frontiers | Frontiers in Sustainable Food Systems

TYPE Original Research
PUBLISHED 20 January 2025
DOI 10.3389/fsufs.2024.1535630

OPEN ACCESS

EDITED BY
Fatima Zahra Jawhari,
Higher Institute of Nursing and Health Techniques, Morocco

REVIEWED BY
Massik Abdelhamid,
Centre Hospitalier Universitaire Hassan II, Morocco
Daoud Daoudi,
Mohammed V University, Morocco

*CORRESPONDENCE
Ashagrie Zewdu
✉ ashagrie.zewdu@aau.edu.et

RECEIVED 27 November 2024
ACCEPTED 24 December 2024
PUBLISHED 20 January 2025

CITATION
Keba A, Girmay G, Nahusenay H and Zewdu A (2025) Spatial analysis of milk and cottage cheese reveals poor microbial quality and contamination with foodborne pathogens in the central highlands of Ethiopia.
Front. Sustain. Food Syst. 8:1535630.
doi: 10.3389/fsufs.2024.1535630

Spatial analysis of milk and cottage cheese reveals poor microbial quality and contamination with foodborne pathogens in the central highlands of Ethiopia

Abdi Keba[1], Gebrerufael Girmay[2], Henok Nahusenay[3] and Ashagrie Zewdu[4]*

[1]Holeta Agricultural Research Center, Ethiopian Institute of Agricultural Research, Holeta, Ethiopia, [2]National Agricultural Biotechnology Research Center, Ethiopian Institute of Agricultural Research, Holeta, Ethiopia, [3]Head Quarter Food Science and Nutrition Research Directorate, Ethiopian Institute of Agricultural Research, Addis Ababa, Ethiopia, [4]Center for Food Science and Nutrition, College of Natural Sciences, Addis Ababa University, Addis Ababa, Ethiopia

Introduction: Foodborne diseases that result from a wide range of illnesses caused by contaminated foods remain a challenge in least-developed countries. The objective of this study was to evaluate microbial quality and safety of milk and cottage cheese and spatial distribution of microbial quality indicators and foodborne pathogens along the dairy value chain in the three regions of the country.

Methods: A cross-sectional study was conducted from December 2020 to May 2021. A total of 912 samples were collected and tested for aerobic plate count, total coliform count, *Escherichia coli* count, *Listeria monocytogenes*, *Salmonella enterica*, and *Campylobacter* spp., according to standard methods of microbial enumerations and isolation procedures.

Results: Microbial quality of milk and cottage cheese in the dairy value chain was found poor quality, as the total bacteria count, total coliform count, and *Escherichia coli* count were estimated to be 98% (95% CI 97.2–98.9%), 61.2% (95% CI 58–64.3%), and 28.6% (95% CI 25.8–31.6%), respectively. Microbial load of milk and cottage cheese samples exceeded the limits set by the Ethiopian Standards Agency. The overall prevalence of samples contaminated by at least one pathogen was 50.3% (95% CI 47.1–53.7%), indicating that raw milk samples collected from milk collectors were predominantly contaminated (OR = 2.1, p = 0.003), followed by milk processors (OR = 1.3, p = 0.003).

Discussion: The spatial analysis revealls that the poor microbial quality standards and distribution of microbial quality indicators and foodborne pathogens were concentrated in the central highlands of Ethiopia, within nearly 100 kilometers radius from Addis Ababa city to surrounding towns. This study offers some insight into the importance of food traceability to prevent food safety threats along the dairy value chain and intervention areas.

KEYWORDS

cottage cheese, central highlands, dairy value chain, milk, quality, safety, spatial analysis

1 Introduction

Foodborne diseases (FBDs) encompass a wide range of illnesses caused by food contamination, posing significant public health challenges worldwide (Bhaskar, 2017). These diseases are linked to human health and have a considerable impact on socioeconomic growth (Gizaw, 2019; Abebe et al., 2020). In 2010, the World Health Organization estimated that there were 600 million foodborne infections and 420,000 related deaths (WHO, 2015).

FBDs are increasing in low and middle-income countries (LMCs), and significant outbreaks occur because of the nutritious and fresh food purchased from informal markets (Grace, 2023). Dairy products have accounted for 20 disability-adjusted life years per 100,000 individuals and contributed approximately 4% of the global foodborne disease burden, while animal sources accounted for 12% of the burden in 2010 (Grace et al., 2020). Dairy products provide favorable conditions for pathogens to survive and multiply, posing a risk to new hosts, including humans (Abebe et al., 2020).

According to the European Food Safety Authority (EFSA), the common foodborne pathogens in dairy products are *Campylobacter* spp., *Salmonella* spp., Shiga-producing *Escherichia coli* (*STEC*), *Bacillus cereus*, *Brucella abortus*, *Brucella melitensis, Listeria monocytogenes, Mycobacterium bovis, Staphylococcus aureus, Yersinia enterocolitica, Yersinia pseudotuberculosis, Corynebacterium* spp., and *Streptococcus suis subsp. zooepidemicus* (Tauxe, 2002; Sugrue et al., 2018).

According to Adugna and Asresie (2015), the microbial quality of dairy products was below international standards, and foodborne pathogens were prevalent and unsafe for human consumption (Keba et al., 2020).

Currently, changes in the global climate are affecting all aspects of food production, handling, marketing, distribution, and consumption habits along the food value chain (Broglia et al., 2019; Gomez-Zavaglia et al., 2020). The shifting of technologies in food processing, food packing and storage, and agricultural practices contributes to the emergence of novel bacteria and their distributions (Hamaideh et al., 2024). Global warming requires a deep understanding of climate-driven emerging risks related to the variability of rainfall, temperature, drought, and moisture (Hellberg and Chu, 2016; Nayak and Waterson, 2019; Duchenne-Moutien, 2021).

Ethiopia is known for its diverse climate and agro-ecological regions, where highland and midlands are characterized by high rainfall and forage production while lowland faces drought (Chamberlin and Schmidt, 2011; Gizaw et al., 2017).

Spatial analysis is important for understanding the distribution of foodborne pathogens and managing associated risks. It helps in developing intervention strategies, efficiently managing resources, and planning (Polonsky et al., 2019; Kanankege et al., 2020; Gething et al., 2023; Jato-Espino et al., 2023).

No previous study has investigated the spatial distribution of milk quality indicators and foodborne pathogen distributions, which is needed for rapid response, decision-making, and management of foodborne diseases. Therefore, the primary aim of this study was to evaluate the microbial load and prevalence of foodborne pathogens in milk and cottage cheese and spatial analysis along the dairy value chain in the three regions of the country.

2 Materials and methods

2.1 Study areas

The study regions were chosen based on milk production potential; accordingly, Oromia, SNNP, and Amhara regions were selected as study areas (CSA, 2019). Each of the regions was represented by 4 study sites based on high dairy production and potential milk flow to the main cities and towns (Figure 1). The selected sites are characterized by the linkage of the dairy value chain activities, including milk production, collection, processing, and milk and cottage cheese retailing practices. Thus, Wolmera, Bishoftu, Asella, and Selale milk shades represented the Oromia region; Hawassa, Yirgalem, Woliata, and Dilla represented the SNPP region; and Debre Berhan, Debre Markos, BahirDar, and Gonder milk sheds represented the Amhara region.

2.2 Study design and sample size determination

A cross-sectional study was conducted, and milk and cottage cheese samples were collected from December 2020 to May 2021. The sample size was calculated based on the following formula (Charan and Biswas, 2013):

$$\text{Sample size} = \frac{Z^2 1-\alpha/2 * P(1-\text{P})}{d^2}$$

Using expected prevalence (P) as 50% and with a standard variation (at 5% type one error) of 1.96 and 5% precision (d), the sample size becomes 384. As we have considered three regions and four value chains, the sample size was increased by two times. Hence, a total of 912 milk and cottage cheese samples were collected from milk producers, milk collectors, milk processors (raw milk pasteurizing), farm markets, and retailers. The raw milk and traditional cottage cheese samples were collected from individual farmers and farm markets ($n = 272$), raw milk collectors ($n = 184$), milk processors ($n = 184$), and pasteurized milk and cottage cheese shops or retailers ($n = 272$).

2.3 Sample collection techniques and GIS

A list of dairy farmers, raw milk collectors, milk processors, retailers, and cottage cheese farm markets were identified from local milk cooperatives or collection centers and administrative districts found in the study areas.

A simple random sampling technique was applied to select raw milk producers and pasteurized retailers from the dataset. However, due to the limited number of milk processors and milk collection sites compared to producers and retailers, we randomly obtained samples from the batch of pasteurized milk and milk cans by varying the sampling days.

Approximately 250 mL of fluid raw milk samples were collected using sterilized plastic bottles for further microbial analysis. Potential cottage cheese farm markets were identified from each study site, and

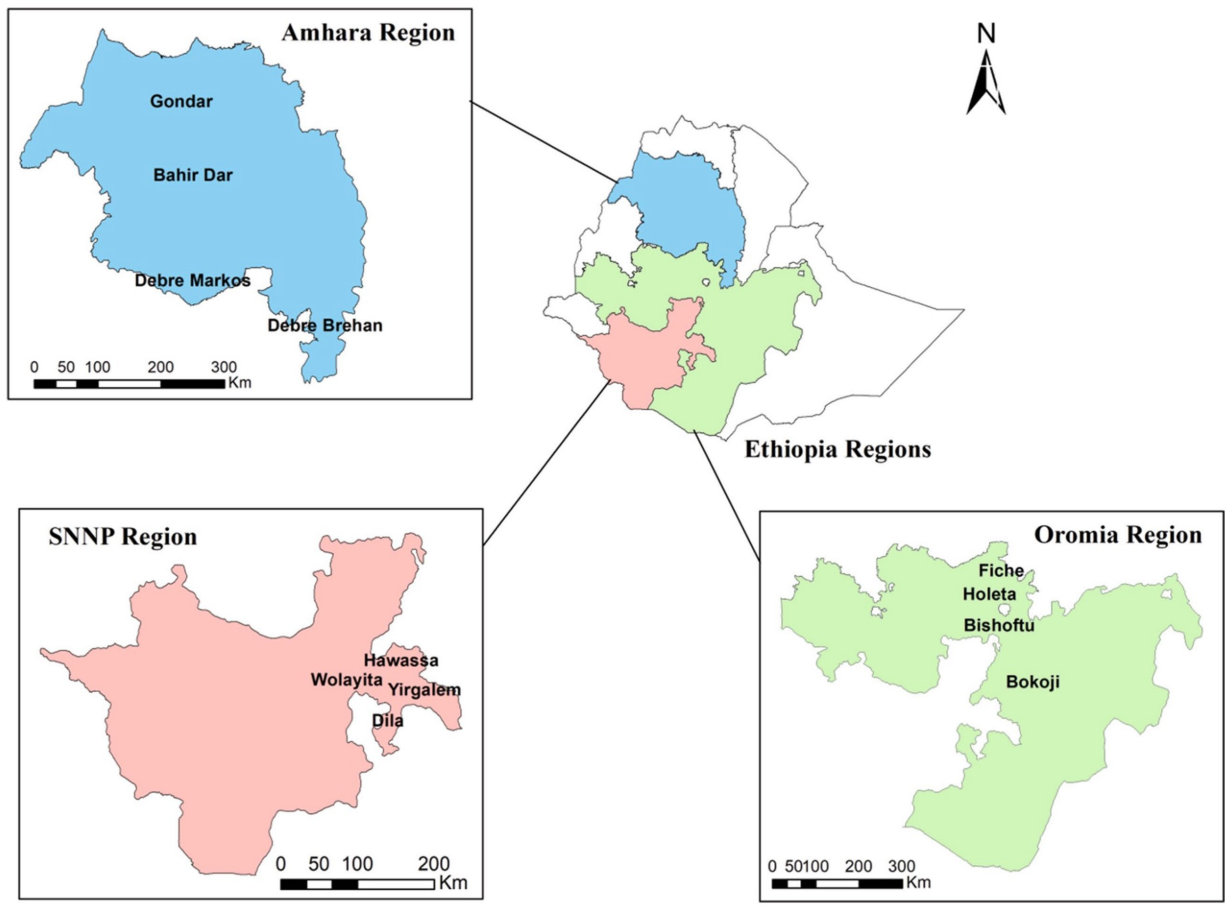

FIGURE 1
Study regions and sites.

250 g of the cottage cheese samples were collected aseptically using sterile stomacher bags. Additionally, pouches of packed pasteurized milk (approximately 500 mL) were collected from milk processors and retailers. The samples were stored in a cold chain and transported using a portable electrical fridge maintained at 4°C, connected to the vehicle's engine. Microbial analyses of the samples were conducted within 24-h of the sample collections.

The geographic locations of each sample were recorded by taking coordinates of altitudes and longitudes (X, Y) using a KOBO Toolbox data collection tool.

2.4 Microbial analyses

2.4.1 Enumerations of aerobic plate count and total coliform/*Escherichia coli* counts

Enumerations of standard plate count (SPC) and coliform/*E. coli* bacteria in milk and cottage cheese were done according to the standard protocols recommended by the Bacteriological Analytical Manual of the FDA (2003) and the 3M Petri film of food safety (3M Food Safety, 2017), respectively. A plate count agar (PCA) was used for the enumeration of APC counts, and 3M Petri film was used for the enumeration of the total coliform/*E. coli* counts. After serial dilutions were performed, 1 mL of the aliquot sample was plated on molten agar and 3M Petri film for the aerobic plate and total coliform/*E. coli* counts, respectively. The full detailed procedure enumeration of the SPC and coliform/*E. coli* was outlined in our previous publication (Mengstu et al., 2023).

2.4.2 Isolation and confirmation of *Listeria* spp.

The isolation and identification of *Listeria* were performed based on the standard detection method (ISO 11290-1, 2017). A 25 mL sample of the fluid milk or 25 g of the cottage cheese was first enriched in 225 mL of the half Fraser broth in filter stomacher bags and incubated at 30°C for 24 h. Then, a 10 µL pre-enriched sample was streaked on the chromogenic Agar Listeria according to Ottaviani and Agosti (ALOA) and Polymyxin-Acriflavin-Lithium Chloride-Ceftazidime-Aesculin-Mannitol (PALCAM) agars using a sterile loop and incubated at 37°C for 24–48 h. For further recovery of *Listeria* spp., 0.1 mL of pre-enriched samples was transferred into a tube containing 10 mL of Fraser broth and incubated at 37°C for 24 h. Then, 10 µL of second enrichment was streaked on ALOA and PALCAM agars and incubated at 37°C for 48 h. Presumptive Listeria colonies (green shiny colonies with diffuse black shadow around them on the PALCAM agar and blue colonies with or without halo around the colonies on the ALOA agar) were transferred to the brain heart infusion agar and further incubated at 37°C for 24 h. DNA of presumptive colonies of *Listeria* isolates was extracted, and PCR

confirmation of *Listeria* spp. and *Listeria monocytogenes* was conducted using specific *iap* and *lmo2234* primers, respectively. The full detailed procedure isolation *Listeria* spp. was outlined in our recent publication (Hassen et al., 2024).

2.4.3 Isolation and confirmation of *Salmonella enterica*

Enrichment and isolation of *Salmonella enterica* from milk and cottage cheese were performed according to ISO 6579-1 (2017). The detailed procedure of *Salmonella* enrichment, isolation, and conformation was outlined in our previous publication (Bedassa et al., 2023). Buffered Peptone Water (BPW) was used for the pre-enrichment of *Salmonella,* and it was incubated for 18 h at 35°C. A pre-enriched sample was transferred into selective media, Muller Kaufmann Tetrathionate (MKTTn) broth and Rappaport Vassiliadis (RV) broth, using a sterile loop and incubated at 41°C and 37°C for 24 h, respectively. Selectively enriched *Salmonella* was plated on xylose lysine deoxycholate (XLD) agar and hektoen enteric (HE) agar. For 24 h, inoculated plates were incubated aerobically at 37°C. Presumptive isolates were further sub-cultured onto the brain heat infusion (BHI) agar and incubated at 35°C for 24 h for molecular conformation. *Salmonella* isolates were confirmed utilizing PCR targeting the *invA gene*. Each electrophoresis run included a 100 bp DNA ladder, as well as positive (*Salmonella enterica ATTCC 35,664*) and negative controls (nuclease free water).

2.4.4 Isolation and confirmation of *Campylobacter* spp.

Samples of *Campylobacter* species were enriched according to ISO 10272-1 (2017) method B. Method B was used because of the anticipated high level of background flora. The detection approach B involved enrichment in Preston broth in a microaerobic environment at 41.5°C for 24 h. After enrichment, the samples were streaked on modified charcoal cefoperazone deoxycholate agar (mCCDA) in a microaerophilic environment at 41.5°C for 44 h, and then a Gram staining or latex agglutination test was performed. Isolates that passed these screenings were confirmed by PCR, which targeted *Campylobacter* spp., the 23S rRNA gene, and genes specific to each *Campylobacter* species. Molecular confirmation was carried out utilizing PCR, as described in full in our previous publication (Admasie et al., 2023).

3 Data analysis

Microbial quality indicators, including standard plate count (SPC), total coliform count (TCC), and *Escherichia Ecoli* (EC) counts, were converted to colony forming units (CFU) prior to data analysis. Based on ESA (2021) standard requirements, the microbial quality of the samples were categorized into below standard or acceptable level (pass), and above standard or unacceptable/more than acceptable level (failed). Then, microbial data was converted to dichotomous variables, where "1" represented the unacceptable/more than acceptable level (failed) or presence of at least one pathogen (either *Listeria monocytogenes, Salmonella enterica* or *Campylobacter* spp.), and "0" represented the acceptable level or absence of the three pathogens. Microbial data were used as the outcome variables, while the dairy value chain and study areas were considered the explanatory variables.

Logistic regression was used to evaluate the association between the microbial data and study areas and the dairy value chain. The strength of association was examined using the odds ratio at 95% confidence interval and $p < 0.05$ using statistical software, STATA version 17. Locations of sample collections were taken using the Global Positioning System (GPS). The Quantum GIS (QGIS) software was applied for the mapping and spatial distributions of the microbial quality indicators and foodborne pathogens. The red (unacceptable/more than acceptable level or failed) and green (acceptable level or pass) dots on the mapping indicate the microbial quality and safety of the samples collected from the different locations.

4 Results

The current study indicated that milk and cottage cheese collected in the three regions were contaminated with foodborne pathogens and high microbial loads, indicating poor microbial quality and safety standards.

Based on the ESA (2021) requirements for the total plate count of bacteria, the majority of the samples collected from the three regions were below the standard requirements. We found no associations between the total plate bacteria load and the regions of the samples collected (Table 1). However, there was a significant difference in total coliform count and *E. coli* bacteria between the regions ($p = 0.01$). The highest risk factors for total coliform count and *E. coli* were found in samples collected from the SNNP region (OR = 1.8) and Oromia region (OR = 2.3), respectively (Table 1).

Distribution of the standard plate count bacteria was found in the Oromia, SNNP, and Amhara regions; the total coliform bacteria were found distributed in the SNNP and Oromia regions, while *E. coli* bacteria were mostly found in the Oromia region (Figures 2–4).

Figure 5 demonstrates difference from total bacteria to *E. Coli* counts distribution in the regions indicating poor hygienic production and handling and fecal contamination of the dairy products, respectively. From the figure below (Figure 2), we can see the high microbial load distributions of the samples collected from Addis Ababa and surrounding sites of the Oromia region, including the rift valley areas, Hawassa and surrounding towns. Controversially, Amhara region sites except Debre Berhan were not identified as spots of the high bacterial load (Figure 2).

Table 2 illustrates some characteristics of dairy value chain types; the pooled prevalence of standard plate count distribution was estimated as 98% (95% CI 97.2–98.9%) across the value chain. The table indicates there was no significant difference in total coliform bacteria of the samples collected across the dairy value chain ($\chi 2 = 0.63$, $p = 0.4274$).

The pooled prevalence of below standard quality for coliform and *E. coli* was estimated as 61.2% (95% CI 58–64.3%) and 28.6% (95% CI 25.8–31.6%), respectively.

Further statistical analysis revealed that raw milk collectors significantly contributed to total coliform ($\chi 2 = 54.1$ $p = 0.000$) (Table 3) and *E. coli* distributions ($\chi 2 = 64.4$, $p = 0.000$) compared to other dairy value chain actors (Table 4). As shown in Table 3, the highest risk factor of total coliform was found at milk collection points (OR = 3.43, 95% CI 60.4–84.8), followed by milk processors (OR = 3.3, 95% CI 59.8–84.4) and retailers (OR = 1.7, 95% CI 44.5–71.8).

From the data indicated in Table 5, it is apparent that 50.3% (95% CI 47.1–53.7%) of the samples collected from the three regions were

TABLE 1 Microbial quality of milk and cottage cheese (standard plate count, total coliform count, and *E. coli*) collected from the three regions.

Regions	Tested samples	Bacteria	Number of positives	Percentages of positives	95% CI	OR	χ^2	*p* value
Amhara	192	TPC	192	100	-	-	-	
SNNP	240		240	100	-	-		
Oromia	480		464	96.7	-	-		
Total	912		896	98.2	97.2–98.9			
Amhara	192	TCC	110	57.3	50.2–64.1	Ref.	13.13	0.01
SNNP	240		170	70.8	43.7–82.8	1.8		
Oromia	480		278	57.9	42.4–72.0	1.0		
Total	912		558	61.2	58.0–64.3			
Amhara	192	EC	35	18.2	13.4–24.3	Ref.	27.41	0.01
SNNP	240		54	22.5	11.1–40.3	1.3		
Oromia	480		172	35.8	20.4–54.9	2.5		
Total	912		261	28.6	25.8–31.6			

Percentages of positive samples in the column are significantly different ($p < 0.05$); χ^2, chi square; OR, odds ratio; CI, confidence interval.

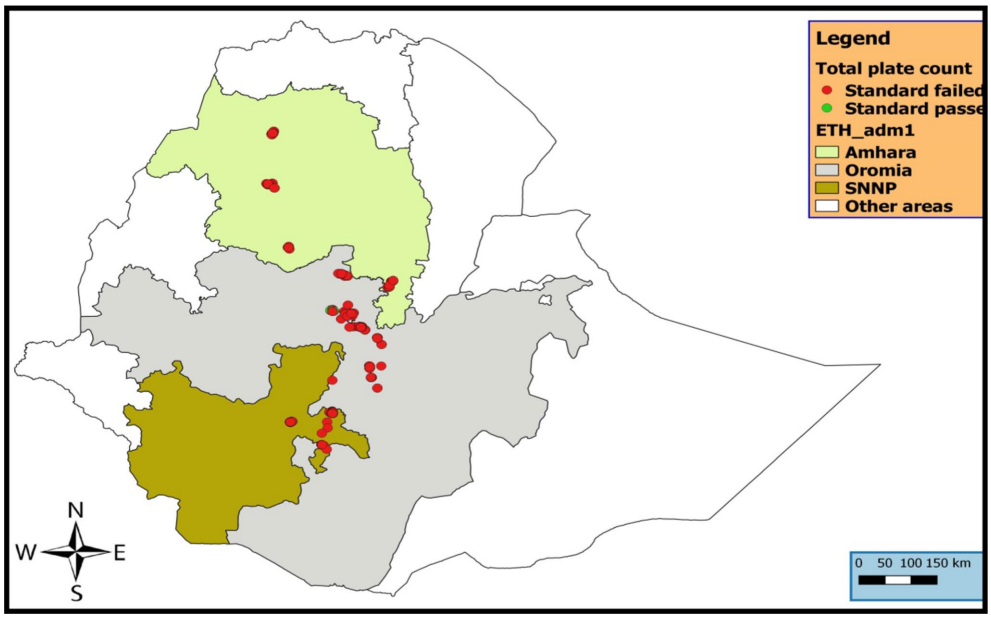

FIGURE 2
Distributions of aerobic plate count bacteria below standard requirements in the three regions.

contaminated by at least one pathogen, for the sample tested for either of *Listeria monocytogenes*, *Campylobacter* spp., or *Salmonella enterica*. The data indicated the significant distribution of the pathogens among the regions ($\chi 2 = 125$, $p = 0.00$). The samples collected from the Oromia region had 5.6 times higher risk of being contaminated by at least one pathogen (OR = 5.6, 95% CI 51.0–80.5) which was followed by the SNNP region (OR = 1.4, 95% CI 20.3–52.4).

The distribution of the foodborne pathogens across the dairy value chain by considering at least one positive pathogen for the tested samples is indicated in Figure 6. Foodborne pathogens distributions across the value chain were observed in similar terms with the microbial indicators as indicated in Table 6. The table indicates the samples collected from milk collectors were contaminated with at least one pathogen by 63.6% of the total samples and had higher risk factor (OR = 2.1, 95% CI 48–74.5%). An important point that emerged from the data was that the risk factor of the pasteurized milk was higher (OR = 1.3, 95% CI 36.6–66.3) than the raw milk at production points (Table 6).

5 Discussion

The current study reveals that the microbial quality and safety of milk and cottage cheese were found below standard requirements and unsafe for human consumption. Based on the microbial load of the standard plate, total coliform, and *E. coli* bacteria counts, the

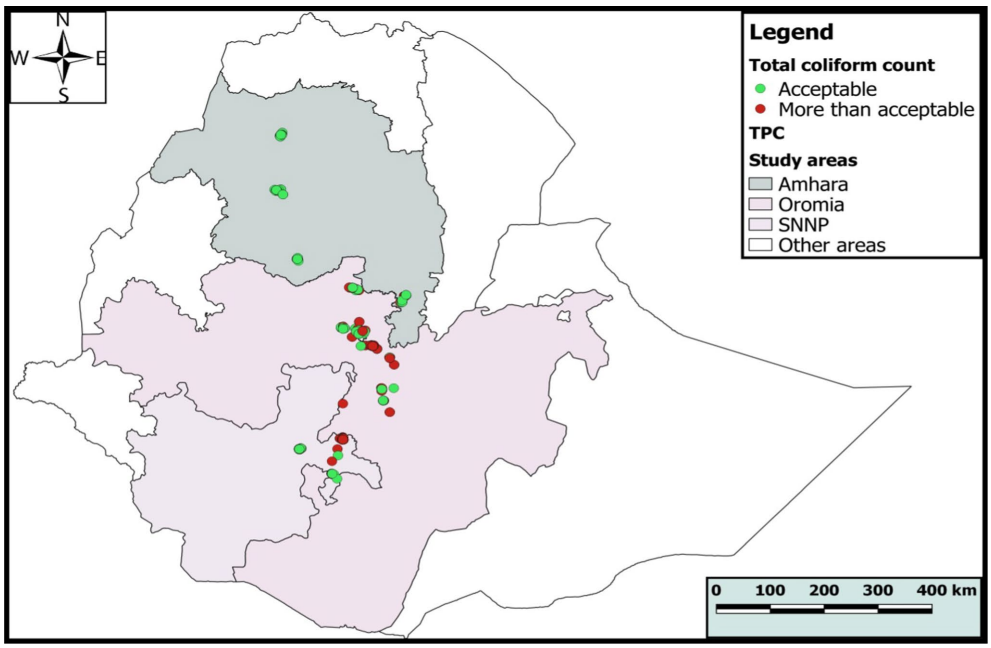

FIGURE 3
Distributions of total coliform bacteria below and above the standard requirements in the regions.

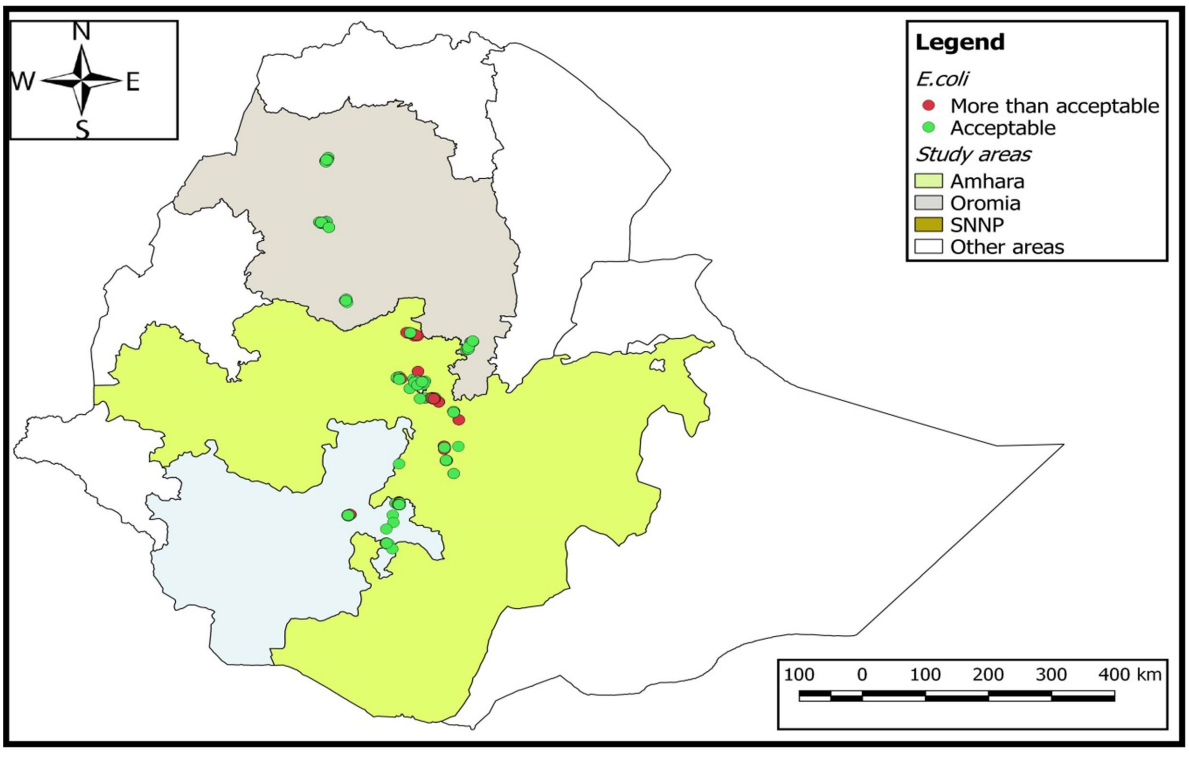

FIGURE 4
Distributions of *E. coli* bacteria counts below and above the standard requirements in the regions.

samples were found below the standards set by ESA (2021). A result comparable to our finding was reported in the central highlands, where the bacteriological quality of dairy product was below the standards set by the Ethiopian Standards Agency (Tola, 2016). The current results indicate dairy products are highly contaminated with foodborne pathogens. The dairy products contaminated with high-load microbial quality indicators of aerobic plate and total coliform were related to the prevalence of

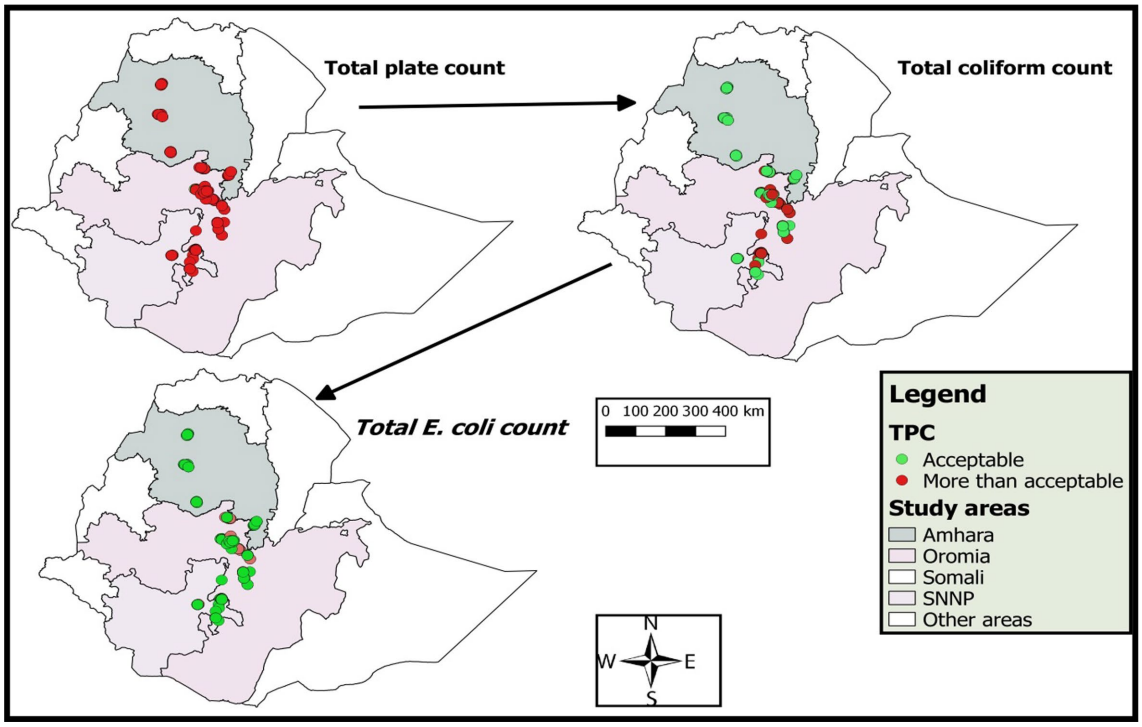

FIGURE 5
Distributions of quality indicators difference from the holistic total bacteria to *E. coli* counts in the regions.

TABLE 2 Distributions of aerobic plate count below standard requirements across the dairy value chain.

Value chain	Samples tested	Number of positives	Percentage of positives	95% CI	OR	χ^2	*p*-value
Producers	272	264	97.0	94.2–98.5	Ref.	0.63	0.4274
Collectors	184	176	96.0	80.0–99.2	0.67		
Processors	184	184	100	-	1.00		
Retailers	272	272	100	-	1.00		
Total	912	896	98.0	97.2–98.9			

Percentages of positive samples in the column are significantly different ($p < 0.05$); $\chi 2$, chi square; OR, odds ratio; CI, confidence interval.

TABLE 3 Distributions of the total coliform bacteria counts below standard requirements across the dairy value chain.

Value chain	Samples tested	Number of positives	Percentage of positives	95% CI	OR	χ^2	*p*-value
Producers	272	125	46.0	40.1–51.9	Ref.	54.11	0.000
Collectors	184	137	74.5	60.4–84.8	3.43		
Processors	184	136	73.9	59.8–84.4	3.3		
Retailers	272	160	58.8	44.5–71.8	1.7		
Total	912	558	61.2	58.0–64.3			

Percentages of positive samples in the column are significantly different ($p < 0.05$); χ^2, chi square; OR, odds ratio; CI, confidence interval.

foodborne pathogens and distributions in the regions. The pooled prevalence of unacceptable levels of aerobic plate count and total coliform count coliform loads indicated that half the tested samples were contaminated with potential foodborne pathogens, which were prioritized by the WHO in the global burden of foodborne disease (Havelaar et al., 2015).

The study also confirms positive associations between microbial load and foodborne pathogens distribution in the regions and dairy value chain. High microbial load and prevalence of foodborne pathogens are mainly found in the Oromia region, followed by the SNNP region. This might be related to milk potential; the Oromia region covers nearly 52% of the country's

TABLE 4 Distribution of *E. coli* bacteria counts below standard requirements across the dairy value chain.

Value chain	Samples tested	Number of positives	Percentage of positives	95% CI	OR	$\chi 2$	*p*-value
Producers	272	70	25.7	20.9–31.3	Ref.	64.41	0.000
Collectors	184	95	51.6	35.4–67.6	3.1		
Processors	184	50	27.2	15.7–42.8	1.1		
Retailers	272	46	16.9	9.3–28.9	0.6		
Total	912	261	28.6	25.8–31.6			

Percentages of positive samples in the column are significantly different ($p < 0.05$); χ^2, chi square; OR, odds ratio; CI, confidence interval.

TABLE 5 Prevalence of foodborne pathogens for at least one of the pathogens (*Listeria monocytogenes, Salmonella enteric*, and *Campylobacter* spp.,) isolated from the three regions.

Study regions	Samples tested	Number of positives	Percentage of positives	95% CI	OR	χ^2	*p*-value
Amhara	192	52	27.1	21.3–33.8	Ref.	125.10	0.000
SNNP	240	83	34.6	20.3–52.4	1.4		
Oromia	480	324	67.5	51.0–80.5	5.6		
Total	912	459	50.3	47.1–53.7			

Percentages of positive samples in the column are significantly different ($p < 0.05$); χ^2, chi square; OR, odds ratio; CI, confidence interval.

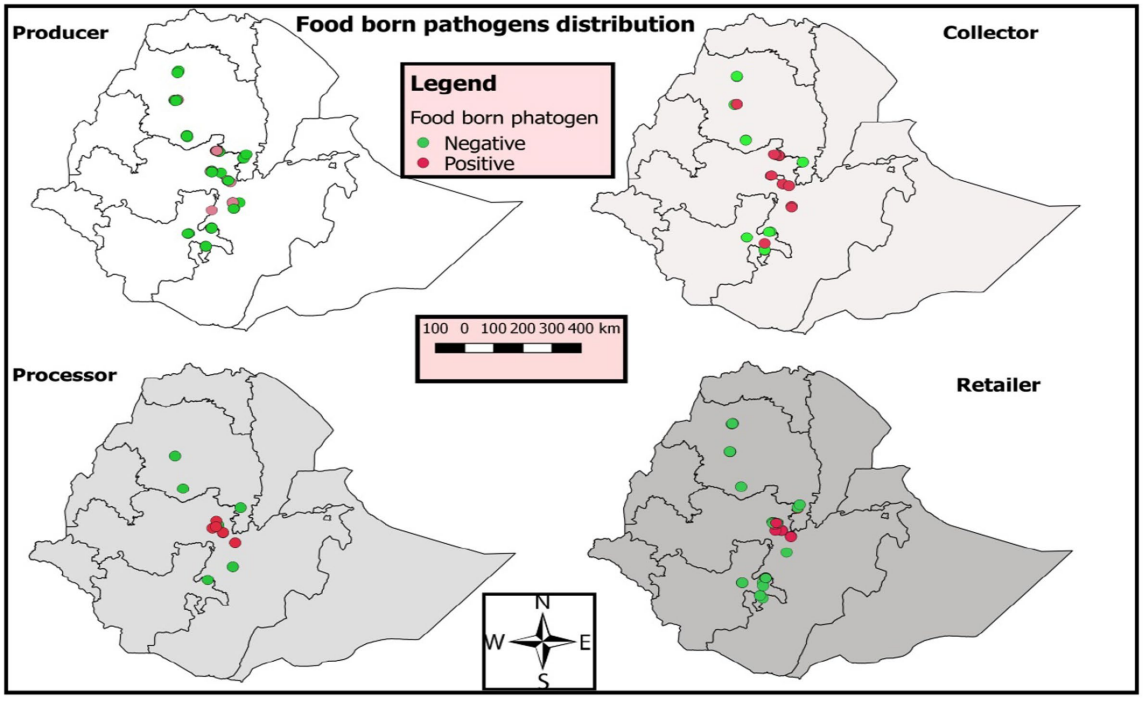

FIGURE 6
Distributions of at least one pathogen (*Listeria monocytogenes, Salmonella enterica,* and *Campylobacter* spp.) across the dairy value chain of the three regions.

milk production (Bereda et al., 2017; CSA, 2019), but also it is geographically located in the central highlands of the country. The study areas are characterized by the conducive climate factors for bacterial growth, optimum temperature, and bimodal rainfall which support the growth of bacteria and promote bacterial proliferation (Alemayehu and Bewket, 2017; Matewos and Tefera, 2019; Brhane et al., 2022; Budusa et al., 2023; Yona et al., 2024).

Dairy product marketing practices are mainly related to urbanization and urban consumers and Oromia and SNNP regions are connected to market outlets (Kenea Amentae et al., 2015; Minten et al., 2020; D'Haene and D'Haese, 2020; Zeleke et al., 2020). High marketing practices can be indicative of high consumer demand for the products, however, practice can give low attention to handling practices, cold chains, and adulteration practices of the food

TABLE 6 Prevalence of foodborne pathogens for at least one of the pathogens (*Listeria monocytogenes, Salmonella enterica*, and *Campylobacter* spp.,) across the dairy value chain.

Value chain	Samples tested	Number of positives	Percentage of positives	95% CI	OR	$\chi 2$	*p*-value
Producers	272	125	46.0	40.1–51.9	Ref.	18.58	0.0003
Collectors	184	117	63.6	48.4–74.5	2.1		
Processors	184	95	51.6	36.6–66.3	1.3		
Retailers	272	122	44.9	31.4–59.1	1		
Total	912	459	50.3	47.1–53.6			

Percentages of positive samples in the column are significantly different ($p < 0.05$); χ^2, chi square; OR, odds ratio; CI, confidence interval.

commodity. The distribution of microbial quality indicators and foodborne pathogens is concentrated in the central highlands of the country within 100 kilometers radius from Addis Ababa city to surrounding areas, including the Rift Valley areas, Hawassa and surrounding towns, which need the dairy safety interventions. This finding supports the work recently initiated by the Oromia Regional State on dairy product quality regulations and marketing systems. Poor hygienic dairy farming practices, absence of clean water resources, and low awareness of dairy safety and quality (Bereda et al., 2017; Yetera et al., 2018; Ndambi et al., 2018; Deddefo et al., 2023; Lijalem et al., 2015).

Although the current study did not collect the temperature and humidity data of the study areas, the result may be explained by the fact that the areas are characterized by the conducive climate factors for bacterial growth and the central part of the Ethiopia, which represented current Oromia region, had an optimum temperature and bimodal rainfall which is support growth temperature and promote bacterial proliferation, (Alemayehu and Bewket, 2017; Matewos and Tefera, 2019; Brhane et al., 2022; Budusa et al., 2023; Yona et al., 2024).

Further study is required to establish the relationship between each of the pathogens and environmental factors of the distribution areas. The results of the study indicate that the raw milk and pasteurized milk collected from milk collectors and pasteurizers significantly contributed to contamination levels, which could be related to unhygienic milk collection practices, lack of testing methods, storage, and transportation practices (Mpatswenumugabo et al., 2019; Andrew et al., 2021), and half of the pasteurized samples were contaminated, which may be due to a lack of adequate milk pasteurization temperature and time, the efficacy of the pasteurizers machine, or post-pasteurization contaminations and poor handling and storage practices (Martin et al., 2018; Fusco et al., 2020; Rosario et al., 2021; Calahorrano-moreno et al., 2022).

6 Conclusion

The following conclusion can be drawn from the present study: consuming raw milk, pasteurized milk, and cottage cheese pose health risks. The central highlands and southern regions are identified as the intervention areas, and understanding the bacteria distributions extends our knowledge of bacteria and environment interactions.

The microbial quality and safety of dairy products are substandard and unsafe for human consumption. Microbial quality standards used by the country should be in place for evaluation and monitoring purposes. Despite its explanatory nature, this study offers some key insight into the importance of food traceability to address the threats across the food value chain and critical intervention areas for future works. The current research was not specifically designed to evaluate factors related to each pathogen isolated from dairy products, and we recommend that further studies might be required to explore complex interactions between pathogens and environments as well as with dairy value chain actors in deep practices.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

Ethics statement

The study was approved and ethical clearance was obtained from the Institutional Review Board (IRB) of the College of Natural and Computational Sciences, Addis Ababa University (CNS-IRB 42/2019). Moreover, both informed and written consent was obtained from the human subjects. Confidentiality of the data was assured by giving a unique personal identification code to each participant and sample.

Author contributions

AK: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing. GG: Conceptualization, Formal analysis, Methodology, Software, Writing – review & editing. HN: Data curation, Writing – review & editing. AZ: Conceptualization, Funding acquisition, Investigation, Project administration, Supervision, Validation, Visualization, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. The project is supported by the Bill and Melinda Gates Foundation and the Foreign,

Commonwealth, and Development Office of the UK through the ENSURE project (grant number INV-008459) awarded to Addis Ababa University.

Acknowledgments

We would like to express our heartfelt gratitude to the people and agricultural development agents (DEAs) of all regions who took the time to facilitate this study. We are particularly grateful to Anwar Hassan, Abdi Bedassa, and Abera Admasie for their assistance with the Listeria, Salmonella, and Campylobacter prevalence analysis in the laboratory.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The authors declare that no Gen AI was used in the creation of this manuscript.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2024.1535630/full#supplementary-material

References

3M Food Safety (2017). 3M petri film *E. coli*/coliform count plate interpretation. Loughborough, UK: 3M Food Safety.

Abebe, E., Gugsa, G., and Ahmed, M. (2020). Review on major food-borne zoonotic bacterial pathogens. *J. Trop. Med.* 2020, 1–19. doi: 10.1155/2020/4674235

Admasie, A., Eshetu, A., Tessema, T. S., Vipham, J., Kovac, J., and Zewdu, A. (2023). Prevalence of Campylobacter species and associated risk factors for contamination of dairy products collected in a dry season from major milk sheds in Ethiopia. *Food Microbiol.* 109:104145. doi: 10.1016/j.fm.2022.104145

Adugna, M., and Asresie, A. (2015). A review on microbiological quality of Ethiopian raw bovine. *Food. Sci. Qual. Manage.* 35, 2224–6088.

Alemayehu, A., and Bewket, W. (2017). Local spatiotemporal variability and trends in rainfall and temperature in the central highlands of Ethiopia. *Geogr. Ann. Ser. A Phys. Geogr.* 99, 85–101. doi: 10.1080/04353676.2017.1289460

Andrew, R., Chusi, T., and Mwembezi, G. P. (2021). Milking hygiene and handling practices among smallholder dairy farmers in Zanzibar. *Eur. J. Agri. Food Sci.* 3, 82–88. doi: 10.24018/ejfood.2021.3.6.422

Bedassa, A., Nahusenay, H., Asefa, Z., Sisay, T., Girmay, G., and Kovac, J. (2023). Prevalence and associated risk factors for Salmonella enterica contamination of cow milk and cottage cheese in Ethiopia. *Food Saf. Risk* 10, 2–11. doi: 10.1186/s40550-023-00101-3

Bereda, A., Yilma, Z., Eshetu, M., Yousuf, M., and Assefa, G. (2017). Socio-economic characteristics of dairy production in the selected areas of Ethiopian central highlands. *J. Vet. Med. Anim. Health* 9, 193–203. doi: 10.5897/JVMAH2017.0588

Bhaskar, S. V. (2017). "Foodborne diseases-disease burden" in Food safety in the 21st century: public health perspective. eds. R. Kumar, G. Dudeja and S. Minhas (New York: Academic Press).

Brhane, H., Amente, G., Ture, K., Legese, G., and Siyoum, T. (2022). Changes in daily rainfall and temperature extremes of upper Awash Basin, Ethiopia. *Sci. Afr.* 16:e01173. doi: 10.1016/j.sciaf.2022.e01173

Broglia, A., Broglia, A., and Kapel, C. (2019). Changing dietary habits in a changing world: emerging drivers for the transmission of foodborne parasitic zoonoses veterinary parasitology changing dietary habits in a changing world: emerging drivers for the transmission of foodborne parasitic zoonoses. *Vet. Parasitol.* 182, 2–13. doi: 10.1016/j.vetpar.2011.07.011

Budusa, M., Tafesse, W., Mekuria, M., Abiyot, G., and Yimer, L. (2023). Spatiotemporal variability and trend of rainfall and temperature in Sidama regional state, Ethiopia. *Theor. Appl. Climatol.* 153, 213–226. doi: 10.1007/s00704-023-04463-8

Calahorrano-moreno, M. B., Ordoñez-bailon, J. J., Baquerizo-crespo, R. J., Dueñas-rivadeneira, A. A., Montenegro, M. C. B. S. M., and Rodríguez-díaz, J. M. (2022). Contaminants in the cow's milk we consume? Pasteurization and other technologies in the elimination of contaminants. *F1000Res* 11:91. doi: 10.12688/f1000research.108779.1

Chamberlin, J., and Schmidt, E. (2011). Ethiopian agriculture: a dynamic geographic perspective. Philadelphia, PA: University of Pennsylvania Press.

Charan, J., and Biswas, T. (2013). How to calculate sample size for different study designs in medical research? *Indian J. Psychol. Med.* 35, 121–126. doi: 10.4103/0253-7176.116232

CSA (2019). Federal democratic republic of Ethiopia central statistical agency agricultural sample survey 2020/21. Addis Ababa: CSA.

D'Haene, E., and D'Haese, M. (2020). Milk in the city: profiles and development paths for urban dairy holders in Ethiopia. *Dev. South. Afr.* 37, 644–660. doi: 10.1080/0376835X.2019.1689099

Deddefo, A., Mamo, G., Asfaw, M., and Amenu, K. (2023). Factors affecting the microbiological quality and contamination of farm bulk milk by *Staphylococcus aureus* in dairy farms in Asella, Ethiopia. *BMC Microbiol* 23:2746. doi: 10.1186/s12866-022-02746-0

Duchenne-Moutien, R. A. (2021). Climate change and emerging food safety issues: a review. *J. Food Prot.* 84, 1884–1897. doi: 10.4315/JFP-21-141

ESA (2021). Bacteriological standards for raw (CES 278) and pasteurized Milk (CES 279). Addis Ababa, Ethiopia: ESA.

FDA. (2003). Bacteriological analytical manual, enumerations of total plate counts in foods. Available at: http://www.foodsafety.gov/~dms/lmrisk.html.

Fusco, V., Chieffi, D., Kabisch, J., Fanelli, F., Logrieco, A. F., Böhnlein, C., et al. (2020). Microbial quality and safety of milk and milk products in the 21st century. *Compr. Rev. Food Sci. Food Saf.* 19, 2013–2049. doi: 10.1111/1541-4337.12568

Gething, P. W., Ayling, S., Mugabi, J., Muximpua, O. D., Kagulura, S. S., and Joseph, G. (2023). Cholera risk in Lusaka: a geospatial analysis to inform improved water and sanitation provision. *PLOS Water* 2:e0000163. doi: 10.1371/journal.pwat.0000163

Gizaw, Z. (2019). Public health risks related to food safety issues in the food market: a systematic literature review. *Environ. Health Prev. Med.* 24, 68–21. doi: 10.1186/s12199-019-0825-5

Gizaw, S., Ebro, A., Tesfaye, Y., Mekuriaw, Z., Mekasha, Y., Hoekstra, D., et al. (2017). Feed resources in the highlands of Ethiopia: a value chain assessment and intervention options. Ethiopia: LIVES Working Paper.

Gomez-Zavaglia, A., Mejuto, J. C., and Simal-gandara, J. (2020). Since January 2020 Elsevier has created a COVID-19 resource Center with free information in English and mandarin on the novel coronavirus COVID-19. The COVID-19 resource Center is hosted on Elsevier connect, the company's public news and information. Available at: https://bookcafe.yuntsg.com/ueditor/jsp/upload/file/20210717/1626492196782000477.pdf.

Grace, D. (2023). Burden of foodborne disease in low-income and middle-income countries and opportunities for scaling food safety interventions. *Food Sec.* 15, 1475–1488. doi: 10.1007/s12571-023-01391-3

Grace, D., Wu, F., and Havelaar, A. H. (2020). MILK symposium review: foodborne diseases from milk and milk products in developing countries — review of causes and health and economic implications. *J. Dairy Sci.* 103, 9715–9729. doi: 10.3168/jds.2020-18323

Hamaideh, S., Olaimat, A. N., Al-holy, M. A., Ababneh, A., Shahbaz, H. M., Abughoush, M., et al. (2024). The influence of technological shifts in the food chain on the emergence of foodborne pathogens: an overview. *Appl. Microbiol.* 4, 594–606. doi: 10.3390/applmicrobiol4020041

Hassen, A., Keba, A., Ebrai, M. S., Mamo, H., Geleta, T. K., Tessema, T. S., et al. (2024). Prevalence of Listeria monocytogenes and Listeria species and associated risk factors for contamination of milk and cottage cheese along the value chains in Ethiopia. *Int. J. Food Microbiol.* p. 111021.

Havelaar, A. H., Kirk, M. D., Torgerson, P. R., Gibb, H. J., Hald, T., Lake, R. J., et al. (2015). World Health Organization global estimates and regional comparisons of the burden of foodborne disease in 2010. *PLoS Med.* 12:e1001923. doi: 10.1371/journal.pmed.1001923

Hellberg, R. S., and Chu, E. (2016). Effects of climate change on the persistence and dispersal of foodborne bacterial pathogens in the outdoor environment: a review. *Crit. Rev. Microbiol.* 42, 548–572. doi: 10.3109/1040841X.2014.972335

ISO 10272-1 (2017). Microbiology of the food chain horizontal method for detection and enumeration of Campylobacter spp., part 1: Detection method. Geneva, Switzerland: International Standardization Organization.

ISO 11290-1 (2017). Microbiology of the food chain — Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. — Part 1: detection method. Geneva, Switzerland: International Standardization Organization.

ISO 6579-1 (2017). Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of Salmonella – Part 1: detection of Salmonella Spp. Geneva, Switzerland: International Organization for Standardization.

Jato-Espino, D., Mayor-Vitoria, F., Moscardó, V., Capra-Ribeiro, F., and Bartolomé del Pino, L. E. (2023). Toward one health: a spatial indicator system to model the facilitation of the spread of zoonotic diseases. *Front. Public Health* 11, 1–17. doi: 10.3389/fpubh.2023.1215574

Kanankege, K. S. T., Phelps, N. B. D., Vesterinen, H. M., Errecaborde, K. M., Alvarez, J., Bender, J. B., et al. (2020). Lessons learned from the stakeholder engagement in research: application of spatial analytical tools in one health problems. *Front. Vet. Sci.* 7, 1–9. doi: 10.3389/fvets.2020.00254

Keba, A., Rolon, M. L., Tamene, A., Dessie, K., Vipham, J., Kovac, J., et al. (2020). Review of the prevalence of foodborne pathogens in milk and dairy products in Ethiopia. *Int. Dairy J.* 109:104762. doi: 10.1016/j.idairyj.2020.104762

Kenea Amentae, T., Gebresenbet, G., and Ljungberg, D. (2015). Characterizing Milk supply and marketing chains and losses in Wolmera and Ejere districts of Ethiopia. *J. Serv. Sci. Manag.* 8, 823–843. doi: 10.4236/jssm.2015.86084

Lijalem, T., Asefa, A., Sharo, A., and Wollaita, E. (2015). Dairy cattle production at smallholder level in Sidama zone selected districts, southern Ethiopia. *Food Sci. Qual. Manag.* 40, 2224–6088.

Martin, N. H., Boor, K. J., and Wiedmann, M. (2018). Symposium review: effect of post-pasteurization contamination on fluid milk quality. *J. Dairy Sci.* 101, 861–870. doi: 10.3168/jds.2017-13339

Matewos, T., and Tefera, T. (2019). Local level rainfall and temperature variability in drought-prone districts of rural Sidama, central rift valley region of Ethiopia. *Phys. Geogr.* 41:850. doi: 10.1080/02723646.2019.1625850

Mengstu, B., Tola, A., Nahusenay, H., Sisay, T., Kovac, J., Vipham, J., et al. (2023). Evaluation of microbial hygiene indicators in raw milk, pasteurised milk and cottage cheese collected across the dairy value chain in Ethiopia Bethlehem Mengistu. *Int. Dairy J.* 136:105487. doi: 10.1016/j.idairyj.2022.105487

Minten, B., Habte, Y., Tamru, S., and Tesfaye, A. (2020). The transforming dairy sector in Ethiopia. *PLoS One* 15, 1–24. doi: 10.1371/journal.pone.0237456

Mpatswenumugabo, J. P. M., Bebora, L. C., Gitao, G. C., Mobegi, V. A., Iraguha, B., and Shumbusho, B. (2019). Assessment of bacterial contamination and milk handling practices along the raw milk market chain in the north-western region of Rwanda. *Afr. J. Microbiol. Res.* 13, 640–648. doi: 10.5897/AJMR2018.8919

Nayak, R., and Waterson, P. (2019). Global food safety as a complex adaptive system: key concepts and future prospects. *Trends Food Sci. Technol.* 91, 409–425. doi: 10.1016/j.tifs.2019.07.040

Ndambi, A., Zijlstra, J., Eupen, M.Van, and Berhanu, T. (2018). Mapping and assessing high potential areas to increase milk production in Ethiopia. Wageningen Livestock Research: Netherlands

Polonsky, J. A., Baidjoe, A., Kamvar, Z. N., Cori, A., Durski, K., John Edmunds, W., et al. (2019). Outbreak analytics: a developing data science for informing the response to emerging pathogens. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 374:20180276. doi: 10.1098/rstb.2018.0276

Rosario, A. I. L. S., Castro, V. S., Santos, L. F., Lisboa, R. C., Vallim, D. C., Silva, M. C. A., et al. (2021). Shiga toxin – producing *Escherichia coli* isolated from pasteurized dairy products from Bahia, Brazil. *J. Dairy Sci.* 104, 6535–6547. doi: 10.3168/jds.2020-19511

Sugrue, I., Tobin, C., Ross, R. P., Stanton, C., and Hill, C. (2018). "Foodborne pathogens and zoonotic diseases" in Raw milk: balance between hazards and benefits. eds. L. A. Nero and A. F. D. Carvalho (New York: Academic Press).

Tauxe, R. V. (2002). Emerging foodborne pathogens. *Int. J. Food Microbiol.* 78, 31–41. doi: 10.1016/S0168-1605(02)00232-5

Tola, A. (2016). Assessment of safety and quality of raw whole cow milk produced and marketed by smallholders in central highlands of Ethiopia. *J. Food Sci. Qual. Manage.* 49, 63–71.

WHO (2015). WHO estimates of the global burden of foodborne diseases. Geneva: World Health Organization.

Yetera, A., Urge, M., and Nurfeta, A. (2018). Smallholder dairy cows husbandry practices in selected districts of Sidama zone, Southern Ethiopia. *Acad. Res. J. Agri. Sci. Res.* 6, 357–369. doi: 10.14662/ARJASR2018.054

Yona, Y., Matewos, T., and Sime, G. (2024). Heliyon analysis of rainfall and temperature variabilities in Sidama regional state, Ethiopia. *Heliyon* 10:e28184. doi: 10.1016/j.heliyon.2024.e28184

Zeleke, B. D., Assegidaw, E. T., and Abiot, D. (2020). Urban and Peri-urban milk market channel choice using multivariate probit approach: evidence from Arsi Highland of Ethiopia. *Res. Sq.* 4, 1–41. doi: 10.21203/rs.3.rs-31191/v1