DATA ARTICLE

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Bacteriological milk quality: possible hygienic factors and the role of *Staphylococcus aureus* in raw bovine milk in and around Gondar, Ethiopia

Betelihem Tegegne¹ and Shimels Tesfaye^{2*}

Abstract

Background: In Ethiopia, around 97% of the annual milk production is accounted by the traditional milk processing system using on-farm traditional milk processing materials that are generally poor in processing capacity, causing high product loss and risky for public consumption. A cross-sectional study was carried out in and around Gondar town, Amhara Regional State of Ethiopia from October 2014 to may 2015 with the objective to assess the bacteriological milk quality, possible hygienic factors and status of *S. aureus* as contamination of bovine raw milk. The study employed questionnaire survey and raw bacteriological load analysis and cow milk samples for isolation and detection of *S. aureus* from raw cow milk. Sixty (60) randomly selected dairy farms were interviewed for the survey-based study of farm hygienic practices and 72 raw milk samples [60 from directly from teats and 12 from collecting tanks (buckets) were aseptically collected and tested for bacteriological load analysis and isolation of *S. aureus*.

Results: The overall average total bacterial count (TBC) were $4.59 \pm 0.118\log_{10} (38,904.51 \text{ cfu/ml})$ and $4.77 \pm 0.23\log_{10} (58,884.37 \text{ cfu/ml})$ for milk samples collected directly from teat during milking and milking buckets at farm level respectively. Accordingly, the count increased by $0.18 \pm 0.23 \log_{10} 0 \text{ or } 19,979.86 \text{ cfu/ml}$ (51.36%) increase from teat to milking buckets. Results showed very significant differences in plate counts (P < 0.05) between the two milk collection points. 73.30% of the milk samples collected directly from the teat were found (>100,000 bacteria per ml), evidence of poor milk hygiene when compared to international standards. In this study hygienic and management factors like udder cleaning, water and soap using for cleaning of udder, hand washing and water and soap using for milking vessels were significantly (P < 0.05) affects the bacteriological count of the milk.

Conclusions: The results of the current study indicated that the cow milk produced and distributed in the study area can generally be considered as substandard in quality for consumption unless pasteurized. Therefore, this risk assessment study with similar different studies reported from different regions in Ethiopia might provide a foundation for the establishment of national milk quality standards that currently do not exist in Ethiopia.

Keywords: Bacteriological quality, Milk hygiene, S. aureus, Total plate count

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Background

Livestock represents major national resources and form an integral part of agricultural production system in Ethiopia (Gebrewold et al. 2000); cows contribute about 95% of the total annual milk produced by dairy cows, goats and camels at national level (CSA 2010).

In Ethiopia, milk production systems can be categorized into urban, peri-urban and rural, based on location (Reda 1998). Dairying constitutes an important sector of the agricultural production system. For smallholder farmers, dairying provides the opportunity to efficient use land, labour and feed resources and generates regular income in Ethiopia (Yitaye et al. 2009). In sub Saharan countries the traditional dairy sector, which is characterized by small herd size dominated by indigenous zebu breeds. These breeds, normally known by their low milk production with very little or no-specialized inputs, accounts 70–80% of Africa's cattle population (Ibrahim and Olaluku 2000).

In Ethiopia around 97% of the annual milk production is accounted by the traditional milk processing system using on-farm traditional milk processing materials (Felleke 2003), which is likewise dominated by indigenous breeds. In almost all areas in Ethiopia, the milk produced are traditionally processed to naturally fermented sour whole milk (ergo), traditional butter (Kibe), butter milk (Arera), cottage cheese (ayib), whey (aguat) and ghee (nitir kibe) dairy products. The traditional milk processing materials used are also similar among different areas which generally poor in quality of processing, includes; plastic container, Bottle gourd (Lagenaria siceraria) and clay pot (Duguma & Janssens 2014; Wafula et al. 2016). Most of the very few enterprises currently operating in and around the capital entirely depend on the traditional sector for their milk intake, while others depend on it for the majority of their intake. These underscore the importance of understanding the traditional sector in order to make improvement interventions. Economically, in Ethiopia Milk and milk products are also very important farm commodities and dairy farming is an investment option for smallholder farmers (Tsehay 2001).

Microbial load is a major factor in determining milk quality (Fatine et al. 2012). It indicates the hygienic level exercised during milking, cleanliness of the milk utensils, condition of storage, manner of transport as well as the cleanliness of the udder of the individual animals. Milk from a healthy udder contains few bacteria but it picks up many bacteria from the time it leaves the teat of the cow until it is used for further processing. These microorganisms are indicators of both manner of handling milk from milking till consumption and the quality of the milk (Lunder and Brehne 1996).

In Ethiopia, the fresh milk is sold unpasteurized to the public either directly from small producers, via informal markets or through dairy farmers cooperatives. This informal marketing system has been a challenge for milk quality control in urban and peri-urban areas at all levels (Godefay and Molla 2000). Awareness and resources aiding for hygienic milk production, storage, and transportation are very limited, especially smallholder production system is under developed when compared with the institutional and urban producers in the in and around Gondar.

The consumption of raw milk, naturally sour/fermented milk (Erego)) and its derivatives is common in Ethiopia (Yilma 2012), which causes for harbouring of milk-transmitted zoonoses, including bovine tuberculosis (bTB), brucellosis and Staphylococcal Food Poisoning (SFP). Raw or processed milk is a well know food medium that supports the growth of several microbes with resultant spoilage of the product or infections (intoxications) in consumers (Oliver et al. 2009).

Staphylococcal Food Poisoning (SFP) is among the most prevalent causes of gastroenteritis worldwide (Wang et al. 2007; European Food Safety Authority 2010; CDC 2016). *S. aureus* has many potential virulence factors and staphylococcus enterotoxin (SE) is one of among several responsible for food poisoning. Ingestion of less than 1.0 μ g enterotoxin causes SFP (Seo and Bohach 2007; Enquebaher et al. 2015). To date, more than 21 different SEs and SE-like super-antigens have been identified and designated classical as enterotoxins SEA/SEB/SEC/SED/ SEE (SEA-SEE), new (SEG-SEI) and new (SEIJ-SEIV) (Bennett and Hait 2011; Hennekinne et al. 2011).

In developing countries like Ethiopia, where high prevalence of clinical and subclinical mastitis mainly caused by S. aureus (Abera et al. 2010; Sori 2011) and high consumption of raw milk is common (Makita et al. 2012), Staphylococcal Food Poisoning (SFP) the most important target for study as risk of milk borne contaminations. Therefore, in order to protect consumers from unhygienic milk consumption and consequently expose to microbial contamination, it was found for us very important to study bacterial load and level of pathogenic microbes such as S. aureus in the milk production and collection. Such surveillance data may provide a basis for risk assessment study as well as give a foundation for the establishment of national milk quality standards that currently do not exist in Ethiopia. Based on these, the present study has been designed to give base line data for bacteriological quality of raw cow milk and status of S. aureus as raw milk contaminant in Northern Gondar, especially in and around the Gondar town.

Methods

Study area, study population and design

The study was conducted in and around Gondar town, which is located 740Km away North of Addis Ababa, the capital of Ethiopia. The town of Gondar is found at latitude of 12 °4'North, longitude of 27°2'east with an altitude of 1800–2500 m above sea level. The annual mean temperature of the area was 20.5 °C (17.2–23.9 °C) and annual rainfall of about 1000 mm (600-1400 mm). The region receives a bimodal rain fall, the average annual precipitation rate being 1000 that comes from the long and short rainy seasons. The short rainy season occurs during March, April, and May, while the long one extends from June through September (CSA 2010).

A cross sectional study was done from October 2014 to may 2015, in and around Gondar town by taking raw milk samples from lactating cows of selected dairy farms directly from teats during milking and milking buckets at farm level. The milk samples were collected from representative cows from each farm for milk samples collected directly from teat. The cow representing one farm was selected by simple random sampling method so that each farm supplying the milk to the local communities have representative.

The study subjects were milk samples collected from teat during milking and milking buckets at farm level and also questionnaire survey on milking personnel and farm attendants. In this study a total of 72 milk samples and 60 personnel for interview were included. The milk samples were collected from 60 farms selected from the sample frame by simple random sampling method and University of Gondar dairy farm included purposively. Two milk collection points (teat during milking and milking buckets at farm level) were considered for bacterial milk load and *Staphylococcus aureus* load too.

The sampling frame for farms selection was taken from agriculture office for Gondar Towns and its surroundings, mainly the members of Lame Bora milk producers association and institutional big farm (University Gondar dairy farm).

Questionnaire survey

Semi-structured questionnaires were used to assess the hygienic practices of dairy farms. Around 60 milking personnel and farm attendants related to the selected farms were interviewed. Consequently, hygienic practices employed in the study farms such as house cleaning, udder cleaning, hand washing practices and milking utensils and collecting vessels (buckets) hygiene and other conditions thought to affect the hygienic quality of raw milk were assessed.

Collection of samples and handling procedures

During sampling of raw milk directly from teats, the udder and teats were cleaned and dried before sampling; each teat end was scrubbed gently with cotton swabs moistened with 70% ethyl alcohol. The first 3–4 streams of milk were discarded, and approximately 10 ml of milk was collected into sterile sampling bottles. Each specimen

was labelled and placed in ice box and transported to Veterinary microbiology laboratory, University of Gondar, Faculty of Veterinary Medicine. After arrival at the laboratory, samples were preserved in refrigerator at +4 °C temporarily for 24 h for processing.

Analysis for bacterial load and detection of Staphylococcus aureus

The raw milk samples were assessed for bacteriological quality using the standard plate count. Total bacteria count was carried out by inoculation of serially diluted milk samples on standard plate count agar (Oxoid, England) and mannitol salt agar (Oxoid, England). All the samples positive for presumptive *S. aureus* contaminations on mannitol salt agar (Oxoid, England) were confirmed using Gram's staining, cultural and biochemical examinations.

Standard plate count

1 ml from each sample of raw milk was transferred to 9 ml sterile distilled water (10%) and thoroughly mixed to give 1:10 dilution. Serial dilutions were made by transferring 1 ml of the previous dilution in 9 ml of sterile distilled water up to 1:10,000 dilutions. Then only 0.1 ml sample from each dilution level was cultured by a glass spread method to the standard plate count agar (Oxoid, England). Total Bacterial Count was made by incubating cultured dilutions of milk samples on Plate Count Agar (Oxoid, England) plates. Colonies were counted after the culture media was incubated at 37 °C for 24 h. Total number of colonies on plates 25 to 250 per plates was selected and colonies were counted (Weldaragay et al. 2012).

Detection of Staphylococcus aureus

Serial dilutions method for total count on plate count agar also followed on Mannitol salt agar (Oxoid, England) for presumptive S. aureus load count. The presumptively identified S. aureus from mannitol salt Agar were subcultured to nutrient agar plate and after 24 h culture colonies of S. aureus was picked by bacteriological loop and placed on clean slide with a small drop of distilled water and emulsified. The test suspension was treated with a drop of rabbit plasma and mixed well with a needle for 5-10 s. Those forming Clumping of cocci were taken as positive (Quinn et al. 2002). Finally, slide coagulase positive samples were cultured on Purple agar base (PAB) (Difco, France) with the addition of 1% maltose and incubated at 37 °C for 24-48 h. The identification was based on the fact that S. aureus rapidly ferment maltose with in 24 h and the acid metabolic products cause the pH indicator (bromocresol purple) to change the medium and colonies to yellow. The rapid fermentation (24 h) was considered as S. aureus isolates (Quinn et al. 2002).

Data management and analysis

The data were entered into excel spread sheet and analysed using a statistical software (SPSS version 20.0). The Log10 transformation of bacterial count was done, before the analysis. Percentages were also used to assess the general farm activities and hygienic practices and to express the proportion of bacterial isolation and milk quality grade based on Indian standards, because we don't have an Ethiopian standard and difficult to follow European and USA standards. Analyzing the effects of hygienic practices on bacteriological count of the milk was also performed by linear Regression analysis. The differences in bacterial load between the raw milk directly from teats and milking buckets were compared by Mean [±S.E] log10 cfu/ml values between the two collecting points. The results were reported as significant for p < 0.05.

Results

Questionnaire survey Back grounds of the farms

Around 60% of the farms were managed under intensive production systems which have milking cows that ranged from 1 to 28 in number. 8.3% of respondents owned <5 head of cows; 51.70% had 5–10 cattle and 40% of respondents had >10 milking cows. All respondents (100%) milk their cows twice a day -early in the morning and at evening. In the study 48.3% of the farms have separate milking barns and 43.3% have continuous water supply for hygiene of floor and equipments. House cleaning intervals of the farms were variable; 88.3% of them clean more than twice per week (Table 1, Fig. 1).

Small holders management and hygienic practices

The study also showed that 75% of respondents' washes the udder before milking and 68.3% washes their hands before milking. Cleaning was performed in all farms using tap water only, while 28.3% of the respondents use towel to dry udder after washing. The cleaning agents used for milking utensils and collecting tanks (buckets) were only in 98.3% of the respondents was water and soap (Table 1).

Linear regression analysis for hygienic Vs milk quality assessment

The result shows the independent variables Udder cleaning, Water and soap using for cleaning of udder, hand washing and Water and soap using for milking vessels were significantly (P < 0.05) affects the bacteriological count of the milk.

| Table 1 Small holder's d | airy production | n system, managem | ient |
|--------------------------|-----------------|-------------------|------|
| and milking practices | | | |

| Hygienic practices frequenc | Frequency (N = 60) | Percentage (in %) | |
|---|---------------------------|----------------------|--------|
| House cleaning interval | Twice a week | 7 | 11.70 |
| | More than twice a week | 53 | 88.30 |
| Udder cleaning | Yes | 45 | 75.00 |
| | No | 15 | 25.00 |
| Cleaning agent for udder | Water | 60 | 100.00 |
| | Water and soap | 0 | 0.00 |
| Towel for drying udder | Yes | 17 | 28.30 |
| | No | 43 | 71.70 |
| Hand washing | Yes | 41 | 68.30 |
| | No | 19 | 31.70 |
| Cleaning agent for | Water | 35 | 58.30 |
| hand wash | Water and soap | 25 | 41.70 |
| Cleaning agent for milking | Water | 1 | 1.70 |
| utensils and collecting tankes (Buckets) | Water and soap | 59 | 98.30 |

N total number of samples, % percentage

Buckets: used as milking utensils in larger farms where tanks used for collection; used as collecting vessels where small no of cows milked

Bacteriological analysis

Tables 2 and 3 Standard Plate Counts (SPC): Total bacterial count and *S. aureus* isolates count from Raw Milk collected directly from teat and milking buckets.

The mean ± standard error for standard plate counts [expressed in log 10 cfu/ml] of raw milk sampled directly from teat during milking and milking buckets at farm level are shown in Table 4. The overall average total bacterial count (TBC) were $4.59 \pm 0.118\log 10$ (38,904.51 cfu/ml) and $4.77 \pm 0.23\log 10$ (58,884.37 cfu/ml) for milk samples collected directly from teat during milking and milking buckets at farm level respectively. Accordingly, the count increased by $0.18 \pm 0.23 \log 10$ or 19,979.86 cfu/ml (51.36%) increase from teat to milking buckets. Results showed very significant differences in plate counts (P < 0.05) between each milk collection points.

There was significant (P < 0.05) milk contamination from direct teat collection to milking buckets (Table 4).

Discussion

Milk is virtually a sterile fluid when secreted into alveoli of udder. However, post-harvest handling like the milking personnel and milk handling containers might generally be source of microbial contamination for raw milk, the three main sources of bacterial contamination; within the udder, exterior to the udder from the surface of teats, milk handling and storage equipments (Abate et al. 2015: Reta et al. 2016).



On average, aseptically drawn milk from healthy udders contains between 500 and 1000 bacteria per ml. But in our study only 5% of individual cows sampled directly from teat had the total bacterial count (TBC) of <1000 cfu/ml which indicates microbiological quality of the raw milk was very poor when compared with Theodore et al. (2016), which reported 95% of the cow milk with TBC <1000 cfu/ml from western Zambia. According to European milk bacteriology standards and USA legal limits for milk collected on the farm level (<100,000 cfu/ml) only 26.70% of the samples can fit this standard. But 73.30% of the milk samples were found high initial counts (>100,000 bacteria per ml), evidence of poor milk hygiene when compared to international standards.

In this study udder milk, had a better bacteriological quality because it was not subjected to further contamination after milking. The milk produced under hygienic conditions from healthy cows should not contain more than 4.7 log10 cfu/ml (O'Connor 1994). The current study revealed mean bacterial counts lower than this standard in which the mean \pm standard error (SE) bacterial count was 4.59 ± 0.12 log10 cfu/ml from milk collected directly from teat and 4.77 + 0.23 log10 cfu/ml in

Table 2 Linear Regression analysis for effect of hygienic practices of farms on bacteriological count of the milk

| Hygienic practices | | Freq. (No = 60) | Percentage | <i>p</i> -value | 95.0% Cl | |
|--|----------------|-----------------|------------|-----------------|----------|--------|
| | | | | | Lower | Upper |
| House cleaning interval | Twice a week | 7 | 11.70 | 0.253 | -1.153 | 0.309 |
| | > twice a week | 53 | 88.30 | | | |
| Udder cleaning | Yes | 45 | 75.00 | 0.050 | -1.059 | 0.002 |
| | No | 15 | 25.00 | | | |
| Cleaning agent for udder | Water | 60 | 100.00 | 0.050 | -1.059 | 0.002 |
| | Water and soap | 0 | 0.00 | | | |
| Towel for drying udder | Yes | 17 | 28.30 | 0.308 | -0.791 | 0.254 |
| | No | 43 | 71.70 | | | |
| Hand washing | Yes | 41 | 68.30 | 0.039 | -1.010 | -0.026 |
| | No | 19 | 31.70 | | | |
| Cleaning agent for hand wash | Water | 35 | 58.30 | 0.202 | -3.009 | 0.649 |
| | Water and soap | 25 | 41.70 | | | |
| Cleaning agent for milking utensils and collecting tanks (Buckets) | Water | 1 | 1.70 | 0.001 | -0.963 | -0.250 |
| | Water and soap | 59 | 98.30 | | | |

^aN total number of samples, % percentage

^bCl confidence interval

| Sample No. | Bacterial load count (CFU/ml) | S. aureus (CFU/ml) | Sample No. | Bacterial load count (CFU/ml) | S. aureus (CFU/ml) | |
|---------------------------------------|-------------------------------|----------------------|---------------------------------------|-------------------------------|----------------------|--|
| Raw Milk collected directly from teat | | | Raw Milk collected directly from teat | | | |
| 1 | 3.36×10 ⁴ | 0 | 38 | 1.60×10 ⁵ | 7.56×10 ² | |
| 2 | 2.74×10 ⁴ | 0 | 39 | 2.03×10 ⁵ | 0 | |
| 3 | 2.10 ×10 ⁴ | 3.4×10 ² | 40 | 4.10×10 ⁴ | 0 | |
| 4 | 2.25 ×10 ² | 0 | 41 | 1.20×10 ⁴ | 0 | |
| 5 | 6.20×10 ³ | 0 | 42 | 8.00×10 ³ | 0 | |
| б | 6.40×10 ⁴ | 2.41×10 ² | 43 | 5.80×10 ³ | 0 | |
| 7 | 2.48×10 ⁵ | 0 | 44 | 2.60×10 ⁴ | 0 | |
| 8 | 7.20×10 ⁴ | 0 | 45 | 9.40×10 ⁵ | 0 | |
| 9 | 2.55×10 ⁶ | 5.00×10 ² | 46 | 2.09×10 ⁶ | 0 | |
| 10 | 2.25 ×10 ⁵ | 0 | 47 | 4.17×10 ⁵ | 2.20×10 | |
| 11 | 7.04×10 ⁵ | 2.50×10 | 48 | 4.86×10 ⁴ | 0 | |
| 12 | 6.49×10 ⁵ | 0 | 49 | 1.37×10 ⁴ | 1.75×10 ² | |
| 13 | 1.84×10 ⁴ | 0 | 50 | 3.00×10 ³ | 0 | |
| 14 | 1.05×10 ⁶ | 3.45×10 ² | 51 | 3.40×10 ⁴ | 0 | |
| 15 | 5.40×10 ⁴ | 8.6×10 | 52 | 7.05×10 ⁴ | 0 | |
| 16 | 4.00×10 ³ | 0 | 53 | 4.30×10 ⁴ | 0 | |
| 17 | 2.30×10 ⁴ | 0 | 54 | 4.20×10 ³ | 0 | |
| 18 | 6.50×10 ³ | 0 | 55 | 8.27×10 ⁴ | 0 | |
| 19 | 2.38×10 ⁶ | 0 | 56 | 4.00×10 ⁵ | 2.50×10 ² | |
| 20 | 4.05×10 ⁴ | 0 | 57 | 2.05×10 ⁴ | 0 | |
| 21 | 9.70×10 ⁴ | 0 | 58 | 3.52×10 ³ | 0 | |
| 22 | 5.94×10 ⁴ | 0 | 59 | 2.45×10 ³ | 0 | |
| 23 | 2.62×10 ⁵ | 0 | 60 | 5.63×10 ⁴ | 0 | |
| 24 | 3.95×10 ⁵ | 0 | Milking bucke | ts | | |
| 25 | 4.92×10 ⁵ | 0 | 61 | 7.10x10 ³ | | |
| 26 | 5.38 ×10 ⁴ | 0 | 62 | 1.17x10 ⁴ | | |
| 27 | 2.09 ×10 ⁴ | 0 | 63 | 2.39x10 ⁵ | | |
| 28 | 1.28×10 ⁴ | 0 | 64 | 2.67x10 ⁴ | 5.25x10 ² | |
| 29 | 9.60×10 ³ | 0 | 65 | 5.10×10 ³ | | |
| 30 | 7.20×10 ² | 0 | 66 | 1.46x10 ⁵ | | |
| 31 | 2.60×10 ³ | 2.76×10 ² | 67 | 5.80x10 ⁴ | 3.12x10 ² | |
| 32 | 1.36×10 ⁴ | 0 | 68 | 4.6x10 ⁴ | | |
| 33 | 1.20×10 ² | 0 | 69 | 2.7x10 ⁶ | 2.3x10 ² | |
| 34 | 3.00×10 ³ | 0 | 70 | 3.1x10 ⁵ | | |
| 35 | 6.22×10 ³ | 0 | 71 | 3.08x10 ⁵ | | |
| 36 | 8.20×10 ³ | 0 | 72 | 6.4x10 ⁴ | | |
| 37 | 6.80×10 ³ | 0 | | | | |

Table 3 Standard Plate Counts (SPC): Total bacterial count and S. aureus isolates count from Raw Milk collected directly from teat and milking buckets

milk collected from milking buckets slightly higher than the standard given. This result is also much lower than the findings of Worku et al. (2012) and Yilma (2012) about 7.59 log10 cfu/ml and 8.87log10 cfu/ml respectively. Even if the means of bacterial counts seem to be lower than the standards given, more than 42% from all samples were found to have greater log10 bacteria counts than the standard stated.

In this study, an increase in the bacterial counts between the two milk collection points which indicates

| Milk collection points | N MIN | MIN | MIN Max | Mean[±S.E] log10 cfu/ml | Log10 increment | Df | 95% CI for mean | | P-value |
|------------------------|-------|-------|---------|-------------------------|-----------------|-------|-----------------|-------|---------|
| | | | | | | lower | upper | | |
| Directly from teat | 60 | 2.079 | 6.407 | 4.5907 [0.117] | 0.18 [0.23] | 1 | 4.352 | 4.832 | 0.011 |
| Milking buckets | 12 | 3.707 | 6.436 | 4.7706 [0.232] | | | 4.369 | 5.206 | |

 Table 4 Descriptive statistics for the standard plate count between two points

Total numbers of samples (N); maximum count (MAX) vs. minimum (MIN) log10 cfu/ml plate counts

Mean [±S.E] log10 cfu/ml = log 10 of colony-forming unit (CFU) above or below standard error (±S.E) in one millilitre of milk sample

Df degree of freedom, I confidence interval

decreasing of the hygienic conditions between milk collection points. Based on the linear regression analysis which was performed to investigate whether certain identified factors i.e., farmers' hygienic practices contributed to the bacteriological quality of the milk or total bacterial counts from raw milk directly from teats and collecting buckets. Udder cleaning, Water and soap using for cleaning of udder, hand washing and Water and soap using for milking vessels were found to be significantly (P < 0.05) affecting the standard plate counts. This is in agreement with the study up on the hygiene measures on raw milk by Abdalla and Elhagaz (2011) in Khartoum state, Sudan who showed that there was a significant effect on application of hygiene practices prior to milking in total bacterial count. Generally, this implies that the sanitary conditions in which milk has been produced and handled are substandard subjecting the product to microbial contamination and multiplication due to lack of and improper cooling systems at milk vending area. It is indicated that total bacterial count is a good indicator for monitoring the sanitary conditions practiced during production, collection, and handling of raw milk (Fatine et al. 2012).

In developing countries like Ethiopia, where high prevalence of clinical and subclinical mastitis mainly caused by S. aureus (Sori 2011) and high consumption of raw milk is common, Staphylococcal Food Poisoning (SFP) the most important target for study as risk of milk borne contaminations. Now days, it is not uncommon to here an extensive outbreak of staphylococcal food poisoning reports from both developed and developing nations from raw milk, powdered skim milk and reconstituted milks (Asao et al. 2003; Ikeda et al. 2005 and Johler et al. 2015). But in Ethiopia SFP outbreak investigations, identification of the causative strain are challenging and scarce data/or information available to estimate its magnitude may be due to limited commercial kits available for diagnosis of causative strains and of enterotoxins (SEs) and week disease outbreak investigation capacity.

Out of 60 samples of raw milk collected directly from teat and 12 collected from milking buckets, 18.33 and 25% were contaminated by *Staphylococcus aureus* respectively, with averages varying between 2.20×10 to 7.56×10^2 cfu/mL, as shown in Table 3. The result is higher than the figure studied by Worku et al. (2012), which was only 7. 29%.

Other lower results were also reported by Shunda et al. (2013) in which about 13.3% of samples were positive for *S. aureus*. According to Wallace (2009), even if the presence *S. aureus* in milk is known to cause spoilage of raw milk, it is not thought to be a frequent contributor to total collecting buckets counts and also he found that this organism is mainly associated with contagious mastitis.

Equipment used for milking, collecting and storage determine the quality of milk and milk products. The use of plastic, tins and traditional containers (clay pots and Bottle gourd) are the dominants in most part of Ethiopia which can be a potential source for the contamination of milk by bacteria, because these allow the multiplication of bacteria on milk contact surfaces during the milking process and their difficult nature for cleaning is also very crucial for contamination of milk. The result in this study also confirmed that 73.30% farmers use plastic containers for milking and collecting milk which can be compared with findings of Abate et al. (2015) which showed over 60% of farms used plastic containers and 40% used pots for milking and collecting milk. Higher figures were also reported by Yilma (2012) in which 81% use plastics the remaining 3.4 and 6.6% used tins and pots respectively.

Maintaining the sanitary condition of milking area is important for the production of good quality milk. The current study showed that about 88.3% of the farms clean the house more than twice per week usually on daily bases but 11.7% of the farms clean the barn twice per week due to shortage of water. Other study Yilma (2012) in Addis Ababa, reported that about 87% of the respondents cleaned their barn on daily basis, while few (9%) of them cleaned only once or twice a week. Contrary to this study Abebe et al. (2012) showed low proportion (47%) of the respondents cleaned the barn three times a week, while 39% cleaned two times and only 11.7% of them reported to clean daily Abate et al. (2015) also report more than 90% farms cleaned their houses once daily.

The study also shows 75% of respondents did not use udder washing before milking and only about 25% of respondents had washed the udder before milking reports. Contrary to the current findings of Weldaragay et al. (2012) in Hawasa reported that >80% households practicing pre milking udder washing (FSA [Food Standards Agency] 2006) reported cleaning of the udder before milking is important to remove both visible dirt and bacteria from the outer surface of the udder and to minimize contamination and produce good quality milk. Cleaning agent used for cleaning the udder in this study was only water with no any detergents. This result has an agreement with the study in Shashemene by Gemechu et al.(2014) in which most of the farms didn't use detergents for cleaning udder but only 2% reported by (Abate et al. 2015).

In this study about 71.1% of farms participated in the survey didn't use separate towels almost similar to the figures (71.79 and 71.0%) found by Gemechu et al. (2014). Hand washing practice before milking of cows in the current study is assessed to be about 68.3% which is not satisfactory with respect to keep the quality of milk. This result is lower than the reports in Jimma (>94%) by Yilma (2012). Most of (98.3%) of the dairy cow owners used water and detergent for cleaning milk handling equipment which is in agreement with the reports of Weldaragay et al. (2012).

Conclusions

The hygienic conditions of the farms studied in Gondar town can be judged as poor, in which most of the farm hygienic practices and parameters like hygienic condition of the milking environment, sanitation of the milk containers, udder and teats cleaning, use of separate towel for each cow and the personal hygiene of the milkers were not fully performed by most of the farm owners. Even if the mean bacterial counts seem to be fair according to the standard, the high proportion of sample having total bacterial counts higher than the USA maximum legal limit (>1.00×10⁵cfu/ml) indicate that the quality of milk produced in the study area had unacceptable levels of contamination with microorganisms that profoundly increase across the milk collection points. This risk assessment study with similar different studies in different regions in Ethiopia might provide a foundation for the establishment of national milk guality standards that currently do not exist in Ethiopia. For more, milk safety increment small scale pasteurization with continuous hygienic education for the farmers should be focused.

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Authors' contributions

ST carried out the conception of the research concept and designed the methodology, data analysis and interpretation and preparation of the manuscript for publication. BT carried out the laboratory work, sample collection and revision of the manuscript. Both authors read and approved the final manuscript.

Competing interests

The authors declare that there is no financial or non-financial competing interest from anybody or institute. We also want to assure that we did not receive any technical assistant in developing the research concept or preparation of the manuscript.

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