



WERABE UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

DEPARTMENT OF BIOLOGY

**MICROBIAL QUALITY OF RAW COW MILK COLLECTED FROM DAIRY
FARMERS AND MARKETED IN KIBAT ADMINISTRATIVE TOWN, SILTE
ZONE, CENTRAL ETHIOPIA REGION, ETHIOPIA**

THESIS SUBMITTED TO DEPARTMENT OF BIOLOGY FOR THE PARTIAL
FULFILLMENT OF THE REQUIREMENTS FOR MASTERS DEGREE IN BIOLOGY
(MICROBIOLOGY)

By

YASIN GETISO

MAJOR ADVISOR: ALEMAYEHU DUGASSA (PhD)

CO-ADVISOR: ABDULMEJID MUTEBA (MSC)

FEBRUARY 2025

WERABE, ETHIOPIA



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Approval sheet- advisor

CERTIFICATE

This is to certify that the thesis, entitled “**MICROBIAL QUALITY OF RAW COW MILK COLLECTED FROM DAIRY FARMERS AND MARKETING IN KIBAT ADMINISTRATIVE TOWN, SILTE ZONE, CENTRAL ETHIOPIA REGION, ETHIOPIA**” submitted to the Department of BIOLOGY, Werabe University, in partial fulfillment of the requirements for the award **MASTER** of the Degree IN MICROBIOLOGY is a record of original research work done by **YASIN GETISO** during the period 2014-2015(E.C) under my supervision guidance and the thesis has not formed the basis for the award of any Degree / Diploma or other similar title of any candidate of any University and it complies with the regulation and accepted standards of the university.

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DECLARATION

I, the under signed postgraduate student in department of Biology, declared that this research thesis is my original work and it has not been presented in my previous works or for any other purpose at any university.

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LIST OF ACRONYMS AND ABBREVIATION

AMR	Antimicrobial Resistance
ANOVA	Analysis of variance
APC	Aerobic plate count
CC	Coliform count
CFU/ML	Colony forming unit per milliliter
CV	Coefficient variation
EAC	East African community standard
ES	Ethiopian standard
FBDS	Food-borne diseases
FCC	Faecal coliform count
MCA	Mac Conkey Agar
MDR	Multidrug Resistance
NA	Nutrient Agar
PDA	Potato Dextrose Agar
SD	Standard variation
SPC	Standard plate count
TAMBC	Total aerobic mesophilic bacterial count
TBC	Total bacterial count
TCC	Total coliform count
VRBA	Violet red bile agar
YMC	Yeast and mold

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ABSTRACT

Milk is a highly nutritious food for humans; however, it also provides an excellent environment for the growth of pathogenic microorganisms. This study aimed to assess the microbial quality of raw cow milk produced by dairy farmers and marketed in Kibate Town, Silte Zone, and Central Ethiopia. A cross-sectional study was conducted in three purposively selected kebele based on their dairy production potential. A total of 105 raw milk samples (35 from each kebele) were collected using random sampling techniques for microbial analysis. The overall mean counts for aerobic mesophilic bacteria, coliforms, and yeasts and molds were 5.4 ± 0.5 , 4.6 ± 0.8 , and 4.1 ± 0.3 log cfu/ml, respectively. Among the pathogenic bacteria identified, *Staphylococcus aureus* (48.7%), *Escherichia coli* (44.8%), and *Salmonella* spp. (6.4%) were detected in the milk samples. Significant variations in total bacterial counts, and yeast and mold counts were observed among the kebele. While the aerobic mesophilic, and yeast and mold counts were within acceptable limits, the coliform counts exceeded recommended levels, likely due to poor hygienic practices during milk handling. Therefore, improving hygiene during milk production, handling, and transportation, along with providing training for dairy producers, is essential to ensure the safety and quality of raw milk.

Keywords: Dairy farmers, Microbial quality, raw milk, susceptibility

1. INTRODUCTION

1.1. Background of the Study

Milk is a natural secretion produced by the mammary glands of lactating mammals. It is a highly nutritious substance that contains both macro- and micronutrients, including proteins, carbohydrates, vitamins, minerals, and various bioactive compounds that contribute to health and disease prevention (Merwan *et al.*, 2018). According to Ayalew (2017), cows account for approximately 95% of Ethiopia's total annual milk production. Globally, milk plays a significant role in supporting the growth of young mammals and in mitigating malnutrition. It is recognized as a complete diet due to its integral components; proteins, lactose, milk fat, vitamins, and minerals. Because these nutrients are highly digestible, milk is recommended as an essential part of the human diet (Preka *et al.*, 2016).

Because it contains the majority of nutrients in the proper mix, balanced food has a high biological value (Scholz *et al.*, 2020). Milk is the first and the only food for the offspring of mammals as is almost complete food. It is complex fluid that contains macro and micronutrients components include water, fat, protein, lactose, mineral substances, organic acids, and miscellaneous other compounds (Franca *et al.*, 2018). Overall milk and milk products have a wide range in providing potable, vacationing, nutritious, economical and well food for humans and animals.

Raw milk is an important vehicle for the transmission of milk borne pathogens to humans, can be easily contaminated during milking and handling of the dairy health problems. The most common pathogenic microbes that spoil milk include *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp.*, *Pseudomonas aeruginosa* and *Proteus spp.* There is no well-documented information about the microbial quality and handling practices of raw cow milk in the study area. Raw milk quality is very important for the quality milk and milk product made it therefore, quality of raw milk should be under control of dairy products with respect to food-borne diseases is a great concern around the world. Therefore, every milk delivery should be inspected with regard to certain quality parameters in the country. According to Terfa *et al.* (2015) the quality of milk is generally free from pathogenic bacteria, sediment and extraneous substances, and harmful toxic substances having good flavor, normal composition, and low bacterial counts.

It also protects shelf life of milk by reducing the number of non-pathogenic microorganisms. Users are known of the quality of milk they need and the problems with using contaminated milk (Ngasala, 2015). Microbiological point of assessment quality of milk is relevant issues. Thus, studies the composition and nature of the raw milk microbiota and its influence on the continuation and quality of the milk and milking up to its transport, storage, and transformation in dairy products and along the phases that allow its transformation (Addis, 2015).

In Ethiopia, around 97% of the annual milk production is accounted by the traditional milk production system. The consumption of raw milk and its products is common in Ethiopia (Gemechu *et al.*, 2015). Contaminated milk and its products have been reported to cause illnesses. This is the reason why milk testing and quality control is important. Although there are several milk venders, no sufficient reports were available on the quality of milk.

There is no well-documented information about the microbial quality and handling practices of raw cow milk in the study area. The study area is known for its intensive dairy farming; however, information about its production systems; specifically feeding practices, breed types, housing systems, husbandry methods, and milk quality handling is not well documented. Therefore, a cross sectional study was designed to fill this knowledge gap by assessing the dairy production systems and evaluating milk quality. Thus, the study was designed to conduct fundamental information on the microbial quality and assess handling practices of raw cow milk and producer's dairy farms in the study area supply their milk directly to consumers. The study particularly focuses on data collected from smallholder farmers and micro-enterprise dairy producers. The evaluation of milk quality includes an assessment of the microbial quality of raw milk.

1.2. Statement of the Problem

The evaluation of the microbial quality of raw cow milk needs special attention due to the problem posed by the presence of pathogenic microorganisms. In Ethiopia, dairying farming is mostly in uncontrolled way and distributed to the users in raw form. The use of raw milk is common in Ethiopia which is unsafe from consumer health point of view it may cause different diseases. Microbiological infections hazards have gained pivotal attention of the researchers to curb the fraudulent food adulteration (Paraffin *et al.*, 2019). The importance of the subject matter in everyday human life, this research conducted to screen various adulterants and determine their impact on the microbiological quality of

marketed dairy farming raw milk. Therefore, the produced and marketed milk under this ways has without any form of pasteurization or quality control measures. As a result, the Milk being marketed under this method has questionable and problematic quality. This area has been very limitation, besides; there are inadequate documents on the microbial quality and load of raw milk. The current study was done to check the microbial quality of raw cow milk and to conduct fill knowledge gaps.

1.3. Objectives of the Study

1.3.1. General objective

The general objective of this study was to evaluate the microbial quality of raw cow milk from dairy farmers and marketed in Kibate Administrative town; Silte Zone, Central Ethiopia Region.

1.3.2. Specific objectives

The specific objectives of this study were to:

- Evaluate the microbial load of raw cow milk from dairy farmers in the study area.
- Identify the possible risk factors for microbial contaminations of raw cow milk in the study area.
- Compare microbial contaminations of raw milk in the study area.
- Asses selected common pathogenic bacteria from dairy farmers and marketed in the study area.
- Investigate the antimicrobial susceptibility pattern of pathogens isolated from raw milk in the study area

1.4. Significance of the Study

This study plays an important role in improving the hygienic quality of milk production. It aims to ensure the provision of safe and wholesome food for consumers by strengthening control measures related to material handling and sanitation practices at all stages from production to consumption. Additionally, it seeks to enhance the overall quality of milk production and address existing knowledge gaps in the study area.

2. REVIEW OF RELATED LITRATURE

Raw milk contains essential nutrients in a balanced way such as lactose, fat, protein, minerals, and vitamins more than most other foods (Hamiroune *et al.*, 2016). This component is not constant, the average coverage of milk elements vary with individual and breeds of animal. Additionally, milk is high quality source of protein, vitamins, minerals such as calcium and phosphorus. Raw milk has a well soft and honey taste and carries hardly any smell (Hamiroune *et al.*, 2016). Initial of microbial infection in milk include microbial entering from the infected or sick milk lactating animal. The next causes of microbial contamination happens along the milk use chain, which may consists contamination when milking, milk handlers, unsanitary utensils and/or milking equipment transportation and raw milk is great mediators for the translation of milk borne infection to humans, as it can be easily contaminated during milking and handling (Addo *et al.*, 2011).

2.1. The Microbiological Quality of Milk

Microbial contamination in milk comes from milk itself because it will be naturally contaminated or comes from infected or sick animal, human, environment and equipment used for milking and storage of milk, animal product, faecal soiling of the udder and water used for cleanliness. The high bacterial count as well as the presence of pathogenic bacteria in milk not only degrades the milk quality and shelf-life of milk or milk related products but, Also poses a serious health threat to consumers. According to Minj and Behera (2012) milk being a wholesome food with high nutritive value is often prone to early contamination and spoilage if not handled properly. The rate of multiplication for microorganisms can be slowed down through cooling the milk and keeping it at the temperature of 4°C and below. The microbiological quality of milk is affected by storage temperatures and time taken in milk transporting. The longer the time taken to transport the milk, the more likely the milk is going to spoil. Quality is an important issue in production of hygienic products especially for safety of consumers in which both microbial and chemical properties of milks in normal state (Gandhi *et al.*, 2020) reported that, the rate of cooling and milk handling procedures during and after milking are also important in affecting the quality of milk. These state that quality of raw milk is poor if it has a coliform count of more than 50 000 cfu/ml and a total bacterial count of more than 2 million cfu/ ml.

In ensuring adherence to clean milk production are set standards of legal limits of bacteria in milk for each country. Jensen *et al.* (2010) proposed that, setting standards technological level and economic constraints of the region is taken into consideration. The European Union countries are the leading with the highest microbiology quality standard count level shows unhygienic standard of milk during milk production and milk storage or milk of a sick animal. The foundation of coliform bacteria particularly *E. coli* in raw milk is a sign of fecal contamination which indicate less hygienic situation and sanitized area since these bacteria are of fecal initial organism that is value as an indication of quality or hygienic status of a food, water, called indicator organisms.

According to Cancino *et al.* (2017) unique composition of raw milk and its properties make milk an excellent medium for the multiplication of various microorganisms. *S. aureus*, *Salmonella* spp. and *E. coli* are the main microbiological hazards associated with raw milk consumption.

2.1.1. Total aerobic mesophilic count

The leaved of a lot of mesophilic bacteria because of milk get on equipment body gives nutrients for growth of bacteria and poor milking area, poor quality of water used for cleanness, unsatisfactory hygiene/sanitation practiced at the farm level, and dirty hands of milker increase total bacteria load of raw milk. Total bacterial count (TBC) is a rough gauge to measure the quality of milk, herd health, efficacy of farm sanitation, milk handling and storage as well as transportation temperature (Melese and Addis, 2015). Because dividing them together are their different ways. All species are starting to grow in foods except at $\text{pH} \leq 4.0$ and water activity ≤ 0.92 ; they are sensitive to low heat treatments and are died by pasteurization. Some can survive at higher temperature (45.5°C). Coliform organisms infects milk from unwashed milkers hands, milking machines, improper preparation of the cow's flecks or dirt, improperly cleaned especially churns manure, hair dropping in to take advantage of when milking, udder washed with unclean water, dirty towels and udder not dried before milking sizable amount of coliform in dairy products indicates that the products are potentially food borne illness (Alganesh, 2016). The presumptive tests are designed to grow the target bacteria and also the media within the confirmed test are accustomed validate the expansion of target bacteria within the presumptive test.

2.1.2. Faecal coliforms

Mostly *E. coli*, along with some *Enterobacter* spp in food microbiology the term “faecal coliform” refer to coliform organisms which grow at 44 or 44.5 °C and ferment lactose to produce acid. Although contains of thermo tolerant coliforms all usually shows faecal contamination. Immediately infecting organism and is reliable indicator of generally in sanitary conditions of water, milk and other dairying products, transmitted to humans primarily through consumption of contaminated foods, such as raw or under cooked raw milk, meat products, and contaminated raw-milk (Alganesh, 2016) reported that, coliform count to determine widely greater than 95% of thermo tolerant coliforms identified from

Milk are the gut organism which is *E. coli*, the finder of this a singe of potential faecal infection of milk and water. The milk sold in raw forms and because of probability of contamination with *E. coli* poses a great hazard to public health.

2.1.3. *Salmonella* species

Facultative anaerobic, non-spore forming rods gram negative, *Salmonella* is the most commonly selected an etiological behaviors associated with new produce related infection. *Salmonella* is a greatly survive pathogen and it is well able to resistance outside the intestine, particularly at water practice between 0.43 and 0.52 (Majowicz, *et al.*, 2010). It passed on to humans when under cooked meats, eggs or milk is consumed. Salmonella may infect environment, and passed in the environment many time. Many time carried by animals like pigs or poultry or insects. Good source of *Salmonella* pathogen and exchanged to vectors like rats, flies and birds during it may shed in their faces for weeks and even months. After direct exchange, main reason for infection transferring animals such as swine's, cows and chickens as source. Eating and drinking contaminated food and water with *Salmonella* through animal reservoirs because human get infected (Newell *et al.*, 2010).

Although, *Salmonella Paratyphoid A* and *Salmonella typhoid* not have animal reservoir and contamination can be happened by feeding the inappropriately handled food by infected food handlers. Many strains of Salmonella can create food borne disease in humans, and the similar symptoms such as gastroenteritis (vomiting and diarrhea). Different bacteria may have arrival to milk from various sources and cause different types of milk-borne disease. Other microorganisms are cause illness to humans and others because spoilage in milk causes it unfavorable for human uses. To prevent consumers and marketers against these milk-borne infections it requires regular hygienic milking. Common bacteria indicated to be identified from milk include *S. aureus*, *Salmonella* spp.

2.2. Sources of Contamination of Milk

According to Serraino *et al.* (2019) reported that, raw milk can be contaminated either endogenously by microorganisms excreted with the milk, or exogenously by microorganisms introduced during or after milking. In the first case, microorganisms are excreted with the milk during or after milking either from the udder skin or from the environment feces, dust, equipment, humans. Chala *et al.* (2017) reported that, microbial contamination in milk may cause milk-borne diseases to humans, while others are known to cause milk spoilage.

Sources of microbial contamination in milk include primary microbial contamination from the infected or sick lactating animal. The secondary causes of microbial contamination occurs along the milk value chain which may include contamination during milking by milkers, milk handlers, unsanitary utensils and/or milking equipment's and water supplies used in sanitary activities (Gurmessa, 2015). Other secondary sources of microbial contamination occur during milk handling, transportation and storage of milk. Initial contamination include disease-causing organisms (pathogens) shedding in milk, infected udder and/or teats, animal skin, faecal soiling of the udder, contaminated milking and storage equipment and water used for cleanliness (Gwandu, 2018).

The major source of milk contamination occurs during milking, from the udder, milking equipment or milking personnel, during transportation and storage of the milk. Therefore, research reveals producers need to pay particular attention to the type as well as the hygienic of milk equipment Debela (2015) ensure hygienic milk handling practices on the household level. Bacterial sources are from air, milkers, handlers, drugs or chemicals used during treatment of animal and from water used for adulteration by unscrupulous and unfaithful workers/sellers who may be infected and may cause more health (Debela, 2015).

Cow: Milk from a healthy cow has a low bacterial count. A cow sheds microorganisms into milk when it is sick. Microorganisms include *Staphylococcus aureus* which is found in the infected udder of mastitis cows. Milk from a sick cow should not be mixed with the one from healthy cows and sick cows should be milked last to avoid contamination of the clean milk. Milkers: They can transmit these diseases through their hands, hair, clothing and aerosols from sneezing or coughing. Must wash their hands with detergent and dip them in disinfectant after milking each animal.

Milk differs in composition due to different factors like species of animal, variety, individuality, lactation's phase, and incidence of milking, age, feed, disease and drugs (Fahmid *et al.*, 2016). Term quality for milk means absence of harmful bacteria, dirt, antibodies, bad flavors, abnormal numbers somatic cell count, chemical analysis to check presence of sufficient amounts of nutrients, removal of fat and other adulterants, verification of hygiene through microbial investigation According to (Fahmid *et al.*, 2016) reviews that, this includes the skin of the animal, feeds, containers and water facilities. Then after, bedding materials must be kept dry and clean at all times. Milk containers used to store milk after milking are not thoroughly cleaned they harbor pathogens and spoilage microorganisms which will contaminate the milk and situation of storage.

2.3. Milk-Borne Infections and Pathogenic Microorganisms

Different bacteria may have got to milk and milk products from different environment and cause different types of milk-borne disease. Sometimes milk and milk products may carry microorganisms or their toxic metabolites toxins. Some of these microorganisms are cause spoilage in milk rendering it unsuitable (unsafe) for human consumption pathogenic and while others cause illness to humans.

Parekh and Subhash (2008) review that; many milk borne epidemics of human diseases are spread through consumption of contaminated milk. Therefore, proper milking, cleaning and sanitizing procedures of equipment's and environments are essential tool to ensure quality of milk. Many countries have implemented laws and regulations concerning the composition and hygienic quality of milk and milk products to protect both the consumers and the sellers (Pandey and Voskuil, 2011). Unfortunately, these laws and regulations are not often adhered in developing countries making milk-borne diseases a higher health risk to public and over 75% of milk marketed in many developing countries is sold raw/unpasteurized through informal channels (Murinda, 2011).

2.4. Hygiene, Handling and Microbial Quality of Raw Milk

Milk is a perishable product and an ideal medium for the growth of a wide variety of bacteria. A healthy udder, raw milk contains only a very few bacteria of about 500 to 1,000 bacteria per milliliter. That count level indicates a very poor hygienic standard of milk during milking and handling or milk of a diseased animal. In developing countries like Ethiopia most of the milk is produced by dairy farmers dominated by local herds of cattle and milking units are widely distributed throughout in rural areas with unhygienic

Infrastructure, while most of the markets and customers are in urban areas (Pandey and Voskuil, 2011). Swai and Schoonman, 2011 proposed that, milk contains a natural inhibitory system or temporary germicidal or bacteriostatic properties which prevent a significant rise in the bacteria count during the first 2 - 3 hours. If the milk original quality and remains safe for processing and consumption it cooled to 4°C within period immediately after milking. To prevent a too high multiplication of bacteria, the milk has to be produced as hygienic as possible and should be cooled or heated at the earliest.

2.5. Milk Hygienic-handling Practices of Producers and Consumers

Operational points likes to the hygienic putting of food and body contacts relating to out of body hygiene and leering. The good handling problems during marketing and producing include the chance that is infected either at the food source, during producing or during transportation to the consumers. Use of detergent and good-quality water for cleaning could be expected to remove microorganisms that affect the microbial quality of milk. As indicated that during milking and handling all the procedures should be kept under hygienic manner to reduce contamination of milk by microorganisms until reaching to processors or consumers. Proper hygienic procedures are of paramount importance to produce good quality milk. The microbial analysis of the milkers hand was exposing for some microorganism, including the some food pathogens, aerobic mesophilic plate counts (Alganesh, 2016).

2.6. Antimicrobial Residues, Bacterial Resistance

Antimicrobial residues occurs when a bacterium acquires the ability to survive exposure to antimicrobials that are intended to kill or stop it from growing, and it has been a global health issue that has put human and animal health at risk (Balamurugan *et al.*, 2018). They are need to improve feed efficiency, addition milk production or as growth promoters. Antibiotic use sometimes occur in response to several challenges include high level of stress, diseases, poor animal genetic potential, poor management, poor nutrition and drought. FAO (2016) reported that, use of antimicrobial agents and the transmission of antimicrobial resistant microbes between humans, animals and the environment are the key causes behind the incidence and spread of AMR.

In developing countries, health services for both humans and animals have been suboptimal, with an increased tendency for animal owners to stock drugs and treat their animals with unskilled people such as farmers and animal attendants. Has a number

of consequences, including a loss of effectiveness and therapeutic efficacy, as well as the treatment of infectious illnesses becoming less successful resulting in productivity losses, lower livelihoods and food security and higher mortality FAO (2016).

2.7. Prevention and Control of Microbial Contamination in Milk

Prevention and control of microbial quality of milk is through differentiating of organisms from human carriers by general improvements in water supplies, public health education, personal and environmental hygiene. Totally, microbial contamination in milk can be minimized through adherence to effective good hygienic practices at farm level; and in order to protect the public against milk-borne infections it is important to screen milk which is informally taken to the market. To prevent contamination of milk by air, outdoor milking should be done in dusty yards. Prevention can also be achieved by thoroughly cleaning and sanitizing milking utensils and/or equipment, controlling flies and insects to prevent the introduction of microorganisms into milk, and removing dung on a regular basis (Hyera, 2015). But, developing countries making milk-borne diseases a higher health risk to public because laws and regulations are not allays adhered. To avoid being a source of infectious diseases, it is also critical for all individuals involved in production of milk to be in healthy condition (Hyera, 2015).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted in Kibate Administration Town which is found in Silte Zone, and Central Ethiopia Regional state. Study period From December 1/2024 to February 1/2025. It is located at 148 km far from capital city of Ethiopia, annual temperature 12co to 26co, Rainfall range from 780mm to 18118mm and 96 km from the regional capital city and 25 km from the zone's town. It is located between 8" 00' and 60"00' N Latitude and 38 19"00' and 60"00' E Longitude (Municipality Office, 2007). 7 55 "N' 38"20' E

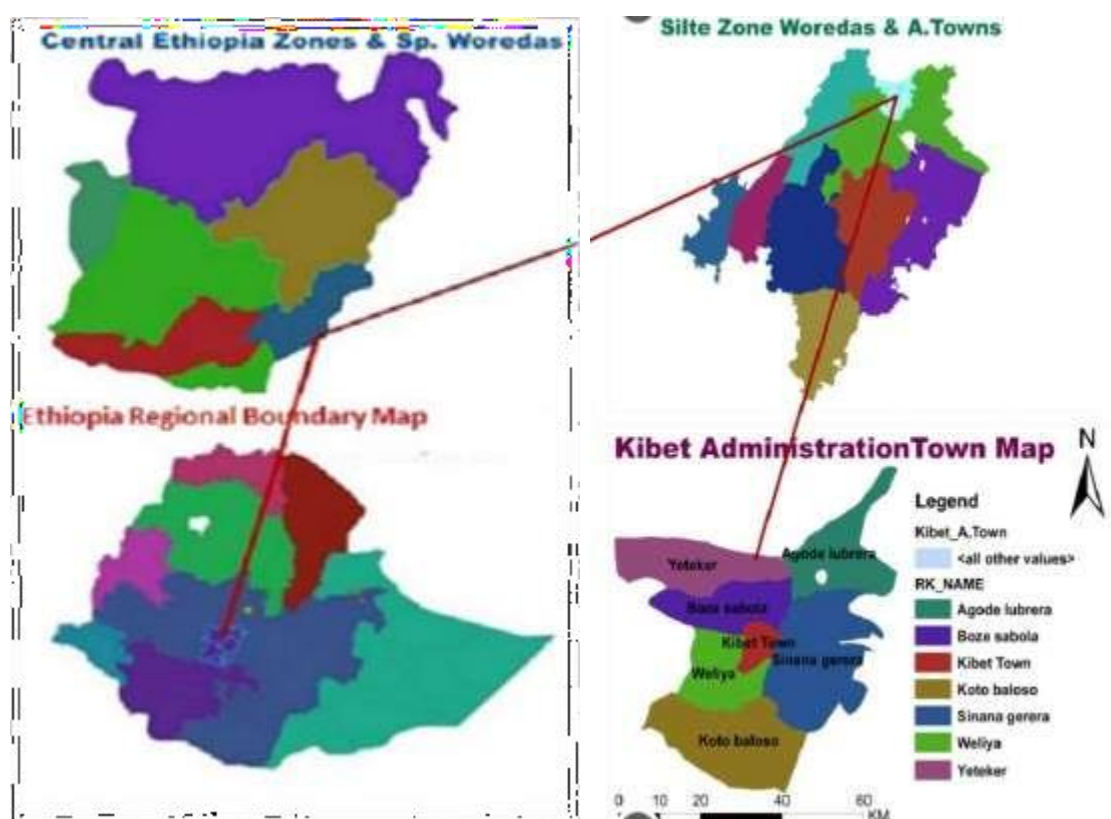


Figure 1: Map of Study Area

3.2. Study Design and Study Population

A cross-sectional study design was used to conduct this study from February to April, 2024. Both primary and secondary data were utilized for this study. Primary data were collected using semi-structured questionnaires administered through interviews with individuals involved in milk production and marketing. Secondary data were collected

From Kibate town agricultural enterprise offices Kibate Town consists of seven kebele of which three were selected based on their milk production potential. From each selected kebele, dairy farmers and marketed 35 milk samples were collected, giving a total of 105 samples ($3 \times 35 = 105$).

3.3. Sampling Techniques

Milk samples were collected from milk dairy farmers and marketed in a separate sterile container for microbiological analysis. All laboratory analyses were conducted in triplicate. Milk samples were purposely selected from each source and collected using simple random sampling technique. A total of 105 raw cow milk samples were collected from dairy farmers' milk containers at farm gate and milk marketing centers. Milk samples were collected aseptically using sterile bottles and immediately kept in an ice box and transported to Werabe University Microbiology Laboratory for analysis. The milk samples were kept in refrigerator at 4°C upon arrival.

3.4. Sample Size Determination

The sample size for this study was used to calculate at 95% confidence interval (CI) which was considered the point of the normal distribution corresponding to the level of significance ($Z=1.96$). Standard deviation (SD) was estimated at 0.15 or 15% and e , is the estimated error and was considered at 0.05 or 5%.

Therefore; the sample size „ n “ was calculated as:

Formula for unknown population (i.e. $n = Z^2 SD^2 / e^2$). Where:

Z = estimated standard variation at ($Z=1.96$).

SD = Standard deviation was estimated at 0.15 or 15% CI = confidence interval 95%

e = estimated error at 0.05 or 5%. Therefore; Sample size „ n “ calculated as:

$n = Z^2 SD^2 / e^2$, $n = (1.96)^2 \times (0.15)^2 = 34.5$ approximately $n = 35$ samples per each area and three selected area (35×3) =105 samples.

3.5. Method of Data Collection

Data were collected through face-to-face interviews with informal dairy farmers using structured questionnaires prepared in both English and the local language (Sltigna). Raw cow milk samples were obtained, and the questionnaires were administered simultaneously to dairy farmers with lactating cows, who served as the study units. In addition, direct

observations were made to assess participants' personal hygiene, the cleanliness of Containers and the general condition of the milking environment all of which were recorded using a checklist

3.6. Milk Sample Collection, Transportation and Sample Preparation

A total of 300 ml of raw milk samples was aseptically collected from dairy farmers and placed into sterile glass bottles. One milliliter raw milk sample was added into sterile test tube containing 9 ml peptone water. Duplicate appropriate decimal dilutions were surface plated and incubated. Milk sample handling all samples were coded with random numbers for identification and stored in a cool box with ice packs during field work. Diluted up to $1:10^{-6}$ and the duplicated samples (1 ml) was poured using 15-20 ml standard plate count agar and mixed thoroughly (Alganesh, 2016).

Sampling 9 glass bottles were prepared sterilized and disinfect with detergent. Study population a total of 105 milk samples was collected from different area of dairy farmers and marketed aseptically collected raw milk sample from container and placed into sterile glass bottles. Then, samples were labeled and put in ice box (4°C) to restrict microbial multiplication and transported as early as possible to the Werabe university, microbiology laboratory for microbial quality and safety of raw milk analysis.

A total of 10 sterile test tubes were dispensed with 9 ml of sterilized Buffered Peptone water (BPW). Using a sterile pipette 25 ml of the milk sample was transferred into a conical flask containing 25 ml of Buffered Peptone water and mixed well. Ten-fold serial dilution of the inoculums from 10^{-1} to 10^{-6} was done into sterile buffered peptone water solution using disposable sterile pipettes tips. The sample was serially dilute up to 10^{-6} . 0.1ml dilution of milk samples were spread plated on 15-20 ml of nutrient agar (Bekele *et al.*, 2020). The dilutions were mixed using a vortex mixer for 5 - 10 seconds. After 15 minutes drying the plates was invert and incubated at 30°C under aerobic condition for 72 hours to allow bacterial growth. Dilutions of 10^{-3} to 10^{-5} were the best for countable range of less than 300 colony forming units per plate (cfu/plate) (Bekele *et al.*, 2020). The dilutions were also used for detection and enumeration of Enterobacteriaceae and *S. aureus*, and the remained initial sample suspensions in the conical flasks were used in the initial preparation for isolation and presumptive identification of *Salmonella spp.*

3.6.1. Total aerobic mesophilic count

Total aerobic mesophilic bacterial counts were determined using sterile standard plate count agar. One ml of milk sample was diluted in 9ml sterile peptone water and serial dilutions were made in sterile peptone water diluents until the expected level of count of 30-300 was obtained. One ml of the milk sample from a chosen dilution was placed on the sterile plate. After sterilization all media were cooled to 45-47°C in a water bath before use. Peptone water was autoclaved at 121°C for 15 minutes and cooled to 30°C were used for serial dilution of the milk samples to determine. Then, plate count agar media (Oxoid, CM0325) of 15-20ml were poured and were mixed with the sample and allowed to solidify for 15 minutes. Plate Count Agar after incubating at 32 °C for 24-48hours aerobic conditions (Motato *et al.*, 2017).

3.6.2. Total coliform count

Violet red bile agar was sterilized by boiling for two minutes. One ml of milk samples was added into a sterile test tube having 9 ml distilled water. Containing 9 ml of 0.1% peptone water and thoroughly mixed using whirling mixed and serial decimal dilutions were prepared. Gently mixed sample were serially diluted up to 10^{-6} and duplicated samples (each with 1ml) were pour plated using sterile 15-20ml violet red bile agar and incubated at 45°C for 24 hours (Motato *et al.*, 2017).

3.6.3. *Enterobacteriaceae* count

Preparation of initial suspension and serial dilutions were prepare for initial suspensions: Inoculation, incubation and isolation two sterile petri dishes were labeled prior to inoculation (Graham *et al.*, 2017). By biochemical tests for five suspected colonies were selected at random from each plate and streaked onto Mac Conkey agar for differentiation of *Enterobacteriaceae*. The plates were inverted and incubated at 37°C for 24 hours selected and colonies were pink to red or purple streaked onto Nutrient agar (Graham *et al.*, 2017).

3.6.4. Yeast and molds count

yeast and mold count were performed using sterile potato dextrose agar. Samples of raw milk were diluted following similar methods as for aerobic plate count but dilutions were surface plated on potato dextrose agar at a pH of 5.6 ± 0.2 . The dried plates were incubated at 25°C for seven days. Colonies with a yellow-white and slightly dark color were counted as yeasts and molds. Other methods as for TBC, but dilutions were surface plated on Potato Dextrose Agar (PDA). The dried plates were incubated at 25 °C for 3 to 5 days. Colonies with a blue green color were counted as yeasts and mold (Bekele *et al.*, 2020).

3.7. Microbial Analysis

3.7.1. Cell morphology

The morphology of microbe isolates and types of bacteria on solid medium was examined based on their cultural characteristics (colony shapes, size, observation and recording results according its standard Bubble formation Mac Conkey agar media different color formation hydrogen peroxide bubble formation observation. Identification of gram positive and negative bacterial was isolation.

3.7.2. Catalase test

The catalase test was conducted by flooding colonies with a 3% solution of hydrogen peroxide (H_2O_2) on a clean microscopic slide. Formation of bubbles indicated a positive test for catalase production and negative for not producing bubble. Gram staining; crystal violet- iodine; 95% Ethanol safrani primary stain mordant decolorize and counter stain respectively used in each step rinse the slide with water observe on microscope different color like pink red color colonies.

3.7.3. Alcohol test and Oxidase Test

The alcohol test was used to diagnose the stability of milk by the presence of flocculation after addition of alcohol at 79%. If the test were positive (+): the milk was unfit for heat treatment and in the absence of flocculation the test was negative (-). Oxidase test was performed and well-isolated colonies were stickling and streaked onto the moistened oxidase detection strips using a sterile plastic loops, and then the strips were observed for colour change within 10 seconds. Appearances of deep blue or purple color were a confirmation for an oxidase positive reaction (Graham *et. al.*, 2017).

3.7.4. Glucose fermentation test

Using sterile loops, selected colonies which negative on Oxidase test were stickled and stabbed into tubes containing glucose agar and then, the tubes were incubated at 37°C for 24 hours were examined for colour change. If a yellow colour develops throughout the tube and sometimes with gas production, it was regarded as a positive reaction counting and calculating the number of *Enterobacteriaceae* colonies (Graham *et. al.*, 2017).

3.8. Detection of Pathogens

Detection of the desired microorganism was done and the respective bacterial colony on the media was stored and used for comparison (colonial morphology) against the test samples. First were analysis for microbial quality of raw milk which involved establishing the total bacterial counts and isolation of common milk-borne bacteria namely *Enterobacteriaceae* (specifically *Salmonella spp*, *S. aureus*).

3.8.1. *Salmonella spp*

For identification of *Salmonella spp*. was used as a pre-enrichment liquid medium and the initial suspensions were incubated at 37°C for 18 hours (Wallace *et al.*, 2020). Parallel with the test samples, positive and negative controls were prepared and used. For a positive control, a drop of *Salmonella* latex reagent was mixed with a smooth suspension of a known *Salmonella spp*. using a sterile loop. The suspected colony were emulsified in a drop of normal saline and then, a drop of *Salmonella* latex reagent was added to the saline suspension and mixed well with a clean sterile loop. After the suspension mixture was examined for agglutination within two minutes along with positive and negative controls, the presence of agglutination indicated positive reaction (Reta *et al.*, 2016).

3.8.2. *Escherichia coli*

Identification and isolation of *E. coli* were carried out according to the protocol of ISO-16654: 2001 standard. Milk was cultured primarily on Mac Conkey agar and incubated aerobically at 37°C for 24 hours. A single, isolated colony was be picked and sub-cultured on Eosin Methylene Blue (EMB) agar for formation of metallic sheen (Abbas *et al.*, 2013). *E. coli* count nine ml of raw cow milk was homogenized in a sterile sample container with saline solution. Decimal dilutions were prepared (10^{-1} to 10^{-6}). Each dilution was used to inoculate Mac Conkey agar using a spread plate method. Plates were incubated at 37°C for 24 hours. The plates were examined for colonies of *E. coli* Pink (Abbas *et al.*, 2013).

3.8.3. *Staphylococcus aureus*

One ml of milk sample was transferred to a test tube containing 9ml of normal peptone water. Serial dilution was repeatedly made until an appropriate dilution was obtained. Then the homogenate was wisely shaken 0.1 ml of the appropriate dilution were spread-plated on the surface of a dry Baird Parker Agar (Oxoid, CM0275) and on blood agar plate using a sterile bent glass rod and incubated at 37°C for 24 - 36hrs (Worku *et al.*, 2012 & Malek *et al.*, 2015). Using gram staining and biochemical tests such as methyl red test, catalase test, coagulase test, and oxidase test the *S. Aureus Count (Sac)* was analyzed. Two ml of milk samples were thoroughly diluted; mixed and white yellow colonies surrounded by clear haloes were considered as colonies belonging to *S. aureus* were counted (Malek *et al.*, 2015).

3.9. Antimicrobial Susceptibility Test of the Isolated Pathogens

The antimicrobial susceptibility test of common milk-borne bacterial pathogens; namely *S. aureus*, *E. coli*, and *Salmonella* spp. were conducted on Mueller Hinton agar (Ovoid CM0337 Basingstoke, England) as described by the Clinical and Laboratory Standards Institute (CLSI, 2015). The plates were inverted and incubated at 37°C for 24 hours. Antibiotics commonly used in human practices were selected for this purpose. The selected antibiotic discs were listed as followed with their concentrations; Tetracycline (TE: 30 µg), Gentamycin (CN: 10 µg), Ciprofloxacin (CIP: 5 µg), and Ampicillin (AMP: 10 µg). After drying the plates with inoculates for few minutes, antibiotic discs were placed over inoculated plates using disc dispenser and sterile forceps. The plates were inverted and incubated under aerobic conditions at 37°C for 24 hours. After incubation period, the

Plates were examined for zones of inhibition around the discs. Diameters of inhibition zones around the discs were measured in millimeter (mm) using a metal caliper, and were recorded and classified as resistant (R) and sensitive (S) (Quinn *et al.*, 2002).

3.10. Statistical Analysis

Quantitative questionnaire data were analyzed using a linear regression interpretation. Descriptive statistics; particularly frequencies, percentages, means and counts from multiple responses analysis were used to determine distribution of variables among the respondents. Relationship between different practices as risk factors for microbial contamination in raw milk were microbial count of total aerobic mesophilic bacterial, coliform, yeast and mold counts statistical significance was established at 95% confidence interval and critical p value of 0.05. The confidence intervals were used to compare proportions at 5% level of significance. Descriptive statistics were used to compute means, standard deviations and range. Analysis of variance (ANOVA) were adopted to compare differences in means of continuous variables by using SPSS software version 21

3.11. Ethical Consideration

A research permission letter to conduct this study was obtained from research and ethical committee of Werabe University. Before going to collect data communication made with the dairy farmers and marketed to accept the study to be conducted in his/her participation in the study on voluntary basis. Verbal consent was obtained from each of the selected heads of study area after explaining the purpose and importance of the study prior to interviews and sampling. Dairy farmers who were willingly agreed to participate in the study were sign the consent form which abides with the rules and regulations of research. The consent form was prepared by researcher and the information was collected from the participants.

4. RESULTS AND DISCUSSION

4.1. Results

4.1.1. Characteristics of the Respondents

A total of 105 dairy-farming households were interviewed. Among the respondents, the majority were single (41.9%) or married (32.4%). In terms of gender, most participants were male (68 respondents, 64.8%) (Table 1)

Table 1: Characteristics of the respondents in the study area

Variable	Category	Frequency	Percent %
Household information	Single	44	41.9%
	Spouse	34	32.4%
	Divorced	21	20.0%
	Windowed	6	5.7%
Genders of respondent	Male	68	64.8%
	Female	37	35.2%

Regarding educational attainment, 62 respondents (59.0%) had completed elementary school, 32 (30.5%) were unable to read and write, and 11 (10.5%) had reached secondary school level. Occupationally, 81% of the respondents were dairy farmers, while 19% were engaged in marketing. The level of education may directly influence milk production, quality, and safety, as well as play an important role in determining milk market standards and farmers' choices of marketing channels (Figure 2).

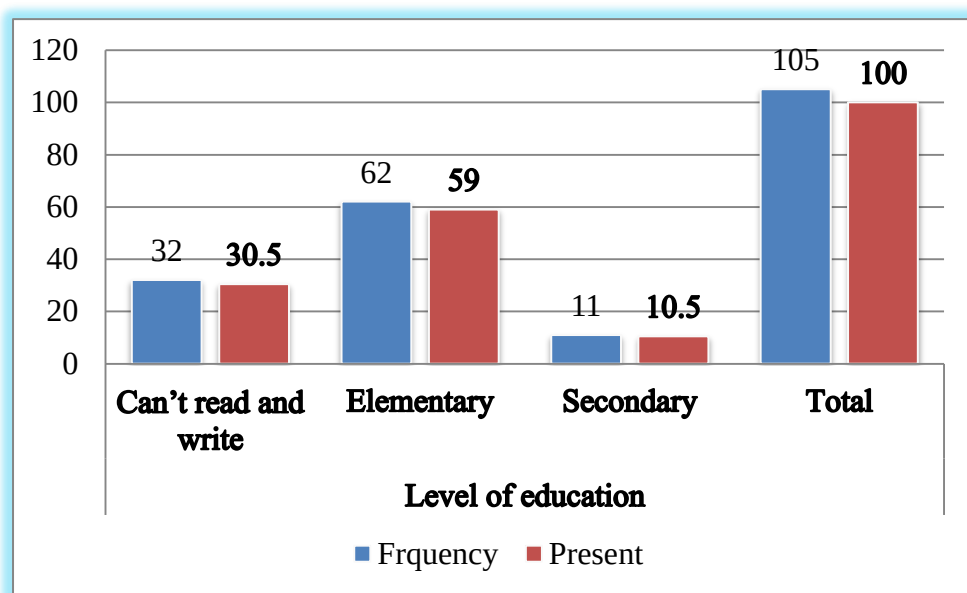


Figure 2: Respondents Levels of education

Regarding age respondent from 20-30 respondents (10.5.0%) from 30 - 40, 32 (34.5%) from 41- 50 (36%) and from 51- 60 (12%) (Figure: 3).

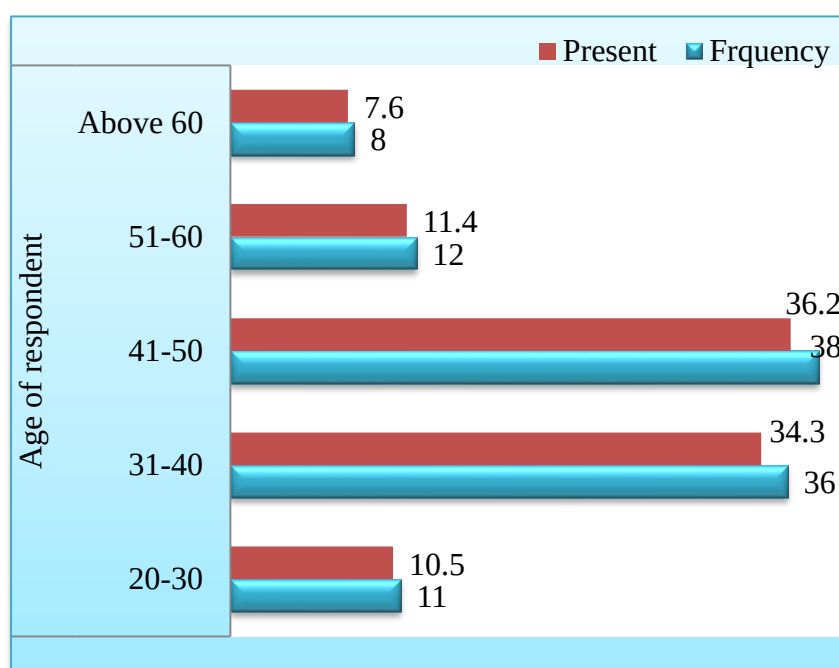


Figure 3: Age of respondents

4.1.2. Cattle housing, milk handling and personal hygienic information

The results obtained from respondents regarding cattle housing and hygiene practices revealed a significant difference among them ($P < 0.05$). The majority of households, 76 (72.4%), reported that they do not keep young and adult animals together, while 29 (27.6%) do. Regarding the type of housing used for cows, 35 households (33.3%) used

block houses, 27 (25%) used stone structures, 23 (21.9%) used wooden houses, and 20 (19%) used concrete houses (Table 3). The findings also indicated a significant variation ($P < 0.05$) among respondents concerning cleaning of animal sheds, washing cows' teats before milking, and other hygienic milk-handling practices on dairy farms. About 81% of respondents cleaned animal sheds regularly, 85.7% washed their hands before milking, while 14.3% did not. For cleaning milk utensils, 94.3% of respondents used tap water, 6% used local rivers, and 5.7% used other sources. Additionally, 84.8% of respondents had received training on producing clean milk, whereas 15.2% had not (Table 2).

Table 2: Cattle housing, milk handling, and personal hygienic information

Variable	Category	Frequency	Present
Floor materials	Concert/cement	20	19.0
	Block	35	33.3
	Wood	23	21.9
	Stone	27	25.7
Numbers of cows	<5	31	29.5
	>5 and <10	57	54.3
	>10	17	16.2
Feeding materials	Grazing	57	54.3
	Purchased fodder	16	15.2
	Grazing & fodder	32	30.5
With what do you clean before milking	Water only	85	81.0
	Water with soap	20	19.0
	Water with disinfect	0	0.0
Water source for clean	Tap water	99	94.3
	Local rivers	6	5.7
Which milking technique used	Hand milking	105	100.0
	Machine milking	0	0.0
Hand washing	Yes	90	85.7
	No	15	14.3

4.1.3. Milk Storage container types and transportation of dairy farm

45.7%) of respondents used plastic bucket, while the rest 13.3 % of them used aluminum-coated utensils for holding and 41% plastic and transporting milk (Table 4).The majority

(67%) private (22.9%) public (9.5%) motorcycle transportation to supply milk for marketing study areas. Majority of milk producers 48(45.7%) were using plastic containers, more near half of respondents 71(67.6%) transported with private (Table 3).

Table 3: Storage container practice and transportation of dairy farm

Variable	Category	Frequency	Present
Dry your animal before milking	Yes	46	43.8
	No	59	56.2
If your cow has an infection/ The udder?	Yes	68	64.8
	No	37	35.2
Milk obtained from cows with udder infection	Discard	98	93.3
	Used	7	6.7
Milking times your animal	Twice a day	99	94.3
	Three times	6	5.7
How do you store your milk	On fridge	7	6.7
	On floor	98	93.3
	Just milking	83	79.0
When do you sell your milk	6hourse	4	3.8
	12hourse	18	17.1
Train on how produce clean milk	Yes	89	84.8
	No	16	15.2
Containers to hold the milk	Plastic bucket	48	45.7
	Plastic	43	41.0
	Aluminum	14	13.3
How do containers are clean before using	hot water	33	31.4
	cold water	72	68.6
	Public	24	22.9
Transportation ways of	Private	71	67.6
	Motorcycle	10	9.5

4.1.4. Marketed milk handling, storage and status of personal hygiene practices

Raw milk was among the widely sold (71.4%). There is significance variation ($P < 0.05$) among the milk marketers. Majority of the milk marketers do not have their own

transportation the farmer deliver the milk to milk marketers which might be contributed to milk and dairy farmer's contamination. Plastic jar containers were materials more used (70.5%) by milk marketers for storage milk and wide necked aluminum vessel (23.8%) and wide necked plastic (5.7%) (Table 5) With regard to milk selling 64.8% were finish their milk within 3 hours of collection while 25.7% were within 6 hours after collection and within 9 hours after collection (9.6%). In line with hygienic practice of marketers before delivery of milk (46.7%) were clean milk utensil, cleaning after delivery of milk 32.3% and twice a day (21%) (Table 4)

Table 4: Marketed milk handling, storage and status of personal hygiene practices

Variables	Category	Frequency	Present
How do you get milk from the farmer	Farmers delvers milk	39	37.1
	Own transport	66	62.9
What type of container(s) do you use for storing milk	Wide necked aluminum	25	23.8
	Wide necked plastic	6	5.7
	Used plastic	74	70.5
What type of container(s) do you use for selling milk	Water bottle	22	21.0
	Steel cup	17	16.2
	Plastic cup	66	62.9
How long does it takes for the milk to be finished	After 3hourse collection	68	64.8
	After 6hourse collection	27	25.7
	After 9hourse collection	9	8.6
How is your cleaning routine for the milk containers	Cleaning before putting	49	46.7
	Cleaning after delivery	34	32.4
	Twice a day	22	21.0

4.1.5. Microbiological count of raw milk

Microbiological count of raw milk on study area was varied types in area and marketed. The mean microbial count for selected raw milk sample in Kibate town was shown Table 6. Total aerobic mesophilic bacterial counts $5.4 \pm 0.5 \log_{10}$ cfu/ml followed by total coliform count was $4.6 \pm 0.8 \log_{10}$ cfu/ml and total yeast and mold counts $4.1 \pm 0.3 \log_{10}$ cfu/ml (Table 6). The maximum aerobic mesophilic bacterial counts were founded in Boze milk sample $5.5 \pm 0.7 \log_{10}$ cfu/ml and minimum counts in weliya milk sample 5.3 ± 0.4 on the other hand there was no marked difference among milk samples collected from Kibate

01 and Boze kebele. The total aerobic mesophilic bacterial count obtained from Kibate 01 and Boze kebele were significantly higher than milk samples collected from Weliya kebele. All samples were significantly variation in microbial counts. The (C.V 12.72%) of Boze kebele > was significantly greater than both Kibate 01 and Weliya kebele. (C.V Kibate 01 and Weliya kebele milk sample were < 10%) with both study area 9.3% and 7.55% respectively (Table 6 and Figure 3). The count of coli form were significantly variations (C.V > 10%) with the sample of all study area. Kibate 01, Boze and Weliya kebele were 13.33%, 20% and 17.02%, respectively overall coli form (C.V 16.78%). But the count of total yeast and mould were significantly variation from other groups of microbial counts (C.V < 10%) with the sample of all study area. Kibate 01, Boze and Weliya kebele were 7.69%, 9.75% and 4.65%, respectively and from yeast and mould counts Boze kebele were greater variation of (C.V 9.75%) (Table 5 and Figure 4)

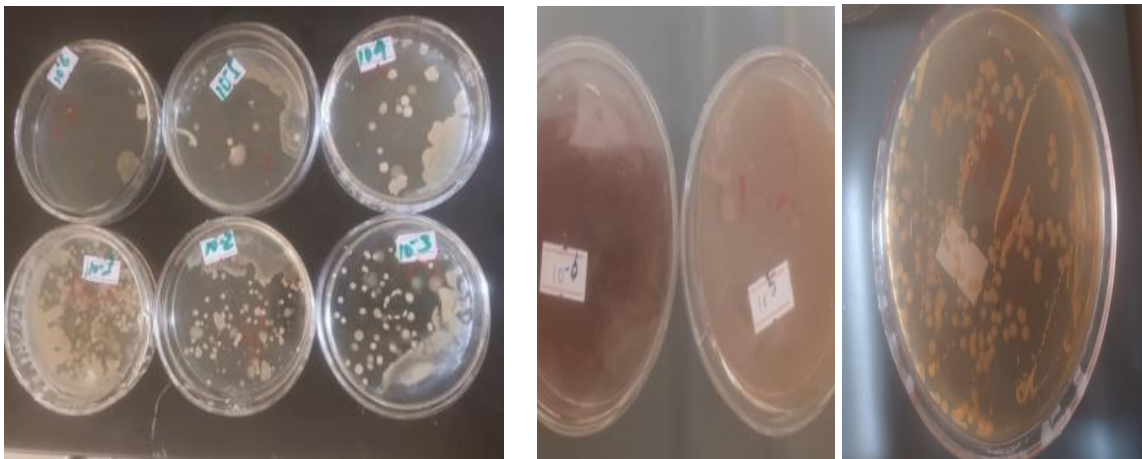


Figure 4: Microbial colony counting

Table 5: Mean microbiological counts (log10 cfu/ml) of selected raw milk samples

Microbi al group	Milk sample Source								
	Kibate		Welieya		Boze		Overall		ES limit
	Mean± SD	% CV	Mean± SD	%CV	Mean± SD	%CV	Mean± SD	CV	
TAMBC	5.4±0.5	9.3	5.3±0.4	7.55	5.5±0.7	12.72	5.4±0.5	9.86	>5.3
TCC	4.5±0.6	13.33	4.7±0.8	17.02	4.5±0.9	20	4.6±0.8	16.78	>3
TYMC	3.9±0.3	7.69	4.3±0.2	4.65	4.1±0.4	9.75	4.1±0.3	7.36	> 4

NOTE: *ES* Ethiopian Standard, *TAMBC*: Total aerobic mesophilic bacterial count, *TCC*: total coliform count, *TYMC*: Total yeast & mold count, *SD*: standard variation, *CV*: Coefficient variation, *cfu/ml*: colony forming unit per milliliter

4.1.6. Frequency distribution of various bacterial groups in milk at different sites

Aerobic mesophilic bacterial count of milk sample analyzed in this study was detected from range 1.9 - 6.9, 1.8 - 5.8 and 3.9-6.7 log₁₀ cfu/ml in Kibate 01, Boze and Weliya, respectively (Table 6). Most of *S. aureus* count range from < 2 – 5.99 log₁₀ cfu/ml, Coli form count were higher range > 3.9 log₁₀ cfu/ml in weliya sample but in Kibate 01 > 2log₁₀ cfu/ml yeast and mould more detected range 3.9-5.9log cfu/ml especially Kibate 01, and Weliya (Table 6).

Table 6: Frequency distribution different bacterial groups in study area

Microbial group	Sample site	Log cfu/ml				
		<2 (%)	2--3.9 (%)	3.9--4.9 (%)	4.9--5.9 (%)	5.9-6.9 %
Total aerobic mesophilic count	Kibate 01	1(2.9)	3(8.57)	11(31.42)	16(45.7)	4(11.42)
	Weliya	20(57.1)	8(22.9)	5(14.3)	2(5.8)	0(0.0)
	Boze	0(0.0)	4(11.42)	10(28.57)	18(51.42)	3(8.57)
Total coliform count	Kibate 01	18(51.4)	7(19.99)	7(20)	3(8.57)	0(0.0)
	Weliya	10(28.57)	7(20)	9(25.7)	6(17.14)	3(8.57)
	Boze	22(57.15)	8(23.1)	3(8.57)	4(11.42)	0(0.0)
Yeast and molds count	Kibate 01	0(0.0)	3(8.57)	15(42.86)	10(28.57)	7(20)
	Weliya	1(2.9)	6(17.14)	18(51.42)	9(25.7)	11(2.9)
	Boze	1(2.9)	14(40)	10(28.57)	7(20)	3(8.57)
<i>Staphylococcus</i> count	Kibate 01	23(65.7)	5(14)	6(17.14)	1(2.9)	0(0.0)
	Weliya	18(51)	4(11)	8(23)	5(14)	0(0.0)
	Boze	20(57.1)	5(14)	2(6)	9(25.7)	0(0.0)

4.1.7. Microbial analysis of raw milk

Gram staining primary stain crystal violet mordant – iodine; decolorize - 95% ethanol counter stain – safrani in each step rinse the slide with water observe on microscope different color like purple violet color colonies isolation morphology *S. aureus* clusters and round shape arranged test and characterization of *S. aureus* catalase and coagulase

positive and appear round form in grape-like clusters, methyl red test positive observation (Figure 5).



Figure 5: Gram staining isolation identification of gram positive bacterial

Eosin-methylene blue agar EMB: commonly used to detect the presence of fermenters lactose sterilize inoculating loop, isolation of single colonies from mixed cultures using the streak plate method Incubate the plates until the next lab Gram staining; crystal violet - iodine; 95% Ethanol safrani primary stain mordant decolorize and counter stain respectively used in each step rinse the slide with on microscope different colour like pink red colonies isolation coliform members *E. coli*. PDA fungal media preparation yellow, white and slightly dark colour formation colonies observation (Figure 6)

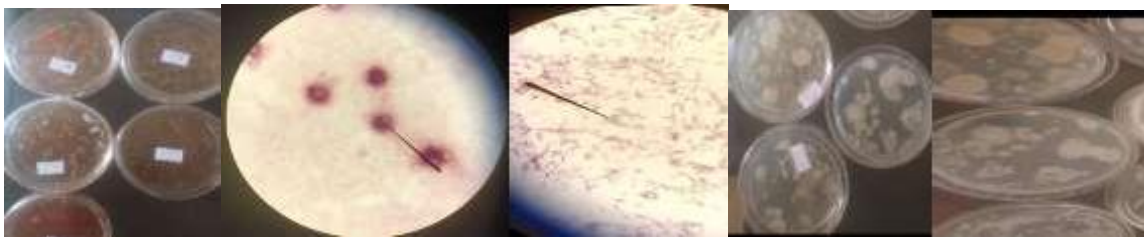


Figure 6: Isolation of gram negatives bacterial and yeast & mould

4.1.8. Frequency of isolation of the isolated pathogens

Among 105 raw milk sample analysed 38 (48.7%) sample were positive for *S. aureus* isolates frequency distribution based *S. aureus* highly prevalent in total aerobic mesophilic bacterial count and were positive for *E. coli* isolate 35 (44.8%) bacteria from contaminated milk were frequency distribution highly prevalent in total coliform count 30 (35.5%) and 5(5.3%) from total aerobic mesophilic bacterial count however *Salmonella* spp. was positive only 5 (6.4%) from total coliform count contaminated raw milk (Table 7).

Table 7: Proportion of bacterial isolated contaminated raw milk

Bacterial count	From TAMBC (94)		From TCC (85)	
	No	(%)	No	(%)
<i>E. coli</i>	5	5.3	30	35.3
<i>S. aureus</i>	22	23.4	16	18.8
<i>Salmonella</i> Spp	0	0	5	5.8
Total	27	28.7	51	60

Note: TAMBC: Total aerobic mesophilic bacterial Count, TCC: Total coliform Count

4.1.9. Antimicrobial susceptibility pattern of the isolated pathogens

A total of 78 bacterial isolates from milk culture samples that were confirmed positive for *S. aureus* (n=38), *Salmonella* spp. (n=5) and *E. coli* (n=35). From the table observed results showed that 84.6% bacteria isolates were resistant to ampicillin but there Were 98.7% sensitive to gentamycin, 34.6% bacteria isolates were resistant to Tetracycline and 100% sensitive to Ciprofloxacin.

Table 8: Drug sensitivity pattern of isolate bacterial

Antimicrobial	(µg)	<i>E. coli</i> (%)		<i>S. aureus</i> (%)		<i>Salmonella</i> (%)	
		S	R	S	R	S	R
Ampicillin	10	14.3	85.7	18.4	81.6	0	100
Ciprofloxacin	5	100	0	100	0	100	0
Tetracycline	30	57.1	42.9	81.6	18.4	0	100
Gentamicin	10	100	0	97.4	2.6	100	0

S= Sensitivity and R = Resistance

Ciprofloxacin and Gentamicin was the most effective drug against *E. coli*, *S. aureus* and *Salmonella* spp (100%). Tetracycline (57.1%) and Ampicillin (14.3%) was also partially effective drug against *E. coli* and (81.6%) Tetracycline and (18.4%) Ampicillin was effective drug against *S. aureus*. On the other hand *S. aureus* and *Salmonella* spp were (81.6%) and (100%) resistant to Ampicillin respectively and (42.9%) Tetracycline was resisted by *E. coli* (Table 9).

4.1.10. Multidrug resistance patterns of isolates

Among four Antimicrobial drug used in this study *Salmonella* showed multi drug resistant to Tetracycline and Ampicillin (60%) and *S. aureus* showed multi drug resistant to Ampicillin and Tetracycline (26.3%)

Table 8: Multiple drug resistance patterns isolated spp

<i>Isolate</i>	<i>Drug resisted Number</i>	<i>Drug resisted</i>	<i>Resistant bacteria</i>	<i>Percent</i>
<i>S. aureus</i> 38	2	(AMP) (TET)	10	26.3 %
<i>Salmonella</i> spp 5	2	(TET) (AMP)	3	60 %

Tetracycline= TET, Ampicillin = AMP

4.2. Discussion

Current study revealed the possible source of contaminated raw milk sample on dairy farmers and marketed. About 70.5% (n = 74) of marketers received milk from the farm in plastic containers. However, using narrow mouthed plastic containers can increase contamination levels as they are difficult to clean properly. Moreover, non-food-grade plastic containers present a challenge for cleaning and disinfect. The survey results showed that all the examined marketers' lack of a cooling system and poor handling practices during transportation could lead to increased microbial contaminants in the milk (Gemechu *et al.*, 2015). Moreover, there was no fridge as milk was being stored on floor (94.3%) while few respondents reported to store milk in refrigerator (6.7%) before selling or other home uses.

In addition, the current study found that cleaning agent used for cleaning the udder was water with no detergents. Most (68.6%) of the dairy cow owners only used cold water to clean milk equipment generally in sanitary conditions of water, milk and other dairying products, transmitted to humans primarily through consumption of contaminated foods. This result was in an agreement with the study in Tanzania (Gwandu *et al.*, 2018). Multiple liner analysis showed that milking without washing hands was nearly 2 times more likely to contaminate the milk and using unclean milk containers could contaminate raw milk nearly 7 times higher. This result was similar to studies carried out in Hawassa (Solomon *et al.*, 2014) and Yabelo (Gurmessa, 2015). It may be due to the fact that the presence of bacteria on the hands and milk container goes through the milk and causes contamination.

This study showed that the count of aerobic mesophilic bacteria range between $5.3 \pm 0.4 \log_{10}$ cfu/ml in Welieya to $5.5 \pm 0.7 \log_{10}$ cfu/ml in Boze kebele milk samples in this area the overall mean total aerobic mesophilic bacterial counts ($5.4 \pm 0.5 \log_{10}$ cfu/ml) were recorded. However, the maximum aerobic mesophilic bacterial counts ($5.5 \pm 0.7 \log_{10}$ cfu/ml) were observed in Boze kebele milk samples. This was not in an acceptable level ($> 5 \log_{10}$ cfu/ml). Contrary to our observation Mesfine *et al.* (2014) reported that the accepted microbiological limit for aerobic mesophilic bacterial counts in raw cow milk intended for human consumption is less than $5.00 \log_{10}$ cfu/ml. This value is nearly consistent with the European Union (EU) standard, which specifies that the total bacterial count of raw cow milk should be below $5.6 \log_{10}$ cfu/ml.

In agreement with the current study higher bacterial counts have been reported in other studies, such as 8.149 log₁₀ cfu/ml in Yabelo (Gurmessa, 2015) and 6.76 log₁₀ cfu/ml in Dire Dawa (Mesfine *et al.*, 2014). According to Garg (2016), poor hygienic milk-handling practices; such as unhygienic milking procedures, unclean udders, and unsuitable storage conditions contribute to high bacterial contamination levels. The study also found that 15.2% of respondents had not received any training, and there was a significant variation ($P < 0.05$) among farms, with 81% of respondents using only water to clean animal sheds and wash cows' teats before milking. These findings indicate poor sanitary conditions and inadequate hygiene practices during milking, collection, and handling of raw milk, as well as improper storage conditions (Garg, 2016).

Total coliform count was hygiene quality indicator of fecal contamination of milk from different source like during transportation, use of poorly cleaned milk containers and improper cleaning of the udder and milking containers. In this study the counts of coliform in all milk samples ranged from 2.0 log₁₀ cfu/ml in Kibate 01 to 6.4 log₁₀ cfu/ml in Weliya kebele milk. Over all mean of total coliform count was 4.6±0.8 log₁₀ cfu/ml. This result was lower than the findings reported by Gurmessa (2015) in Yabelo (6.3 ± 0.028 log₁₀ cfu/ml), by Tadele *et al.* (2016) (7.3 ± 0.07 log₁₀ cfu/ml), and by Ibrahim *et al.* (2015), who reported 5.16 log₁₀ cfu/ml in raw milk.

In contrast, the coliform count of the current study was greater than study reported by Wanjala *et al.* (2016) from Kenya (4.00 ± 0.66 log₁₀ cfu/ml), Tanzania (4.44 log₁₀ cfu/ml). Moreover, when compared with the recommended values of American public health standard which should be less than 100 cfu/ml for grade A milk and 101-200 cfu/ml for grade B milk the coliform counts in our milk samples were higher. This could be attributed to the hygienic status of milkers and the contamination of bacteria from milk containers, particularly during transportation and washing water for milking materials.

Most of milk samples (>94%) in the current study showed yeast and mold counts ranged from 2log₁₀ cfu/ml to 5log₁₀ cfu/ml. The overall mean of yeast and mold counts (YMC) were 4.1±0.3 log 10cfu/ml. It was significantly different between kebele. These were greater than study reported by Duguma (2020) and Sisay *et al.* (2012) in Bench Maji Zone, South-western Ethiopia who reported 3.902 ± 0.477 log₁₀ cfu/ml However, it was lower than the yeast (5.1 log₁₀ cfu/ml) and mold (3.7 log₁₀ cfu/ml) counts reported from Jimma (Alebel *et al.*, 2013). Mulugojjam & Adugna (2015) YMC found in barn dust, feeds, manure, and unclean utensils. The obtained YMC (3.9 - 4.9 log cfu/ml) in all samples were

Higher than the recommended maximum limit set by Teshome & Tesfaye (2016). This might be due to the contamination of cow milk from environment such as from air, unclean containers and poor personal hygiene of milk handler (Merwan *et al.*, 2018).

The presence of *E. coli*, *S. aureus* and *Salmonella* spp. in milk samples are dangerous to consumers. *S. aureus* was most frequently isolated (48.7%) followed by *E. coli* (44.8%) from milk samples. The load of *S. aureus* in milk samples in present study was greater than studies conducted in Tanzania (0.9%) by Emil (2015) and in Borana (7.29%) by Worku (2012). Also, it was greater than the results reported from Iran (41.66%) Fadaei (2014) and from Hawassa (35.2%) (Solomon, *et al.*, (2014). this high load of *S. aureus* revealed that poor milk handling practices during storage and transportation of milk to marketed sites.

Similarly,

Greater *E. coli* load (44.7%) was recorded than the study conducted in Hawassa (8.8%) by Solomon *et al.* (2014) and in Mekelle (17.6%) by Ahmedsham (2018). However, the result was lower than that study reported in Tanzania (90.67%) and Jigjiga, Ethiopia (58%) (Reta *et al.*, 2016). This could be attributed to on how to produce clean milk often clean containers or hadn't better handling practices in the study area during production and storage. However, we obtained less *Salmonella* spp count 5 (6.4 %) than studies reported from Tanzania, (37.4%) by Lubote (2014), Ethiopia (33 - 83%) by Mulugojjam *et al.* (2013) in milk samples collected from small, medium and large-scale dairy farms, and Borana (11.5%), by Worku (2012). These variations among the prevalence could be due to sample collection and the high proportion of those bacteria in milk related to the level of poor hygienic practices and respondents use only water for this purpose without any disinfectant.

The drug resistance patterns of all isolated pathogens viz., *E. coli* (85.7%), *S. aureus* (80.6%), and *Salmonella* spp. (100 %) showed that highly resistant to Ampicillin. These were comparable with the report of Abrha, and Marjan *et al.* (2014) and report from Egypt and Bangladesh by Sadek, and Marjan *et al.* (2014). Overall, prevalence of multidrug resistance was 25.6%. The result of the present study was less as compared to (55.2%) in Jigjiga reported by Reta *et al* (2016. Multidrug resistant of *S. aureus* were 39.5%. This result was greater than 34.8% result reported from Egypt by (Sadek *et al.* 2014). This implies that multidrug resistant common milk-borne pathogens are spread in the current study.

5. CONCLUSION & RECOMMENDATIONS

5.1. Conclusion

- Most of milk dairy farmers and marketers were used plastic buckets for milk collection and tap water for cleaning of milk containers and stored milk at room temperature before selling.
- The high poor quality of milk samples collected from marketers possibly because further contamination after initial contamination.
- Poor hygienic condition of the milking environment, sanitary condition of the milk containers indicate overall microbial count increased from dairy farm to markets.
- Washing hands of the milker, cleaning utensils with hot water and detergents is the advisable method of avoiding milk contamination as *E. coli* can easily be killed with high temperature and using hot water.
- Narrow necked plastic containers/glass bottles are not easily washed especially in the inner corner and this lead to sticking of residues.
- Most of *Staphylococcus aureus* 38 (48.7%), *E. coli* 35 (44.8%), and *Salmonella* spp. 5 (6.4 %) were resistant to ampicillin but 98.8% of them were sensitive to gentamycin and 20.5% were resistant to Tetracycline
- Lack of clean milk containers is significantly associated with milk contamination.

5.2. Recommendations

- Before milking, the udder should be washed and dried animal house should be cleaned.
- Clean the milk containers and hands properly before start milking.
- Dairy farmers should follow the set standards procedures for milking and proper milk storage.
- Good husbandry practices must be applied during milking and handling practices until it reaches the marketed area.
- The available laws and regulations need to be developed where they are not available to take care of possible sources of spoilage like replacement of plastic milk storage containers with the use of stainless steel cans for milk storage.
- Local regulatory organization controlling bodies should consider drug resistance during the treatment of patients with diseases mediated by milk.
- Without doubt understandable manuals, leaflet and posters should be prepared and distributed at large in local languages to make responsiveness on improved milk handling practices and the associated health benefits.
- Governmental bodies should regularly monitor the overall hygienic conditions of the milk production and conduct frequent inspections of milk produced and marketed in Kibate town to check whether or not compliance with the minimum legal standards.

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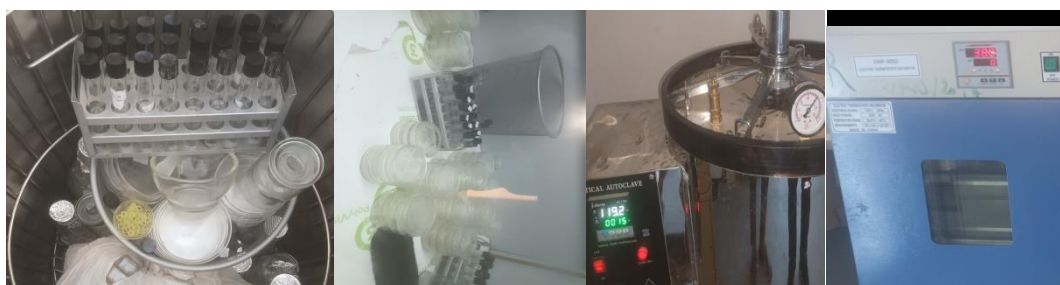
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APPENDIX



Material and equipment's used for laboratory



Dairy animals, housing floors and feeding materials



Milk containers both dairy farmers and marketed place



Sample collection and taken ice bag strile cup and put in cold chain box.



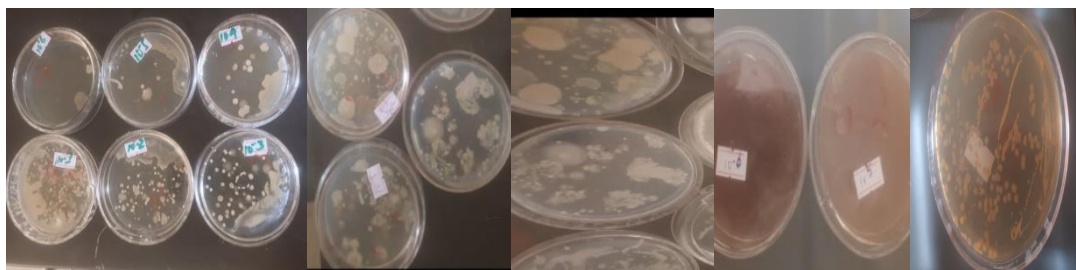
Milk Sample, media and peptone water on serial equipment's



Serial dilution, dispensing sterilization and pouring media



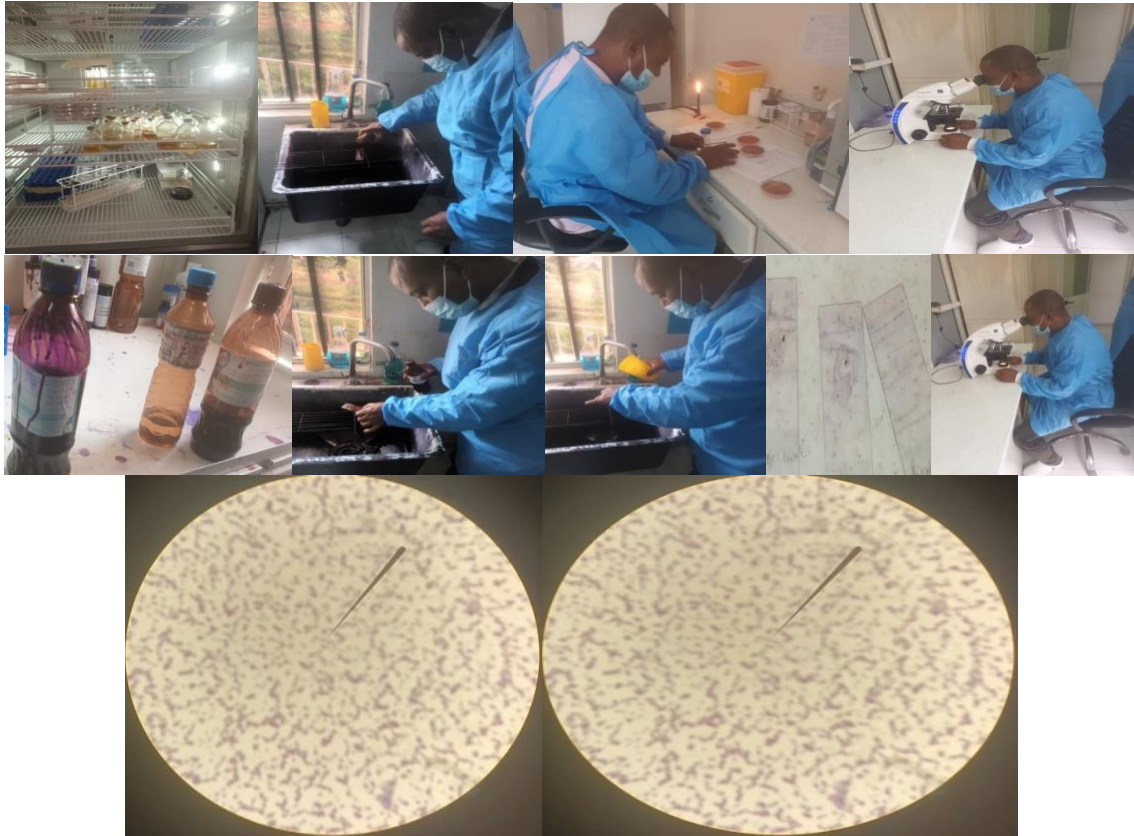
Colony counting and recording



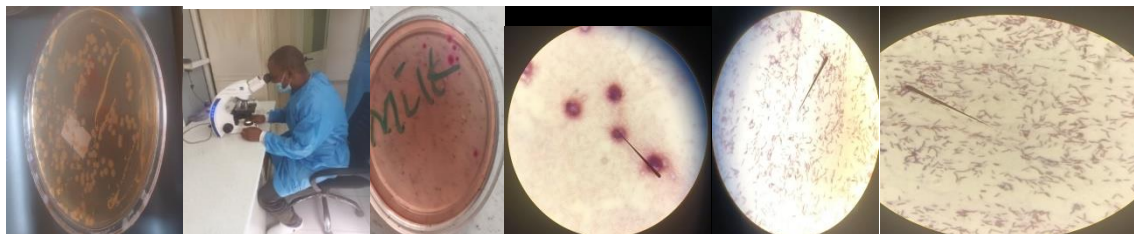
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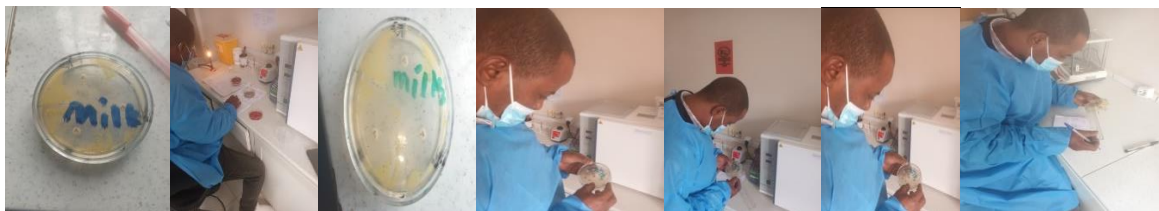
TCC



Gram staining and microscopically isolation identification of gram positive bacterial e.g. *S. aureus*



Gram staining and microscopically gram negative bacterial isolation of colour and shape e.g. *E. coli*



Antimicrobial susceptibility test of isolated bacteria/antibiotic test

ANOVA Table

		Sum of Squares	Df	Mean Square	F	Sig.
Yeast and Mould count * with what do you clean	Between Groups	5.700	1	5.700	1.064	.305
	Within Groups	551.846	104	5.358		
	Total	557.546	105			
<i>E. coli</i> Kibate * with what do you clean	Between Groups	.095	1	.095	.062	.804
	Within Groups	158.053	104	1.534		
	Total	158.148	105			
<i>Salmonella</i> Kibate 01* with what do you clean	Between Groups	10.571	1	10.571	15.263	.000
	Within Groups	71.336	104	.693		
	Total	81.907	105			
<i>Staphylococcus aureus</i> * with what do you clean	Between Groups	11.149	1	11.149	6.433	.013
	Within Groups	178.513	104	1.733		
	Total	189.662	105			
<i>Staphylococcus aureus</i> kibate01 kebele * how do you store your milk	Between Groups	9.296	1	9.296	3.763	.055
	Within Groups	254.450	104	2.470		
	Total	263.746	105			
Total coli form * how often do you clean	Between Groups	43.620	1	43.620	7.173	.009
	Within Groups	626.338	104	6.081		
	Total	669.958	105			
Yeast and Mould * Do you wash your hands	Between Groups	17.560	1	17.560	3.350	.070
	Within Groups	539.986	104	5.243		
	Total	557.546	105			

Descriptive Statistics for standard plat count

Model	Sum of Squares	Df	Mean Square	F	Sig.
Regression	2.835	1	2.835	6.482	.012 ^b
Residual	45.042	104	.437		
Total	47.877	105			

ANOVA Table

		Sum of Squares	df	Mean Square	F	Sig.
<i>Staphylococcus aureus</i> kibate01 kebele * how do you store milk	Between Groups	9.296	1	9.296	3.763	.055
	Within Groups	254.450	104	2.470		
	Total	263.746	105			
<i>E. coli</i> woliya kebele *how do you store milk	Between Groups	6.111	1	6.111	7.414	.008
	Within Groups	84.895	103	.824		
	Total	91.006	104			
<i>E. coli</i> boze kebele *how do you store milk	Between Groups	16.013	1	16.013	7.761	.006
	Within Groups	212.529	103	2.063		
	Total	228.543	104			
Salmonella boze kebele * how do you store milk	Between Groups	7.883	1	7.883	6.672	.011
	Within Groups	121.686	103	1.181		
	Total	129.569	104			
Total Aerobic mesophilic bacterial count how do you store	Between Groups	1.715	1	1.715	3.827	.053
	Within Groups	46.161	103	.448		
	Total	47.877	104			
Yeast and Mould count * Type of container storing milk	Between Groups	27.755	1	27.755	5.396	.022
	Within Groups	529.791	104	5.144		
	Total	557.546	105			
Coli form * level of education		6.129	2	3.064	5.416	.006
	Between Groups	3.973	1	3.973	7.023	.009
		2.156	1	2.156	3.810	.054
	Within Groups	57.707	104	.566		
	Total	63.836	105			

ANOVA^a

Model		Sum of Squares	Df	Mean Square	F	Sig.
1	Regression	83.770	30	2.792	2.393	.001 ^b
	Residual	86.335	75	1.167		
	Total	170.105	105			

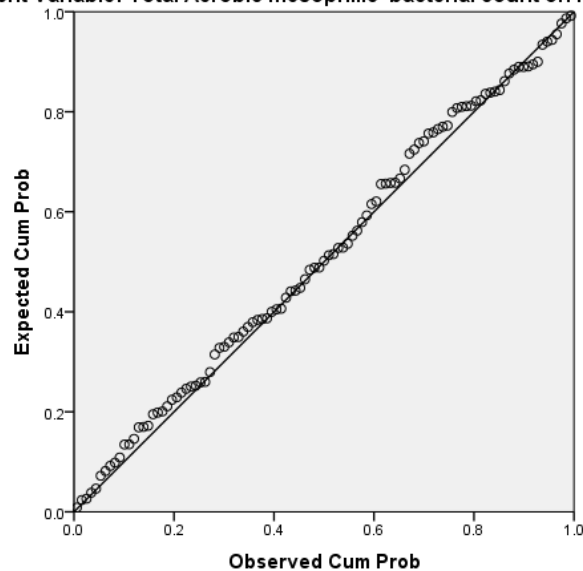
a. Dependent Variable: Staphylococcus

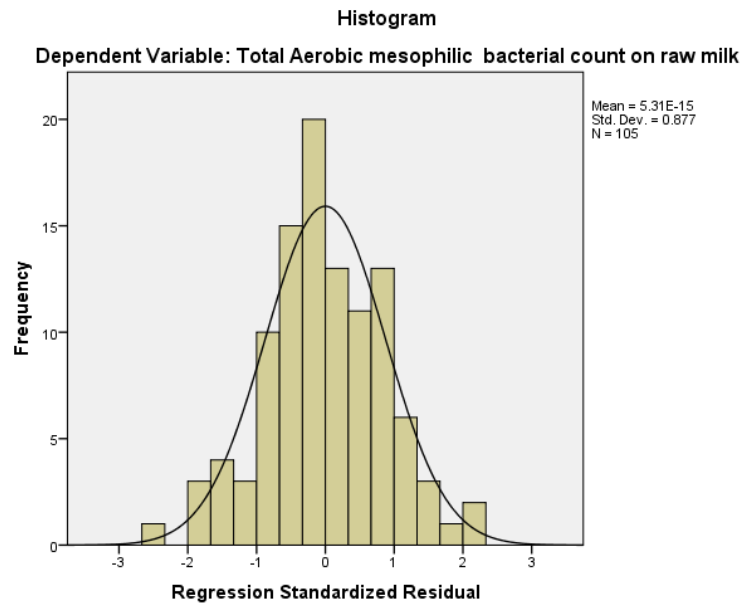
Model		Sum of Squares	Df	Mean Square	F	Sig.
1	Regression	2762.660	6	460.443	555.754	.000 ^c
	Residual	82.022	99	.829		
	Total	2844.682 ^d	105			

a. Dependent Variable: Total Aerobic mesophilic bacterial count on raw milk

Normal P-P Plot of Regression Standardized Residual

Dependent Variable: Total Aerobic mesophilic bacterial count on raw milk

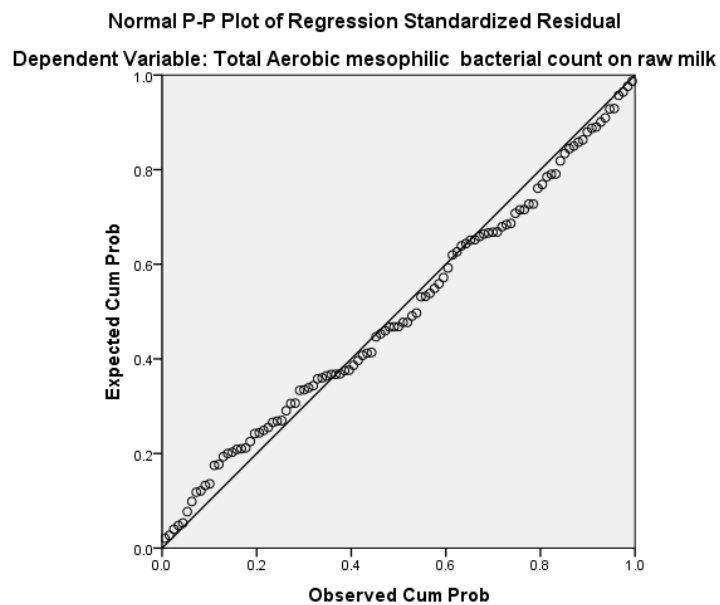
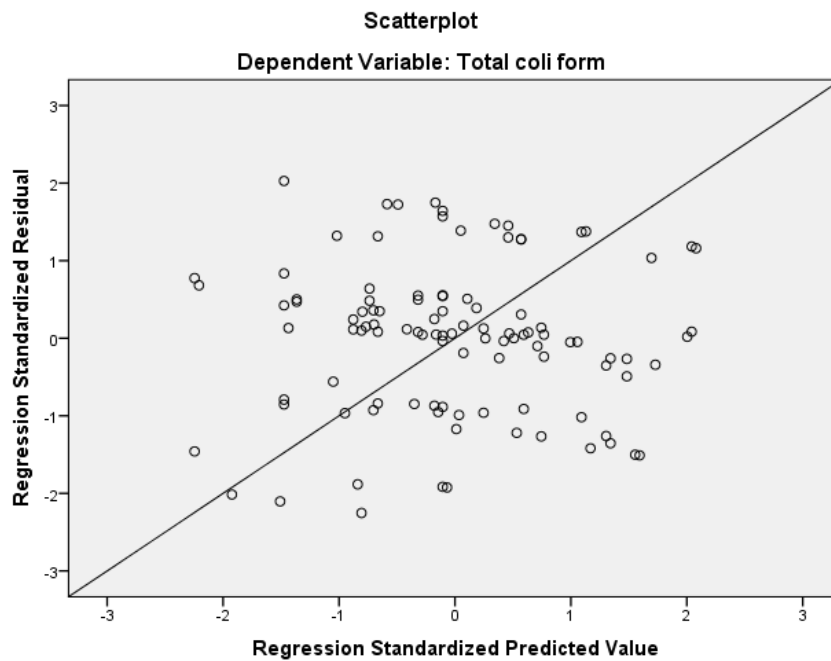




ANOVA^a

Model		Sum of Squares	Df	Mean Square	F	Sig.
1	Regression	83.770	30	2.792	2.393	.001 ^b
	Residual	86.335	74	1.167		
	Total	170.105	104			

a. Dependent Variable: Staphylococcus



Model Summary

Mod	R	R	Adjusted	Std. Error	Change Statistics
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el		Square	R Square	of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change
1	.649 ^a	.422	.229	2.22861	.422	2.188	26	78	.004

ANOVA

Model	Sum of Squares	Df	Mean Square	F	Sig.
Regression	282.554	26	10.867	2.188	.004 ^b
Residual	387.404	78	4.967		
Total	669.958	104			

a. Dependent Variable: Total coli form

ANOVA^a

Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	227.494	26	8.750	2.068	.008 ^b
Residual	330.052	78	4.231		
Total	557.546	104			

a. Dependent Variable: Yeast and Mould count of raw milk

Model Summary

R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
				R Square Change	F Change	df1	df2	Sig. F Change
.639 ^a	.408	.211	2.05705	.408	2.068	26	78	.008

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.692 ^a	.479	.305	.65317	.479	2.755	26	78	.000

ANOVA^a

Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	30.559	26	1.175	2.755	.000 ^b
Residual	33.277	78	.427		
Total	63.836	104			

a. Dependent Variable: Staphylococcus aureus

Antibiotic Susceptibility Pattern of *Escherichia coli* isolated from raw milk produced in Kibate town

Antimicrobial Agent	Disk content (µg/ml)	Resistance		Intermediate		Sensitive	
		Number	%	Number	%	Number	%
Ampicillin(AMP)	10	30	85.7	1	2.9	4	11.4
Ciprofloxacin(CIP)	5	0	0	0	0	35	100
Tetracycline(TET)	30	0	0	0	0	35	100
Gentamicin(GM)	10	0	0	0	0	35	100

Antibiotic Susceptibility Pattern of *Staphylococcus aureus* isolated from raw milk

Antimicrobial	Disk content	Resistance		Sensitive	
Agent	(µg/ml)t	Number	%	Number	%
Ampicillin(AMP)	10	31	81.6	7	18.4
Ciprofloxacin(CIP)	5	0	0	38	100
Tetracycline(TET)	30	7	18.4	31	81.6
Gentamicin(GM)	10	37	97.4	1	2.6

Salmonella spp

Antimicrobial	Disk content	Resistance		Intermediate		Sensitive	
Agent	(µg/ml)	Number	%	Number	%	Number	%
Ampicillin(AMP)	10	5	100	0	0	0	0
Ciprofloxacin(CIP)	5	0	0	0	0	5	100
Tetracycline(TET)	30	5	100	0	0	0	0
Gentamicin(GM)	10	0	0	0	0	5	100

Source: Werabe compressive specialized hospital result 2024

Drug	Concentration	Sensitive	Intermediate	Resistance
Tetracycline (TE	30 µg	>15	12-14	<11
Ciprofloxacin (CIP:	5 µg	>21	16-20	<15
Gentamycin (CN	10 µg	>15	13-14	<12
Ampicillin (AMP	10 µg	>17	14-16	<13

Variables	Response	Fry	%	Sig.	95.0% CI	
How do clean before milking	Water only	85	81.0	.043	-1.098	-.017
	Water & soap	20	19.0			
Water source for clean	Tap water	99	94.3	.024*	.107	1.479
	Local rivers	6	5.7			
Dry animal before milking	Yes	46	43.8	.035*	-.631	-.023
	No	59	56.2			
Hand washing	Yes	90	85.7	.024*	.107	1.479
	No	15	14.3			
How do you store your milk	On fridge	7	6.7	.001*	-.491	-.136
	On floor	98	93.3			
Containers clean before use	Hot water	33	31.4	.007*	.136	.813
	Cold water	72	68.6			
Containers to hold the milk	Plastic bucket	48	45.7	.001*	-.491	-.136
	Plastic	43	41.0			
	Aluminum	14	13.5			

Dairy Farmers and Marketed Questionnaire

1. Socio-demographic characteristic of the respondents

- 1) **Household information** Single ☐ Spouse ☐ Divorced ☐ Widowed ☐
- 2) **Educational level** illiterate ☐ High or secondary school ☐ Primary School ☐
Degree/college ☐

2. Cattle housing information and hygiene and practices

- 1) How many cows do you keep? _____ ≤ 5 ☐ > 5 ☐ >10 ☐ others
- 2) Do you keep young and old animals together? Yes ☐ No ☐
- 3) Floor material; Concrete or cement ☐ Block house ☐ Mud ☐ Wood ☐ Stones ☐
Plastic
- 4) How often do you clean? Once a day ☐ Twice a day ☐ Three time a day ☐ Twice
a week
- 5) With what do you clean? water only ☐ Water with soap ☐ Water with
disinfect
- 6) What is the water source for your cattle herd? a) Tap water b) Local Rivers c)
Flood water
- 7) Which milking technique do you use? a) Hand milking b) Machine milking

3. Feeding Source of fodder Grazing ☐ Purchased fodder ☐ Grazing + fodder ☐

4. Milk Handling, storage, status of personal hygiene and production practices

- 1) Do you wash your hands? A yes B no
- 2) Do you dry your animal before milking? A yes B no
- 3) Do you wash your hands? a) Water only b) Water with soap c) Water,
disinfectant and soap
- 4) Can you recognize if your cow has an infection/ a problem in the udder? a) Yes b)
No
- 5) What do you do with the milk obtained from cows with udder infection?
a. a) Discard b) Consume in the household c) Sale to the market d) Feed to
calves
- 6) How many times do you milk your animal per day? A. Twice B. Thrice
- 7) How do you store your milk? A keep the fridge B on the floor

- 8) When do you sell your milk? A. Soon after milking B. 12 hours after milking C. Others
- 9) Have you attended any course on how to produce clean milk? A yes B no
- 10) What type of containers do you use to keep the milk? A plastic bucket B plastic C aluminum cans
- 11) How do you ensure that containers are clean before using them? A. rinsing with hot water B. rinsing with cold water
- 12) How long does it take from point of collection to the selling point? A 1 hour B. 2 hours
- 13) What type of transport do you use to carry your milk to selling Centre? A. public transport B. private car C. Motorcycle

Marketers

1. What type of milk and milk products do you sell? Raw milk ☐ Raw + Fermented ☐
2. From where do you get your milk? From: More than 2 farmers in the same village ☐ A farmer from neighboring village ☐ More than 2 farmers in the neighboring village ☐
3. How do you get milk from the farmer(s)? a. Farmer(s) delivers the milk ☐ busing own transport ☐
4. What type of container(s) do you use for storing milk? (Observe if applicable)
5. a. Wide necked-aluminum vessels ☐ b. Wide necked-plastic vessels ☐ c. Used plastic water bottles ☐
6. What type of container(s) do you use for selling milk? (Observe if applicable)
- a. Glass bottle ☐ c. Used water bottles ☐ b. Steel cups ☐ d. Plastic cup ☐
7. How long does it takes for the milk to be finished? a. 3 hrs. After collection ☐ b. 6hrs after collection ☐
- c. 9 hrs after collection ☐ d. 12 hrs after collection ☐ e. other(s) specify ☐
8. How is your cleaning routine for the milk containers?
- a. Cleaning just before putting in milk ☐ b. After delivery of milk ☐ c. twice a day ☐ d. other(s)

