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Original Article

Assessment of microbial and physicochemical qualities of cow milk in selected districts of Borena Zone, Oromia Regional State, Ethiopia

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ABSTRACT

This research study was initiated to evaluate the microbial and physicochemical qualities of randomly selected cow milk samples in three districts of Borana Zone (Gomole, Elwaye and Dubluk). The microbial and physicochemical analyses were performed following standard protocols. Milk samples collected from Elwaye Town showed the maximum standard plate and total coliform count values of 8.5 log10 CFU mL^{-1}, and faecal coliforms have been detected in 98% of the samples across the three districts. The maximum mean counts of Salmonella (mean prevalence: 39.9%) and Shigella (mean prevalence: 95.8%) of 5.5 (Elwaye) and 8.4 (Elwaye & Dubluk) log10 CFU mL^{-1}, respectively, were obtained showing potential faecal contamination of milk. Yeast and mold test (100% prevalence) revealed that Gomole District samples (4.8 log10 CFU mL^{-}1) were significantly higher (<0.0001) than samples of the other districts. In analysis for physicochemical parameters, fat (7.5%) (Dubluk), protein (3.4%) (Gomole and Elwaye), lactose (5.1) (Gomole, Elwaye & Dubluk), salts (0.8%) (Gomole and Elwaye), Solid-Not-Fat (9.2 -9.4) (Dubluk – Gomole) and 0.0% added water (all samples) were recorded; with fat, lactose and SNF being better as compared to earlier reports. In general, microbial population levels exceeded the set standard rendering it unsafe for raw consumption. Among the causes may be prevalence of a

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serious hygienic problem in the process of milk production and subsequent handling in the study areas expressed in terms of inadequate cleanliness of the milking personnel, milking materials, udder, teat and concern of health of the cows. Thus, the pastoral society should be given awareness through training regarding the general hygienic practices required and the health hazards associated with production and consumption of low standard cow milk.

Keywords: Cow milk, microbial quality, physicochemical quality

INTRODUCTION

Ethiopia is believed to have the largest livestock population in Africa (Tilahun and Schmidt, 2012; CSA, 2013), which is estimated to be about 54 million cattle. Cow milk is among the most important nutritional components in human diet. It is naturally a rich source of protein (3.2-3.4g/100g), fat (3.1-3.3g/100g), energy (247-274 kJ), water (87.3–88.1g/100gm), lactose (4.5-5.1g/100gm) and valuable vitamins (Vitamin A (μg RE): 61; E (mg): 0.08) and minerals like calcium (91-120mg/100g), potassium (132-155mg/100g), phosphorus (84-95mg/100g) and others (FAO, 2013). This property on the other hand makes milk and milk products an ideal media for the growth and proliferation of an array of microorganisms of various taxonomic origin with some of them being beneficial and others undesirable and also pathogenic (Fernandese, 2009). Microbiology of milk is among the principal indicators of its quality. Milk drawn from a healthy mammary gland may be contaminated by a number of microorganisms entering the udder through the teat duct. Gram-positive cocci, streptococci, staphylococci and micrococci, lactic acid bacteria (LAB), *Pseudomonas* spp., *corynebacteria* and yeast are most frequently found in milk drawn aseptically from the udder (Fernandese, 2009). In cases of infection of the udder (mastitis), large number of microorganisms can be shed into milk. In general, microbial contamination of raw milk can emanate from a variety of sources, *viz,* air, cleanliness of milking equipment, feed, soil, faeces and grass (Coorevits *et al*., 2008). Previous surveillance systems had reported that 2-6% of bacterial food-borne outbreaks are shared from milk and milk products (De Buyser *et al*., 2001). Some wild type molds, such as, *Aspergillus* are capable of producing mycotoxins (Aflatoxin B1, M1, G1) that are secondary metabolites with carcinogenic, teratogenic and mutagenic effects (Frisvad *et al*., 2005).

Milk production obtained from the subsistence farmers of Ethiopia contributes to about 98% of the annual milk production of the country. Moreover, dairy processing in the country is mostly restricted to the smallholder level which experience low hygienic quality (Alganesh *et al.*, 2009). Weldearegay *et al.* (2012) reported maximum values of 10.28, 6.52, and 7.13 log10 CFU of aerobic mesophilic bacteria, coliforms and yeasts and molds, respectively per gram of sample in their study of hygienic practices and microbiological quality of milk produced under different farm sizes in Hawassa city. Similarly, milk and milk products quality assessment in terms of microbiology from Debre Zeyit, Adama and Jimma areas reported that mean total bacterial count ranged from 8.3 to 10 Log CFU/mL. and the mean coliform count could reach 4 Log CFU/mL (Zelalem, 2009). These values far exceeded the quality standard level of $2.0X10^6$ CFU/mL (Kiiyukia, 2003) set previously.

On the other hand, various studies investigated the microbial population of yoghurt and confirmed that it was mainly dominated by lactic acid bacteria with *Lactobacillus casei* and/or *Lactobacillus plantarum* being the most prevalent inhabitants (Kassaye *et al.*, 1991). Mogessie (1995) had studied the microbial development of 'Ergo' formed from raw milk collected from different dairy farms of Hawassa city. The result showed the presence of lactic acid bacteria, coliforms and yeasts in different proportions. *Lactococcus, streptococcus, Leuconostoc and Lactobacillus* were identified as the culturing bacterial population in 'Ergo' as reported by Almaz *et al.,* (1999).

The microbial and physicochemical characteristics of milk and milk products obviously vary depending on the season of the year, agro-ecology, hygienic practices exercised, processing and storage conditions (Ali, 2011; Yilma, 2012), local cultural setting and so on. Moreover, most of the very few currently operating enterprises that process milk almost entirely depends on the traditional sector for the majority of their milk intake (Yilma, 2012). Lack of adequate information and facts mentioned above necessitate the need for periodic investigation into the quality status of sample milk and milk products produced in those areas. Thus, this research activity was initiated to assess the microbial quality and the physical and chemical characteristics of cow milk in the selected districts of Borena zone.

MATERIALS AND METHODS

Description of the study area

Borena zone is one of the zones in Oromia Regional State, Ethiopia, situated in the southern part of the region. It is bordered by Southern Nations and Nationalities and Peoples Region (SNNPR), Guji zone and Somali region to the West, North and East, respectively. This zone is characterized by arid and semiarid climate with an area of 50,000 km^2 (Homann *et al.*, 2008) having an average annual rainfall range of 350 to 900 mm. It possesses a bimodal rainfall type with 60% occurring between March and May as a main rainy season. The second peak appears between September and November (short rainy season) (Coppock, 1994).

The study was conducted in three districts (Gomole, Elwaye and Dubluk) of the zone in the year 2019/2020 covering areas with an altitudinal range of 1124 to 1659 masl. Two kebeles (the smallest administrative unit) were selected as sampling sites in each of the districts in consultation with the respective Regional Bureau and Zonal Office.

Sample collection

The study was conducted during the year 2019/20 in three purposively selected districts, namely, Gomole, Elwaye and Dubluk, Borena zone, Oromia Regional State, Ethiopia, following the procedure employed by Belli *et al.*, (2013). A total of 47 raw milk samples (Kela Qufa (10); Bildim (8); Elwaye town (8); Golba (8); Dambala Badana (7) and Bokosa Gudda (6)) were collected from randomly picked households. The samples (approximately 200 ml each) were collected aseptically and immediately placed in sterilized screw capped sampling bottles and put into an ice box (1-5 ^{o}C) to ensure the suppression of the growth and proliferation of microorganisms (ET ISO 707, 2012) and immediately transported to laboratory for analysis.

Microbiological analysis

Each of the collected raw milk samples were analysed for microbial quality in terms of standard plate count (SPC), total coliform count (CC), *Salmonella* count, *Shigella* count and yeast and mold count (YMC). Each analytical unit consisted of 25 ml of the product.

Standard plate count (SPC)

Samples were serially diluted in test tubes containing 1% peptone water up to 10^{-7} concentration and 1 mL of the dilution with appropriate concentration was transferred into an empty petri dish and 10-15 Ml of molten (45 ^{o}C) standard plate count agar (Himedia laboratories LTD) was poured onto the dishes containing the inoculums, in duplicate. The culture was incubated at 35$\pm$2 ^{o}C for 24 hrs and plates with acceptable number of colonies (30-300) were considered for enumeration using a colony counter (Richardson, 1985). The estimated number of colonies per mL of sample were calculated by:

$$\mathrm{N} = \frac{\Sigma C}{(1*n1)+(0.1*n2)} xd$$ (Maturin and Peeler, 2001)

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.

Total Coliform count (TCC)

The procedure followed in the SPC analysis was employed for specimen dilution. One mL of the samples was pour plated onto Violet Red Bile Agar (VRBA) (Himedia laboratories LTD) and incubated at 35$\pm$2 ^{o}C for 24 hrs. Following growth of the culture, confirmatory test was conducted to assure the identity of the coliforms. Four to five typical colonies were incubated further in tubes containing 2% Brilliant Green Lactose Bile at 35 °C for 48 hrs (Richardson, 1985).

Salmonella and Shigella count

Salmonella and *Shigella* are assumed to remain in viable but non-culturable (VBNC) state or in stressed condition in the environment (Olive, 2005; Rahman and Noor, 2012). Therefore, enrichment technique was employed for the isolation and identification of these bacteria. One ml sample was added to 9 ml selenite cysteine broth (SCB) (Difco Laboratories, Detroit, Mich.) and the suspension was incubated for 4 hrs at 37°C with shaking at 100 rpm (Rahman and Noor, 2012). Then a 10 fold serial dilution was prepared up to 10^{-7}. Thereafter, 0.1 mL from each of the enriched broth was spread over Salmonella-Shigella agar (SSA) and incubated at 37 **°C** for 12-24 hours. After incubation characteristic colonies were detected as follows and enumerated. The black centered colony on *Salmonella*/*Shigella* agar was considered as *Salmonella* spp. and the colorless colony on the same agar plate was noted as *Shigella* spp.

Yeast and mold count (YMC)

Samples were serially diluted following the procedure employed for SPC and TCC analysis and 0.1 mL of the diluted sample was plated using a pour plate technique on potato dextrose agar (PDA) (Himedia laboratories LTD). The medium was acidified using 50% lactic acid to prevent growth of contaminant bacteria. The plates were incubated at 25°C for 3-5 days (Yousef and Carlstrom, 2003).

Physicochemical analysis

Some physicochemical parameters such as fat, protein, lactose, solids-not-fat (SNF), added water, salts and density were analysed using Lactoscan (ultrasonic milk analyser, Milkotronic LTD).

Data management and statistical analysis

Microbial count from each district was transformed into logarithmic values (log 10 CFU mL^{-1}) and subjected to statistical analysis using SAS version 9.0 and SPSS version 20. Variability among the data was separated using Duncan Multiple Range Test (DMRT) at 0.05 α level of probability (in the case of district wise comparison). Means, maximum and minimum values were calculated in descriptive statistics.

RESULTS AND DISCUSSION

Microbial quality of milk samples

Standard plate count (SPC)

Standard plate count is an important microbial quality parameter meant to evaluate the hygienic status of a food product. Microbiological analysis showed that in Gomole district, the highest mean log10 CFU mL^{-1} value of 6.0 (range: 5.5 to 6.6) was recorded at Kela Qufa Kebele. Whereas samples collected from the second Kebele, Bildim, had a mean log10 CFU mL^{-1} value of 4.2 (range: 3.7 to 4.4) (Table 1). The finding in Kela Qufa kebele was in agreement with the result obtained by Hawaz *et al*., (2015) where the mean standard plate count in cow milk samples collected from 4 districts of Eastern Hararghe was 6.25 log10 CFU mL^{-1}. However, it was slightly lower than the findings of Teshome and Tesfaye (2016) in three districts of Bench Maji Zone (Southern Nations and Nationalities and Peoples (SNNP) Region) with mean total bacterial count of 7.1 log10 CFU mL^{-1} and 7.3 log10 CFU mL^{-1} in Dawa Chefa District of Amhara region (Solomon *et al*., 2015). Result of the current study showed that only 5.6% of the samples collected from the district had exceeded the East African Community Standards (EAS 67:2007) which is 2 x 10^6 CFU mL^{-1} (Table 7) with regard to SPC.

In Elwaye district, a slightly higher level of standard plate count was obtained. Samples collected from Town had the highest mean value of standard plate count of 8.5 log10 CFU mL^{-1} which was 2.0 log10 CFU mL^{-1} value higher than that of the second kebele, Golba (Table 1). The study made it clear that standard plate count exceeded the acceptable standard in almost 93.8% of the milk samples collected from this district rendering it unsafe for raw consumption. Equivalent magnitude of mean total bacterial loads (8.7 and 8.6 log10 CFU mL^{-1}) had been reported in selected Sub-Cities of Addis Ababa (Akaki, Nifas Silk Bole and Yeka) by Fufa *et al*. (2019) and Biruktawit (2016) (Akaki-Kality, Nifas Silk-Lafito and Bole), respectively. Moreover, Wanjala *et al*. (2017) had come across a mean value of 8.1 log10 CFU mL^{-1} in samples collected from Nairobi area.

The analysis of milk samples from Dambala Badana Kebele of Dubluk district had been found to show the mean log10 CFU mL^{-1} standard plate count value of 8.2 with nearly comparable mean value of 8.4 recorded from samples of Bokosa Gudda Kebele of the district (Table 1). Standard plate counts in all the samples from the district are out of acceptable range making the samples below standard for use without appropriate treatment. Reports of Fufa *et al*. (2019) and Biruktawit, (2016) had proved to be comparable to the mean values obtained in those two Kebeles of the district.

Among the three districts, the maximum mean log10 CFU mL^{-1} value of 8.3 was recorded in Dubluk which was significantly higher than that of Elwaye (7.5) which in turn had significant superiority over Gomole's samples (5.0 log10 CFU mL^{-1}) (Fig. 1).

These results were considerably lower as compared to 9.8 log10 CFU mL^{-1} obtained in Ezha District of Gurage Zone, Southern Ethiopia (Abebe *et al.*, 2012). The values of total viable count obtained from samples collected from Dubluk and Elwaye were found to surpass the standard. Whereas the milk samples collected from Gomole district showed a mean log10 CFU mL^{-1} value of 5.0 which was below the standard set value of $2.0x10^6$ CFU mL^{-1} (6.3 log10 CFU mL^{-1}) by East African Community Standards (EAS 67:2007) hence acceptable even for raw consumption. In general, 61.7% of the samples across the three districts have failed to qualify for safe consumption (Table 7).

Table 1. Standard plate count (mean ±SE) in milk samples (log10 CFU mL^{-1})

District	Kebele	n	Mean	Minimum	Maximum	P value	CV (%)
Gomole	Kela Qufa	10	6.0±0.155	5.5	6.6		6.3
	Bildim	8	4.2±0.090	3.7	4.4		6.1
Elwaye	Golba	8	6.5±0.045	6.3	6.6	<0.0001	2.0
	Town	8	8.5±0.039	8.3	8.6		1.3
Dubluk	Dambala Badana	6	8.2±0.374	7.4	8.6		4.5
	Bokosa Gudda	7	8.4±0.080	8.0	8.6		2.3
Overall mean			7.0±0.700	6.5±0.711	7.2±0.694		

Note: CV=coefficient of variability; SE=standard error

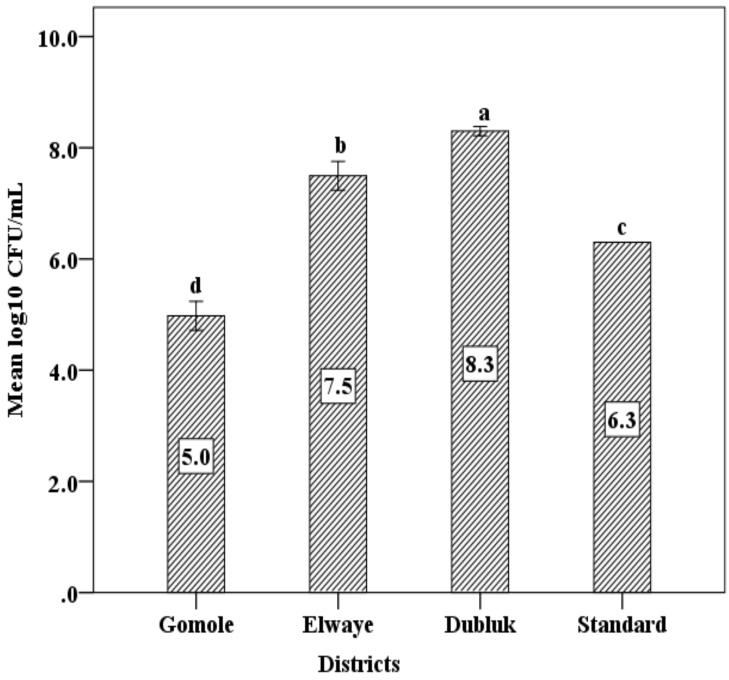

Figure 1. Standard plate count in milk samples across districts
Bars with the same letters are non-significantly different (p≤0.05)

Total Coliform Count

All the milk samples collected from across the three districts have been tested for the magnitude of indicator microorganisms, the coliforms. The presence of indicators in the food samples is a warning signal that there is a probability of occurrence of pathogenic species in the food lot and its magnitude helps in the decision making for whether the food and/or its products is/are microbiologically safe for consumption or not.

At Kela Qufa Kebele of Gomole District, the highest mean value of 6.0 log10 CFU mL^{-1} of total coliforms had been recorded. The average value obtained at this kebele was almost over 33% higher than that reported from the second Kebele of the same district, Bildim, with the mean log10 CFU mL^{-1} of 4.0. From the study in the district, it could be seen that 22.2% of the samples examined was significantly higher in terms of total coliforms than the recommended level of 5.0×10^4 CFU mL^{-1}. (Table 2).

The highest mean coliform count (8.5 log10 CFU mL^{-1}) was recorded in milk samples collected from Elwaye Town. Moreover, in Dambala Badana and Bokosa Gudda Kebeles of Dubluk District, TCCs were nearly equivalent to the highest value with mean log10 CFU mL^{-1} values of 8.3 and 8.4, respectively (Table 2). The coliform contamination level of samples in all the Kebeles except Bildim was found to be considerably higher as compared to several studies in Ethiopia. Asaminew and Eyassu (2011), Tadele *et al*., 2016, Teshome and Tesfaye (2016) and Fufa *et al*., (2019), had reported overall mean values of 4.5, 4.2, 5.1 and 4.9 log10 CFU mL^{-1}, respectively. In Tanzania and Bangladesh, Happy (2014) and Khaton *et al*., (2014) recorded mean total coliform counts of 6.45 and 2.7 log10 CFU mL^{-1}, respectively. In addition, in rural areas of Nairobi (Wanjala *et al*., 2017) and Banaskantha district of India (Nalwaya *et al*., 2018), comparatively lower total coliform counts of 4.56 and 3.47 log10 CFU mL^{-1}, respectively, were reported.

Comparison of the means of coliform counts for the three districts showed that the highest mean coliform count value of 8.4 log10 CFU mL^{-1} was obtained from samples of Dubluk district. Elwaye district took the second place with a mean log10 CFU mL^{-1} value of 7.2 which was significantly lower than that of Dubluk (Fig. 2). Coliform counts recorded from both of the districts were beyond the standard set. In this investigation, all samples (100%) from Elwaye and 92.3% from Dubluk Districts had TCC value significantly exceeding the recommended standard of 5.0×10^4 CFU mL^{-1} (4.699 log10 CFU mL^{-1}) (Table 7). However, a mean log10 CFU mL^{-1} value of 4.661 was reported from Gomole district, which was below the threshold level 4.669 log10 CFU mL^{-1} set by EAS (67:2007).

Table 2. Total coliform count (mean ±SE) in milk samples (log10 CFU mL^{-1})

District	Kebele	Mean	Minimum	Maximum	P value	CV (%)
	Kela Qufa	6.0±0.208	5.4	6.4	<0.0001	6.9
Gomole	Bildim	4.0±0.136	3.3	4.3		9.7
	Golba	6.0±0.062	5.7	6.3		2.0
Elwave	Town	8.5±0.048	8.3	8.7		1.6
	Dambala Badana	8.3±0.074	8.2	8.6		2.2
Dubluk	Bokosa Gudda	8.4±0.068	8.1	8.5		2.0
Overall mean		6.9±0.632	6.5±0.703	7.1±0.612		

CV=coefficient of variability; SE=standard error

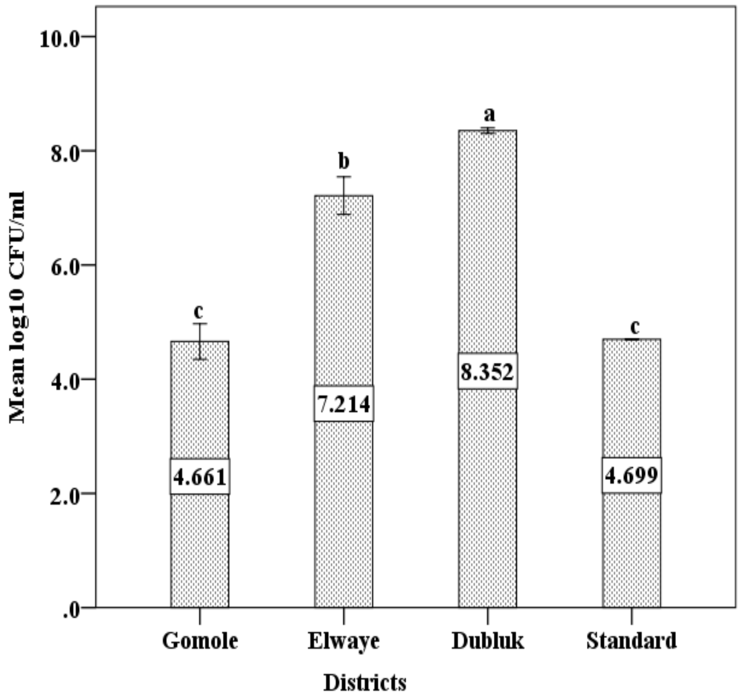

Figure 2. Total coliform count in milk samples across districts
Bars with the same letters are non-significantly different (p≤0.05)

Salmonella species count

Salmonella species, as one of the cow milk contaminants have been detected only in the two Kebeles of Gomole and Golba Kebele of Elwaye districts with an overall mean prevalence of 39.9 % (Table 6). In the rest of the sampling Kebeles, the cultures ranged from no growth (NG) to too few to count (TFTC) level (Table 3). Laboratory analysis revealed that samples from Dubluk district and Elwaye Town similarly ranged from NG to TFTC (Table 3). The highest mean log10 CFU mL^{-1} value of 5.5

was recorded at Golba Kebele, whereas, the lowest mean log10 value of 3.4 mL^{-1} was detected in milk samples from Bildim Kebele of Gomole district (Table 3). In general, Elwaye district samples had significantly higher log10 CFU mL^{-1} value (5.5) of *Salmonella* count as compared to the samples from Gomole (3.8 log10 CFU mL^{-1}) (Fig. 3). In the study conducted by Hawaz *et al*. (2015) at Harar milk shed of Eastern Ethiopia, a similar value of *Salmonella* (5.13 log10 CFU mL^{-1}) to that in the Golba Kebele was reported. However, in Nigeria Oladipo *et al*. (2016) obtained a little higher value (6.04 log10 CFU mL^{-1}).

Table 3. *Salmonella* count (mean ±SE) in milk samples (log10 CFU mL^{-1})

District	Kebele	n	Mean	Minimum	Maximum	P value ($\leq$0.05)	CV (%)
Gomole	Kela Qufa	10	5.3±0.148	5.1	5.6	0.0008	4.8
	Bildim	8	3.4±0.131	3.1	3.6	0.002	6.8
Elwaye	Golba	8	5.5±0.209	5.1	5.8	0.001	2.0
	Town	8	NG -TFTC	-	-	-	-
Dubluk	Dambala Badana	6	NG	-	-	-	-
	Bokosa Gudda	7	NG -TFTC	-	-	-	-
Overall mean			4.7±0.699	4.4±0.667	5.0±0.702		

NG-TFTC= no growth - too few to count; SE=standard error

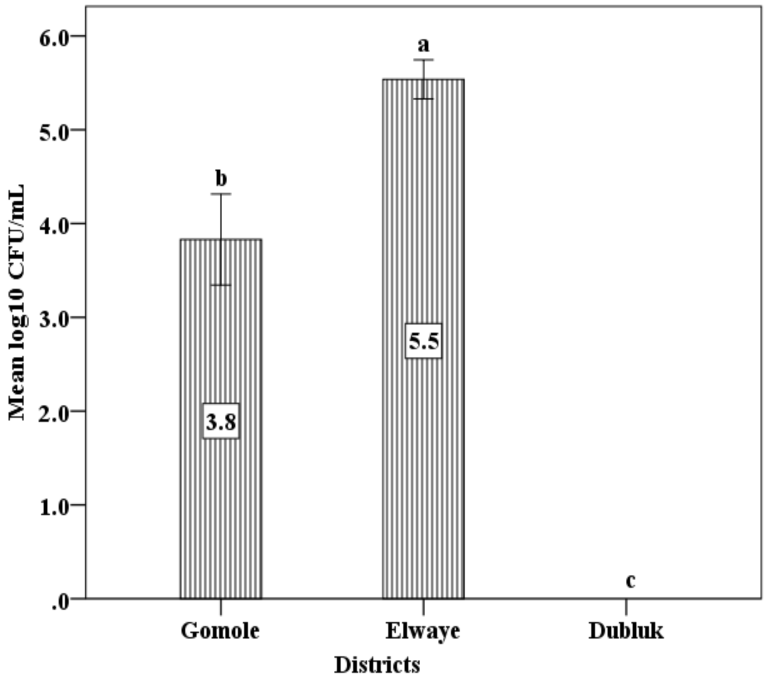

Figure 3. Salmonella species count in milk samples across districts
Bars with the same letters are non-significantly different (p$\leq$0.05)

***Shigella* species count**

Shigella species was one of the pathogenic bacteria for which the milk samples were tested as potential contaminants. The data revealed that the highest mean log10 CFU mL^{-1} (8.4) was recorded in samples collected from Elwaye Town and Dambala Badana Kebeles of Elwaye and Dubluk Districts, respectively (Table 4). Whereas, in Gomole District in Bildim Kebele, mean log10 CFU mL^{-1} of 3.8 was detected. The rest of the Kebeles in the three Districts had a mean log10 CFU mL^{-1} value above 5 (Table 4). Table 6 shows that approximately 95.8 % of the samples were contaminated by *Shigella* species. District wise, 7.5 log10 CFU mL^{-1} was recorded in Dubluk which was significantly higher than that obtained from Elwaye (7.3) and Gomole (5.0) (Fig. 4).

Table 4. *Shigella* count (mean ±SE) in milk samples (log10 CFU mL^{-1})

District	Kebele	Mean	Minimum	Maximum	P value	CV (%)
	Kela Qufa	5.4±0.075	5.2	5.9	<0.0001	4.1
Gomole	Bildim	3.8±0.132	3.5	3.9	0.0012	6.1
	Golba	6.0±0.052	5.8	6.2	<0.0001	2.3
Elwaye	Town	8.4±0.074	8.1	8.7	<0.0001	2.5
	Dambala Badana	8.4±0.127	8.2	8.9	<0.0001	3.4
Dubluk	Bokosa Gudda	7.6±0.146	7.2	8.0	<0.0001	4.7
Overall mean		6.6±0.755	6.3±0.752	6.9±0.794		

NG-TFTC= no growth - too few to count; SE=standard error

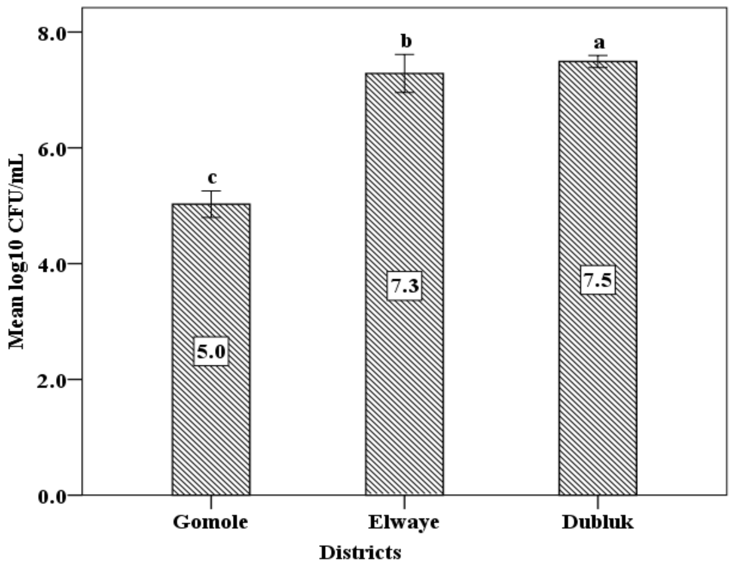

Figure 4. Shigella species count in milk samples across districts
Bars with the same letters are non-significantly different (p≤0.05)

Yeast and mold count

Milk samples from Bildim kebele of Gomole District exhibited the highest level of contamination by yeast and mold with the mean log10 CFU mL^{-1} value of 4.8. Kela Qufa Kebele from the same district had taken the second place in yeast and mold contamination with mean log10 CFU mL^{-1} value of 4.5 (Table 5). Samples from Kebeles of Elwaye and Dubluk had shown values ranging from 3.7 to 4.4. Among the districts thus, Gomole exhibited higher contamination (4.8 log10 CFU mL^{-1}) with significant difference from the rest of the districts (Fig. 5). In Menz district of Amhara Region, Lina *et al.*, (2018) recorded a higher overall mean yeast and mold count of 1.83 x 10^6 (6.3 log10 CFU mL^{-1}) in butter. Additionally, Teshome and Tesfaye (2016) found a comparatively lower overall mean value of yeast and mold counts (3.9 log10 CFU mL^{-1}). Higher yeast and mold count in a food product contributes to the spoilage hence shorter shelf life. Moreover, some molds are of critical public health concern due to their production of mycotoxins which may not be easily destroyed during processing including cooking (Wouters *et al.*, 2002). The current study revealed that all the milk samples from Kebeles in the districts exhibited a yeast and mold count that was higher than the acceptable limit set by the Malaysia food quality standard of yeast and mould in raw milk sample which should be below 2.1 log cfu/mL (Torkar and Vengust, 2008).

Table 5. Yeast and mold count (mean±SE) in milk samples (log10 CFU mL^{-1})

District	Kebele	Mean	Minimum	Maximum	P value (≤0.05)	CV (%)
Gomole	Kela Qufa	4.5±0.144	3.9	5.0		8.4
	Bildim	4.8±0.195	3.8	5.4		11.5
Elwaye	Golba	3.7±0.116	3.1	4.0		8.2
	Town	3.9±0.130	3.4	4.4	<0.0001	9.3
Dubluk	Dambala Badana	4.4±0.115	3.7	4.6		6.9
	Bokosa Gudda	4.2±0.095	3.9	4.6		5.5
Overall mean		4.3±0.165	3.6±0.131	4.7±0.198		

NG-TFTC= no growth - too few to count; SE=standard erro

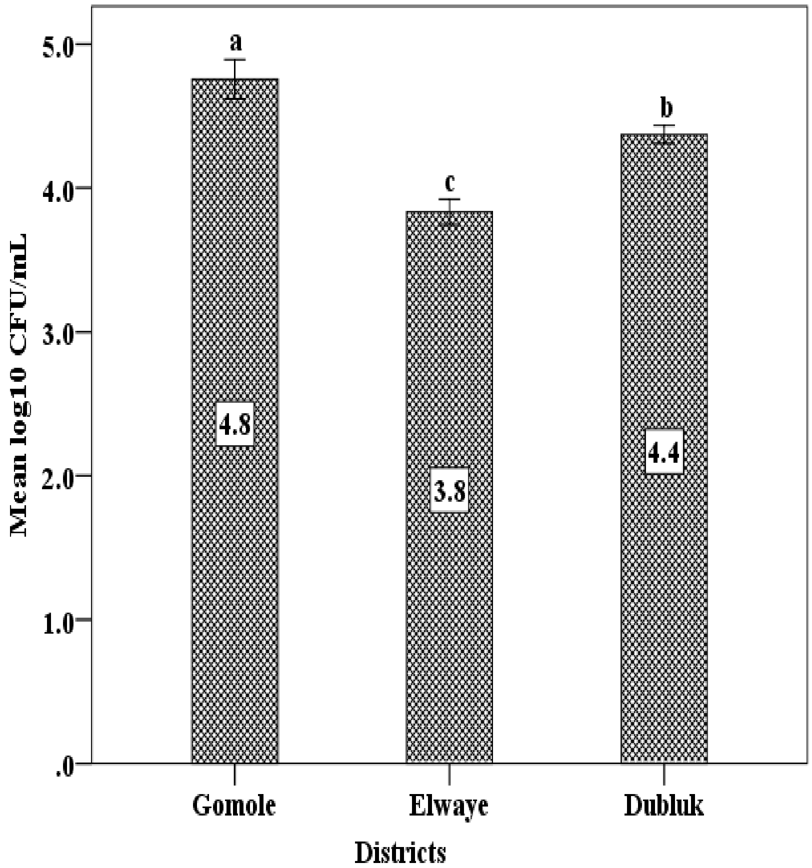

Figure 5. Yeast and mold count in milk samples across districts
Note: bars with the same letters are non-significantly different ($p \leq 0.05$)

Prevalence of the microbial contaminants in the milk samples

Total coliforms, and yeasts and mold were detected in all the samples collected from all kebeles of the three districts. In addition, faecal coliforms were noted in all samples except in Kela Qufa kebele in which a prevalence of 90% was recorded (Table 6). One the contrary, only 10, 16.7 and 37.5% of the samples were positive for *Salmonella* in Kela Qufa, Bokosa Gudda and Elwaye Town, respectively. Over eighty seven per cent *Salmonella* growth prevalence was reported in milk samples from Bildim and Golba of Gomole and Elwaye districts, respectively. *Shigella* species were noticed in all samples from kebeles except that 75% of the samples taken from Bildim were contaminated by this microorganism (Table 6).

Table 6. Prevalence (%) of microorganisms from different categories in milk samples

District	Kebele	n	Total coliform		Faecal coliform		*Salmonella* species		*Shigella* species		Yeast and mold	
			KM	DM	KM	DM	KM	DM	KM	DM	KM	DM
Gomole	Kela Qufa	10	100	100	90	95	10	48.8	100	87.5	100	100
	Bildim	10	100		100		87.5		75		100	
Elwaye	Golba	8	100	100	100	100	87.5	62.5	100	100	100	100
	Town	8	100		100		37.5		100		100	
Dubluk	Dambala Badana	7	100	100	100	100	0	16.7	100	100	100	100
	Bokosa Gudda	6	100		100		16.7		100		100	
Overall mean			**100**	**100**	**98.3**	**98.3**	**39.9**	**42.7**	**95.8**	**95.8**	**100**	**100**

DM=District Mean; KM=kebele mean; N=number

Table 7. Proportion of milk samples exceeding EAS (67:2007) standard in standard plate and total coliform counts

District	Number of samples analysed	Samples exceeding EAS in SPC (6.3 log10 CFU/mL)		Samples exceeding EAS in Total CC (4.70 log10 CFU/mL) (%)	
		Number of samples	Proportion (%)	Number of samples	Proportion (%)
Gomole	18	1	5.6	4	22.2
Elwaye	16	15	93.8	16	100.0
Dubluk	13	13	100.0	12	92.3
Total	**47**	**29**	**61.7**	**32**	**68.1**

EAS: East African Standards; CFU=colony forming units; SPC=standard plate count; CC=coliforms count

Physicochemical qualities

Each of the cow milk samples collected from across the three districts was subjected to analysis for a number of physical and chemical qualities including fat content, solid-non-fat (SNF), density, protein content, lactose content, added water and total salts as presented in Table 8. According to the data, mean fat content ranged from 5.85

to 7.60% and samples from Dambala Badana kebele in Dubluk district were noted as having the maximum (7.60%) fat content. Figure 6 shows that samples from Dubluk district had the highest fat content of 7.5% being significantly higher ($p \leq 0.05$) than the rest of the districts. The finding from the current study showed that most of the samples from the study districts contained higher fat content compared to previous reports such as Hawaz *et al*. (2015) (5.17%), Teshome *et al*. (2015) (4.5%), Teshome and Tesfaye (2016) (6.024%) and Legesse *et al*. (2017) (5.54%). According to the European Union Quality Standards, for unprocessed whole milk, fat content should not be less than 3.5% (Tamime, 2009). East African Standard recommends that proportion of fat should not be below 3.25% ((EAS 67:2007)). Thus, all samples collected from the three districts had a much higher proportion of fat content as compared to both the milk quality specifications.

Mean solids-not-fat (SNF) had ranged from 9.17 to 9.43% across the Kebeles of the districts (Table 8). However, there was non-significant difference ($p \leq 0.05$) among the districts. East African Standards recommends that cow milk SNF should not be less than 8.50%. Accordingly, all the samples from the three districts had an SNF content above the set standard.

With regard to density, milk samples from Bildim and Kela Qufa had shown to have a density above 30.0 kg/m^3. There was non-significant statistical difference ($p \leq 0.05$) among the districts in milk density. Samples from Gomole showed the highest density (30.1 kg/m^3), with Elwaye and Dubluk in the second (29.1 kg/m^3) and third (28.4 kg/m^3) places, respectively (Fig. 6). Analysis of milk samples for protein and lactose contents revealed almost equivalent values across the samples from all the Kebeles ranging from 3.32 to 3.43% and 5.03 to 5.16%, respectively. Similarly, samples from all the districts were nearly equal in the case of protein content which ranged from 3.3 to 3.4% (Fig. 6). They were exactly equal in lactose content (5.1%) (Fig. 6) too. The protein content of the milk samples was a little bit lower than some of the previous reports such as Debela *et al*. (2015) (3.94%), Hawaz *et al*. (2015) (3.51%) and Teshome and Tesfaye (2016) (3.98%). However, the current result was in agreement with 3.43% which was reported by Teshome *et al*. (2015). On the contrary, mean lactose content of the samples across the districts was considerably higher than earlier research results of the same authors obtaining 4.53% (Debela *et al*., 2015), 3.79% (Hawaz *et al*., 2015) and 4.43% (Teshome *et al*., 2015). Likewise, samples from all the kebeles were almost equivalent in total salts content ranging from 0.74 to 0.77%. From this investigation, all the milk samples were free of added water and this agrees with the standard set by EAS which states that there shouldn't be any added water in the milk samples.

Table 8. Physicochemical parameters (mean±SE) of milk samples

Physico-chemical parameters		**Gomole**		**Elwaye**		**Dubluk**	
		Kela Qufa	***Bildim***	***Golba***	***Town***	***Dambala Badana***	***Bokosa Gudda***
Fat (%)	Mean	6.31±0.6	6.52±0.4	6.87±0.5	5.85±0.6	7.60±0.3	7.45±0.5
	Min.	3.73	5.13	5.22	4.43	6.56	5.96
	Max.	9.62	8.45	8.71	9.37	8.56	8.61
	CV	27.4	16.6	20.2	30.1	10.7	15.7
SNF (%)	Mean	9.37±0.1	9.43±0.2	9.17±0.1	9.40±0.1	9.17±0.1	9.27±0.2
	Min.	8.88	8.72	8.83	8.87	8.69	8.82
	Max.	10.08	10.56	9.45	9.66	9.54	9.95
	CV	4.0	6.0	2.7	2.6	3.3	4.4
Density (kg/m^3)	Mean	30.04±0.5	30.21±0.8	28.85±0.6	29.38±1.2	28.2±0.3	28.67±0.8
	Min.	27.93	26.83	27.00	21.78	27.08	25.96
	Max.	33.23	33.47	31.13	32.42	29.2	32.04
	CV	5.0	7.1	6.2	11.6	2.8	6.8
Protein (%)	Mean	3.41±0.04	3.43±0.1	3.33±0.0	3.40±0.0	3.32±0.0	3.36±0.1
	Min.	3.22	3.16	3.20	3.23	3.15	3.18
	Max.	3.65	3.83	3.44	3.51	3.45	3.62
	CV	3.9	5.98	2.9	2.8	3.3	4.5
Lactose (%)	Mean	5.14±0.1	5.16±0.1	5.03±0.0	5.16±0.0	5.03±0.1	5.08±0.1
	Min.	4.87	4.78	4.84	4.87	4.77	4.83
	Max.	5.53	5.79	5.18	5.29	5.23	5.46
	CV	3.9	5.8	2.7	2.63	3.3	4.38
Added water (%)	Mean	0	0	0	0	0	0
	Min.	-	-	-	-	-	-
	Max.	-	-	-	-	-	-
	CV	-	-	-	-	-	-
Salts (%)	Mean	0.76±0.0	0.77±0.1	0.74±0.0	0.76±0.0	0.74±0.0	0.75±0.0
	Min.	0.72	0.71	0.71	0.72	0.70	0.71
	Max.	0.81	0.86	0.77	0.78	0.77	0.81
	CV	3.8	5.9	2.9	2.6	3.5	4.6
P(≤0.05)		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

SNF=solids-not-fat; CV=coefficient of variability; Max=maximum; Min=minimum; SE=standard error

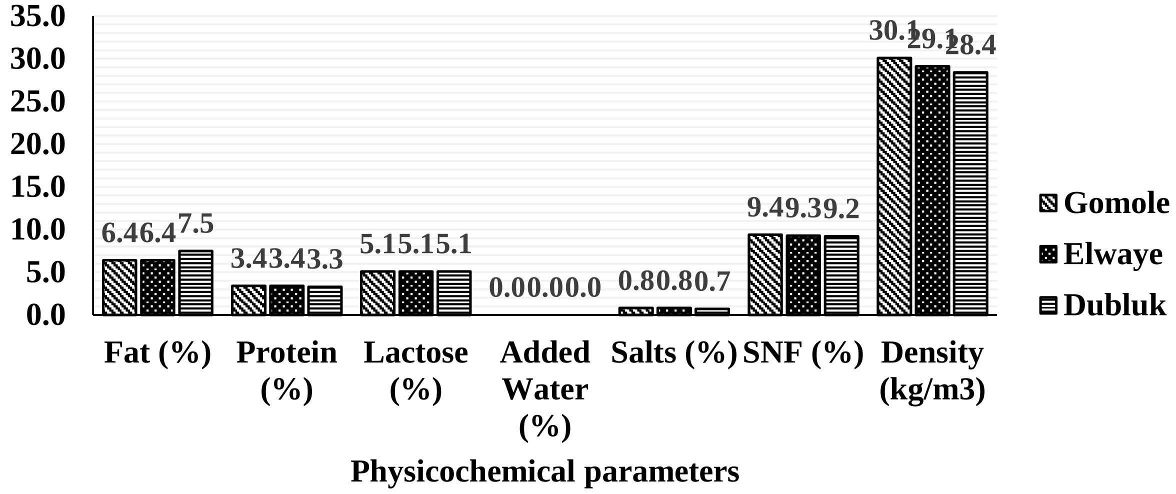

Figure 6. Physicochemical quality parameters of the cow milk samples

Conclusion and Recommendation

Bacteriological analysis of the milk samples in the three Districts showed that over 60% of the samples collected were found to be below standard set by East African Community Standards (EAS) with regard to standard plate count. This depicts that there is a serious hygiene problem in the process of milk production and subsequent handling in the study areas which emanates from inadequate cleanliness of the milking personnel, milking materials, udder and teat and the barn in general. This might be the result of lack of knowledge and awareness for the role of hygiene in the milk quality and its safety for consumption and further processing.

Similarly, more than 68% of the milk samples were substandard due to very high load of indicator microorganisms (total coliforms) considerably exceeding the maximum acceptable count set by EAS. The level of total coliforms contamination is an important signal for the presence of pathogenic bacterial species in the milk samples. This poses a potential health hazard and warns for a safety concern in consumption of cow milk without heat treatment in the study areas. However, most of the samples collected from Kebeles of Gomole District have been proved to be of acceptable quality in terms of low counts of standard plate and total coliforms. This is a reflection of better hygienic production practices in the specific district and other good cultural practices which probably contributed to this quality should also be studied for possible extension to the other areas.

However, further detailed investigations need to be made to create a clear understanding of the root cause as to why higher proportion of the milk being produced is of low microbial quality with corresponding higher health risk. Any ways, it requires that training should be delivered to the pastoral society to create awareness on the critical importance of hygienic practices in the production and handling of cow milk which plays a key role in assuring the production of enhanced quality milk for local consumption as well as increase its wider acceptability for market and industrial processing. The safety issues and health hazards related to the consumption of low standard raw cow milk should also be thought to the society.

From the analysis of physical and chemical qualities, it could be perceived that cow milk being produced in the study areas are of better quality in terms of fat, protein, lactose and SNF content with most of the parameters being even better than the standards set by East African Standards (EAS 67:2007). This might be attributed to higher feed diversity and quality of the cattle breed in the area and needs further scientific investigation.

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