Evaluation of microbial hygiene indicators in raw milk, pasteurized milk and cottage cheese collected across the dairy value chain in Ethiopia

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Abstract

Microbial hygiene of raw and pasteurized milk and cottage cheese samples was assessed along the value chain in three regions of Ethiopia between December and May 2020. A total of 912 samples (368 raw milk, 368 pasteurized milks and 176 cottage cheese) were collected from producers, collectors, processors, and retailers. Raw milk and pasteurized milk samples were examined for their total aerobic mesophilic bacterial count (APC) and total coliforms (TCC). Additionally, prevalence data was collected on all sample types for generic *Escherichia coli* (*E. coli*) and for TCC in cottage cheese. Significant interactions were observed between region and value chain for APC, TCC, and generic *E. coli* in raw and pasteurized milk samples. Data on microbial counts for most samples were above the Ethiopian Standard Authority (ESA) standard, which indicates poor food quality, suggestive of poor hygienic practices. Data indicate a need to improve hygienic milk handling in Ethiopia.

Keywords: Total aerobic mesophilic bacteria, *E. coli,* coliforms, dairy, milk, cottage cheese, food quality

1. Introduction

Ethiopia has the largest livestock population in Africa estimated at about 70.79 million head of cattle. Additionally, 11.4 million milking cows are currently producing 3,044,977 tons of milk annually. The Ethiopian dairy cattle population is distributed across the country, yet the four regions with the highest number of milking cows are Oromia (44%), Southern Nations Nationalities and People's or SNNP (Southern Nations, nationalities and peoples region; 22%), Amhara (17%) and Tigray (9%). Of these regions, the Oromia region produces an estimated 52% of the milk produced in the country (CSA, 2011).

Ethiopia has a complex dairy value chain, with both formal and informal channels. Additionally, formal milk markets are commonly limited to peri-urban areas and the capital city, Addis Ababa (Zegeye, 2003). Over the past decade, the formal market system appears to have been expanding, with more of the private sector entering the dairy processing industry in larger urban areas, such as Addis Ababa (SNV, 2008). However, currently, only 2% of the national milk reaches the final consumers through formal value chain, whereas 98% of the milk is unprocessed and marketed through informal channels (Tekelyesus, 2015). Input suppliers, milk producers, milk processors and consumers compose the formal milk value chain. Milk provides a typical example of a value-added product in Ethiopia with a growing demand and a limited local supply (Beyene, et al., 2015). Formal milk marketing of pasteurized milk and milk products accounts for less than 30% of total milk sales in Addis Ababa, despite these products being hygienically prepared and are generally considered safe for human consumption (Giangiacomo, 2000; Tekliye and Gizaw, 2017). However, inadequate, or faulty pasteurization does not destroy all foodborne pathogens (Pal et al., 2012) and post pasteurization contamination of milk can occur when pathogens are not adequately controlled in the food processing environment.

Foodborne diseases and food poisoning are widespread and a great public health concern for individuals and countries of the modern world, particularly in developing countries (Carbas et al., 2012). Milk-borne pathogens cause human diseases ranging from gastrointestinal disturbances characterized by diarrhea and vomiting to life-threatening food borne illnesses (Oliver et al., 2009). The same authors indicated that foodborne diseases also have economic importance. The consumption of raw milk and milk products creates public health concerns because it is a common reservoir or a vehicle for transmission of several foodborne pathogens (FBP) such as *Campylobacter* spp., *Listeria monocytogenes*, *Salmonella* spp., and *Escherichia coli* (Zelalem and Faye, 2006).

Assessment of bacterial levels is a frequently used procedure to measure the microbial quality of milk. Furthermore, poor microbial quality is associated with unhygienic milk production, which increases the food safety risk (Disassa et al., 2017). The total plate count and total coliform counts are universal methods to estimate the total aerobic mesophilic and coliform bacterial concentrations in raw milk (Fatine, 2012; Tamirat, 2018). *E. coli* is a subgroup of the coliform bacteria and is classified as a fecal coliform. Although most *E. coli* bacteria are harmless, they are enteric microorganisms associated with human and animal fecal contamination, which increases the risk for contamination of foods with enteric pathogens (Quinn, 2002; Zemenu, 2017).

It has been estimated that most of the microbial contamination of raw milk occurs during milking, collection, handling, processing, and distribution (Dorward et al., 2004). Only a few research reports, such as a meta-analysis of the prevalence of *E. coli* O157:H7 in raw animal products, include data on *E. coli* in milk products in Ethiopia (Keba et al., 2020). Several earlier research publications have reported the total coliform counts in milk and cottage cheese and revealed the seriousness of the dairy microbial contamination problem in Ethiopia (Zelalem et al., 2005; Ashenafi, 2006; Binyam, 2008; Seifu et al., 2013; Alganesh, 2016). Additionally, in Ethiopia, several studies have been conducted on the prevalence and

antimicrobial resistance patterns of *E. coli* from various clinical sources that might be associated with consumption of milk contaminated with E. coli (Disassa et al., 2017; Zemenu, 2017). Tigabu et al (2015) indicated that there is no standard milking protocol that is recommended to be followed by the smallholder farmers in Ethiopia. This same report indicates the need for a standard milk quality assurance system, that focuses on the safety of milk intended for human consumption. However, there are limited research reports that provide insights into the safety of dairy products along the value chain. Moreover, data describing the counts of hygiene indicator coliform and pathogenic *E. coli* is scarce. This data is essential to provide necessary feedback to the policy makers and regulatory bodies to take measures. Thus, the objective of this current study was to detect and quantify microbial indicators of milk hygiene for raw milk, pasteurized milk, and cottage cheese across the value chain in Oromia, Southern Nation's Nationalities and Peoples (SNNP), and Amhara regional states of Ethiopia.

2. Materials and methods

2.1. Sample Origin

A cross sectional study design was used to investigate the contamination of raw milk collected from producers and collectors, pasteurized milk collected from processing factories, and retailers, and cottage cheese collected from farm markets and retail shops. Samples were collected between December 2020 and May 2020, across areas with high potential for dairy production, in three regions of Ethiopia (Oromia, SNNP, and Amhara).

In Oromia region, samples were collected from Selale, Welmera, Debre Zeit and Asella. In SNNP region, samples were collected from Hawassa town, Yirgalem, Dilla and Wolaita areas. For Amhara region, samples were collected from Debre Birhan, Debre Markos, Bahir Dar and Gondar areas. Initially it was planned to collect samples also from the Tigray region; however, due to a violent conflict in the region, sample collection was not possible.

2.2. Sample size determination and participant enrollment

A representative number of milk and cottage cheese samples were determined based on previously published prevalence of microbial load in milk and milk products. The sample size was calculated using the statistical formula: $N = Z^2 P (1-P)/D^2$ (Daniel, 1999), where N is the minimum sample size required, Z is 1.96 at 95% confidence interval, D is margin of sampling error (5% marginal error was used), P is an estimate of the prevalence rate for the population. Since the overall prevalence of APC, TCC and *E. coli* in the value chain for milk and dairy product in the three regions is not known, P was taken to be 50% for the calculation (Mulaw, 2017).

Not all regions in Ethiopia have equal milk production capacity. For example, Oromia produces an estimated 52%, SNNP 23%, and Amhara 20%, from the total milk production volume in Ethiopia (Tigray is responsible for the final 5%; Beyene, 2015). Hence, the sample size for each region was proportional to milk production capacity across the studied regions. Therefore, a total of 384 milk samples and 96 cottage cheese samples were collected from Oromia, 192 milk samples and 48 cottage cheese samples were collected from SNNP, and 160 milk samples and 32 cottage cheese samples were collected from Amhara regions (Table 1). Sample collection in Tigray was not possible during the time of the study due to conflict within the region.

Table 1. Number of raw, pasteurized milk, and cottage cheese samples collected across the dairy value chain in the dry season in Oromia, Southern Nations Nationalities and People's (SNNP), and Amhara regions of Ethiopia.

	Milk Value Chain					**Cottage Cheese Value Chain**		
	Raw Milk		**Pasteurized milk**		**Total**	**Cottage Cheese**		**Total**
Region	Producers	Collectors	Processors	Retailers		Farm markets	Retail shops	
Oromia	96	96	96	96	384	48	48	96
SNNP	48	48	48	48	192	24	24	48
Amhara	40	40	40	40	160	16	16	32
Total	184	184	184	184	736	88	88	176

Lists of the members of the respective dairy cooperative unions who deliver raw milk were purposively obtained from the milk collection centers. 'Kebeles' (the smallest administrative units in Ethiopia) and households were selected based on their milk production potential at the time of cross-sectional study. A list of farm households in each region was compiled with the help of local development agents at respective sites and milk collection centers and households were randomly selected from the compiled list for inclusion in the study. The households were members of dairy cooperative unions which owned milk collection centers. In some cases, the milk collectors had their own milk processing factories. The entire lists of the milk processing factories were obtained from the Ethiopian milk and meat institute. Moreover, the lists of the retailers who pay government taxes were obtained from the market authority. A multi-stage random cluster sampling method was used to select sampling sites within each sub-city or 'kebele' of the town to select the retailers for pasteurized milk and cottage cheese sampling. Consent was asked to interview for the cross-sectional study. After obtaining, the selected study participants, the consent of each participate was asked and each of them signed on the consent form. The contents of the consent included providing samples for microbiological analyses, allowing access to their property for observational survey, and answering questions listed in a questionnaire survey that was approved by the Addis Ababa University Ethics Committee.

2.3. Sample collection, handling and transportation

Sample handling and transportation was implemented in accordance with milk and milk products sampling guidance of ISO 707:2008, as adapted by Ethiopian Standard Authority (ESA).

2.3.1. Raw milk sample collection

Raw milk samples were collected from smallholder farmers (producers), milk collectors that were enrolled in the study as outlined above. At the production level, one liter

of fresh raw milk was collected and purchased, whereas at collection level, representative samples were collected from the 50 L capacity aluminum bulk milk containers for 2-3 consecutive days. Samples were homogenized through mixing and collected in 200 ml-capacity sterile bottles. Immediately prior to sample collection, sterile cotton wipe was soaked in 70% ethanol and used to disinfect hands to reduce chances of cross contamination. Samples were placed in an insulated box/jet cooler and immediately transported to the Addis Ababa University Center for Food Science and Nutrition (AAU CFSN) microbiology laboratory for analysis. In the laboratory, samples were stored at 4°C until further microbial analysis of total aerobic mesophilic bacteria, total coliforms, and *E. coli*. The microbial analysis of samples was completed within 48 hours after the samples arrived in the laboratory.

2.3.2. Pasteurized milk sample collection

Pasteurized milk samples were collected from enrolled milk processors and retailers. Processors obtained their raw milk from either their own farm or individual farmers that were members of the cooperative unions that own the milk collection centers. Processors that participated in the study carried out pasteurization and distributed pasteurized milk to retail shops. Participating retail shops were located in urban and peri-urban areas and were registered as taxpayer in the office of income and revenue. In each region, the numbers of factories were limited. Plastic sachets (pouches) containing half liters of pasteurized milk samples that were processed consecutively were purchased from milk processors for 2-3 consecutive days until representative and required numbers of samples were obtained. However, since the numbers of retail shops in each sub city and or towns were enough identical brands of pasteurized milk samples were purchased from randomly selected retail shops. The sachets of pasteurized milk samples were immediately placed into sterile stomacher bags that were immediately placed into insulated box/jet cooler and transported to the AAU CFSN microbiology laboratory for analysis. In the laboratory, samples were stored at 4°C until further microbial analysis of total aerobic mesophilic bacteria, total coliforms, and

E. coli. All microbial analysis of samples was completed within 48 hours after the samples arrived in the laboratory.

2.3.3. Cottage cheese sample collection

In Ethiopia, cottage cheese (ergo) is a produced through a fermentation process, utilizing natural non-starter lactic acid bacteria species found naturally in the milk. Smallholder milk producers collect small amounts of milk daily and allow the milk to accumulate for three to four days. The collected milk is left at room temperature—on average 23^0C—and allowed to naturally ferment (souring) and coagulate. The fermented milk is churned using local vessels, such as bottle gourds and clay pots. After separating the butter milk and butter, the butter milk is slowly heated to 55^0C until the whey and cottage cheese separate. After cooling the buttermilk and whey, the whey is drained off using muslin cloth or local strainers and the cottage cheese process is complete. Cottage cheese samples were randomly purchased from producers in the farm marketplaces.

Samples at the retailer's level were purchased from urban and peri-urban areas from randomly selected retailers who were registered as taxpayer in the office of income and revenue. One kg of each of cottage cheese samples were purchased from each of randomly selected smallholder producers and retail shop and were transferred to sterile sample bottles. All collected samples were subsequently transferred to sterile stomacher bags. Prior to transferring the samples, sterile cotton wipe soaked in 70% ethanol was used to disinfect hands to reduce the chances of cross-contamination. Samples were placed in insulated box/jet cooler and immediately transported to the AAU CFSN microbiology laboratory for analysis. In the laboratory, samples were stored at 4°C until further microbial analysis of total aerobic mesophilic bacteria, total coliforms, and *E. coli*. The microbial analysis of samples was completed within 48 hours after the samples arrived in the laboratory.

2.4. Enumeration of total aerobic mesophilic bacteria in milk and cottage cheese

The aerobic plate count (APC) was applied to enumerate total aerobic mesophilic bacteria in milk and cottage cheese samples by following the protocol for the pour plate method recommended by the Bacteriological Analytical Manual (BAM) (FDA, 2001). Briefly, 1 ml of milk sample was serially diluted into 9 ml of sterile saline solution (0.85 % NaCl) up to five or seven dilutions for pasteurized and raw milk samples, respectively. One ml of each dilution was aseptically transferred to the respective petri dish along with 15-20 ml of molten standard plate count agar (Oxoid, UK) tempered in a water bath at 47°C. The sample and the agar were thoroughly mixed by alternating clockwise and counterclockwise rotations and then were left to solidify under safety hood for about 30 minutes, followed by incubation at 32 ± 1°C for 48 hours. After incubation, colonies were counted using a colony counter for a plate that was within a countable range of 30 – 300 CFU. Counts were used to calculate CFU/ml of milk.

2.5. Enumeration of total coliforms and generic *E. coli* bacteria in milk

Enumeration of total coliforms and generic *E. coli* from milk and cottage cheese samples were performed on *E. coli*/coliform count (ECC) Petri film following manufacturer protocol (3M Food Safety, 2017). One milliliter of milk sample was serially diluted into 9 ml of saline solution (0.85% NaCl) up to three or five dilutions for raw and pasteurized milk samples, respectively. One ml of dilution was transferred to the respective ECC Petri film. Ten grams of each cottage cheese sample were aseptically weighed and transferred to stomacher bags and subsequently diluted into 90 ml of saline solution. Samples were manually mixed until the suspension was homogeneous. One ml of homogenate was transferred to the respective ECC Petri film. All EEC count plates were incubated at 35 ± 1°C for 24 ± 2 hours. After incubation, red and blue colonies with a gas bubble were counted to obtain a total coliform count and blue colonies with gas bubbles were counted to obtain an *E.*

coli count (Food Safety, 2017). The colony counts were used to calculate the concentration of total coliforms and *E. coli* per ml or g of tested food.

2.6. Prevalence of total coliforms and generic *E. coli* in cottage cheese

Methods utilized to quantify TCC and generic *E. coli* in milk samples (outlined in section 2.5.) were not suitable for quantification of TCC and generic *E. coli* in cottage cheese samples, due to very low levels of TCC and generic *E. coli*. Consequently, for cottage cheese samples, Petri films were utilized to identify samples that were positive for TCC and generic *E. coli* but were considered below the threshold for accurate quantification

2.7. Statistical Analysis

A two-way ANOVA with interactions is employed and all statistical models were fitted using the GLIMMIX procedure of SAS (version 9.4, SAS Institute, Cary, NC). Estimated least square means and corresponding standard errors and/or 95% confidence intervals (CI) are presented for all data.

For quantification data, *sample type* served as the experimental unit (raw milk, pasteurized milk, and cottage cheese) and data were analyzed utilizing a general linear mixed model with three x three factorial (region x value chain) design. The response variable was log CFU per ml for (raw milk and pasteurized milk) or log CFU per g (for cottage cheese), and the linear predictors included the fixed effects of region (SNNP, Oromia, Amhara), value chain (producer, collector, retailer) and all two-way interactions.

For prevalence data (i.e., presence vs. absence data) of *E. coli*, a generalized linear mixed model was fitted to a binary response. The experimental unit was again *sample type*. The logit link function was used to connect the Bernoulli probability of detection with a linear predictor that included the fixed effects of a region (SNNP, Oromia, Amhara), value chain (producer, collector, retailer) and all two-way interaction. Differences were considered to be significant at $\alpha \leq 0.05$.

3. Results and Discussion

3.1. Result Comparisons

It is important to note that inferences from this data were confined by sample type, as comparisons across sample types are not appropriate due to differences in the number of points in the value chains for each sample type. For example, raw milk was collected from producers and collector, while pasteurized milk was collected from processors and retailers, and cottage cheese was collected from producers and retailers.

3.2. Aerobic Plate Count Results

APC was determined for each sample of milk (raw and pasteurized) and cottage cheese, collected at each point in the value chain (i.e., producers, processors, collectors, and retailers), and in three regions (Oromia, SNNP, and Amhara; Table 2). Statistical analysis revealed a significant interaction between region and value-chain for each sample type (raw milk p= 0.002; pasteurized milk p = 0.004; and cottage cheese p = 0.0008), whereby differences in estimated APC levels at different value chain points were specific to each region.

The average APC for raw milk samples collected from producers in Oromia, SNNP and Amhara regions significantly differed at $p < 0.05$ and the values were 7.10±0.10, 10.53±0.14 and 8.35±0.15 log CFU/ml, respectively. In that order, the average APC for raw milk samples collected from collectors also significantly differed at $p < 0.05$ and the values were 7.25±0.10, 9.70±0.14 and 8.36±0.15 log CFU/ml, respectively. For raw milk, there was a significant difference in APC levels between SNNP producers and collectors ($p < 0.05$) and significant differences in APC could be observed by region, with SNNP having the highest levels, followed by Amhara, and Oromia. However, within region with the exception of SNNP, comparisons did not show evidence of a difference in APC levels between processors and collectors.

Table 2. Total aerobic plate count (APC) in raw milk, pasteurized milk, and cottage cheese samples collected along the value chain in Oromia, Southern Nations Nationalities and People's (SNNP), and Amhara regions of Ethiopia. Results are least square means ± standard deviation in log CFU/ml.

	Region	Estimated level (log CFU/ml or g)		
		Producers	Mean	SEM
Raw Milk	Oromia	Producers	7.10[d]	0.10
		Collectors	7.25[d]	0.10
	SNNP	Producers	10.53[a]	0.14
		Collectors	9.70[b]	0.14
	Amhara	Producers	8.35[c]	0.15
		Collectors	8.36[c]	0.15
Pasteurized Milk	Oromia	Processors	5.72[d]	0.93
		Retailers	6.16[c]	0.93
	SNNP	Processors	8.64[a]	0.13
		Retailers	8.99[a]	0.13
	Amhara	Processors	6.93[b]	0.15
		Retailers	6.59[b]	0.13
Cottage Cheese	Oromia	Producers	6.11[d]	0.10
		Retailers	6.11[d]	0.10
	SNNP	Producers	8.44[a]	0.14
		Retailers	9.38[b]	0.14
	Amhara	Producers	6.54[c]	0.16
		Retailers	6.72[c]	0.18

Means with different letters within a row (lowercase letter) indicate statistical difference among regions with a significance level of $P < 0.05$.

The average APC for pasteurized milk samples collected from processors in Oromia, SNNP and Amhara regions were significantly different, and the values were 5.72±0.93, 8.64±0.13 and 6.93±0.15 log CFU/ml, respectively. Similarly, the average APC for pasteurized milk samples collected from retailers in the three regions also significantly differed and were 6.16±0.93, 8.99±0.13 and 6.59±0.13 log CFU/ml, respectively. Data for pasteurized milk showed similarities with the data for raw milk samples. For example, significant differences were observed in APC levels between regions, with SNNP maintaining the highest levels, followed by Amhara, and Oromia. However, within region differences were significant for pasteurized milk ($p < 0.05$).

Finally, data collected from cottage cheese followed the same trend in regional differences in APC. The average APC for cottage cheese samples collected from producers in Oromia, SNNP and Amhara regions were significantly different, and the values were 6.11±0.10, 8.44±0.14 and 6.54±0.16 log CFU/ml, respectively. In that order, the average counts for samples collected from retailers were also significantly different for regions ($p < 0.05$) and counts were 6.11±0.10, 9.38±0.14 and 6.72±0.18 log CFU/ml, respectively. APC was approximately two logs higher in cottage cheese samples collected from producers and retailers in SNNP, as compared to those collected in Amhara, and approximately three logs higher compared to samples collected from producers and retailers in Oromia.

In SNNP, there was a significant difference in APC in cottage cheese samples collected from producers compared to retailers. However, there was no evidence of significant differences in APC in samples from producers compared to retailers in Amhara and Oromia. It is worth noting that APC counts for cottage cheese did not account for the natural flora of the cottage cheese, and therefore should not be considered as a hygienic indicator for this samples type.

The Ethiopian Standard Authority (ESA) monitors the microbial quality of raw and pasteurized milk based on established limits for APC. Raw milk with APC ≥ 6.301 log CFU/ml is categorized as *very poor* milk quality (ESA, 2009), while raw milk with APC ≤ is categorized at *very good* milk quality. In this study, the average APC in raw milk samples collected from producers and collectors from all regions were higher than the standard established by ESA. Based on the bacteriological quality rating of ESA, only 0.3% and 4.1% of the raw milk samples fell within the categories of very good and good, respectively. While, the majority of the samples, 9.8% and 85.9% felt within the categories of *poor* (6.000-6.301 Log CFU/ml) and *very poor*, respectively.

For pasteurized milk, ESA established that samples with APC > 5 log CFU/ml *shall be rejected* (ESA, 2009). In this study, the average APC in pasteurized milk samples

collected from milk processors and retailers from all regions were higher than 5 log CFU/ml. Only 4.9% and 4.4% of the pasteurized milk samples collected from processors and retailers would be categorized as *good* (4.699-5.00 Log CFU/ml) and *very good* (≤4.699 Log CFU/ml), respectively. However, 90.8% of the tested pasteurized milk samples would be categorized as should be rejected.

Our results indicated that most of the tested raw and pasteurized milk samples collected from the three regions had poor sanitary quality. Interestingly, APC results from this study are higher than previously reported studies for both raw and pasteurized milk (Tola, 2002; Asaminew, 2010; Tassew and Seifu, 2011 and Worku et al., 2012; Shunda et al., 2013; Solomon et al., 2013). The reason for this is unknown but could be due to differences in methodologies or differences in handling practices of stakeholders enrolled in the study. Furthermore, bacterial contamination post-pasteurization could be due to defects associated with the pasteurization, poor handling conditions, post-pasteurization contamination, and/or maintenance of substandard hygienic practices by working personnel (ICMSF, 1998).

3.3. Total Coliform Count Results

Results from total coliform counts (TCC) in raw and pasteurized milk samples tested in this study are presented in Table 3. Similar to the APC data, a significant interaction between region and value-chain for each sample type was observed (raw milk $p < 0.0001$ and pasteurized milk $p = 0.004$), whereby differences in estimated TCC levels at different value chain points were specific to each region. The average TCC for raw milk samples collected from producers in Oromia, SNNP and Amhara regions significantly differed ($p < 0.05$), at levels of 5.53±0.12, 6.04±0.15 and 3.52±0.15 log CFU/ml, respectively. In that order, the average TCC for raw milk samples collected from collectors were statistically similar except for those collected in SNNP ($p<0.05$), at levels of 5.84±0.10, 6.32±0.17 and 5.72±0.15 log CFU/ml, respectively.

Table 3. Total coliform bacterial counts in raw and pasteurized milk samples collected along the supply chain in Oromia, Southern Nations Nationalities and People's (SNNP), and Amhara regions of Ethiopia.

			Estimated level (log CFU/ml)[a]	
	Region	**Producers**	**Mean**	**SEM**
Raw Milk	Oromia	Producers	5.53^{c}	0.12
		Collectors	5.84^{bc}	0.10
	SNNP	Producers	6.04^{ab}	0.17
		Collectors	6.32^{a}	0.17
	Amhara	Producers	3.52^{d}	0.15
		Collectors	5.72^{bc}	0.15
Pasteurized Milk	Oromia	Processors	3.23^{c}	0.20
		Retailers	4.99^{a}	0.19
	SNNP	Processors	4.49^{a}	0.25
		Retailers	5.00^{ab}	0.27
	Amhara	Processors	3.71^{c}	0.30
		Retailers	4.03^{bc}	0.36

[a]Means with different letters within a row (lowercase letter) indicate statistical difference with a significance level of $P < 0.05$.

Raw milk samples obtained from collectors had higher levels of TCC than those obtained from producers in SNNP and Amhara ($p<0.05$). The highest levels of TCC were observed in raw milk samples collected in SNNP from milk collectors and the lowest levels were observed in raw milk samples collected from producers in Amhara. However, there was no evidence of differences between TCC in raw milk samples obtained from collectors and producers in Oromia.

As defined by the ESA. of raw milk samples collected from producers, collectors, only 7.3 % and 18.2 % were categorized as good and very good based on their TCC. While, 29.4 % and 45.0 % of the samples felt within the poor and very poor category, respectively. Previous studies on TCC have reported lower (4.09 - 4.63 log CFU/ml), similar (5.47 log CFU/ml), or higher (6.94 log CFU/ml) counts in raw milk (Weleragegay et al., 2012; Amentie et al., 2015; Korma et al., 2018; Mikru et al., 2021).

Within region comparisons of TCC from Oromia and SNNP regions showed that there was no statistical difference ($p < 0.05$) between raw milk samples collected from producers

and collectors. Likewise, no statistical difference in TCC ($p < 0.05$) was observed between pasteurized milk samples from processors and retailers in SNNP and Amhara regions. However, statistical differences were observed in TCC counts between raw milk samples collected from producers and collectors in Amhara, and pasteurized milk obtained from processors and retailers of Oromia region.

The average TCC for pasteurized milk samples collected from processors in Oromia, SNNP and Amhara regions significantly differed at $p < 0.05$ and the values were 3.23±0.20, 4.49±0.25 and 3.71±0.30 log CFU/ml, respectively. In that order, the average TCC for pasteurized milk samples collected from retailers from Oromia and Amhara were statistically similar except for that of the SNNP ($p<0.05$) and the values were 4.99±0.19, 5.00±0.27 and 4.03±0.36 log CFU/ml, respectively.

Coliform data for raw and pasteurized milk showed more variation in between and within region differences when compared to APC, with the highest levels being observed in raw milk. Only 15.2 % of all pasteurized milk samples met the standard set by ESA for coliforms (≤1 Log CFU/ml), while 84.8 % of the milk samples were substandard (≥1 Log CFU/ml). This provides significant opportunity for improvements for value-chain actors and regulatory bodies.

TCC results from this study were lower than previously reported (Aberra, 2010; Asaminew and Eyasu, 2011; Korma et al., 2018). Nonetheless, the TCC along the milk value chain for the three regions failed to meet ESA standards. Among the possible reasons for the high TCC are fecal contaminations during milking, absence of cold chain, low level of awareness of milkers and milk handlers, and unhygienic water supply.

3.4. Prevalence Results

Prevalence data is presented for *E. coli* for all sample types and for TCC in cottage cheese. This is due to a low number of samples having quantifiable results of *E. coli*. Results for the comparison of prevalence of *E. coli* in raw milk and pasteurized milk are presented in

Table 4. The prevalence, represented in percent, of *E. coli* in raw milk samples collected from producers in Oromia, SNNP and Amhara regions were 40.63±0.05%, 45.83±0.072% and 2.5±0.025%, respectively. In that order, the percentage prevalence of *E. coli* in raw milk samples collected from milk collection centers were 64.58±0.049%, 54.17±0.072% and 17.50±0.060%, respectively.

Table 4. Prevalence of *Escherichia coli* in raw and pasteurized milk samples collected along the supply chain in Oromia, Southern Nations Nationalities and People's (SNNP), and Amhara regions of Ethiopia.

			Estimated prevalence (%)[a]		
	Region	**Producers**	**Mean**	**95 CI (lower, upper)**	**SEM**
Raw Milk	Oromia	Producers	40.63[b]	31.26, 50.73	0.050
		Collectors	64.58[a]	54.52, 73.51	0.049
	SNNP	Producers	45.83[b]	32.37, 59.93	0.072
		Collectors	54.17[ab]	40.07, 67.63	0.072
	Amhara	Producers	2.50[c]	0.35, 15.82	0.025
		Collectors	17.50[c]	8.55, 32.47	0.060
Pasteurized Milk	Oromia	Processors	28.13[b]	20.03, 37.95	0.046
		Retailers	43.75[a]	34.17, 53.82	0.051
	SNNP	Processors	50.00[a]	36.18, 63.82	0.072
		Retailers	8.33[c]	3.15, 20.26	0.040
	Amhara	Processors	*	*	*
		Retailers	2.3[c]	0.32, 14.53	0.022

*Values were below detectable level. [a]Means with different letters within a row (lowercase letter) indicate statistical difference among regions with a significance level of $P < 0.05$.

The percentage prevalence of *E. coli* in pasteurized milk samples collected from processors in Oromia, SNNP and Amhara regions were 28.13±0.046% and 50.00±0.072% and 2.5±0.025%, respectively. In Amhara region, the values were below detectable limit. In that order, the percentage prevalence of *E. coli* in pasteurized milk samples collected from retailers were 43.75±0.051%, 8.33±0.040% and 2.30±0.022%, respectively. The possible reason for the lower prevalence of *E. coli* in pasteurized milk samples collected from Amhara and SNNP regions could be due to smaller proportions of samples collected from the region as compared to that of Oromia. The pasteurized milk samples in Amhara region were collected from different batches of pasteurized milk processed in one or two factories as

compared to the nine factories in Oromia region from which pasteurized milk samples were collected.

Prevalence data for total coliforms and *E. coli* data for cottage cheese is presented in Table 5. Analysis of prevalence data also showed a significant interaction between region and value-chain for each sample type (raw milk $p < 0.0001$ and pasteurized milk $p < 0.0001$), whereby differences in estimated prevalence of *E. coli* at different value chain points were specific to each region. For raw milk, the highest prevalence of *E. coli* was observed in samples from collectors in Oromia (64.6 %) and SNNP (54.2 %). Additionally, collectors and producers from SNNP had a similar prevalence of *E. coli* (54.2 % and 45.8 %, respectively) as compared to Oromia producers (40.6 %). Data for pasteurized milk had fewer similarities, with *E. coli* being most prevalent in samples from SNNP processors and Oromia retailers. This data supports further evaluation of factors that may influence microbial contamination within the SNNP region. The reason for highest APC and TCC in the samples from the SNNP region could also likely be due to its low-land environment and warmer climate. Conversely, samples from Amhara and SNNP retailers had the lowest prevalence. This may indicate that handling practices of cottage cheese may be more compliant with standards, or that the cottage cheese as a matrix is less suitable for *E. coli* survivability and growth. Further research to investigate both possibilities may be warranted.

Table 5. Total *E. coli* prevalence for cottage cheese samples collected from farm markets and retail shops in Oromia, Southern Nations Nationalities and People's (SNNP), and Amhara regions of Ethiopia.

		Estimated prevalence *E. coli* (%)[a]			Estimated prevalence TCC (%)[a]		
	Producers	**Mean**	**95 CI (lower, upper)**	**SEM**	**Mean**	**95 CI (lower, upper)**	**SEM**
Region	Oromia	3.13[a]	1.00, 9.31	1.776	37.50[a]	28.35, 47.63	4.941
	SNNP	4.20[a]	1.03, 15.32	2.884	10.42[b]	4.40, 22.81	4.408
	Amhara	3.13[a]	0.43, 19.33	3.076	3.13[b]	0.43, 19.33	3.076
Value-Chain	Retailers	*[a]	*	*	18.60[a]	11.68, 28.31	4.196
	Producers	6.67[a]	3.01, 14.12	2.629	28.89[a]	20.43, 39.13	4.778

*Values were below detectable level. [a]Means with different letters within a row (lowercase letter) indicate statistical difference among regions with a significance level of $P < 0.05$.

Finally, prevalence data for cottage cheese was evaluated for coliforms and *E. coli.* Statistical analysis did not show significant interaction between region and value chain for cottage cheese (coliform $p < 0.1964$ and *E. coli* $p < 0.9995$). Additionally, the prevalence of *E. coli* in cottage cheese did not differ by region or by value-chain (main effect: $p < 0.9445$ and $p = 0.970$, respectively). However, estimated prevalence of coliforms in cottage cheese differed by region (main effect: $p=0.004$), with the highest prevalence being observed in Oromia. No evidence of value-chain differences was apparent.

The possible reason for the difference in the prevalence of *E. coli* in cottage cheese samples could be due to the incomparable number of samples collected from the three regions (96 samples from Oromia VS 48 samples from SNNP and only 32 samples from Amhara region). The other possible reason could be that cottage cheese samples from producers from Oromia region were collected from marketplaces from open local packaging materials with possibility of more contamination. While, in the Amhara region freshly made cottage cheese samples which might have had less possibility of contamination were collected from households. This was because cottage cheese samples were not found in local marketplaces in the region.

4. Conclusion

This study aimed to fill a gap in knowledge and evaluate contamination points in raw, pasteurized milk, and cottage cheese along the dairy value chain in three regions of Ethiopia. The study provides both quantitative and qualitative data for total aerobic mesophilic bacteria, total coliforms, and *E. coli* in raw, pasteurized milk, and cottage cheese collected across the value chain in Oromia, SNNP and Amhara regions. Results demonstrated significant interactions between region and value chain for APC, TCC, and generic *E. coli* in raw and pasteurized milk samples, indicating that bacterial contamination is highly influence by region and point in the value-chain.

Results also showed that 86 % and 90 % of the raw and pasteurized milk samples were substandard, based upon ESA standards. Raw and pasteurized milk coliform data showed more statistical similarities than APC. Numerically, the SNNP region had the highest bacterial counts across all sample types. Prevalence data for TCC varied substantially in cottage cheese samples, with the highest prevalence in Oromia and lowest prevalence in Amhara. Finally, *E. coli* prevalence showed no differences between retailers and producers, nor was their observable differences across each of the three regions. Additionally, mean prevalence for *E. coli* in cottage cheese for each region were relatively low, with samples from retailers being below the limit of detection. It can be concluded that there is lack of process control for microbial contamination in all sample types. There is a need for the application of stronger preventive and control measures, such as regular washing and sterilization of milk utensils, personnel hygiene of milkers and milk handlers, cleaning and disinfection of udder and teats of milking cows, culling of diseased animals from time to time, health and hygiene of milking cows, cold chain management along the value chain, proper and regulated pasteurization of milk.

Data availability statement: All data used in the analyses presented here are available in the Supplementary Material.

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