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Research Article

Determination of Microbial Hygiene Indicators of Raw Cow Milk in Assosa District, Ethiopia

Abebe Bereda Fereja [iD],[1] Nigussu Fekade Aboretugn,[2] and Nigatu Qiltu Bulti[2]

[1]*Federal Technical and Vocational Training Institute, Addis Ababa, Ethiopia*
[2]*Assossa University, Assosa, Ethiopia*

Correspondence should be addressed to Abebe Bereda Fereja; ababfereja@gmail.com

The study was conducted to evaluate raw cow milk microbial quality in the Assosa district from January to March 2021. Sixty farmers were chosen from 200 surveyed households for milk samples to determine microbial analysis using standard procedures. From each farmer, 450 mL of the fresh morning raw cow milk sample was collected aseptically using a sterile sampling bottle. The milk samples were analyzed in duplicates immediately after 12 hrs upon laboratory arrival. Milk microbial analysis, sampling, transportation, and handling followed standard procedures. The average aerobic mesophilic bacterial count, *E. coli*, *Staphylococcus aureus*, yeast and mold count, and coliform count were 9.0, 5.3, 7.8, 9.2, and 6.8 $\log_{10}$ CFU/mL, respectively. However, significant differences between urban and rural locations were observed except for the last parameter. The counts of all microbial parameters, including the pathogen *Staphylococcus aureus* found in this study, were higher than the acceptable limit set by the Ethiopian standard authority. This indicates that smallholder milk was highly contaminated and may have originated from a single source. Consequently, the hygienic quality of milk is not safe for consumption; instead, it can cause different types of foodborne pathogens. Therefore, identification of the contamination sources of milk and urgent remedial actions will be taken to protect consumers' health.

1. Introduction

Ethiopia has the most extensive livestock inventory in the region, with 70 million cattle, of which female cattle account for around 56%. Livestock accounts for 16.5 percent of the national GDP and 40% of the agricultural GDP, respectively. The number of dairy cows is anticipated to be 7.5 million, with an annual milk production of around 4.69 billion liters [1].

In Ethiopia, just 2% of milk reaches end customers through the legal milk marketing channel, while the remaining 98% is traditionally processed, unprocessed, and marketed through its counterpart, the informal marketing chain [2]. This shows how significant milk and milk products are for household consumption and revenue sources. As a result, goods derived from raw milk must be of high hygienic quality. Ethiopia, in particular, and underdeveloped countries generally lack adequate circumstances for manufacturing safe and high-quality dairy products. Milk can be contaminated by various microorganisms, including pathogens, which can cause foodborne diseases in consumers due to unsanitary handling procedures used by farmers and following chain actors in Ethiopia [3, 4].

Foodborne infections pose a significant public health danger to individuals and governments, especially in developing countries where food is routinely produced in unsanitary conditions. The primary public health problems associated with raw milk and its products are contaminated with pathogens such as *E. coli*, *Salmonella* spp., *Campylobacter* spp., and *Listeria monocytogenes* [5–7]. As a result, bacteria are potential causes of human digestive disturbances, such as vomiting and diarrhea, which can lead to life-threatening infections [8].

The presence of bacteria in milk is a crucial predictor of its quality, and the concentration of total coliform and aerobic mesophilic bacteria in raw cow milk is widely used to

measure this [9]. For example, milk produced under optimal sanitary conditions and with healthy milking animals has a maximum amount of 5×10^5 bacteria per milliliter (mL) of milk. *Escherichia coli* is an Enterobacteriaceae species common in human and animal intestines, but only a few strains are enterohemorrhagic pathogens. Furthermore, *E. coli* is a common sign of fecal contamination because it might originate from and/or animals [10]. Although there has been little research on milk and milk products in Ethiopia, it is important to note the 10.7% prevalence finding of enterotoxigenic *E. coli* in the Tigray region [7], and Keba et al. [11] also indicated the meta-analysis prevalence of *E. coli* in raw animal products including dairy products.

In many parts of the world, staphylococcal food poisoning (SFP) is the most common cause of foodborne infections such as gastroenteritis. *Staphylococcus aureus* enterotoxication, for example, is ranked third, fourth, and second in the United States of America, Europe, and China, respectively [12–14]. Although the country lacks solid statistics on SFP prevalence, a fragmented research report reveals a high prevalence of *Staphylococcus aureus* [7, 15]. *Staphylococcus aureus* was found in 21.2% of the people in southern Ethiopia [16], 22.2% in Gondar [17], and 19.6% in Sebeta [18]. Although *Staphylococcus aureus* is found everywhere, people and animals are the principal reservoirs. Heat treatment and practically all sanitizing chemicals are effective at killing *Staphylococcus aureus*. The presence of high levels of *Staphylococcus aureus* indicates insufficient sanitation. Worse, raw cow milk intake is widespread in Ethiopia [19].

According to Zemenu [10], milk ready for human consumption requires a strict standard quality and safety assurance system. However, research findings in Ethiopia are unrepresentative and concentrated in the country's central highlands, providing fragmented information on dairy product quality and safety across the dairy value chain. As a result, data on the counts of hygiene indicators, aerobic mesophilic bacteria, coliform count, *E. coli*, yeast and mold count, and *Staphylococcus* aureus are rare in the current research district. Data generation, in this respect, provides critical input to customers, vendors, milk processors, regulatory authorities, and policymakers to take appropriate remedial measures. Therefore, the study's objective was to evaluate the microbial quality of raw cow milk in Assosa district, Benishangul-Gumuz regional state of Ethiopia.

2. Materials and Methods

2.1. Description of the Study Area.
The research was carried out in the Assosa district of Benishangul-Gumuz regional state (BGRS), western Ethiopia. The district is $10°46'0''$ N latitude, $35°32'0''$ E longitude, and 1,581 meters above sea level. The annual average rainfall in the district ranges from 850 to 1200 mm, with minimum and maximum average yearly temperatures of 20 and 32°C, respectively. Rainfall has bimodal patterns that typically occur from May through October, with the most rain falling between July and August. Assosa is Benishangul-Gumuz regional state's capital city, 661 kilometers from Ethiopia's capital city, Addis Ababa.

Crop-livestock mixed agricultural techniques characterize the district. The main food crops grown in the district are maize, sorghum, finger millet, teff, and pulses. The overall number of cattle in the region is anticipated to be 592,228 heads, with Assosa accounting for 69,440 heads [1]. The average number of indigenous milking cows, dry cows, heifers, and calves in both locations per household was 2.29. 0.88, 1.37, and 1.23, respectively, while the corresponding crossbreed numbers were 1.93, 3.02, 1.15, and 3.5, respectively. The primary feed resources available in Assosa district were natural pasture, crop residues, collected fodder, stubble grazing, and trees and shrubs. Farmers access veterinary services predominately from the government. Farmers used artificial insemination and bulls as a mating method for the animals. The rural farmers used an open-grazing system, while the animals were indoors in urban locations.

2.2. Sample Collection and Handling Procedures.
The study was conducted between January and March 2021 to assess the sanitary indicators of raw cow milk produced in Assosa district's rural and urban neighborhoods. Out of 200 homes with at least one nursing cow recruited for the last survey, 60 were chosen randomly for milk samples. A total of 60 raw cow milk samples were obtained (30 from urban areas and 30 from rural areas). ISO 707:2008, as adopted by the Ethiopian Standard Authority [20], was followed for milk sampling, transportation, and handling. The laboratory analysis was performed at the regional microbiology laboratory in Benishangul-Gumuz. Each sample farmer provided 450 mL of the fresh morning raw cow milk sample, collected aseptically using sterile sampling bottles. To avoid cross-contamination of the milk sample, 70% alcohol was used to disinfect the hands before sample collection. The milk sample bottles were closed and labeled in permanent markers before being placed in an ice box cooler with ice packs and promptly transferred to the Assosa Regional Microbiology Laboratory for analysis. The milk samples were kept at 4°C for 12 hours after arrival to test total coliforms, aerobic mesophilic bacteria, yeast and molds, *Staphylococcus aureus*, and *E. coli*. Every sample was analyzed twice.

2.3. Total Aerobic Mesophilic Bacterial Counts.
According to the Bacteriological Analytical Manual [21], the pour plate method was used to calculate total aerobic mesophilic bacteria in milk samples. The media were normally prepared according to the directions provided by individual manufacturers and labeled on the bottle. Peptone water was autoclaved at 121°C for 15 minutes before cooling to 30°C and utilized for serial dilution of milk samples to determine each TAMBC, TCC, and YMC microbiological parameter. To get the required counts of 30–300 colony-forming units (CFUs) per mL, 1 mL of the raw milk sample was serially diluted into 9 mL of peptone water up to eight times. Using the glass spread method, 0.1 mL of the milk sample from the specified dilution was grown on solidified standard plate count agar (Oxoid, UK). Colonies were counted using a colony counter after 48 hours of incubation at 32°C in an inverted orientation.

2.4. Total Coliform and Escherichia coli Counts. The total coliform count (TCC) was applied to enumerate the total coliform bacterial concentrations in raw milk samples following the procedure endorsed by the Bacteriological Analytical Manual [21]. In brief, 1 mL of the milk sample was serially diluted into 9 mL of peptone water up to six dilutions of the raw milk sample following thorough mixing using a vortex mixer. Then, 0.1 mL of each dilution was aseptically transferred to the Petri dish along with 15–20 mL of solidified violet red bile agar (Oxoid, UK). The agar and sample dilutions were mixed with glass spread methods. The culture media were incubated at 32°C for 24 h. All counts were made on duplicate plates. After incubation, typically dark red or purplish-red colonies appearing on the plates were counted using a colony counter within a 15–150 CFU/mL countable range. Counts were used to calculate CFU/mL of milk. Milk samples (25 ml) were diluted in buffered peptone water (225 ml); serial dilution of 10^{-1}, 10^{-2}, and 10^{-3} was applied to quantify *E. coli*. Thereby, 0.1 mL of the sample was taken from the chosen dilution spread onto the surface of sorbitol-MacConkey agar (HiMedia Pvt. Ltd. M 043, India). The inverted inoculated plates were incubated at 35°C for 24 hours.

2.5. Yeast and Mold Counts. Yeast and mold counts (YMCs) were determined using sterile Sabouraud dextrose agar (SDA) supplemented with streptomycin and chloramphenicol. One mL of the raw milk sample was added into a sterile test tube containing 9 mL of sterile peptone water. After thoroughly mixing, the suspension was serially diluted up to 10^{-7} and a duplicate sample of 0.1 mL was plated on predried surfaces of the media-containing SDA. The plates were then incubated at room temperature 25°C for 3–5 days. Creamy to white/gray colonies were counted as yeasts, whereas filamentous (fuzzy) colonies of various colors (yellow, green, and light brown) were counted as molds [22], with 10–150 colonies used for determining yeast and mold counts.

2.6. Staphylococcus aureus. One mL of the raw milk sample was added into a sterile tube with 9 ml sterile peptone water, and a serial dilution of the sample was made up to 10^1; 0.1 ml aliquot from this dilution was transferred to properly labeled mannitol salt agar (MSA) plates. The plates were spread and incubated (inverted) at 37°C for 48 hrs, typical *Staphylococcus aureus* colonies appeared as golden yellow, smooth, circular of 2-3 mm diameter, convex, and moist and surrounded by an opaque halo, and clear zones were counted. For confirmation, five positive presumptive *Staphylococcus aureus* colonies on mannitol salt agar (MSA; Oxoid, England) were confirmed using Gram-staining tests.

2.7. Statistical Analysis. The microbial count data were coded on an Excel sheet and analyzed using the Statistical Package for Social Science (SPSS) version 25. The variations in the bacterial count between raw milk obtained from urban and rural smallholder farmers were compared by average values, and their corresponding standard errors are presented for all data. The variations were considered significant at $p \leq 0.05$. The data of microbial counts were expressed as CFU/mL, and then, the bacterial counts were $\log_{10}$-transformed to normalize the distributions before statistical analysis. They were analyzed using *T*-test analysis. The total number of CFU per milliliter of the milk sample was calculated using the formula provided by IDF [23]:

$$N = \frac{\sum C}{\{(1 x n1) + (0.1 x n2)\}d}, \tag{1}$$

where N is the number of CFU per milliliter of the milk sample, ΣC is the sum of colonies on all plates counted, $n1$ is the number of plates in the first dilution counted, $n2$ is the number of plates in the second dilution counted, and d is the dilution from which the first counts will be obtained.

3. Results

3.1. Microbial Quality of Raw Cow Milk

3.1.1. Rural and Urban Locations. Statistical analysis results of the aerobic mesophilic bacterial count demonstrated a significant interaction between locations ($P = 0.004$) (Table 1). Although the average values are higher than the established limits of the Ethiopian Standard Authority, significantly ($P < 0.05$) higher average TAMBC for raw cow milk samples was obtained in rural farmers (9.9) than in urban farmers (8.1). The average values of TCC and *E. coli* are presented in Table 1; however, no significant ($P > 0.05$) variation was observed in the mean values of TCC between the two locations, unlike *E. coli*, which significantly differed ($P < 0.05$). Like most of the other parameters, significant variations ($P < 0.05$) in counts of YMC were found between urban (8.6 $\log_{10}$ CFU/mL) and rural (9.7 $\log_{10}$ CFU/ml) locations (Table 1). Average *Staphylococcus aureus* found in this study was 7.8 $\log_{10}$ CFU/mL; however, a significantly higher average number of *Staphylococcus aureus* was observed in rural locations than in urban locations (Table 1).

4. Discussion

According to [20], raw milk with TAMBC greater than 6.3 $\log_{10}$ CFU/mL is categorized as very poor milk quality. The mean AMBC in raw cow milk samples obtained from smallholders in both locations was higher than the standard recognized by ESA. The high aerobic mesophilic bacterial count in milk indicates that the high levels of contamination could have originated from the external surfaces of the udder, poor personal hygiene, and inappropriate milking utensils [6]. Production of clean raw cow milk by farmers is crucial for all actors involved in the dairy supply chain, such as consumers, milk-processing plants, vendors, and dairy cooperatives, including farmers [24]. AMBC is a marker for monitoring the sanitary conditions practiced during milking and successive raw milk handling [25]. No or scarce research findings were available for raw cow milk samples with higher AMBC than the current results of this study, but lower AMBC values (6.09–7.36 $\log_{10}$ CFU/mL) were noted by

TABLE 1: The mean (±SE) of microbial load of raw cow milk ($\log_{10}$ CFU/mL).

Parameters	Location		Overall mean ($N = 60$)	P value
	Rural ($n = 30$)	Urban ($n = 30$)		
Aerobic mesophilic bacterial count	9.9 (3.7)[a]	8.1 (1.3)[b]	9.0 (2.9)	0.004
Coliform count	6.5 (3.07)[a]	7.1 (2.3)[a]	6.8 (2.6)	0.540
Escherichia coli	6.3 (1.6)[a]	4.3 (2.01)[b]	5.3 (1.7)	0.002
Staphylococcus aureus	8.1 (1.2)[a]	7.5 (3.6)[b]	7.8 (2.6)	0.002
Yeast and mold count	9.7 (1.3)[a]	8.6 (3.7)[b]	9.2 (2.6)	0.001

Superscripts with different letters in the same row show significant differences ($P > 0.05$).

Haile et al. [26], Shunda et al. [27], and Tadele et al. [28]. Reasons for such differences might be associated with variations in handling conditions of milk during milking and subsequent milk chain that correspond to water quality, animal health management, type of milk utensils, storage temperature, and farmers' awareness about handling practices of milk and milk products.

Escherichia coli is a common contaminating microorganism as a result of fecal contamination. In addition, *E. coli* in food indicates the likely occurrence of enteropathogenic organisms, which could constitute a public health hazard. Enteropathogenic *E. coli* leads to mild to severe vomiting and diarrhea. ESA [13] set a maximum of 3.0 $\log_{10}$ CFU/mL of TCC for good-quality raw milk, and the amount exceeded this highest limit in dairy products, indicating that products are not safe for consumers [6]. The average TCC in the current study was higher than the ESA defined for good-quality milk. According to ESA, the category of the current TCC results is inferior. Earlier studies have reported a lower TCC of 4.18–4.99 $\log_{10}$ CFU/ML [6, 29–31], and more or less similar TCC of 7.0 $\log_{10}$ CFU/mL was found at selling points of milk in southwest Ethiopia [32] compared to the current study. The results of TCC and *E. coli* failed to meet ESA standards, which might be due to farmers not following all recommended sanitary procedures during milking and storage of milk, and in the study district, as observed physically during sample collection, the farmer's hands were soiled or contaminated with animal feces. Besides, the higher coliform count observed in milk indicates cows with coliform mastitis (clinical and subclinical).

The average values observed in the current finding were much higher than the reports of previous studies 4.66–5.1 $\log_{10}$ CFU/mL [6, 26, 33], but similar average values (8.3 $\log_{10}$ CFU/mL) of YMC were observed in traditional fermented cow milk in the central highlands of Ethiopia [3]. However, the value of YMC observed in the current finding was above the acceptable limit (<4 $\log_{10}$ CFU/mL) established for milk [34]. Yeast and molds are not only contaminants of foods of dairy products but also can produce mycotoxins that are not destroyed by heat [35]. In addition, yeast and molds cause objectionable changes in the product; therefore, care should be taken for milk contamination with dust, air, and soil starting from production and reach to consumers [36].

Staphylococcus aureus is the most common cause of dairy production disease, especially mastitis, which causes significant milk loss while negatively impacting animal comfort [16]. Higher levels of *Staphylococcus aureus* in rural regions indicate poor hygiene during milking. Previous studies in Ethiopia found lower numbers of *Staphylococcus aureus* (1.34–2.89 $\log_{10}$ CFU/mL) [37] and 4.35 $\log_{10}$ CFU/mL [38]. Maintaining clean milking locations, milk utensils, and effective dairy animal illness management are critical to reducing the prevalence of *Staphylococcus aureus* and preventing its transmission to humans [37, 39]. Furthermore, fresh milk should be thoroughly heated before it is fit for ingestion. SFP was produced by consuming 100 ng of *Staphylococcus* enterotoxin (SE) [40]. The toxin was produced by *Staphylococcus aureus* populations more significant than 5.0 $\log_{10}$ CFU/mL [21]. According to the current study, it is beyond the maximum limits for the possibility of producing SE that is thermostable and resistant to low pH and freezing. *Staphylococcus aureus* was identified in healthy people's nasal passages, hair, throat, and skin [41].

5. Conclusions

The current study concluded that the quality of raw cow milk received from the two locations was inadequate, as harmful microorganisms such as *Staphylococcus aureus* and *E. coli* were found in high concentrations in the samples. *Staphylococcus aureus* and *E. coli* contamination will render the milk unsafe for human consumption since many of these germs will cause illness and intoxication. As a result of the current study's findings, more stringent preventive measures may be required following the identification of the conditions that cause milk contamination. The magnitude of the problem of a high microbial load merits more public health attention and comprehensive studies from milk production to consumption, as well as holistic preventive strategies to protect against unsafe milk consumption and ensure that milk remains free of pathogens and spoilage microorganisms.

Data Availability

The data used in this study are available from the corresponding author upon reasonable request.

Ethical Approval

Farmers were asked to sign a consent form before milk samples were collected and used for microbial analysis.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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