



MEKELLE UNIVERSITY

COLLEGE OF VETERINARY MEDICINE

DEPARTMENT OF VETERINARY PUBLIC HEALTH AND FOOD SAFETY

ZOONOSES AND FOOD SAFETY



**ASSESSMENT OF MICROBIAL QUALITY, AND PHYSICOCHEMICAL
PROPERTIES OF RAW COW MILK ACROSS DAIRY SUPPLY CHAINS OF
AGULAE TOWN, TIGRAY, ETHIOPIA**

By:

Hailay Gebretnsae Berhane

(ID No.CVM/PR002/13)

**A Thesis Submitted to the College of Veterinary Sciences, Mekelle University, in
Partial Fulfillment of the Requirements for the Degree of Master of Science in
Zoonosis and Food Safety (ZOFS-800)**

June, 2025

Mekelle, Ethiopia

Declaration

I declare that this thesis presents the work carried out by myself and does not incorporate without the acknowledgement of any material previously submitted for a degree or diploma in any university; and to the best of my understanding, it does not contain any materials previously published or written by another person except where due reference is made in the text; all substantive contributions by others to the work presented including jointly authored publications, is clearly acknowledged.

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(ID No.CVM/PR002/13)

BOARD OF EXTERNAL EXAMINERS

1. **Chairperson:** _____

2. **Internal:** _____

3. **External Examiner:** _____

Main advisor

1. **Dr. Abrha Bsrat (DVM, MSc), and Associated Professor: Signature:** _____

Co- Advisors

2. **Dr. Weldemelak Girmay (DVM, MSc), Assistant professor: Signature:** _____
3. **Dr. Tesfay Hailu (DVM, MSc), Assistant professor: Signature:** _____
4. **Dr. Gidey Embaye (DVM, MSc): Signature:** _____

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ACKNOWLEDGMENTS

I would like to express my sincere gratitude to the Norwegian Agency for Development Cooperation (NORAD) for providing me with the financial support to undertake this research project. Their generous funding has been instrumental in enabling me to pursue my academic endeavors and contribute to the advancement of knowledge in this field.

I am deeply indebted to Dr. Abrha Bsrat, who played a pivotal role in connecting me with the NORAD opportunity. His guidance, mentorship, and unwavering support as my main advisor, have been invaluable throughout the entire process. I am truly grateful for his unwavering commitment to fostering academic excellence and creating opportunities for students like me. I would also like to extend my heartfelt appreciation to Dr. Weldemelak Girmay, Dr. Tesfay Hailu, and Dr. Gidey Embaye my co-advisors, for their invaluable expertise, constructive in supporting me throughout the execution of this thesis. I also thank, Dr Yohannes Hagos, Dr. Enqibahr Kassaye and Dr. Mebrahtu Berhe for their constructive review and feedbacks.

Furthermore, I would like to acknowledge the contributions of the members, and staff from the College of Veterinary Sciences, College of Animal Science Laboratory staffs, and Tigray Bureau of Agriculture and Natural Resource laboratory (BoANR) staff for their technical and material support, who have provided me with this MSc study, their expertise, resources, and support during the course of this study. Their collective efforts have been crucial in facilitating the successful completion of this research.

Finally, I would like to thank my family, friends, colleagues, and peers for their unwavering support, understanding, and encouragement throughout this journey. Their constant motivation and belief in my abilities have been a constant source of inspiration, enabling me to overcome the challenges and achieve the successful completion of this thesis.

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LIST OF ABBREVIATIONS

°C:	Degree Celsius
ADMY	Average Daily Milk Yield
ANOVA:	Analyses of variance
CCA	Coliform Count Agar
CFU:	Colony Forming Unit
CSA	Central Statistical Agency
ES:	Ethiopian Standard
EU:	European Union
FAO:	Food and Agriculture Organization
Log10:	Logarithm in base ten
MOA:	Ministry of Agriculture
PCA:	Plate Count Agar
pH :	Hydrogen ion concentration
SD:	Standard Deviation
SG:	Specific Gravity
SMA	Sabourad Maltose Agar
SNF:	Solid Not-Fat
SPC:	Standard Plate Count
SPSS:	Statistical Package for Social Science
TBC:	Total Bacterial Count
TCC:	Total Coliform count
TS:	Total Solid
WHO:	World Health Organization
YMC:	Yeast and Mould Count

ABSTRACT

Raw cow milk is a key food source in Ethiopia, but it poses significant health risks due to microbial contamination and poor handling practices. The aim of this study was to assess the microbial and physicochemical quality of raw cow milk across milk supply chains (farms, vendors, and cafeterias) in Agulae town, Tigray. A cross-sectional study was conducted from June 2024 to April 2025; involving 83 raw milk samples collected using a proportional cluster sampling technique. Microbial loads (total bacterial count, total coliform count, and total yeast and mold count) and physicochemical properties were analyzed using standard laboratory techniques. The results revealed a concerning escalation of bacterial contamination as milk moved through the supply chain. Total Bacterial Count increased significantly ($p < 0.001$) from farms ($6.67 \log_{10}$ CFU/mL) to vendors ($7.07 \log_{10}$ CFU/mL) and cafeterias ($7.37 \log_{10}$ CFU/mL). Similarly, Total Coliform Count was significantly ($p < 0.001$) higher in cafeterias ($6.33 \pm 0.31 \log_{10}$ CFU/mL) and vendors ($6.09 \pm 0.70 \log_{10}$ CFU/mL) than in farms ($5.04 \pm 0.85 \log_{10}$ CFU/mL). Additionally, total yeast and mold counts were also high (overall mean = $4.05 \pm 0.41 \log_{10}$ CFU/mL) but did not differ significantly ($p > 0.05$) across the milk supply chain. The overall mean (mean \pm SD%) of raw cow milk physicochemical parameters, including added water content ($0.19 \pm 0.84\%$), temperature of milk ($18.49 \pm 3.30^{\circ}\text{C}$), freezing point ($-0.55 \pm 0.02^{\circ}\text{C}$), pH (7.04 ± 0.19), fat ($4.17 \pm 1.36\%$), lactose ($4.70 \pm 0.23\%$), solids-not-fat (SNF) ($8.57 \pm 0.41\%$), protein ($3.15 \pm 0.18\%$), total solids ($12.75 \pm 0.96\%$), and ash content ($0.70 \pm 0.03\%$), have no significant ($p > 0.05$) variations across the chains. Several risk factors linked to higher bacterial counts, including sex, educational status, marital status, types of milk supply chains, hand washing before milking, and time of milk, were identified. These findings expose critical hygiene and handling gaps, particularly at the vendor and cafeteria levels, driving substantial microbial contamination. Targeted interventions, such as hygiene milk handling training and infrastructure improvements, are strongly recommended to bolster the safety of the dairy farms and protect consumers.

Keywords: Dairy Supply Chain; Microbial Quality; Physicochemical Properties; Raw Cow Milk; Tigray

1. CHAPTER I: INTRODUCTION

1.1. Background and Justification

Since ancient times, milk has been a basic diet, offering a variety of easily absorbed nutrients, including proteins, lipids, carbs, vitamins, and minerals, as well as critical support for the immune system. Its nutrient composition makes it more effectively absorbed than many other foods, contributing to its global significance (Fusco *et al.*, 2020; Kakati *et al.*, 2021; Tadesse *et al.*, 2023). For smallholder milk producers, milk is not only a nutritional asset but also a critical source of daily income (Dugdill *et al.*, 2013; Adesogan & Dahl, 2020).

However, the same nutrient-rich composition that makes milk beneficial also creates an environment conducive to the growth of pathogenic microorganisms, posing serious risks to human health and accounting for nearly 90% of dairy-related health issues (Berhe *et al.*, 2020; Tadesse *et al.*, 2023). While milk from a healthy udder is initially microbiologically safe, contamination can occur at various stages from milking to processing due to internal factors like cow health and external factors such as equipment, handling, and storage conditions (Demissie *et al.*, 2018; Adzitey *et al.*, 2022; Tadesse *et al.*, 2023). Additionally, environmental contaminants, such as grass, soil, feed, and air, further exacerbate these risks, particularly in developing countries where regulatory oversight is weak (Adesogan & Dahl, 2020; Tadesse *et al.*, 2023).

The microbiological quality of milk, typically assessed through bacterial load, is a critical indicator of its safety. Elevated bacterial counts not only compromise the quality and shelf life of milk but also pose significant health risks to consumers (Paraffin *et al.*, 2019). In developing nations like Ethiopia, poor hygiene during production, storage, and transportation worsens these risks, contributing to unsafe dairy products (Tadesse *et al.*, 2023).

Globally, there is an increased risk of foodborne infections due to the lack of consistent sanitary control over milk and dairy products in many poor nations (Ndahetuye *et al.*,

2020). In these countries, where milk production and processing usually occur in unsanitary conditions and with inadequate methods, this problem becomes especially serious (Berhe *et al.*, 2020; Tadesse *et al.*, 2023). Respecting the law is essential to safeguarding the health of people who eat milk and dairy products. Certain legal limitations may differ depending on the technology and economic conditions of various places, even though these standards might be universal (Jensen *et al.*, 2010; Tadesse *et al.* & Minten *et al.*, 2023).

Countries have established criteria for nutritional content and microbial contamination to ensure raw milk safety and quality (Khan *et al.*, 2008; Kakati *et al.*, 2021). In Ethiopia and similar developing nations, informal dairy sectors dominate, often lacking pasteurization and regulatory oversight. These challenges highlight the need for improved safety practices along the dairy value chain (Fusco *et al.*, 2023).

Worldwide, consumer worries about food safety are growing, especially with regard to milk and dairy products. Thus, it is crucial to maintain quality along the entire dairy value chain (Mohamed & Farah, 2018; Tadesse *et al.*, 2023; Jemal, 2023). The sale of unpasteurized milk through small-scale establishments that usually lack adequate refrigeration and regulatory oversight is a common practice in the informal sector, which is vital to the milk and dairy market in many African nations, especially Ethiopia (Odero & Waitituh, 2017; Blackmore *et al.*, 2022).

In Ethiopia, studies highlight concerns about poor hygiene in milk production, storage, and transportation, contributing to microbial contamination and adulteration. Adulteration practices, such as adding water or harmful chemicals like formalin to increase volume or preserve milk attributes, are common due to a lack of regulation and proper oversight. Accurate assessment of milk physicochemical properties, such as pH, fat content, and Solid Not Fat (SNF) levels, is essential to prevent such practices and ensure milk safety for Ethiopian consumers (Kakati *et al.*, 2021; Tadesse *et al.*, 2023).

Although several studies have investigated milk quality in Ethiopia, there remains a significant gap in understanding the microbial quality, and physicochemical properties of raw cow milk across the dairy supply chain, particularly in Agulae town of Tigray, which

lacks comprehensive data on these parameters. This gap hinders efforts to identify critical control points in the supply chain and address key issues affecting milk quality. Therefore, the purpose of the study was to assess the microbial quality, and physicochemical properties of raw cow milk in the study area. By examining microbial and physicochemical analysis, socio-demographic factors, and hygienic practices across the supply chain, which in turn provide evidence-based recommendations to improve milk quality. Ultimately, these findings will contribute to consumer health protection and the strengthening of the region's dairy sector.

1.1.1. Research objectives

1.1.1.1.General objective

The main objective of this study was to investigate the microbial safety level and physicochemical characteristics of raw cow milk across milk supply chains in Agulae town of Eastern Zone, Tigray, Ethiopia.

1.1.1.2.Specific objectives

- To evaluate the microbiological quality of raw cow milk in the study area
- To analyze the physicochemical composition of raw cow milk quality
- To identify the possible risk factors associated with contamination of raw cow milk

1.2. Literature Review

1.2.1. Overview of dairy production system in Ethiopia

Ethiopia boasts the largest cattle population in Africa, estimated at approximately 59.5 million head, with about 98.2% comprising local breeds. The remaining cattle consist of hybrid and exotic breeds, accounting for roughly 1.62% and 0.18%, respectively (CSA, 2017). Consequently, milk production in Ethiopia predominantly relies on indigenous breeds, particularly cattle, goats, camels, and sheep. Cattle are the primary contributors to national milk output, accounting for 81.2% of the total annual production, followed by goats at 7.9%, camels at 6.3%, and sheep at 4.6% (CSA, 2014). The estimated total cow milk production in rural sedentary areas of Ethiopia is approximately 3.06 billion liters (CSA, 2017).

The average daily milk yield (ADMY) of indigenous cows is 1.85 liters per day, with yields ranging from 1.24 liters in the rural lowland agro-pastoral systems of Mieso to 2.31 liters in the highland dairy production systems of Fogera (Azage *et al.*, 2013). In contrast, hybrid cows produce between 8 and 10 liters per day (Tadesse *et al.*, 2015). Currently, per capita milk consumption in Ethiopia is relatively low, estimated at about 19 liters per person annually; however, urbanization is driving an increase in consumption, particularly in Addis Ababa, where it reaches approximately 52 liters per person per year (Azage, 2018).

Dairy production represents a vital subsector of livestock farming in Ethiopia, providing essential sources of food and income for many households, despite the sector not being fully optimized or promoted. Similar to many tropical dairy production systems, Ethiopia's dairy industry encompasses a wide range of farm sizes and operational types, from subsistence to market-oriented enterprises. Various classifications have been employed to characterize the dairy production systems in the country, which can broadly be categorized into three main types: urban, peri-urban, and rural dairy production systems (Abebe, 2021).

Most dairy farmers in Ethiopia are predominantly situated in rural areas, while a significant portion of dairy markets operate within urban centers. The highly perishable nature of dairy products, coupled with their potential to transmit zoonotic diseases, pathogens, and toxins, complicates the ability of dairy farmers to engage effectively in urban markets (Bekuma *et al.*, 2018). Consequently, a comprehensive approach that encompasses the entire supply chain, including consumer education, is imperative. In the absence of viable market outlets for milk and dairy products, producers primarily retain these goods for household consumption, resulting in suboptimal production levels (Seifu *et al.*, 2014; Tadesse *et al.*, 2023). Over 85% of milk produced by rural households is consumed within the producer households, with less than 7% reaching the market (Bereda *et al.*, 2014). Rural production systems account for 97% of the national milk output, predominantly generated by smallholder farmers. This rural milk production system heavily relies on the low productivity of indigenous zebu cattle breeds, which typically yield between 400 and 680 liters of milk per cow per lactation period. The rural dairy production system encompasses pastoralists, agro-pastoralists, and mixed crop-livestock producers (Abebe, 2021; Tadesse *et al.*, 2023).

In contrast, peri-urban dairy cattle production systems are primarily located on the outskirts of urban areas, benefitting from relatively better access to the urban centers where dairy products are in high demand (Tegegne *et al.*, 2013). This production system is characterized as a semi-intensive crop-livestock farming model. Due to a steadily increasing demand for milk consumption, peri-urban dairy farms are expanding around cities and towns (Galmessa *et al.*, 2013). The majority of dairy producers in these areas rely on hybrid cows, implementing supplementary concentrate feeding practices (Gebresellasi, 2019). The peri-urban sector hosts a variety of animal breeds, ranging from 50% crosses to high-grade Friesian, despite contributing only 2% to Ethiopia's total milk production. This sector is home to a considerable proportion of the country's improved dairy stock (Gobena, 2016). Unlike the rural dairy production systems, peri-urban dairy systems are typically located along accessible roads close to urban centers, with producers actively participating in the fluid milk market (Nigatu *et al.*, 2012).

In many Ethiopian towns, urban dairy cattle production systems operate with limited land resources for the production and sale of milk. This system is the most market-oriented compared to other dairy production systems (Bekele *et al.*, 2015). Producers in urban areas typically utilize crossbred and high-grade dairy animals. However, only 1% of the country's total dairy cattle population is maintained within urban dairy production systems (Gezu and Zelalem, 2018). Cattle are housed in improved shelters constructed from locally available materials. The primary feed resources in urban dairy systems include concentrates, roughages, and non-conventional feeds. Additionally, roadside grazing, plant fruits, and agricultural by-products are also utilized (Gurmessa *et al.*, 2015).

Urban dairy production systems benefit from intensive management practices, providing better access to inputs and services from both public and private sectors compared to other dairy systems (Gebresellasia, 2019; Gobena, 2016). Producers have access to animal health services and employ more intensive production methods. Milk is sold to consumers and processing plants primarily through informal markets; however, milk supply remains low due to the small number of dairy cattle kept under this system (Abebe, 2021).

Ethiopia is recognized as having the largest livestock population in Africa, contributing significantly to the national economy and holding promise for continued economic development (Gebregziabher, 2021). Livestock products and by-products, including meat, milk, honey, eggs, cheese, and butter, provide essential animal protein that enhances the nutritional status of the population. Furthermore, livestock play a crucial role in generating export commodities, such as live animals, hides, and skins, which contribute to foreign exchange earnings. Draught animals are vital for cultivating smallholdings and for crop threshing throughout the country, serving as essential transportation modes for farmers and their families, facilitating the movement of agricultural products to markets, and transporting household necessities. Livestock also offer a degree of security during crop failures, acting as a “near-cash” capital asset. Additionally, livestock provide farmyard manure, which is commonly applied to enhance soil fertility and serves as a source of energy.

Despite the abundance of agricultural products, Ethiopia remains reliant on imports of substantial quantities of semi-processed and processed foods that have the potential to be produced domestically (FAO, 2021). The Ministry of Agriculture has developed a strategy aimed at improving milk marketing and processing at the village level. This strategy seeks to create an environment that enables smallholder dairy farmers to respond promptly to market demands. Specifically, it aims to develop markets for existing sellable surpluses, regardless of quantity, thereby gradually encouraging producers to meet market needs. The establishment of a robust marketing structure is believed to incentivize production improvements (Benti, 2022).

The primary constraints to the intensification of livestock, particularly dairy production, include the unavailability of adaptable high-yielding genetics, inadequate feed resources, animal diseases, and insufficient animal health, extension, and market services. In Ethiopia, efforts to improve the genetics of indigenous breeds through crossbreeding and upgrading have been underway for over 40 years, following the establishment of the National Artificial Insemination Center (NAIC). However, the number of improved breeds remains insufficient to transform the current subsistence-based smallholder dairy system into a market-oriented commercial dairy sector capable of meeting current and projected future domestic demands (Gebregziabher, 2021).

1.2.2. Milk composition and characteristics

Milk is a yellowish-white liquid secreted by the mammary glands of all mammals and serves as the primary source of nutrition for their offspring before they transition to other food sources. It is compositionally balanced, containing essential macro and micronutrients needed for the growth and maintenance of both human and animal bodies (Pandey *et al.*, 2011; Tadesse *et al.*, 2023). As a highly nutritious substance, milk is rich in proteins, fats, carbohydrates, vitamins, minerals, and bioactive compounds that contribute to health protection. Key components such as milk proteins, fats, and lactose provide significant energy sources (Merwan *et al.*, 2018; Tadesse *et al.*, 2023).

The chemical composition of milk, particularly its fat content, serves as a primary criterion for quality assessment. Important nutritional constituents of milk include solids,

which are crucial for both dietary intake and the production of various shelf-stable dairy products. Determining the major chemical components is essential for processing milk into these products (Goff, 2010). According to Tadele & Gebreananya (2024), the Tigray raw cow milk is composed of approximately 87.4% water, 13.67% milk solids, 9.46% solids-not-fat, 4.20% fat, 4.00% protein, 4.80% lactose, and 0.66% ash. The constituents can vary significantly due to genetic factors, cow breed, individual animal differences, as well as environmental influences such as milking intervals, stages of lactation, age, diet, and health status.

Recent physicochemical analyses have highlighted variations in fat, protein, and lactose content, with seasonal factors playing a notable role (Kazeminia *et al.*, 2023). Furthermore, many milk samples analyzed failed to meet safety standards for consumption, revealing the necessity for enhanced dairy management practices (Sebsibe *et al.*, 2023).

Milk pH: The pH of milk is a crucial indicator of its hygienic condition, ideally ranging between 6.6 and 6.8 at a temperature of 20 °C. Maintaining this pH level aid, in reducing bacterial growth, as cooling milk mitigates risks associated with higher temperatures that promote bacterial proliferation (Bruktawit, 2016; Tadesse *et al.*, 2023; Tadele & Gebreananya, 2024). A pH exceeding 6.8 may signal mastitis in the milk, while values below 6.6 indicate increased acidity due to bacterial activity (O'Connor, 1995). Additionally, pH levels typically decrease with rising temperatures. Variations in pH and buffering capacity among individual samples of fresh milk can highlight underlying compositional differences (McCarthy, 2002; Tadesse *et al.*, 2023; Tadele & Gebreananya, 2024).

Total solid content: Total solids refer to the non-water components of milk, including proteins, fats, lactose, and minerals. Measuring total solids is vital for assessing the quality of milk and milk products. This can be accomplished indirectly through specific gravity and fat content measurements or directly via gravimetric analysis, where a sample is heated until all moisture evaporates, allowing for the calculation of the percentage of dry solids (Berhe & Teklit, 2018; Tadesse *et al.*, 2023; Tadele & Gebreananya, 2024).

Solids non-fat content: Solid non-fat is an important criterion of milk selection for further processing. Milk solids nonfat would include nitrogenous substances, milk sugar, and mineral matter. The fluid milk contains a minimum of 8.25 percent SNF. The determination of solid non-fat is done by taking a lactometer reading at 40 °C. Solids-not-fat (SNF) content was determined by the following formula (Harris & Bachman, 2003; Tadesse *et al.*, 2023; Tadele & Gebreananya, 2024).

$$\text{SNF content (\%)} = \text{TS (\%)} - \text{Fat (\%)} \dots\dots\dots (1)$$

Fat content: Milk fat is a significant component, influencing not only the nutritional value but also the sensory and physical characteristics of dairy products. With a highly complex fatty acid profile, over 400 unique fatty acids have been identified within milk fat, though approximately 90% is comprised of 15 to 20 predominant fatty acids. Most of these are saturated straight-chain fatty acids ranging from 4 to 18 carbons, alongside monounsaturated and polyunsaturated fatty acids, each contributing distinct flavors to milk products (McCarthy, 2002; Tadesse *et al.*, 2023; Tadele & Gebreananya, 2024).

Protein content: Milk proteins, which include casein, lactoalbumins, and lactoglobulins, account for significant nutritional value. Roughly 82% of milk's protein content is casein, which binds to calcium to form calcium caseinate in colloidal form. This complex can be precipitated by acid, rennet, alcohol, or heat (Guetouache *et al.*, 2014). Milk proteins are of high biological value, providing all essential amino acids necessary for growth and tissue maintenance, though sulfur amino acids like methionine and cysteine are present in lower amounts (Guetouache *et al.*, 2014; Tadesse *et al.*, 2023; Tadele & Gebreananya, 2024).

Ash content: The ash content reflects the total mineral content remaining after the organic mass is eliminated through heating in oxidizing environments. Analytical methods such as dry ashing, wet ashing, and low-temperature plasma dry ashing are employed to quantify the mineral content, capitalizing on the properties of minerals that resist destruction during heating and exhibit low volatility compared to other food components (McCarthy, 2002; Tadesse *et al.*, 2023; Tadele & Gebreananya, 2024).

1.2.3. Hygiene and handling practices of milk in Ethiopia

Milk and milk products are essential components of the human diet globally. While milk synthesized in a healthy cow's udder is virtually sterile, it rapidly becomes contaminated with microorganisms upon exposure to the external environment, leading to spoilage prior to consumption or further processing (Tollessa, 2016). Given its liquid form and nutrient-rich composition, milk serves as an ideal medium for microbial growth, necessitating stringent hygienic practices. Effective milk handling requires attention to the cleanliness of the milking environment, the hygiene of the milker, and the sanitation of utensils used for collection and storage (Tadesse *et al.*, 2023).

In Ethiopia, the standards for milk hygiene and handling are often inadequate, primarily due to ineffective pre-milking practices. Insufficient sanitation measures, such as failing to wash the udder with clean water, neglecting to clean the milking barn, improper drying techniques, and inadequate hand washing by milkers, contribute to the risk of contamination. Additionally, the use of low-quality, non-boiled water for cleaning exacerbates these issues. Post-milking handling practices also fall short; poor hygiene of storage equipment, prolonged storage durations, and unsatisfactory transportation methods further predispose milk to microbial contamination (Tadesse *et al.*, 2023).

The use of plastic containers for milk handling significantly increases contamination risks. Greater reliance on plastic leads to a higher likelihood of microbial invasion, particularly since many plastic materials are not suited for milk storage. Furthermore, traditional clay pots, commonly used for milk storage, are difficult to clean hygienically and can harbor bacteria that promote spoilage (Tsedey and Asrat, 2015).

Dairy producers sometimes employ unconventional cleaning methods, such as washing with cold or hot water, followed by fumigation with aromatic plants like *Olea africana* and *Juniperus procera*. This traditional practice aims to flavor the milk and extend its shelf life while reducing microbial load through disinfection (Tsedey and Asrat, 2015). Nonetheless, approximately 55.6% of producers do not utilize refrigeration for milk storage, which fosters bacterial proliferation and spoilage (Shewangzaw *et al.*, 2016). Collectively, these substandard handling practices lead to elevated bacterial counts,

increasing the risk of spoilage and foodborne illnesses, thus posing significant health hazards to consumers (Tolessa, 2016).

1.2.4. Milk quality concerns in Ethiopia

Despite its high nutritional value, milk is susceptible to contamination by physical, chemical, and microbiological hazards. Milk quality encompasses various characteristics—chemical, physical, microbial, and aesthetic—that enhance product acceptability, while milk safety is defined by the absence of pathogenic organisms and harmful contaminants (Merwan *et al.*, 2018; Tadesse *et al.*, 2023). Key indicators of milk quality and safety include specific gravity, chemical composition, and microbial integrity, with the latter two being pivotal for milk products (Merwan *et al.*, 2018).

In Tigray, Ethiopia, the quality and safety of milk and milk products are significantly compromised due to various factors, including poor hygienic practices, contamination, and adulteration. Research indicates that over half of the raw milk samples tested in Tigray exhibited bacterial contamination, with a prevalence of 52% among commercial producers (Berhe *et al.*, 2020). Common pathogens identified include *Escherichia coli* and *Staphylococcus aureus*, which pose serious health risks (Weldeabezgi *et al.*, 2020). Additionally, the nutritional quality of milk is undermined by practices such as water adulteration and the presence of chemical contaminants like aflatoxins and heavy metals, often exceeding international safety standards (Zebib & Zewdu, 2020; Gemechu, 2017). The informal sector dominates dairy production, contributing to substandard quality and safety measures (Gemechu, 2017).

1.2.5. Microbial quality of milk in Ethiopia

Milk harvested from a healthy cow's udder is initially free from significant bacterial contamination. However, it becomes susceptible to microbial invasion from the moment it exits the teat, accumulating bacteria from various sources, including the cow itself, ambient air, feed, milking equipment, and the milker. Once introduced, these microorganisms proliferate rapidly in the milk (Tadesse *et al.*, 2023).

The microbial content of milk serves as a key indicator of hygiene during the milking process, reflecting the cleanliness of utensils, proper storage and transportation methods, and the overall health of the cow's udder. Common assessments of microbial quality in milk and dairy products include the total bacterial count (TBC) or standard plate count (SPC), coliform count (CC), and yeast and mold counts, along with monitoring for Enterobacteriaceae (Tamirat, 2018; Fufa *et al.*, 2019; Tadesse *et al.*, 2023).

Total aerobic bacterial count (TABC): According to the Ethiopian Standard Agency (ESA, 2009), the acceptable bacteriological quality of raw cow milk is defined as follows: less than 2×10^5 CFU/ml for very good quality, 2×10^5 to 1×10^6 CFU/ml for good quality, 1×10^6 to 2×10^6 CFU/ml for bad quality, and above 2×10^6 CFU/mL for very bad quality. However, numerous studies indicate that the actual quality of milk in Ethiopia consistently falls below these standards. For instance, Merhawit (2014) reported an average total aerobic bacterial count of 2.3×10^9 CFU/mL in milk samples from cafeterias, followed by milk vending shops 4.9×10^8 CFU/mL, and lower counts occur in dairy farms with 6.37×10^7 CFU/mL. Similarly, Fufa *et al.* (2019) found average counts of 8.55 log₁₀ CFU/ml, 8.99 log₁₀ CFU/ml, and 3.12 log₁₀ CFU/ml in milk from dairy farms, vendors, and restaurants, respectively.

Other studies, including Tadesse *et al.* (2023), revealed mean total bacterial counts of 6.14 log₁₀ CFU/ml for farm samples, 7.86 log₁₀ CFU/ml for milk vendor samples, and 8.64 log₁₀ CFU/ml. Additionally, Mekonine and Mengistu (2017) recorded 6.99 log₁₀ CFU/ml for raw milk from farmers and 6.87 log₁₀ CFU/ml from dairy cooperatives. Tsedey and Asrat (2015) reported mean counts of 6.73 log₁₀ CFU/ml for producers and 7.15 log₁₀ CFU/ml for consumers.

These elevated bacterial levels can be attributed to unhygienic milking practices, such as inadequate udder cleaning, the use of non-sanitized local containers, unclean milking environments, poor personal hygiene among milkers and handlers, and the absence of milk cooling systems (Debela, 2015; Mekonin, 2018; Tamirat, 2018; Tadesse *et al.*, 2023).

In the Tigray region, the microbial quality of milk is particularly concerning, with studies indicating total bacterial counts ranging from 2.15×10^8 to 5.96×10^8 CFU/ml across various production and handling points. Notably, contamination prevalence is higher in milk from vendors (64.7%) and cafeterias (58.9%) compared to dairy farms (33.3%) (Berhe *et al.*, 2020). Common pathogens identified include *Escherichia coli* and *Staphylococcus aureus*, posing serious health risks (Merhawit, 2014). Poor hygiene practices during milking and handling significantly contribute to these high microbial loads (Angaw, 2015; Gebeyew, 2020).

According to the Ethiopian Standards Agency, the bacteriological grades of quality of whole/raw cow milk based on total enumeration of bacterial plate count (CFU/mL) are shown as follows in table 1 (Tamirat, 2018; Tadesse *et al.*, 2023).

Table 1: Grade of raw cow milk based on Plate Count Agar in raw cow milk

Bacterial count CFU/MI	Grade
Not exceeding 200,000	Very good
200,000-1,000,000	Good
1,000,000-2,000,000	Bad
>2,000,000	Very bad

Source: (Tamirat, 2018; Tadesse *et al.*, 2023).

Total coliform count (CC): Coliforms are a significant bacterial group that influences milk quality and reflects hygiene practices during milking and subsequent handling (Tadesse *et al.*, 2023). According to the Ethiopian Standard Agency (ESA, 2009), good quality milk should exhibit total coliform counts not exceeding 0-1,000 CFU/ml. However, numerous studies have reported coliform levels that surpass this standard. For example, Tadesse *et al.* (2015) found coliform counts of 5.98 log₁₀ CFU/ml in raw milk from farms, 7.48 log₁₀ CFU/ml for samples collected from vendors, and 7.52 log₁₀ CFU/ml from cafeterias. Similarly, Habtamu *et al.* (2018) reported mean counts of 5.58

log₁₀ CFU/ml for household samples, 6.63 log₁₀ CFU/ml for dairy farm samples, and 7.24 log₁₀ CFU/ml for pasteurized milk. Fufa *et al.* (2019) recorded total coliform counts of 5.91, 5.77, and 2.17 log₁₀ CFU/ml for milk from dairy farms, vendors, and restaurants, respectively.

The elevated coliform counts are often attributed to contamination during milking, stemming from inadequate hygiene practices of milkers or fecal contamination from the udder and lower abdomens of cows, particularly via bedding materials. The presence of coliforms is a safety concern, indicating a need for stringent adherence to minimum recommended levels in milk products (Alganesh, 2016; Fufa *et al.*, 2019; Tadesse *et al.*, 2023).

According to the Ethiopian Standards Agency, the bacteriological grades of quality of whole/raw cow milk according to the total number of CFU/mL are shown as follows in Table 2. These standards are more or less similar to other international standards, according to different works of literature (Tadesse *et al.*, 2023).

Table 2: Grade of raw cow milk based on Violet Red Bile Agar (VRBA) in raw cow milk

Coliform count CFU/MI	Grade
Not exceeding 1,000	Very good
1,000-50,000	Good
50,000-500,000	Bad
>500,000	Very bad

(Tamirat, 2018; Tadesse *et al.*, 2023).

Yeast and mold counts: Yeasts and molds commonly associated with milk and dairy products include *Saccharomyces* spp., *Penicillium* spp., *Rhizopus* spp., *Aspergillus* spp., *Geotrichum candidum*, *Alternaria* spp., and *Cladosporium* spp. (Vishweshwar & Krishnaiah, 2005). In a study assessing 78 milk samples collected from the udder, storage containers at the farm level, and distribution containers in Hawassa, the overall mean yeast and mold counts were recorded at 3.03, 4.65, and 7.13 log₁₀ CFU/ml, respectively

(Haile *et al.*, 2012). Similarly, yeast and mold counts from milk samples in Jimma were reported at 5.1 and 3.7 log₁₀ CFU/ml (Alebel *et al.*, 2013). The same study conducted in Jimma showed the total yeast and mold count at farms at 3.94 log₁₀ CFU/ml, milk vendors at 4.10 log₁₀ CFU/ml, and cafeterias at 4.48 log₁₀ CFU/ml (Tadesse *et al.*, 2023).

These microorganisms can originate from inadequately sanitized equipment or as airborne contaminants, thriving in environments such as soil, barn dust, feed, manure, and unclean utensils. They are capable of producing toxic metabolites, exhibit resistance to freezing conditions, and contribute to off-odors and off-flavors in foods. The presence of these fungi can compromise the shelf life of milk and pose serious health risks to consumers (Tadesse *et al.*, 2023).

1.2.6. Sources of microbial contamination in milk value chain

Microbial contamination in milk can originate from various sources, including the milk itself, infected animals, humans, the environment, water, and equipment used in milking and storage. Pathogenic organisms may be shed in milk from infected udders or teats, contaminated animal skin, and fecal matter. Additionally, contamination can arise from unclean milking and storage equipment, as well as water used for sanitation. Airborne contaminants, milkers, handlers, and drugs or chemicals administered during animal treatment further contribute to bacterial presence. Unscrupulous practices, such as adulteration by dishonest workers, exacerbate the risk of contamination and pose significant health threats (Swai & Schoonman, 2011).

A study conducted in Bahr Dar showed that there was a statistically significant difference between milk producers who wash their hands before milking (85.8%) and those who do not wash (14.2%) their hands before milking. Besides, this study revealed that only 36% and 23.1% of dairy producers washed udders and teats in urban and peri-urban production systems, respectively. The majority (85.9%) of milk producers weren't practicing drying udders and teats after washing, whereas only 14.1% of milk producers were practicing drying udder and teats with individual towels (4.7%) and common towels (9.4%). A significant number of milk producers (47.8% and 68.8%) used gourds as milk

storage equipment in peri-urban and rural areas, respectively, while plastic containers were dominant (84%) in urban production systems (Yeserah *et al.*, 2020).

Exposure to these contamination sources can compromise milk quality, and recontamination may occur post-processing due to inadequate hygiene practices and improper handling during consumption (Parekh & Subhash, 2008; Yeserah *et al.*, 2020). Overall, milk quality is adversely affected by factors such as adulteration, contamination during and after milking, udder infections, mastitis (inflammation of the mammary gland), and drug residues from treatment. These issues not only raise public health concerns but also lead to substantial economic losses in the dairy industry globally (Mdegela *et al.*, 2009; Yeserah *et al.*, 2020).

2. CHAPTER II: MATERIALS AND METHODS

2.1. Study Area

Agulae Town, situated in Ethiopia's Tigray Regional State ($13^{\circ}41'39''\text{N}$, $39^{\circ}35'9''\text{E}$) at an elevation of 2,018 meters, experiences an annual rainfall of 606.4 mm and mean monthly temperatures ranging from $8\text{--}12.3^{\circ}\text{C}$ (min) to $18.6\text{--}28.8^{\circ}\text{C}$ (max) (EMA, 2021). The area practices mixed crop-livestock farming, with approximately 240 dairy farms managing 850 cows, including 106 lactating cows. Dairy producers are organized into three cooperatives, enabling efficient milk distribution to Mekelle City and contributing to local livelihoods, food security, and the regional economy (TBoANR, 2024).

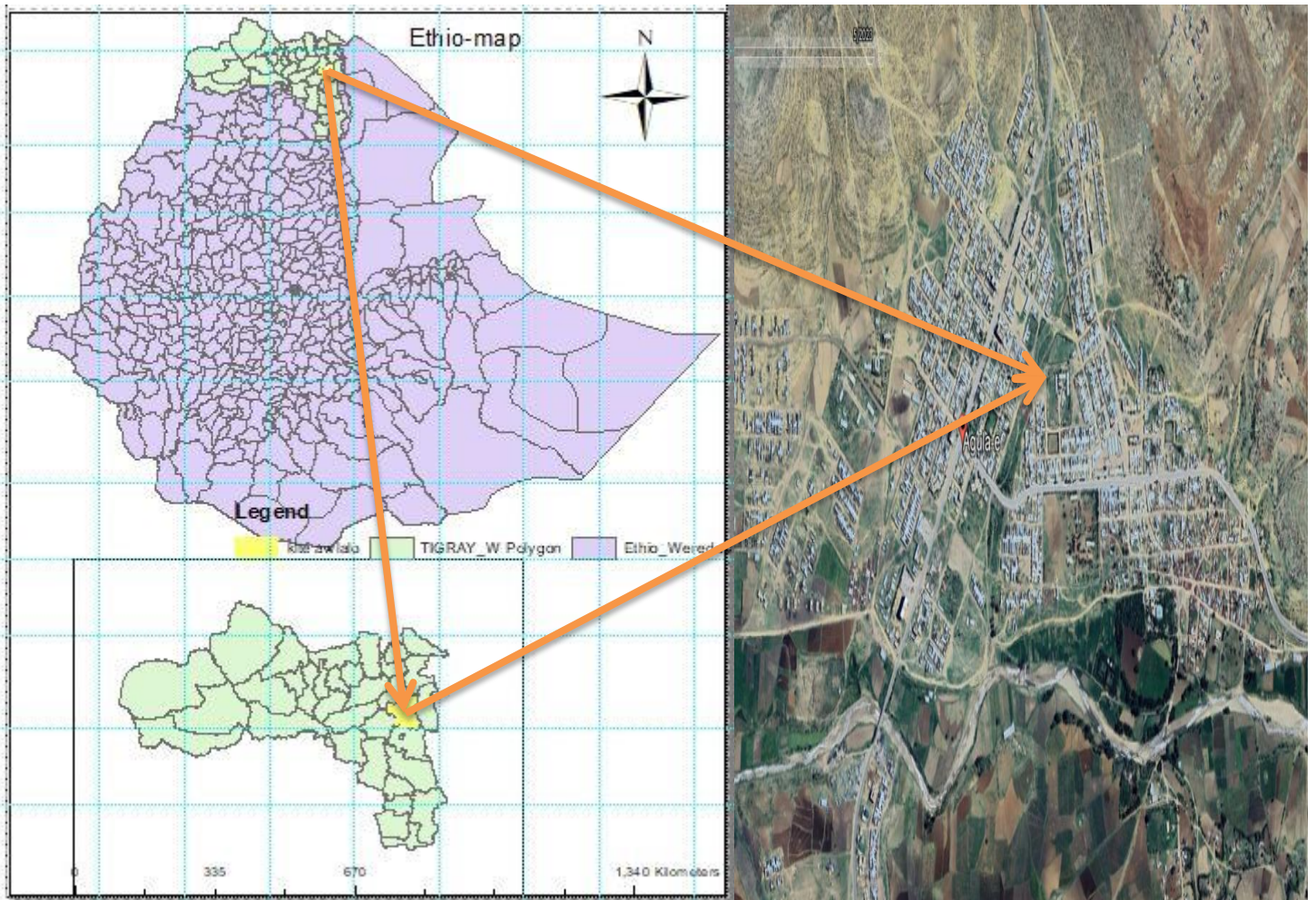


Figure 1: The location on map and satellite image of Agulae town

2.2. Study Design and Sampling Techniques

A cross-sectional study design has been conducted from June 2024 to April 2025, to investigate the microbial quality, and physicochemical characteristics of raw cow milk across different stages in the milk supply chain from Agulae town of Eastern Zone, Tigray, Ethiopia. This approach provides a snapshot of data on milk quality across various handling stages.

Agulae town has been chosen purposively due to its significance in the regional dairy supply chain and its reliance on smallholder dairy farming. Dairy farms, milk vendors, and cafeterias were randomly selected using proportional stratified sampling from finite population of 106 active in milk production and market were stratified into three groups based on their characteristics: farmers (81), vendors (5), and cafeterias (20).

2.3. Sample Size Determination

To determine the appropriate sample size for conducting a study on the microbial quality, and physicochemical properties of raw cow milk, the available population in the study area was first determined. Then proportional stratified sampling method has been used. Based on the above information, about the available stratified target populations, the total sample size calculated using Cochran's formula for finite population was 83, with a 95% confidence level and a 5% margin of error. Based on the population proportions, the sample size was calculated as follows: 63 farmers, 4 vendors, and 16 cafeterias. This method ensures that the sample is representative of the overall population, maintaining a balanced distribution among the groups for accurate data collection and analysis (Cochran, 1977). The formula used:

$$n = [N \cdot Z^2 \cdot p(1-p)] / [(N-1)e^2 + Z^2 \cdot p(1-p)]$$

Where: N = Total population (106)

Z = Z-value for 95% confidence (1.96)

p = Estimated proportion (0.5)

e = Margin of error (0.05)

2.4. Apparatus and Equipment

Sterile milk-collecting container, ice box with ice packs, Disposable gloves, 70% alcohol, Permanent marker, Sterile pipettes and pipette tips, Petri dishes, Flasks, incubators, sterile test tubes, autoclaves, Colony counter, Vortex mixer, safety cabinet, Water bath, Analytical balance, Bunsen burner, distilled water, Dry hot oven sterilizer, Graduated cylinders and beakers, A refrigerator for sample storage and a hot plate for boiling media were used as equipment during the study.

2.5. Chemicals and Reagents

Plate count agar (10453 (PM 014)), Sabouraud Maltose Agar (Oxoid-CM41a), Buffer Peptone Water (CONDA ISO 6579, ISO 22964, ISO 6887, ISO 19250), and Coliform Culture Agar/Harlquin® E. coli/coliform culture agar (NEOGEN NCM1002A) solutions were used during the experiments.

2.6. Sample Collection and Sampling Procedures

For microbial and physicochemical analysis, 50 mL of raw cow milk from each identified sampling point in the supply chain, including dairy farms, milk vendors, and cafeterias, were collected aseptically using a sterile container after the raw milk was homogeneously mixed (Stella *et al.*, 2022). Each sample was labeled with the type of supply chain, location, date and time of collection, and then placed properly in a cooler icebox with ice bags and transported as immediately as possible to the Tigray Bureau of Agriculture and Natural Resource laboratory (BoANR) for microbial milk quality, and physicochemical analysis within 1hr. and then, after arrival, it was stored in a +4⁰C refrigerator. Finally, the microbiological and physicochemical analyses were conducted within 24hrs. of collection to ensure accurate and reliable results (Yeseraah *et al.*, 2019; Stella *et al.*, 2022).

2.7. Microbial Quality, and Physicochemical Analysis of Raw Cow Milk

2.7.1. Sample preparation

Serial dilutions were prepared according to the ISO 6887-1 procedure (ISO, 1999). To obtain 15% Buffered Peptone Water (BPW), 15g of BPW powder was dissolved in 1 liter of distilled water according to the manufacturer's instructions (OXOID® Ltd., Basingstoke, and Hampshire, England) and sterilized in an autoclave. Samples were removed from cold storage and allowed to sit for 30 minutes to attain room temperature. They were then thoroughly shaken, and using a sterile pipette, 1 mL of the sample was transferred into a sterile Falcon tube containing 9 mL of BPW (10^{-1} dilution), which was followed by serial dilutions. The procedure was repeated up to 10^{-7} dilution, and in the last dilution, 1 mL of inoculum was discarded. The dilutions were mixed thoroughly using a vortex mixer before they were used to enumerate TBC, TCC, and TMC (Mogotu *et al.*, 2022).

2.7.2. Total bacterial count

One measure of milk quality is the bacterial content of the raw milk. This is often termed the total bacterial count or the standard plate count. Total bacterial counts were enumerated as per ISO 4833 (ISO, 2001). The standard plate count agar (PCA) was boiled, autoclaved at 121°C for 15 minutes, and cooled to 45-50 °C before pouring. Appropriate serial dilutions of 10^{-5} to 10^{-7} samples were selected that gave the expected total number of colonies on a plate (Mogotu *et al.*, 2022). Then 1 mL of the sample from the appropriate decimal dilution was placed on a petri dish in duplicate, and then plate count agar medium (15-20 mL) was poured onto the petri dish. Plates were covered, gentle sufficient shaking was done, and after drying, they were inverted and incubated at 37°C for 24 hours. A colony counter was used to count plates with colonies ranging from 30 to 300, which were expressed as colony-forming units per ml of the sample (CFU/ml) (MMAF, 2012).

2.7.3. Total coliform count/TCC

The TCC is a test that estimates the number of bacteria that originate from manure or a contaminated environment. To do this first, the Coliform Culture Agar/Harlquin® E. coli/coliform culture agar medium was prepared by suspending 36.6 gm. of the powder in 1 liter of distilled water. Then heat the medium with frequent agitation and boil for one minute to completely sterilize it by autoclave at 121°C for 15 minutes and cool to 45-50 °C. Dilutions of 10^{-2} to 10^{-4} of duplicate samples (0.5mL) were spread on Coliform count agar using a glass spreader. Then incubated at 37°C for 18-24 hours, and the plates with 15–150 colonies were selected, and all dark blue to violet colonies as presumptive E. coli and all rose-pink colonies as presumptive coliforms were counted totally, and CFU/mL calculated (Baylis & Patrick, 1999).

2.7.4. Total yeast and mold count/TYMC

Sabouraud maltose agar was used as the established method for enumerating yeasts and molds in raw cow milk samples collected from farms, vendors, and cafeterias (Harrigan, 1998). To prepare the media, 65 g of Sabouraud maltose agar (SMA) powder per liter of deionized or distilled water was dissolved, and the mixture was autoclaved at 121°C for 15 minutes to ensure sterility (Atlas, 2010). The cooled agar, at approximately 45-50°C, was aseptically poured into sterile Petri dishes and allowed to solidify at room temperature (Vanderzant & Splittstoesser, 1992). The 0.1 ml of inoculum sample dilution was spread with glass spreaders (Downes & Ito, 2001; Compendium of Methods for the Microbiological Examination of Foods, 2015). The dried plates were then incubated at 25°C for 3 to 5 days. Colonies with filamentous, cotton-like white, or cream, pink to various shades of green, brown, or black (i.e., powdery) appearances (Tournas *et al.*, 2020) were counted as yeasts and molds (APHA, 2001; Beuchat & Cousin, 2001).

2.7.5. Physicochemical properties analysis

Analyses of the physicochemical properties of cow milk were done at the Mekelle University Animal Production and Technology Laboratory. To conduct a thorough analysis of raw milk, an instrument, a lacto Scan ultrasonic milk analyzer, examined

physicochemical properties such as fat, protein, lactose, solids-not-fat (SNF), total solids (TS), milk salt, and water added content. The lacto-scan also measured specific gravity, temperature of the milk, freezing point, and milk density, providing insights about the milk. The digital pH meter determined acidity in the milk, which was crucial for evaluating its freshness and quality (Tadele & Gebreaninya, 2024).

To ensure consistency and standardized evaluation, all tests and instruments adhered to the ISO scoring system. This approach guaranteed reliable and comparable results, allowing for accurate assessments of raw milk quality (Suprayogi *et al.*, 2022).

2.8. Questionnaire Survey and Observational Data Collection

To gather quantitative data on hygienic practices, both questionnaires and observational checklist methods were employed. All selected owners (dairy farmers, milk vendors, and cafeteria operators) were interviewed using semi-structured questionnaires. These questionnaires included a mix of closed-ended and open-ended questions to assess hygienic practices related to milking, storage, and transportation of raw cow milk. Face-to-face interviews were employed to facilitate in-depth discussions and ensure clarity.

Furthermore, direct observations were conducted using standardized checklists (FAO, 2008; Codex Alimentarius Commission, 2003). These checklists focused on

Personal hygiene of dairy farm practitioners: "Good" was assigned if the practitioner had visibly clean hands, wore clean outerwear, covered any cuts or sores, and was observed washing their hands before handling milk containers and milking cows while avoiding behaviors that could contaminate the milk. "Poor" was assigned if the practitioner had soiled clothing, no hair covering, dirty hands, and was observed coughing or sneezing without covering their mouth or nose, followed by touching equipment without washing hands.

Cleanliness of milk containers: "Good" was assigned if all milk cans, buckets, and other containers were free from visible milk residue, dirt, or rust, and the surrounding area was clean and free from debris. "Poor" was assigned if milk containers had caked-

on milk residue, visible mold growth, or cracks and damage, and the surrounding area was cluttered and dirty.

Cleanliness of the environment: "Clean" was assigned if the milking parlor was well-maintained with clean floors, walls, and ceilings. Manure was properly stored and managed, the surrounding area was free from excessive dust and debris, and animals appeared clean and healthy. **"Dusty"** was assigned if the milking parlor had dirty floors covered in manure, cobwebs hanging from the ceiling, dust accumulation on surfaces, manure was improperly stored and managed, and the surrounding area was muddy with stagnant water and scattered debris.

2.9. Data Entry and Analysis

The microbial counts were initially transformed to logarithmic values (log₁₀) before conducting statistical analyses. The transformed microbial count data along with the physicochemical characteristics were evaluated using the multiple regression analysis procedure in SPSS version 26 to see the possible risk factors. Mean comparison was performed using one-way analysis of variance (ANOVA) across the milk supply chains, and a one-sample t-test was also used to compare the sample means with the national and international standards. The level of significant differences between means was set at $p < 0.05$.

2.10. Ethical Considerations

An official letter was obtained from Mekelle University College of Veterinary Sciences and presented to the sample collection sites in need. While verbal consent was secured from the participants before they were approached for an interview, assuring that all of the personal information gathered from each study participant was kept confidential.

3. CHAPTER III: RESULTS

3.1. Socio-Demographic Findings

The research study examined the characteristics of 83 individuals involved in milk supply chains across three groups: dairy farms (n=63), milk vendors (n=4), and cafeterias (n=16), as presented in Table 3. The majority of participants were male (52; 62.7%), and most fell within the 38-75 years age range (54; 68.7%). Educational attainment varied with the largest proportion having completed 1th - 8th grades (58; 69.9%), while a smaller percentage (7; 8.4%) were illiterate. A notable finding was the disparity in training experience, with (46; 55.4%) of dairy farm participants reporting training related to dairy production, compared to none among milk vendors or cafeteria staff. While nearly half of the total participants (29; 40.0%) of the dairy farms did not know about milk-borne diseases.

Table 3: Descriptive Statistics of the Household Characteristics of Dairy Supply Chain respondents in Agulae Town, Eastern Zone, Tigray.

Variable	Category	Farms (n=63)		Vendors(n=4)		Cafeterias(n=16)		Overall(n=83)	
		Count	%	Counts	%	Count	%	Count	%
Sex	Male	41	65	1	25	10	62.5	52	62.6
	Female	22	35	3	75	6	37.5	31	37.3
Age	1-20	3	4.8	0	0	0	0	3	3.08
	20-37	18	28.6	3	75	5	31.2	26	31.3
	38-75	42	66.4	1	25	11	68.8	54	68.7
Level of Education	Illiterate	6	9.5	0	0	1	6.2	7	8.4
	1-8 Grade	44	69.8	0	0	14	87.5	58	69.9
	9-12 Grade	11	17.5	2	50	1	6.3	14	16.9
	Diploma	2	3.2	2	50	0	0	4	4.8
Marital status	Single	8	12.7	0	0	0	0	8	9.6
	Married	47	74.6	4	100	15	93.8	66	79.5
	Divorced	6	9.5	0	0	1	6.2	7	8.4
	Widowed	2	3.2	0	0	0	0	2	2.5
Training	Yes	47	74.6	0	0	0	0	47	56.6
	No	16	25.4	4	100	16	100	36	34.4
Knowledge of milk-	Yes	49	77.8	1	25	4	25	54	65.1
	No	14	22.2	3	75	12	75	29	34.9
Total		63	75.9	4	4.8	16	19.3	83	100

3.2. Microbial Quality in Raw Cow Milk

Based on microbial analysis of raw cow's milk across Agulae Town's dairy supply chain, Total Bacterial Count (TBC) and Total Coliform Count (TCC) increased significantly ($p < 0.001$) from farms (lowest levels) to vendors and cafeterias, with cafeterias showing the highest mean counts (see Figure 2). Post-hoc tests confirmed farms had significantly lower TBC and TCC compared to both vendors and cafeterias. However, Total Yeast and Mold Count (TYMC) levels remained statistically similar ($p > 0.05$) and stable across all stages (farms, vendors, cafeterias).

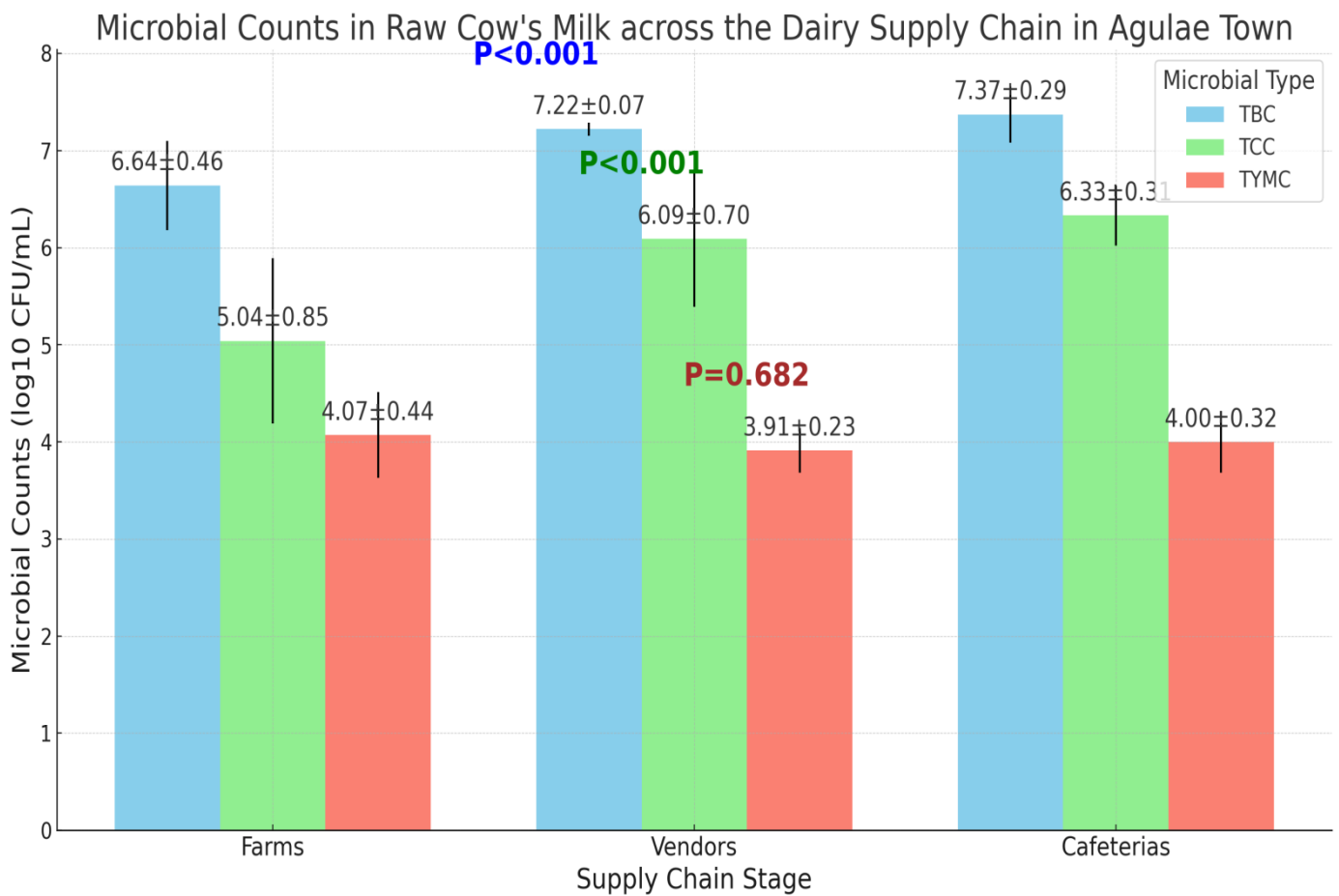


Figure 2: Microbial Counts (log₁₀ CFU/mL) in Raw Cow's Milk across the Dairy Supply Chain in Agulae Town

3.3. Physicochemical Analysis of Raw Cow Milk Findings

3.3.1. Physical properties of raw cow milk

Analysis of physical properties of revealed no statistically significant differences ($p > 0.05$) across the dairy supply chain segments for added water content ($0.19 \pm 0.84\%$), milk temperature ($18.49 \pm 3.30^\circ\text{C}$), freezing point ($-0.55 \pm 0.02^\circ\text{C}$), or pH (7.04 ± 0.19), indicating these parameters remained uniform from farms through vendors to cafeterias (see Table 4).

Table 4: Mean (\pm SD) Physical Properties of Raw Cows' Milk Samples Collected across the Dairy Supply Chains in Agulae Town.

Variables	Mean \pm SD (95% CI) of the physical properties				One-way ANOVA test
	Farms (%)	Vendors (%)	Cafeterias (%)	Total (%)	P-value
Added Water (%)	0.18 ± 0.80 (-0.02–0.39)	0.00 (0.00–0.00)	0.28 ± 1.11 (-0.31–0.87)	0.19 ± 0.84 (0.01–0.38)	0.834
Temperature ($^\circ\text{C}$)	18.34 ± 3.66 (17.42–19.27)	19.25 ± 1.99 (16.08–2.42)	18.87 ± 1.68 (17.98–19.76)	18.49 ± 3.30 (17.77–9.21)	0.765
Freezing Point ($^\circ\text{C}$)	-0.55 ± 0.02 (-0.55–0.54)	-0.54 ± 0.02 (-0.57–(-0.52))	-0.55 ± 0.02 (-0.56–0.54)	-0.55 ± 0.02 (-0.55–0.54)	0.922
pH	7.05 ± 0.20 (7.00–7.10)	6.93 ± 0.16 (6.68–7.18)	7.05 ± 0.19 (6.95–7.16)	7.04 ± 0.19 (7.00–7.08)	0.488

Note. N = sample size; SD = standard deviation; CI = confidence interval.

*Note: ** $p < 0.01$ highly significant, $p < 0.05$ significant, $p > 0.05$ not significant.

3.3.2. Chemical composition findings

The chemical composition parameters of the raw cow's milk remained consistent across the supply chain, with no statistically significant differences ($p > 0.05$) observed in fat ($4.17 \pm 1.36\%$), lactose ($4.70 \pm 0.23\%$), solids-not-fat ($8.57 \pm 0.41\%$), protein ($3.15 \pm 0.18\%$), total solids ($12.75 \pm 0.96\%$), or ash content ($0.70 \pm 0.03\%$) among farms, vendors, and cafeterias, indicating minimal variation in these parameters regardless of the collection point (see Table 5).

Table 5: Mean (\pm SD) Chemical Composition of Raw Cow's Samples Collected across the Dairy Supply Chain in Agulae Town.

Variables	Mean \pm SD (95% CI) of the chemical composition				One-way ANOVA test
	Farms (63)	Vendors (4)	Cafeterias (16)	Total (83)	<i>P-value</i>
Fat (%)	4.17 \pm 1.33 (3.84-4.50)	5.09 \pm 1.33 (2.98-7.20)	3.93 \pm 1.48 (3.14-4.72)	4.17 \pm 1.36 (3.87-4.47)	0.316
Lactose (%)	4.70 \pm 0.23 (4.64-4.76)	4.58 \pm 0.16 (4.32-4.84)	4.70 \pm 0.26 (4.56-4.84)	4.70 \pm 0.23 (4.64-4.75)	0.590
SNF (%)	8.58 \pm 0.42 (8.48-8.69)	8.37 \pm 0.28 (7.93-8.80)	8.59 \pm 0.44 (8.36-8.82)	8.57 \pm 0.41 (8.48-8.66)	0.593
Protein (%)	3.16 \pm 0.19 (3.11-3.21)	3.06 \pm 0.11 (2.89-3.24)	3.14 \pm 0.17 (3.05-3.23)	3.15 \pm 0.18 (3.11-3.190)	0.578
Total Solid (%)	12.76 \pm 0.97 (12.52-13.01)	13.04 \pm 0.35 (12.49-13.59)	12.64 \pm 1.06 (12.07-13.21)	12.75 \pm 0.96 (12.54-12.96)	0.750
Ash content	0.70 \pm 0.03 (0.69-0.71)	0.69 \pm 0.02 (0.66-0.72)	0.71 \pm 0.03 (0.69-0.72)	0.70 \pm 03 (0.70-0.71)	0.655

Note. *N* = sample size; *SD* = standard deviation; *CI* = confidence interval.
Note: ***p* < 0.01 highly significant, *p* < 0.05 significant, *p* > 0.05 not significant.

3.4. Microbial and Physicochemical Compliance with the Standards

3.4.1. Microbial compliance

The raw cow's milk exhibited alarmingly high microbial contamination, with overall mean Total Bacterial Count (TBC: 6.82 \pm 0.49 log₁₀ CFU/mL, *p* < 0.001) and Total Coliform Count (TCC: 5.34 \pm 0.93 log₁₀ CFU/mL, *p* < 0.001) significantly exceeding both Ethiopian (ES: TBC < 5.6, TCC < 3.0) and European Union (EU: TBC < 5.3, TCC < 2.3) standards. Furthermore, the overall mean Total Yeast and Mold Count (TYMC: 4.05 \pm 0.41 log₁₀ CFU/mL) surpassed the EU standard (< 2.1 log₁₀ CFU/mL, *p* < 0.001), indicating contamination levels were substantially higher than acceptable regulatory limits nationally and internationally.

Table 6: Comparison of Mean (\pm SD log₁₀ CFU/mL) Microbial Load of Raw Cow's Milk Samples with National and International Standards.

Variables	Mean \pm SD	ES	<i>P</i> -value	EU	<i>P</i> -value
TBC	6.82 \pm 0.49	<5.6	<i>P</i> <0.001	<5.3	<i>P</i> <0.001
TCC	5.34 \pm 0.93	<3	<i>P</i> <0.001	<2.3	<i>P</i> <0.001
TYMC	4.05 \pm 0.41	NA	NA	<2.1	<i>P</i> <0.001

Note. SD = standard deviation; ES = Ethiopian Standards; EU = European Union Standards; *Note:* ***p* < 0.01 highly significant, *p* < 0.05 significant, *p* > 0.05 not significant.

3.4.2. Physical properties compliance of raw cow's milk

Significant deviations from regulatory standards were observed in physical properties parameters, including added water content (0.19 \pm 0.84%), exceeded the 0.00% limit of both ES and EU (*p* < 0.001); temperature (18.49 \pm 3.30°C), surpassed the EU threshold (\leq 10°C, *p* < 0.001); and pH (7.04 \pm 0.19), exceeded the acceptable range (6.6–6.8) for ES and EU (*p* < 0.001). While the freezing point (-0.55 \pm 0.02°C) fell within the ES range (-0.547 to -0.590°C), it still showed a statistically significant deviation (*p* < 0.001).

Table 7: Comparison of (Mean \pm SD%) Physical Properties of Raw Cow's Milk Samples with National and International Standards

Variables	Mean \pm SD	ES	<i>P</i> -value	EU	<i>P</i> -value
Added Water	0.19 \pm 0.84	0.00	<i>P</i> < 0.001	0.0	<i>P</i> < 0.001
Temperature	18.49 \pm 3.30	NA	NA	NA	NA
Freezing Point	-0.55 \pm 0.02	-0.547 to (-0.590)	<i>P</i> < 0.001	-0.520 to (-540)°C	<i>P</i> < 0.001
pH	7.04 \pm 0.19	6.6-6.8	<i>P</i> < 0.001	6.6-6.8	<i>P</i> < 0.001

Note. SD = standard deviation; ES = Ethiopian Standards; EUS = European Union Standards; *Note:* ***p* < 0.01 highly significant, *p* < 0.05 significant, *p* > 0.05 not significant.

3.4.3. Chemical composition compliance of raw cow's milk

The chemical composition quality parameters of raw cow milk, including fat content ($4.17 \pm 1.36\%$), fell within the ES range (2.5–7.0%) but significantly deviated from both ES and EU thresholds ($P < 0.001$); lactose ($4.70 \pm 0.23\%$) exceeded the lower limits of both standards ($P < 0.001$); SNF ($8.57 \pm 0.41\%$) surpassed ES minimums but was outside the EU range ($P < 0.001$); and protein ($3.15 \pm 0.18\%$) though within absolute ranges for ES and EU still showed statistically significant deviations ($P < 0.001$). Total solids ($12.75 \pm 0.96\%$) met ES criteria but differed significantly from EUS ($P < 0.001$), while ash content also significantly diverged from both standards ($P < 0.001$), indicating widespread deviations from national and international benchmarks (Table 8).

Table 8: Comparison of (Mean \pm SD%) Chemical Compositions of Raw Cow's Milk Samples with National and International Standards

Variables	Mean \pm SD	ES	P-value	EU	P-value
Fat	4.17 ± 1.36	2.50-7.0	$P < 0.001$	3.5-6.0	$P < 0.001$
Lactose	4.70 ± 0.23	3.60-5.50	$P < 0.001$	4.2-5.5	$P < 0.001$
SNF	8.57 ± 0.41	>8.0	$P < 0.001$	8.25-10.5	$P < 0.001$
Protein	3.15 ± 0.18	2.90-5.0	$P < 0.001$	2.73-5.0	$P < 0.001$
Total Solids	12.75 ± 0.96	10.50-14.50	$P < 0.05$	12.5-14.5	$P < 0.001$
Ash	0.70 ± 03	0.60-0.90	$P < 0.001$	0.7-0.8	$P < 0.001$

*Note. SD = standard deviation; ES = Ethiopian Standards; EUS = European Union Standards;
Note: ** $p < 0.01$ highly significant, $p < 0.05$ significant, $p > 0.05$ not significant.*

3.5. Hygienic Practice Findings

3.5.1. Hygienic practices in milk operations

The study observed the highest bacterial contamination in milk transport on foot ($6.89 \pm 0.54 \log_{10}$ CFU/mL), followed by car ($6.83 \pm 0.55 \log_{10}$ CFU/mL) and motorcycle ($6.70 \pm 0.51 \log_{10}$ CFU/mL) (Table 9).

Table 9: General Milk Handling Practices and Transportation Methods across the Dairy Supply Chain of Agulae Town

Variables	Categories	Microbial contamination (mean \pm SD \log_{10} CFU/mL)			
		Farms (63)	Vendors (4)	Cafeterias (16)	Total (83)
Means of milk transport	Car	6.75 ± 0.53	NA	7.45 ± 0.0	6.83 ± 0.55
	Bicycle	6.74 ± 0.26	NA	NA	6.74 ± 0.26
	Bajaj/motorcycle	6.57 ± 0.48	7.17 ± 0.07	7.29 ± 0.09	6.70 ± 0.51
	On foot	6.65 ± 0.46	7.27 ± 0.04	7.40 ± 0.35	6.89 ± 0.54
	Total	6.64 ± 0.46	7.22 ± 0.75	7.37 ± 0.29	6.81 ± 0.52
Time to finish milk.	One day	6.98 ± 0.0	7.24 ± 0	7.22 ± 0.0	6.81 ± 0.72
	Two days	5.65 ± 0.46	7.21 ± 0	7.38 ± 0.30	6.81 ± 0.52
	Total	6.64 ± 0.46	7.22 ± 0.04	7.37 ± 0.29	6.81 ± 0.52
How do you store milk?	By smoking	7.25 ± 0.46	NA	7.56 ± 0.27	7.41 ± 0.52
	By the fridge	NA	7.20 ± 0.07	7.02 ± 0.23	7.21 ± 0.19
	Total	6.95 ± 0.46	7.20 ± 0.07	7.29 ± 0.29	6.81 ± 0.52

Note. *N* = sample size; *SD* = standard deviation; *CI* = confidence interval; *NA* = not available

3.5.2. On-farm hygiene and pre-milking practices

Table 10 illustrated on-farm hygiene and pre-milking practices in Agulae town, focusing on their impact on microbial contamination levels (mean \pm SD \log_{10} CFU/mL) across the supply chains. Cleaning utensils with cold water resulted in the highest contamination (7.38 ± 0.24), while using soap and hot water significantly reduced contamination (6.80 ± 0.51), underscoring the importance of proper cleaning methods. Water sources also influenced contamination, with tap water showing lower levels (6.87 ± 0.46) compared to well water (7.41 ± 0.0) and river water (6.47 ± 0.64). Handwashing before milking reduced contamination (6.60 ± 0.45) compared to not washing hands (6.96 ± 0.47), and washing cow udders slightly lowered contamination (6.63 ± 0.46 vs. 6.66 ± 0.47).

Table 10: On-Farm Hygiene and Pre-Milking Practices in Agulae Town

Variables	Categories	Microbial contamination (mean \pm SD log ₁₀ CFU/mL)			
		Farms (63)	Vendors (4)	Cafeterias (16)	Total (83)
How do you clean utensils?	Cold water	7.47 \pm 0.30	7.12 \pm 0.00	7.45 \pm 0.00	7.38 \pm 0.24
	Soap and cold water	6.38 \pm 0.38	7.24 \pm 0.00	7.44 \pm 0.00	6.70 \pm 0.58
	Soap and hot water	6.62 \pm 0.40	7.26 \pm 0.06	7.47 \pm 0.28	6.80 \pm 0.51
	Only hot water	6.64 \pm 0.50	0	7.22 \pm 0.32	6.76 \pm 0.52
	Total	6.64 \pm 0.46	7.22 \pm 0.07	7.37 \pm 0.29	6.81 \pm 0.52
Sources of water used for cleaning?	Tape water	6.70 \pm 0.40	7.22 \pm 0.07	7.35 \pm 0.28	6.87 \pm 0.46
	Well water	7.41 \pm 0.0	NA	NA	7.41 \pm 0.0
	River water	6.37 \pm 0.54	NA	7.81 0.0	6.47 \pm 0.64
	Total	6.64 \pm 0.46	7.22 \pm 0.07	7.37 \pm 0.29	6.81 \pm 0.52
Do you wash your hands before milking?	Yes.	6.60 \pm 0.45	NA	NA	6.60 \pm 0.45
	No	6.96 \pm 0.47	NA	NA	6.96 \pm 0.47
	Total	6.64 \pm 0.46	NA	NA	6.64 \pm 0.46
Do you wash a cow's udders before milking?	Yes	6.63 \pm 0.46	NA	NA	6.63 \pm 0.46
	No	6.66 \pm 0.47	NA	NA	6.66 \pm 0.47
	Total	6.64 \pm 0.46	NA	NA	6.64 \pm 0.46
Cleaning barn practice	Daily	6.64 \pm 0.45	NA	NA	6.64 \pm 0.45
	Every two days	6.67 \pm 0.60	NA	NA	6.67 \pm 0.60
	Every three days	6.45 \pm 0.20	NA	NA	6.45 \pm 0.20
	Total	6.64 \pm 0.46	NA	NA	6.64 \pm 0.46

Note. *N* = sample size; *SD* = standard deviation; *CI* = confidence interval; *NA* = not available

3.5.3. Observational findings of the different dairy supply chains

The overall mean (mean \pm SD log₁₀ CFU/mL) of observational findings of hygiene and cleanliness across various dairy supply chains showed the highest scores in both personal hygiene appearance (7.17 \pm 0.07) and cleanliness of milk containers (7.17 \pm 0.07). Cafeterias also scored highly in cleanliness of the environment (7.34 \pm 0.29) and container cleanliness (7.31 \pm 0.31). In contrast, farms had slightly lower hygiene scores, with personal hygiene appearance (6.62 \pm 0.44) and cleanliness of containers (6.61 \pm 0.43) reflecting more variability. Overall, the total mean for all categories was consistent, with a score of 6.81 \pm 0.52.

Table 11: Observed Hygiene and Cleanliness across the Dairy Supply Chain in Agulae Town.

Variables	Categories	Microbial contamination (mean \pm SD log ₁₀ CFU/mL)			
		Farms (63)	Vendors (4)	Cafeterias (16)	Total (83)
Personal hygiene appearance	Looks good.	6.62 \pm 0.44	7.17 \pm 0.07	7.31 \pm 0.57	6.75 \pm 0.49
	Looks poor.	6.73 \pm 0.57	7.27 \pm 0.04	7.53 \pm 0.21	7.05 \pm 0.57
	Total	6.64 \pm 0.46	7.22 \pm 0.07	7.37 \pm 0.29	6.81 \pm 0.52
Cleanness of the milk container	Looks good.	6.61 \pm 0.43	7.17 \pm 0.07	7.31 \pm 0.31	6.75 \pm 0.49
	Looks poor.	6.76 \pm 0.60	7.27 \pm 0.04	7.52 \pm 0.21	7.04 \pm 0.58
	Total	6.64 \pm 0.46	7.22 \pm 0.07	7.37 \pm 0.29	6.81 \pm 0.52
Cleanliness of the environment	Clean	6.62 \pm 0.45	7.19 \pm 0.06	7.34 \pm 0.29	6.77 \pm 0.51
	Dusty	6.93 \pm 0.53	7.30 \pm 0.0	7.63 \pm 0.26	7.18 \pm 0.51
	Total	6.64 \pm 0.46	7.22 \pm 0.07	7.37 \pm 0.29	6.81 \pm 0.52

Note. N = sample size; SD = standard deviation; CI = confidence interval

3.6. The Possible Risk Factors Affecting Raw Cow Milk

The regression analysis identified several significant risk factors, as shown in Table 12. River water usage for utensil cleaning unexpectedly reduced contamination ($B = -0.264$, $p < 0.05$), while not washing hands before milking increased bacterial counts ($B = 0.412$, $p \leq 0.05$). Effective utensil cleaning with soap and cold water significantly lowered contamination ($B = 0.446$, $p < 0.001$), underscoring hygiene protocol importance. Transportation via Bajaj/motorcycle ($B = -0.181$, $p < 0.05$) and refrigeration ($B = -0.353$, $p < 0.05$) reduced contamination, whereas dusty environments increased risk ($B = 0.272$, $p < 0.05$). Notably, formal training showed no significant impact ($p > 0.05$), suggesting targeted hygiene interventions rather than general education are key to mitigating bacterial load in the supply chain.

The regression analysis identified several significant risk factors as shown in (Table 12) influencing the bacterial count in raw cow milk, underscoring critical variables that contribute to milk contamination. Among demographic factors, sex and marital status emerged as key determinants. Specifically, being female was associated with a decrease in bacterial contamination ($B = -0.218$, $p < 0.007$), while being widowed was linked to a

significant increase in bacterial count ($B = 0.810$, $p < 0.001$). Educational attainment demonstrated a strong inverse relationship with bacterial load, with lower levels of education (1-8th grade, 9-12th grade, and diploma or above) significantly reducing contamination, as reflected by the negative coefficients ($B = -0.844$, $p < 0.001$; $B = -0.742$, $p < 0.001$; $B = -1.006$, $p < 0.001$, respectively). These findings suggest that education may play a crucial role in promoting hygienic practices, thereby reducing the bacterial load in raw milk.

Supply chain-related factors also contributed significantly to bacterial contamination levels. Milk produced in cafeterias ($B = 0.881$, $p < 0.001$) and by vendors ($B = 1.081$, $p < 0.001$) exhibited higher bacterial counts compared to milk from farms, indicating that practices within these supply chains may be more prone to contamination. In contrast, the variable "training taken" showed no significant effect on bacterial count ($B = 0.125$, $p > 0.05$), suggesting that general training did not mitigate contamination risks in this context.

Water source used for cleaning utensils was another critical factor, with river water ($B = -0.264$, $p < 0.05$) significantly correlating with lower bacterial counts. Conversely, the method of cleaning utensils using soap and cold water was positively associated with reduced contamination ($B = 0.446$, $p < 0.001$), highlighting the importance of effective cleaning protocols in dairy production. Hand washing before milking was also found to be a significant factor, with individuals who did not wash their hands before milking showing an increase in bacterial contamination ($B = 0.412$, $p < 0.05$).

Transportation methods and milk preservation techniques further influenced contamination levels. The use of Bajaj or motorcycles for transportation ($B = -0.181$, $p < 0.05$) was linked to a reduction in bacterial counts, suggesting that specific transportation methods may help maintain milk hygiene. Additionally, preserving milk using a fridge was associated with lower contamination ($B = -0.353$, $p < 0.05$), indicating the importance of proper storage in controlling microbial growth. Environmental hygiene, specifically when environments were described as dusty, showed a positive association

with bacterial contamination ($B = 0.272$, $p < 0.05$), emphasizing the role of surrounding cleanliness in maintaining milk safety.

Table 12: The Regression Analysis Identified Several Significant Risk Factors in Raw Milk Bacterial Count (log10)

Variables	Categories	Unstandardized Coefficients		Standardized Coefficients	t-test	Sig.
		B	Std. Error	Beta		
	(Constant)	7.495	.128		58.419	.000
Sex	Female	-.218	.079	-.204	-2.769	.007
Marital status	Widowed	.810	.231	.241	3.507	.001
Education	1-8 th Grade	-.844	.123	-.751	-6.861	.000
	9-12th grade	-.742	.145	-.539	-5.110	.000
	Diploma and above	-1.006	.212	-.418	-4.741	.000
Supply chain	Cafeterias	.881	.146	.674	6.029	.000
	Vendors	1.081	.259	.449	4.166	.000
Training taken	No	.125	.100	.120	1.254	.214
Sources of water-cleaning utensils	river water	-.264	.093	-.197	-2.847	.006
Methods of cleaning utensils	Soap & cold water	.446	.123	.224	3.640	.001
Handwashing before milking	No	.412	.145	.207	2.837	.006
Means transport	Bajaj/Motorcycle	-.181	.079	-.168	-2.285	.025
Preserve milk.	using the fridge	-.353	.159	-.249	-2.220	.030
environmental hygiene	Dusty	.272	.118	.147	2.316	.024

The overall Model Fit include $R^2 = 0.893$, R Square = 0.797, Adjusted $R^2 = 0.631$, and $p > 0.001$.
*Note: ** $p < 0.01$ highly significant, $p < 0.05$ significant, $p > 0.05$ not significant.*

4. CHAPTER IV: DISCUSSION

The results revealed significant variations in bacterial load between different stages of the supply chain, with high contamination levels at all stages, exceeding both Ethiopian and European Union standards. The analysis revealed a concerning high TBC and TCC at all stages, exceeding both Ethiopian and European Union standards.

4.1. Microbial Load Variations

The microbiological assessment revealed that the overall mean microbial contamination levels in raw cow's milk across the milk supply chains were found to be high. The mean total bacterial count (TBC) was $6.81 \log_{10}$ CFU/mL, the total coliform count (TCC) was $5.34 \log_{10}$ CFU/mL, and the total yeast and mold count (TYMC) was $4.05 \log_{10}$ CFU/mL, all exceeding the Ethiopian and European Union safety limits. This high microbial level may be due to the lack of training about hygienic milk operations, lack of handwashing before milking, and using unhygienic river water for cleaning utensils.

Mean total bacterial count (TBC) at the farm level was $6.669 \log_{10}$ CFU/mL, which closely parallels values reported in prior regional studies, including $6.8 \log_{10}$ CFU/mL in the Ethiopian central highlands (Abebe *et al.*, 2018) and $6.42 \log_{10}$ CFU/mL in Oromia (Eshetu *et al.*, 2019). Notably, this microbial load substantially exceeds the maximum permissible thresholds set by both the Ethiopian Quality Standards ($\leq 5.3 \log_{10}$ CFU/mL) and the European Union Standards ($\leq 5.6 \log_{10}$ CFU/mL). Such elevated counts are indicative of substandard hygienic practices, inadequate milking protocols, and the absence of cold chain infrastructure at the production level. These findings are consistent with broader African dairy supply chain studies, where microbial contamination is frequently traced to poor udder hygiene, unsanitized milking equipment, and the use of biologically unsafe water for cleaning (Berhe *et al.*, 2020; Mekonnen, 2020). As milk progressed along the supply chain, bacterial contamination intensified significantly, with the mean TBC at the vendor level increasing to $7.073 \log_{10}$ CFU/mL ($p < 0.001$). This escalation mirrors results from Eshetu *et al.* (2019) and Oumer *et al.* (2017), who documented comparable or higher TBC values (7.49 and $8.01 \log_{10}$ CFU/mL,

respectively) in vendor-sourced milk. The sharp rise in microbial load at this stage may be largely attributed to the lack of cold storage facilities, extended transportation periods under ambient conditions, and continued exposure to unhygienic handling practices. The peak contamination was observed in cafeteria milk samples, with a mean TBC of 7.374 \log_{10} CFU/mL, reinforcing findings by Amentie *et al.* (2016), who reported values reaching 8.82 \log_{10} CFU/mL in Eastern Ethiopia.

Similarly, coliform bacteria serve as critical indicators of fecal contamination and reflect the overall sanitary quality of milk throughout the dairy supply chain. In the present study, the mean Total Coliform Count (TCC) at the farm level was recorded at 5.036 \log_{10} CFU/mL, escalating to 6.088 \log_{10} CFU/mL in vendor samples and peaking at 6.331 \log_{10} CFU/mL in cafeteria samples. These values far exceed the maximum permissible thresholds set by both Ethiopian standards ($\leq 3 \log_{10}$ CFU/mL) and European Union standards ($\leq 2.3 \log_{10}$ CFU/mL), indicating widespread microbial contamination throughout the supply chain. The progressive increase in TCC was found to be statistically significant ($p < 0.001$), highlighting the cumulative effect of inadequate sanitary practices during milking, transportation, and storage. Similar patterns have been reported in other regions of Ethiopia: for instance, Jemal (2023) in Jimma and Oumer *et al.* (2017) in Mersa documented comparably elevated coliform levels, which were strongly correlated with the use of contaminated water sources, insufficient hand hygiene, and improperly sanitized milk handling equipment. These findings are further supported by Asresie *et al.* (2020), who attributed elevated coliform levels in dairy markets across Ethiopia to direct contact with manure, the reuse of inadequately cleaned containers, and the absence of effective pasteurization protocols.

Additionally, the TYMC in farm milk samples was 4.068 \log_{10} CFU/mL (SD = 0.442), with levels decreasing slightly to 3.914 \log_{10} CFU/mL in vendors and 4.053 \log_{10} CFU/mL in cafeterias. Unlike bacterial and coliform contamination, no significant increase ($p > 0.05$) in TYMC was observed along the supply chain. However, these values are higher than the EU standard of $\leq 2.1 \log_{10}$ CFU/mL, suggesting potential risks associated with fungal contamination in raw milk. Higher yeast and mold counts can be attributed to prolonged exposure to moisture, improper milk storage conditions, and

inadequate sanitation of containers. Similar findings were reported by Amentie *et al.* (2016), who observed TYMC exceeding safe limits in milk supply chains in Eastern Ethiopia, particularly where milk was stored in plastic containers and exposed to humid conditions. The findings indicate that controlling milk storage temperatures and improving container hygiene are critical in reducing fungal contamination.

The risk factor identification revealed that several socio-demographic and operational determinants significantly contributed to elevated microbial contamination in raw cow milk. Notably, milk sourced from male producers, individuals with limited educational backgrounds, and widowed respondents exhibited significantly higher bacterial counts. These findings align with previous research from Bench Maji Zone, Southwest Ethiopia (Tadesse *et al.*, 2023), and Gondar (Demissie *et al.*, 2018), which linked insufficient hygiene awareness and poor sanitation practices to increased microbial load in milk. Moreover, operational factors such as evening milking sessions, inadequate hand hygiene, and milk obtained from vendors and cafeterias were identified as strong predictors of microbial contamination. These associations are corroborated by studies across Ethiopia (Berhe *et al.*, 2020), which have consistently reported the adverse impact of substandard post-harvest handling, lack of cold chain infrastructure, and poor environmental hygiene on milk quality. The high microbial loads in the raw cow milk make it unsafe for consumption and pose severe foodborne disease risks, particularly for vulnerable populations such as infants, pregnant women, and the elderly. Ethiopian studies, such as those in Tigray (Berhe *et al.*, 2020) and East African research (Nyaribo *et al.*, 2022), have linked raw milk consumption to outbreaks of foodborne illnesses, including those caused by *E. coli* O157:H7, *Staphylococcus aureus*, and other enteric pathogens that can lead to diarrhea and gastrointestinal infections. Beyond health implications, the economic impact is substantial, ranging from increased healthcare costs and loss of productivity to reduced consumer trust and diminished market values of the raw cow milk. Internationally, the adoption of pasteurization and strict regulatory controls has dramatically reduced the incidence of milk-borne diseases, as seen in Europe and North America (Oliver *et al.*, 2005). These contrasts highlight the urgent need for targeted training for farmers and vendors, improved hygiene infrastructure, and the promotion of milk pasteurization in Ethiopia.

4.2. Physicochemical Properties of Milk

The physicochemical properties of milk, including pH, fat content, total solids (TS), solids-not-fat (SNF), and lactose content, are key indicators of milk quality. The pH values of milk samples (Table 4) did not significantly vary, though they were slightly above the normal range (6.6-6.8) of both ES and EUS, with means of 7.046 ± 0.197 (farms), 6.928 ± 0.158 (vendors), and 7.053 ± 0.194 (cafeterias), Ethiopian studies typically report lower pH values: for example, Jalel et al. (2021) found pH values of 6.41–6.28 along milk value chains in Oromia and Gemechu & Amene, (2021) from Bench Maji-Zone, Southwestern Ethiopia, and other studies confirm that fresh cow's milk should have a pH between 6.6 and 6.8 Eshetu et al. (2019). Marketed milk in Ethiopia often shows pH values below the standard, likely due to fermentation during storage and transport (Fita, & Fekata, 2024). The added water content was $0.18 \pm 0.80\%$, 0.00% and 0.28 ± 1.11 for farms, vendors, and cafeterias, respectively, showing there were addition of water at some farms and cafeterias this may be due to lack of awareness these findings are similar with Tadele & Gebreanya (2024) in Tigray Adwa. The freezing point of the milk samples was within the standard range (-0.548 ± 0.024) (Tadele & Gebreanya, 2024) and other studies in Ethiopia (Azeze and Tera, 2015).

Protein content across sources was $3.159 \pm 0.186\%$ (farms), $3.063 \pm 0.111\%$ (vendors), and $3.144 \pm 0.168\%$ (cafeterias) (Table 2), showing no significant difference ($P > 0.05$). The overall mean ($3.151 \pm 0.179\%$) aligns with the European Union standard ($\geq 2.9\%$), but slightly greater than the findings from Sebeta and bushoftu ($3.05 \pm 0.04\%$) by Shibru *et al.* (2019).

Fat content was highest in vendors ($5.090 \pm 1.327\%$) compared to farms ($4.170 \pm 1.326\%$) and cafeterias ($3.931 \pm 1.477\%$), with an overall mean of $4.169 \pm 1.359\%$, accepting both the Ethiopian and European Union standards but exceeding the findings from Sebeta and bushoftu ($3.50 \pm 0.23\%$) by Shibru *et al.* (2019). Indicating the milk is sourced from different breeds with greater fat.

Total solids (TS) content was $12.764 \pm 0.970\%$ (farms), $13.042 \pm 0.346\%$ (vendors), and $12.641 \pm 1.064\%$ (cafeterias), with an overall mean of $12.754 \pm 0.964\%$ (Table 8). These values comply both standards, indicated good nutritional quality, though lower than the 13.67% reported by Tadele & Gebreanya (2024) in Tigray Adwa but exceeding from the findings in Sebeta and bushoftu (11.81 ± 0.26) by Shibru *et al.* (2019). Higher TS content enhances milk's nutritional value and sensory appeal (Yayota *et al.*, 2013), influencing processing suitability for cheese-making versus fluid milk products (Abd El-Gawad & Ahmed, 2011).

Solids-not-fat (SNF) content was $8.580 \pm 0.416\%$ (farms), $8.565 \pm 0.276\%$ (vendors), and $8.591 \pm 0.437\%$ (cafeterias), with an overall mean of $8.572 \pm 0.414\%$, meeting the ES EU standards and almost nearly to Tamime (2009) findings (8.5%) and Shibru *et al.* (2019) findings (8.31%).

Lactose content was highest in milk from farms ($4.701 \pm 0.230\%$) and cafeterias ($4.701 \pm 0.257\%$) compared to vendors ($4.578 \pm 0.163\%$), with an overall mean of $4.70 \pm 0.23\%$, within the ES ($\geq 3.60\%$) and EU ($\geq 4.2\%$) standards slightly lower than findings from bushoftu ($4.91 \pm 0.12\%$) (Shibru *et al.*, 2019). The ash content with overall mean $0.70 \pm 0.03\%$ also complies the Ethiopian and European standards as shown in Table 8.

4.3. Limitations of the Study

The small sample size, particularly for vendors ($n=4$), limits the generalizability of these findings. Future research requires a larger, more representative sample across all actor groups. Furthermore, detailed information on training content and its impact on actual practices related to hygiene and food safety is crucial.

5. CONCLUSION AND RECOMMENDATIONS

The current study findings have shown a significant increase in bacterial contamination as milk moved through the supply chain from dairy farms to end users through vendors and cafeterias. Total bacterial counts and total coliform counts were notably higher at the vendor and cafeteria stages, indicating high contamination due to inadequate hygiene and poor handling practices, which compromised the microbial safety of the milk. Several risk factors of bacterial contamination of raw cow milk were identified, including male gender of the milk handlers, illiteracy level of education, widowhood marital status, lack of handwashing before milking, sourcing the milk from vendors and cafeterias, and evening milking as the most significant factors. On the other hand, elevated yeast and mold counts, although consistent across all stages, further highlighted concerns about microbial spoilage. Interestingly, several risk factors linked to higher bacterial counts, including sex, educational status, marital status, types of milk supply chains, hand washing before milking, and time of milk, were identified. These findings expose critical hygiene and handling gaps, particularly at the vendor and cafeteria levels, driving substantial microbial contamination. In contrast, physicochemical properties showed no significant differences in most parameters across the milk supply chain and were largely compliant with the Ethiopian and European Union quality standards. However, slight variations in milk composition were observed, water adulteration at the farms and vendors supply chains. Generally, these findings indicated that while the nutritional quality of the raw cow milk remained intact, its microbial safety was critically compromised, posing substantial public health risks and economic challenges for the local dairy sector.

Based upon the above conclusions the following recommendations were provided:

- Strengthening hygiene practices during milking, handling, and storage by using clean equipment, washing hands before and after milking, and regularly cleaning containers and the environment.
- Ensuring immediate cooling to reduce microbial growth and minimizing the use of plastic or unclean containers that harbor contaminants.

- Establish community-based awareness creation programs about milk handling practices across the milk value chain actors.
- Introduction and implementation of mandatory milk hygiene inspections, laboratory tests, and certification systems to the value chain.
- Promote cooperative-based milk collection centers with proper testing, refrigeration, and safety controls to reduce contamination before distribution.
- Further research should be conducted to better understand the specific sources of microbial contamination in milk production.

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APENDIX-I
MEKELLE UNIVERSITY
College of Veterinary Science
Department of Veterinary Public Health
MSc in Zoonosis and Food Safety
Questionnaires survey

Dear respondent,

The aim of this questionnaire is to collect data for MSc research work to gather information on the handling practices of milk and the prevalence of bacterial contamination among dairy farmers, milk vendors, and cafeteria owners in Tigray, Ethiopia. Your collaboration in providing genuine response to the following questions is highly important for the success of this study; it is only academic purpose your response would be kept confidentially.

Thank you in advance

Instruction

No need to write your name

Put tick (√) mark in the box next to the given alternatives and write your answers on the space provided.

Section 1:- The Sample Profiles

1. Date of sample collection _____
2. Address _____
3. breeds _____
4. Name of the farm _____
5. Sample code _____

Section 2: Socio-Demographic Information

1. Residence: _____ 2. Age: _____ 3. Sex: _____
4. Level of Education: : _____ 5. Marital Status: _____ 6. Type of dairy supply chain: _____

Section 3: Concerning Hygienic Practices and handling During Milk Operations

1. Have you ever taken any training on hygienic milk production and handling?
2. Do you have knowledge on milk borne diseases? _____
3. What do you use for Cleaning of utensils?? (Select all that apply)
 Cold water Soap and cold water Soap and hot water Only
 hot water
4. Do you wash your hands before milking? _____
 4.1. If 'yes', how do you wash your hands? (Select all that apply)
 Cold water Soap and cold water Soap and hot water Only
 hot water
5. Do you wash the udders of lactating cows before milking? _____
 If 'yes', how do you wash the udders? (Select all that apply)
 Cold water Soap and cold water Soap and hot water Only
 hot water
6. What type of containers do you use? (Select all that apply)
 Aluminum vessel/Steel Plastic vessel Wood Traditional
 pot
7. How do you transport milk? (Select all that apply)
 Car Bicycle Bajaj or Motorcycle On Foot By
 Animal
8. How long do you take for transportation of milk?: Less than 1 hour 1–2
 hours More than 2 hours
9. Do you Mix milk from different sources? _____
10. What is your source of water for cleaning? _____
11. How do you store milk? (Select all that apply)
 Refrigerator Room temperature
 Boiling and room temperature Boiling and refrigeration
12. How do you store milk?: By smoking By fridge
13. How long does it take to finish the milk after collection? _____
14. Have you encountered milk spoilage? _____
15. How often do you clean the barn? _____

Section 4: Milk Collection Practices

1. Time of Milk Collection: When do you collect milk? (Select all that apply)
 Evening Early morning Both
2. Sources of Milk: Where do you source your milk from? (Select all that apply)
3. Own farm Other one farm Other two farm more than two farms Other three or more farms Milk vendors
4. Type of Milk Sold: What type of milk do you sell? (Select all that apply)
 Raw milk Boiled milk Yogurt
 Boiled milk and yogurt Raw milk, boiled milk, and yogurt

Section 5: Observational Findings

1. Personal hygiene appearance: Looks poor looks good
2. Cleanliness of milk container: Looks poor looks good
3. Cleanliness of the environment: Clean sty

Thank you for your participation in this survey. Your responses will contribute to understanding the bacterial contamination of milk and improving public health in the region.

For any questions or concerns, please contact:

Hailay G/tnsae Berhane (Dr.)

Email: hailaygbrtns@gmail.com

Phone No.: +251914-26-88-32/+251949-837379

APENDIX-II

Images taken during the field and laboratory works



Measuring the media and the distilled water for media preparation



Boiling and thoroughly mixing the media to homogeneously dilute it

Population unit and sample unit verification

No	Household	Sample Unit	Population	Sample Unit	Population	Sample Unit	Population					
01	0001	ncu	11	1	2	1	2					
02	0002	ncu	11	2	2	1	2					
03	0003	ncu	11	3	1	1	2					
04	0004	ncu	11	4	1	1	2					
05	0005	ncu	11	5	2	2	1	1	1	1	1	1
06	0006	ncu	11	6	2	2	1	1	1	1	1	1
07	0007	ncu	11	7	1	1	1	1	1	1	1	1
08	0008	ncu	11	8	1	1	1	1	1	1	1	1
09	0009	ncu	11	9	1	1	1	1	1	1	1	1
10	0010	ncu	11	10	1	1	1	1	1	1	1	1
11	0011	ncu	11	11	1	1	1	1	1	1	1	1
12	0012	ncu	11	12	1	1	1	1	1	1	1	1
13	0013	ncu	11	13	1	1	1	1	1	1	1	1
14	0014	ncu	11	14	1	1	1	1	1	1	1	1
15	0015	ncu	11	15	1	1	1	1	1	1	1	1
16	0016	ncu	11	16	1	1	1	1	1	1	1	1
17	0017	ncu	11	17	1	1	1	1	1	1	1	1
18	0018	ncu	11	18	1	1	1	1	1	1	1	1
19	0019	ncu	11	19	1	1	1	1	1	1	1	1
20	0020	ncu	11	20	1	1	1	1	1	1	1	1
21	0021	ncu	11	21	1	1	1	1	1	1	1	1
22	0022	ncu	11	22	1	1	1	1	1	1	1	1
23	0023	ncu	11	23	1	1	1	1	1	1	1	1
24	0024	ncu	11	24	1	1	1	1	1	1	1	1
25	0025	ncu	11	25	1	1	1	1	1	1	1	1
26	0026	ncu	11	26	1	1	1	1	1	1	1	1
27	0027	ncu	11	27	1	1	1	1	1	1	1	1
28	0028	ncu	11	28	1	1	1	1	1	1	1	1
29	0029	ncu	11	29	1	1	1	1	1	1	1	1
30	0030	ncu	11	30	1	1	1	1	1	1	1	1

Sample Collection, Survey and observational data gathering



Dairy vendors participating in different processing





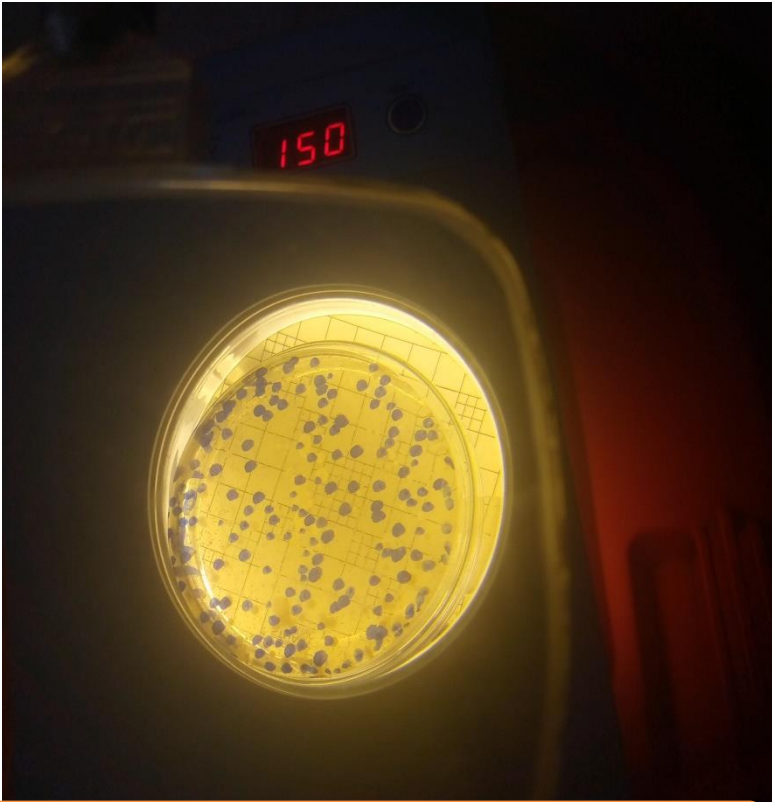
Serial dilution preparation



Boiling of the sterile media after checked for any growth



Everything ready within the safety cabinet for culture and sample is placed on the plates



Colony count under the colony counter



Colony in the plate count agar media

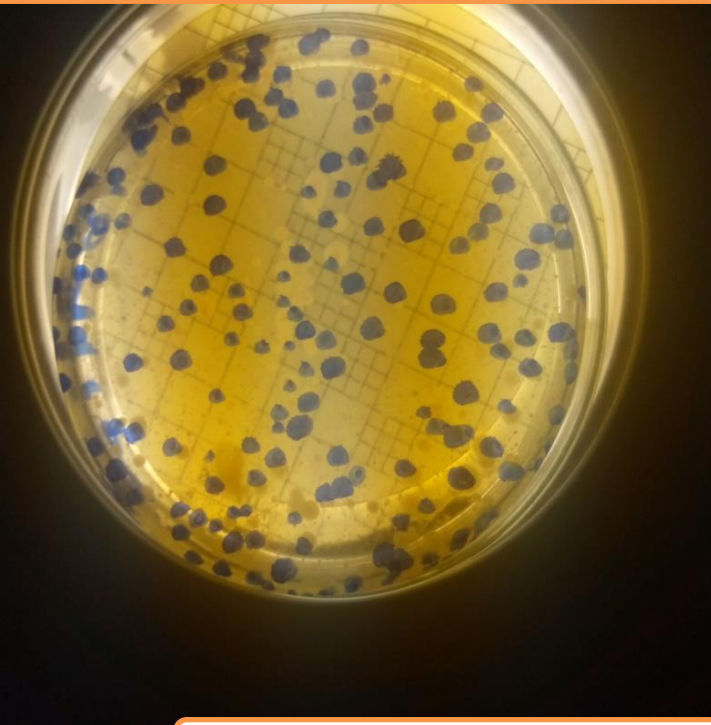
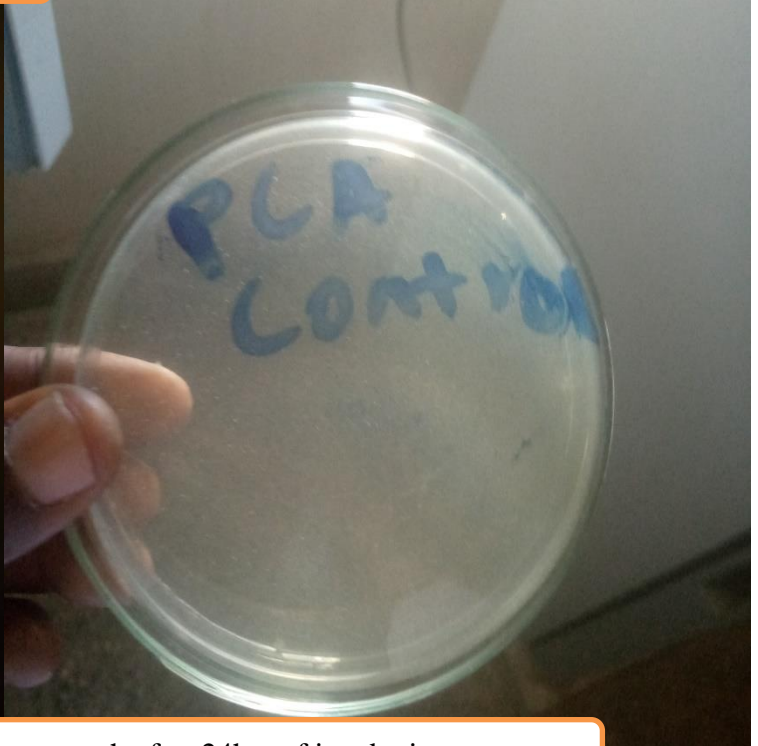
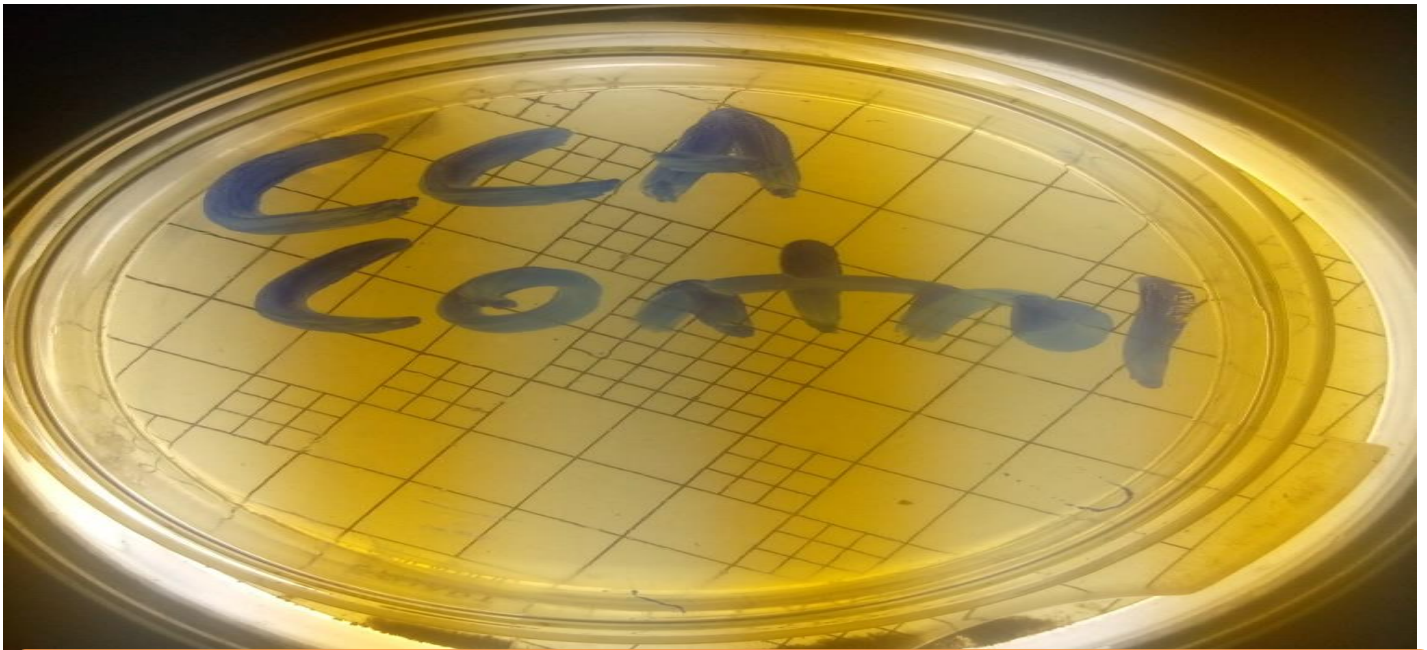


Plate count agar control with no growth after 24hrs of incubation





Coliform colonies under the coliform culture agar



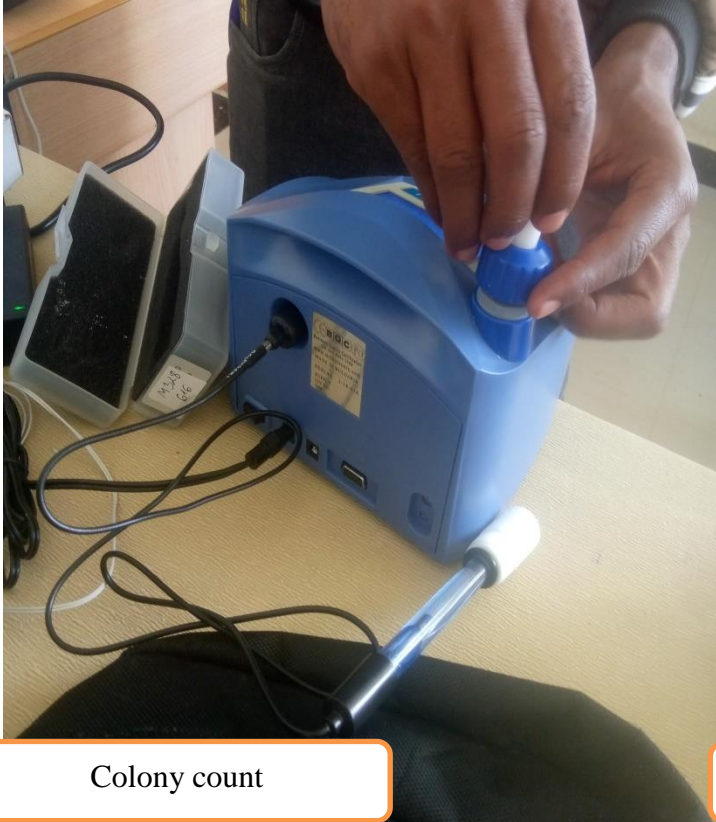
No growth on control of the coliform culture agar after 24 hrs of incubation

Colonies with filamentous, cotton-like having white, cream, pink to various shades of green, brown, or black (i.e., powdery) appearance of yeasts and molds



Yeast and mold culture using sabourade maltose agar for 5days at room temperature

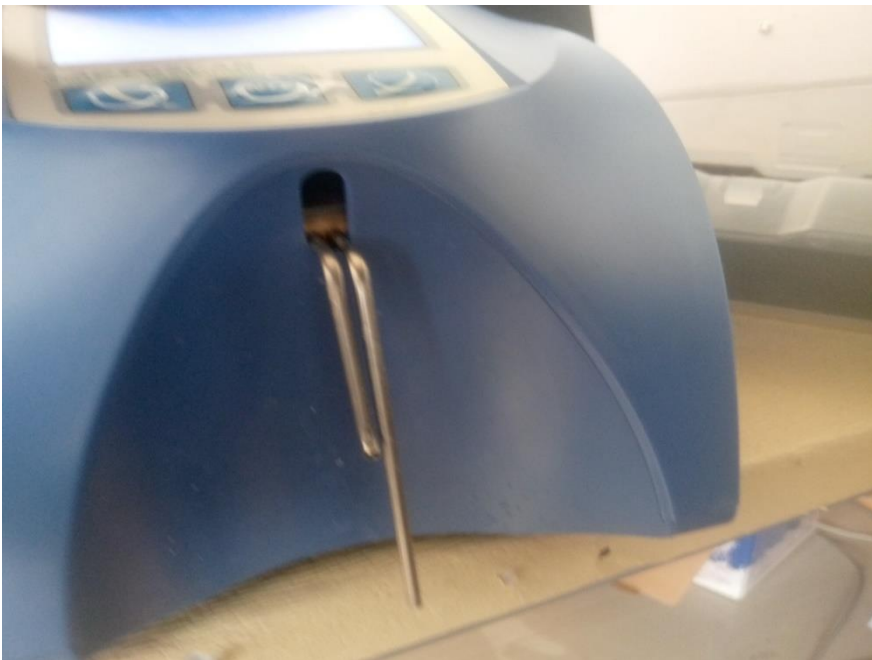




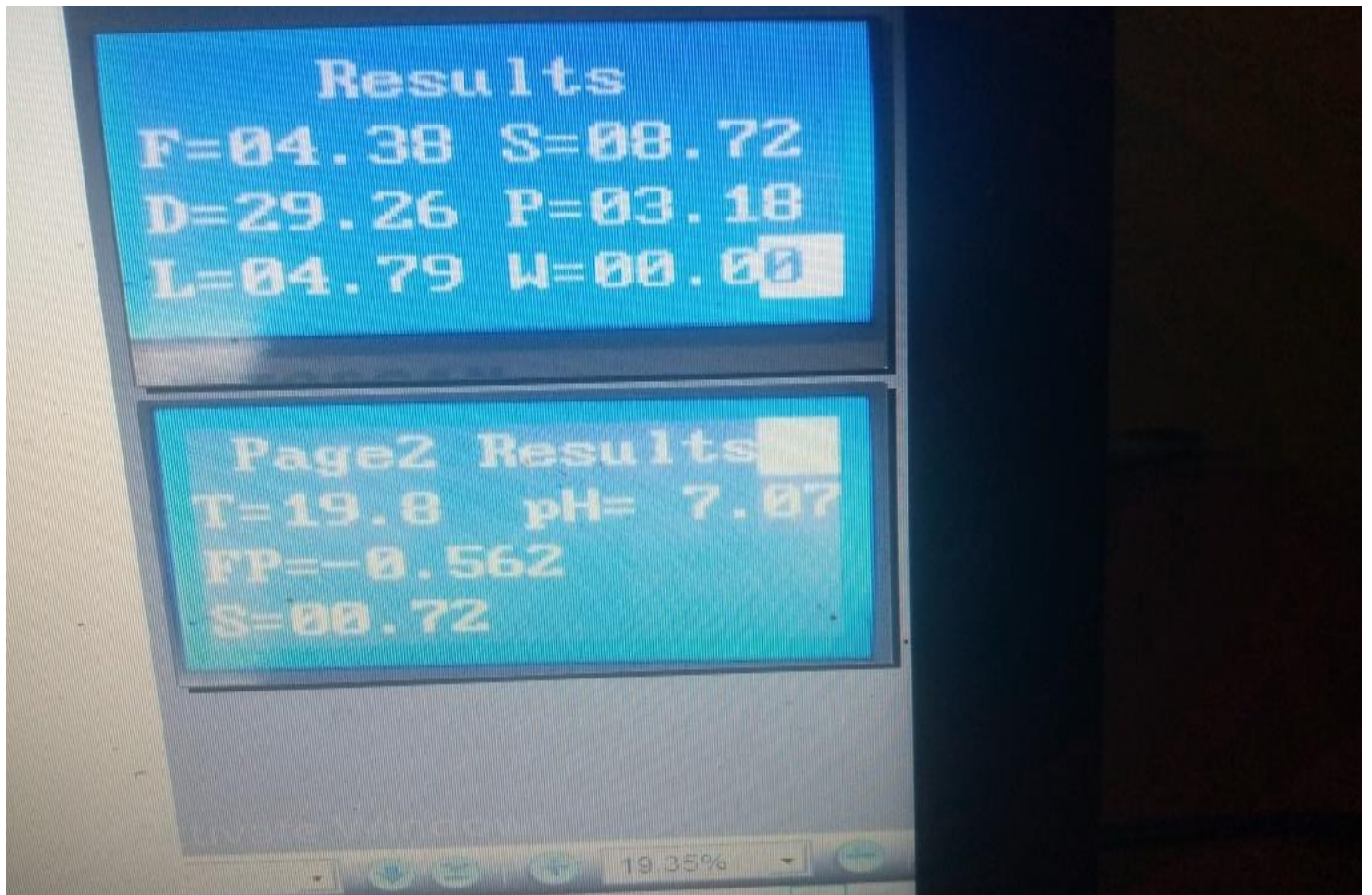
Colony count



SMA used as control and no growth after 5 days



Lacto scan ultrasonic milk analyzer



Lacto scan ultrasonic milk analyzer displaying result after analysis.