57.0	59.0	55.2	53.6
Clean towel	3.2	2.2	1.4	3.9	4.2	5.6	6.2	1.04	3.6
Not at all	40.8	49.8	51.4	45.1	40.8	37.4	34.8	43.8	43.0

1= Debre Berhan, 2= Sheno, 3= Sendafa, 4= Chancho, 5= Fiche, 6= Degem, 7= Debre Zeit, 8= Asella.

Table 2. Hygienic practices followed to clean cows' udder in the study areas (% respondents)

Parameters	Study sites (N=320)								Overall mean
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	
Udder washing									
Before milking	90.0	76.3	100	95.0	85.7	100	85.0	93.5	90.7
Before and after milking	10.0	7.5	0.0	5.0	0.0	0.0	15.0	6.5	5.5
Not wash udder	0.0	16.2	0.0	0.0	14.3	0.0	0.0	0.0	3.8
Udder drying									
Common towel	22.2	30.7	57.6	58.2	48.7	40.3	35.5	43.3	42.1
Individual towel	41.6	20.8	22.4	10.9	20.5	15.5	21.0	4.3	19.6
Bare hand	36.2	48.5	20.0	30.9	30.8	44.2	43.5	52.4	38.3
Water type used for cleaning									
Warm water	89.5	55.0	78.8	58.7	75.0	69.0	80.0	73.8	72.5
Cold water	10.5	45.0	21.2	41.3	25.0	31.0	20.0	26.2	27.5

1= Debre Berhan, 2= Sheno, 3= Sendafa, 4= Chancho, 5= Fiche, 6= Degem, 7= Debre Zeit, 8= Asella.

Hygienic Conditions of Milk Utensils

The majority (82.5 - 100%) of the households used plastic containers for milking and milk storage (Table 3). About 78% of the respondents cleaned milk utensils immediately after use; while 22% cleaned the equipment prior to use. About 53.8% of the households' clean milk utensils with cold water and detergent mainly ajax whereas, the remaining 46.2% cleaned using warm water and detergents.

After cleaning and drying, milk utensils were smoked using a few species of trees and shrubs. *Woira* (*Olea africana*) was the most frequently (96%) used plant to fumigate milk containers with the objective to impart desirable flavor and aroma, and to reduce the microbial loads. Other plant species such as *Tid* (*Juniperous procera)* and *Tossegn (Thymus vulgari)* were also used to smoke milk containers.

Most (72.9%) of the respondents filtered the milk before delivering to milk collection centers to remove visible dirt that entered into the milk (Table 3). The filters used were muslin cloth (78.8%) and sieve (19.4%). Most of the respondents usually sell morning milk immediately and 82.5% stored evening milk at room temperature until sold the following morning. About 40.6% of the respondents in Debre Zeit placed evening milk inside cold water overnight to sale the next morning. Milk left for fermentation is stored at ambient temperature in all study areas.

Microbial Quality and Safety of Raw and Fermented Milk

All raw milk samples had high Aerobic Mesophilic Bacterial Count, Coliform Count, Yeast and Mould Count and Lactic Acid Bacterial Count (Table 4). However, except yeast and mould and lactic acid bacteria, significantly lower counts were reported for fermented milk samples. All the raw and fermented milk samples analyzed were negative for *Salmonella*. *Listeria monocytogenes* was identified in few of raw and fermented milk samples (Table 4).

Constraints related to Milk Handling

Lack of clean water (42.0%) and electric and cooling facilities (24.0%) were the two most frequently reported challenges of milk handling. Other constraints as mentioned by respondents were limited awareness of

milk handling, lack of transport, and cost and availability of milk utensils (Figure 1).

Table 3. Type of milk utensils and milk handling practices in the study areas (% respondents)

Variables	Study sites (N=320)								Overall mean
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	
Milking utensils									
Plastic	98.9	97.9	98.0	98.3	97.3	98.2	94.0	94.7	97.2
Metal	1.1	2.1	2.0	1.7	2.7	1.8	6.0	5.3	2.8
Milk treatment									
Filtration	85.0	69.0	85.8	92.5	60.0	64.3	55.9	71.3	72.9
Not at all	15.0	31.0	14.2	7.5	40.0	35.7	44.1	28.7	27.1
Material used for filtration									
Muslin cloth	75.0	71.4	85.0	95.0	65.6	100	67.5	70.6	78.8
Sieve	10.0	28.6	15.0	5.0	34.4	0.0	32.5	29.4	19.4
Both	15.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.9
Storage condition									
At ambient T°	83.0	90.0	79.0	88.6	87.8	84.2	53.2	93.8	82.5
In cold water	15.0	10.0	19.2	9.2	10.3	15.0	40.6	2.0	15.2
Refrigerators	2.0	0.0	1.8	2.2	1.9	0.8	6.2	4.2	2.4
Utensils used for milk fermentation									
Plastic	90.0	94.5	100	100	100	90.0	84.5	82.5	92.7
Clay	10.0	5.5	0.0	0.0	0.0	0.0	15.5	0.0	3.9
Metal	0.0	0.0	0.0	0.0	0.0	10.0	0.0	17.5	3.4
Type of water and use of detergent for cleaning utensils									
Cold water and soap	80.0	77.2	68.4	17.0	14.2	46.0	69.7	58.0	53.8
Warm water and soap	20.0	22.8	31.6	83.0	85.8	54.0	30.3	42.0	46.2
Cleaning schedule									
After use	77.0	82.0	73.0	82.5	77.8	74.9	83.4	73.5	78.0
Before use	23.0	18.0	27.0	17.5	22.2	25.1	16.6	26.5	21.9

1= Debre Berhan, 2= Sheno, 3= Sendafa, 4= Chancho, 5= Fiche, 6= Degem, 7= Debre Zeit, 8= Asella.

Table 4. Microbial quality and safety of raw and fermented milk samples of the study area

Parameters	Products (N=80 each)		p-value
	Raw milk	**Fermented milk**	
Aerobic Mesophilic Bacterial Count (log cfu per mL)	6.76 (0.12)[a]	5.29(0.03)[b]	0.001
Coliform Count (log cfu per mL)	3.50(0.01)[a]	2.51(0.08)[b]	0.001
Lactic Acid Bacterial Count (log cfu per mL)	2.87(0.06)[a]	3.40(0.21)[b]	0.001
Yeast and Mould count (log cfu per mL)	5.06(0.01)	5.00(0.07)	0.565
Listeria monocytogenes (%)	7.50	2.50	
Salmonella (%)	0.00	0.00	

Different letters in the same row show significant difference at $p<0.05$.

Discussion

Hygienic Practices during Milking

To minimize contamination during milking, effective hygienic practices need to be applied to the udder of the animals, the milking equipment, the handlers and the general environment such as reducing faecal sources of contamination (Getachew, 2003). Washing hands without detergent may not improve the hygienic conditions of milk and milk products (Zelalem, 2010). Drying of hand with any cloth available to milkers and poor drying practices observed in the present study was also reported by Mezgeb (2012) in the central highlands of Ethiopia. Poor drying practices following hand washing and use of old and unclean clothes for other farm activities is a risk factor for milk contamination (Mezgeb, 2012). Water used for cleaning of hands, udder of the cows and milk utensils should be appropriate for the purpose, such that it will not result in contamination of milk. Major water sources used for cleaning purpose in the present study area are variable, and sources other than tap are likely to be unsanitary contributing to the poor quality of milk and its products (Zelalem, 2010).

As indicated by FSA (2006) pre-milking udder washing is important to remove both visible dirt and bacteria from the outer surface of the udder. The practice of cleaning cow udder prior to milking by the present respondents should be encouraged and scaled out to areas where this practice is less. Previous study in Hawassa showed that 82.5% of smallholder farmers practice pre-milking udder washing (Haile *et al.,* 2012). In contrast, Abebe *et al.* (2012) observed that respondent farmers in Guraghe Zone of Southern

Ethiopia did not practice udder washing prior to milking.

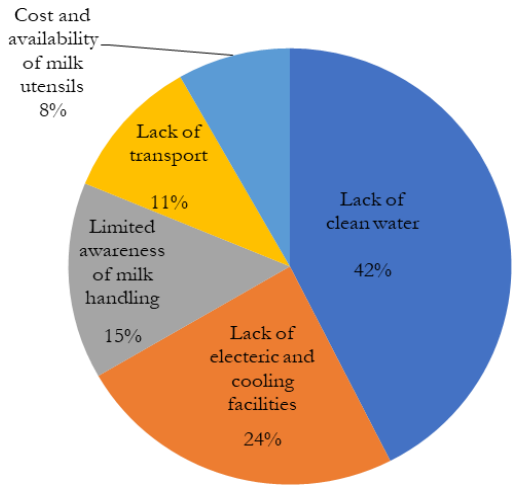

Figure 1. Major constraints of milk handling.

Following udder washing, drying of udder with a clean towel designated for individual milking cows is essential to limit cross contamination and thus microbial load (Haile *et al.,* 2012). However, the practice of using common towel by the majority of the sample respondents negatively affect milk quality and also lead to cross contamination of udder in cases where there are animals with diseased udder/teats (Zelalem, 2010). Similar to the results of the present study (38%), about 36 - 73% of the respondents in the Ethiopian central highlands used the same towel for all cows to dry the udder (Zelalem, 2010; Mezgeb, 2012). This practice can predispose the udder and milk for microbial contamination. Ruegg (2006) revealed that wet teats allow bacteria to get easy access into the mammary gland.

Foremilk (initially drawn small quantity of milk) from each teat should be discarded or collected separately and not used for human consumption since it contains many bacteria. Observing foremilk using a strip cup and discarding it is recommended to improve the microbial quality of milk. Since all of the respondents in the present study did not follow this principle, it is imperative to provide training to dairy farmers to improve microbial quality of milk and udder health. Following milking, it is essential to filter milk to remove visible dirt that may have entered into milk during milking. This practice is properly implemented by respondents of the current study before supplying to milk collection centers unlike reports from other areas of the country (Asrat, 2009).

Hygienic Conditions of Milk Utensils

Equipment associated with milk handling introduces high number of microorganisms in raw milk (Fook *et al.*, 2004). Most of the respondent's used plastic containers for milking and milk fermentation. In agreement with this finding, plastic containers (82.5-100%) were the most frequently used materials for milking and milk fermentation in different parts of the country (Mezgeb, 2012; Tsadkan and Amaniel, 2016). The use of plastic containers and traditional clay pot can be a potential source for contamination of milk due to difficulty of removing all milk residues from such porous containers by common cleaning method. Hence, they may result in increased microbial load of milk, which in turn acidifies and results in undesirable fermentation (Pandey and Voskuil, 2011). Producers should therefore pay particular attention to the type as well as cleanliness of milk equipment.

Using hot water and detergent to clean milk vessels helps to effectively remove fat residuals of previous batch from the milk vessels (O'Connor, 1995). The use of cold water without detergent results in to insufficient cleaning of containers which serve as sources of milk contamination (Pandey and Voskuil, 2011). Only 46% of the respondents in the present study used warm water and detergent to clean milk utensils. Mezgeb (2012) reported that 70% of the sample farmers used warm water to wash milk utensils, whereas only few (26.7%) of the women in Delbo area of Wolayta used hot water (Rahel, 2008). The later author indicated that producers who used hot water increased to 80% following training on hygienic milk production indicating the importance of creating awareness among producers about how to produce milk under hygienic condition.

Utensils used for milk handling should be cleaned using good quality water and detergents immediately after use (FAO and WHO, 1997). About 22% of the sample respondents cleaned the milk utensils prior to use, not immediately after use. Delay in cleaning milk containers gives microorganism's adequate time to multiply and increase in number to the level that may be difficult to reduce to acceptable amount during cleaning. This could result into high microbial counts in milk kept in these containers and hence accelerated microbial spoilage leading to post harvest losses of the milk.

Similar to the present finding, *Olea africana* (*Woira*) was the most commonly used smoking plant in different parts of the country not only to enhance desirable flavour and aroma but also to increase the shelf life of fermented milk (Zelalem, 2010; Abebe *et al.,* 2012; Tsadkan and Amaniel, 2016). As proved by different researchers, smoking of containers tends to markedly retard the growth of bacteria including pathogens such as *Salmonella typhimurium, Escherichia coli* O157:H7 and *Listeria monocytogenes* due to the antimicrobial properties of the smoke (Feyissa *et al.*, 2008). Smoking containers also proved to slow the growth of lactic acid bacteria and impart desirable and slow development of flavor components (Tsadkan and Amaniel, 2016).

Storage conditions are also the basic determinants of milk quality (Fook *et al.,* 2004). After milking, milk should be delivered as quickly as possible to milk collection centers or properly stored in clean containers and kept in a cool and shady place where contaminat-

ion is minimal (Pandey and Voskuil, 2011). Hence, use of this practice is important to keep the morning milk safe until it is delivered to milk collection centers or private traders in the following morning. Keeping fermented milk at room temperature in the present study is similar to that reported by Zelalem and Faye (2006).

Microbial Quality and Safety of Raw and Fermented Milk

Aerobic mesophilic bacterial count (AMBC): Production of raw milk of good sanitary quality by farmers is important to farmers, milk processing companies and consumers (Kunda *et al.,* 2015). Total bacterial count is a good indicator for monitoring the sanitary conditions practiced during production and handling of raw milk (Richard, 2002). The average AMBC obtained in the current study failed to comply with the acceptable limit given for raw milk intended for processing (5 log cfu/mL) (Bodman and Rice, 1996). Previous studies also provided evidence of high AMBC (6.36 - 9.82 log cfu/mL) in raw milk taken from different areas of the country (Zelalem, 2010; Abebe *et al.,* 2012; Haile *et al.,* 2012). Reports of similar studies conducted in other countries in the region such as Zambia, Malawi and Uganda showed similar high bacterial counts (5.6 - 7.5 log cfu/mL) in raw milk (Shitandi and Kihumbu, 2004; Grimaud *et al.,* 2007; Yambayamba and Zulu, 2011).

The low ($P < 0.001$) counts of aerobic mesophilic bacteria recorded in fermented milk (*Irgo)* samples compared to raw might be attributed to the increase in acidity of fermented milk. The mean AMBC of homemade fermented milk in the current study is comparable to results of other studies (Abdalla and Nabi-Ahmed, 2010; Jermen *et al.,* 2016). However, higher values of AMBC (7.1 log cfu/mL) were also reported in the central highlands of Ethiopia (Zelalem and Faye, 2006; Feyissa *et al*., 2008). The high load of bacteria present in milk and milk products indicates that the level of contamination was very high. This high contamination could be a result of initial contamination originating from the udder surface, unhygienic milking equipment and poor personal hygiene as well as failure to cool milk rapidly.

Coliform count (CC): The presence of coliform in large number in dairy products shows that the products are potentially hazardous to the consumers' health. The average CC (3.5 log cfu/mL) obtained in the present study exceeds the values reported for excellent quality milk (<1 log cfu/mL) (Reinemann *et al.,* 2000). Higher CC values of 4.03 - 4.84 log cfu/mL were also reported in different parts of the country (Rahel, 2008; Abebe *et al*., 2012). The lower ($P<0.001$) CC (2.51 log cfu/mL) recorded in fermented milk samples may be an indication of the beneficiary effect of fermentation and could be attributed to the growth inhibition of the coliforms. This makes the traditional fermented milk relatively safe for human consumption. However, the mean counts of coliform bacteria in *Irgo* in the current study failed to meet the international acceptable standard of 10 cfu/mL set for yoghurt (USDA, 2001). However, it is much lower than the result of the study by earlier workers (Kumbhar *et al.,* 2009; Zelalem, 2012; Jermen *et al.,* 2016) who reported CC ranging from 4 to 6 log cfu/mL. The presence of coliform bacteria in high numbers in milk indicates that the milk has been contaminated with fecal materials, unclean udder and teats of lactating cow's, insufficient cleaning of milking containers, poor hygiene of the milking environment, contaminated water, and/or cows with subclinical or clinical coliform mastitis (O'Connor, 1995).

Lactic acid bacterial count (LABC): Fermented milk has received extensive microbiological works and it has been found that lactic acid bacteria dominate all other microorganisms followed by yeast and mould (Robert and William, 2008) since these organisms are acid tolerant as compared to the other groups (Feyissa *et al*., 2008). Therefore, high values of lactic acid bacteria and yeast and mould are expected in fermented milk samples.

The mean LABC observed in fermented milk samples (3.4 log cfu/mL) is significantly higher ($P<0.05$) than that of raw milk samples (2.8 log cfu/mL). This could be explained by the general observation that lactic acid bacteria are acid tolerant and responsible for the fermentation of raw milk. Zelalem and Faye (2006) and Feyissa *et al.* (2008) reported higher LABC (7.68 log cfu/mL) than obtained in the present study in fermented milk collected from the central highlands of Ethiopia. Due to the spontaneous nature of fermentation, the traditionally fermented milk has varying taste and flavor often with poor hygienic quality and as a result it does not meet the acceptable limit set by various regulatory agencies (Lingathurai *et al.,* 2009) as observed in the present study.

Non-lactic acid bacteria are generally considered as contaminants in fermented milk products (Feyissa *et al*., 2008). The growth of other bacteria in fermented milk samples used in the present study was low as compared to the raw milk. This could be attributed to the low pH and high acidity of fermented milk, which has bacteriostatic effect on contaminant bacteria in milk.

Yeast and mould count (YMC): The occurrence of yeast and mould in milk and its derivatives is undesirable even in few numbers as they can result in objectionable changes that render the products of an inferior quality and reduce shelf life (Abdelhameed, 2011). The overall average YMC (5.06 log cfu/mL) observed in the present study is beyond the acceptable limit set for milk (<4 log cfu/g) and yoghurt (50 cfu/mL), which could potentially be injurious to human health (USDA, 2001; Cocoline *et al.,* 2002). The current finding coincides with earlier reports (4.66 - 5.1 log cfu/mL) for milk samples from other areas of Ethiopia (Haile *et al.,* 2012; Alebel *et al.,* 2013).

Likewise, Akabanda *et al.* (2010) and Abdalla and Nabi-Ahmed (2010) reported mean YMC that ranged from 4.97 to 6.63 log cfu/mL in Sudanese and Ghanaian fermented dairy product. A study conducted by Zelalem (2012) also showed higher YMC (8.3 log cfu/mL) in fermented milk samples collected from the central highlands of Ethiopia.

Yeast and mould are primary contaminants of fermented product such as yoghurt. Fungi growing in fermented milk utilize some of the acid favoring the growth of putrefactive bacteria (Oyeleke, 2009) and other pathogenic microorganisms such as *Staphylococcus aureus* and *Listeria monocytogenes* (Makut *et al.,* 2014). Moreover, fermented milk by nature is a high acidic product which is a highly selective environment or medium favoring the growth of yeast and mould as spoilage microorganisms whose presence in fermented milk is an indicator of poor handling practices (Oyeleke, 2009). Yeast and mould can contaminate many foods and produce toxic metabolites (mycotoxins), which are not destroyed during food processing and cooking (Chimezie *et al.,* 2015) and may represent potential health risks. Conversely, yeast contributes to the enhancement of the flavor of fermented milk as different yeast species assimilate different milk substrate (Gadaga *et al.,* 2001). Contamination of milk and its products by yeast and mould might originate from air, feed, inadequately cleaned milk utensils and poor personal hygiene of milk handlers (Chimezie *et al.,* 2015).

Listeria monocytogenes: *Listeria monocytogenes* is the most commonly reported pathogen in recent dairy product related food-borne outbreaks throughout the world. In this study, about 7.5% of the milk and 2.5% of the fermented milk samples were positive for *Listeria monocytogenes*. However, the presence of *Listeria monocytogenes* at any point during the shelf life of ready to eat foods is unacceptable (European Commission, 2007). Relatively lower percentage of *Listeria monocytogenes* is observed in fermented milk and this might be attributed to the antimicrobial properties of lactic acid bacteria against the organism.

In the present study, the occurrence of *Listeria monocytogenes* in raw milk and traditional fermented milk was low; while an earlier study by Firehiwot (2007) showed no *Listeria monocytogenes* in milk samples. Haile *et al.* (2012), however, reported prevalence of *Listeria monocytogenes* in 19.6% of the milk samples obtained from Hawassa town. Detection of *Listeria monocytogenes* in raw milk samples is also reported in many other countries such as China (Chao *et al.,* 2007), India (Sharma *et al.,* 2012) and Tanzania (Kanyeka, 2014). The presence of *Listeria* spp. particularly *Listeria monocytogenes* in raw milk is a public health concern because of the ability of the organism to survive through the various milk processing stages to the final product (Kanyeka, 2014).

Salmonella: *Salmonella* species are known pathogenic microorganisms that can cause food poisoning through consumption of contaminated milk and milk products. In the present study, none of the samples were positive for *Salmonella*. In agreement with the current finding, Liyuwork *et al.* (2013) did not detect *Salmonella* species in fermented milk produced by spontaneous fermentation using traditional utensils in Addis Ababa, Ethiopia. However, several researchers documented the prevalence of *Salmonella* in milk. For instance, about 23.6% prevalence of *Salmonella* was reported for raw milk collected from Debre Zeit, Ethiopia (Tesfa and Assefa, 2016). *Salmonella* are destroyed or inactivated during fermentation of high acidic products such as yoghurt in which pH value is less than 4.55. The difference in prevalence between different studies might be associated with difference in the hygienic and farm management practices (Tesfa and Assefa, 2016).

Constraints related to Milk Handling

The most frequently mentioned milk handling related constraints in the study areas were lack of clean water (42.0%), absence of electricity and cooling facilities (24.0%) and limited knowledge or awareness of milk handling (15.0%). These results are in agreement with that reported by Tsadkan and Amaniel (2016). Zelalem (2012) noted the top-ranking constraints related to milk quality in the central highlands of Ethiopia to be limited awareness on hygienic handling, shortage of capital, lack of access to clean water, and poor type of barn. The same author also reported that lack of knowledge about clean milk production and uses of unclean milking equipment were factors contributing to the poor hygienic quality of milk produced.

Conclusion

From the results of the present study it can be concluded that the microbiological quality of raw and fermented milk produced in the study areas were of poor quality. This is evidenced by the qualities of these products that did not meet standards (minimum acceptable limits) set for quality milk and milk products. High microbial counts and the occurrence of pathogens are likely to affect the keeping quality and safety of raw milk as well as products derived from it. Therefore, in addition to the need for establishing a functional quality control system it is important to lift the knowledge and skill of personnel engaged in the handling of milk and milk products, on the importance as well as ways of ensuring clean milk production, processing, and handling. Moreover, creating awareness among handlers of milk and milk products on the nutritional, health as well as income benefits of hygienic milk production, processing and further handling of milk and milk products will add value.

Conflict of Interests

The authors declare that they have no competing interests.

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