ASSESSMENT ON MILKING AND HANDLING PRACTICES, MICROBIAL QUALITY AND SAFETY OF RAW COW MILK IN KOLFEKERANIO AND LEMIKURA SUB-CITIES OF ADDIS ABABA, ETHIOPIA

M.Sc. THESIS

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JUN, 2023

WOLKITE UNIVERSITY, WOLKITE, ETHIOPIA

ASSESSMENT ON MILKING AND HANDLING PRACTICES, MICROBIAL QUALITY AND SAFETY OF RAW COW MILK IN KOLFEKERANIO AND LEMIKURA SUB-CITIES OF ADDIS ABABA, ETHIOPIA

A THESIS SUBMITTED TO THE DEPARTMENT OF ANIMAL SCIENCE

COLLEGE OF AGRICULTURE, SCHOOL OF GRADUATE STUDIES

WOLKITE UNIVERSITY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTERS OF EDUCATION IN ANIMAL SCIENCE

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DEDICATION

This thesis is dedicated to my beloved kids Zetseat and Eaftah Molalign.

STATEMENT OF AUTHOR

I declare that this thesis is my work and that all sources of materials used for this thesis have been properly acknowledged. This thesis is my original work under the supervision and guidance of Melesse Etifu (Asst.prof.) and Helen W/Michael (PhD) and has been submitted to Collage of Agriculture and Natural resource Department of Animal Science in partial fulfillment of the requirement of MSc. degree at Wolkite University. I truly declare that this thesis is not submitted to any other institution wherever for the award of any other academic degree, diploma, or certificate.

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BIOGRAPHICAL SKETCH

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ACKNOWLEDGEMENTS

Above all, I bow with gratefulness before Almighty God for having bestowed upon me his grace and blessing, giving me the strength to bring out this humble piece of work into the light. I would like to express my deepest gratitude to Melesse Etifu (Asst. Prof.), my major advisor and Dr. Helen Weldemichael, Co-advisor, for their guidance, technical advice, suggestions and constructive criticisms. I would like to express my highest gratitude and appreciation to the Federal ministry of Agriculture for providing me all the support, resources and facilities to do my research work. I am very grateful to Dr. Nardos Tefera from Animal product, Veterinary drug and Feed quality Assessment Center microbiology directorate director for his, encouragement and guidance that made it possible for this work to reach completion. It is my pleasure while I take chance to extend my gratitude towards Dr. Zerihun Beyene, Dr. Misrak Netsere, Dr. Fediko Tolosa and Miss Sinke Ararso who helped me in the laboratory work and for all the sub-cities and district livestock experts who helped me in survey work as well as milk sample collection. Thanks are also owing to all the sample dairy farmers in Lemikura and Kolfekeranio sub-cities for their thoughtful help in giving valuable information to this study. Words fall short to express my adoration to my beloved kids for their patience and endurance in making me free to devote my time to my study in the due course of the entire research work. The complete list of direct and indirect contributors is too long for illustration here and I can only ask the staff of Dairy at Ministry of Agricultural as a whole to accept my sincere thanks for their general support and for their valuable inputs during the course of the study. Last but not the least, My families; my mother, brothers and sisters were supporting me a lot in different ways hence it is my pleasure to say thank you.

LIST OF ABBREVIATIONS AND ACRONYMS

APC	Aerobic Plate Count
APVDF, QAC	Animal Product, Veterinary Drug and Feed Quality Assessment Center
AMR	Antimicrobial Resistance
AST	Antimicrobial Susceptibility Test
CSA	Central Statistics Agency
CC	Coliform count
CES	Compulsory Ethiopian Standard
Cfu/ml	Colony-forming unit per milliliter
ESA	Ethiopian Standard Agency
ES_ISO	Ethiopian Standard- International Organization for Standard
FAO	Food and Agricultural Organization
GN	Gram Negative
GP	Gram Positive
HACCP	Hazard Analysis and Critical Control Point
ILRI	International livestock Research Institute
LAIA	Latin American Integration Association
MSA	Mannitol Salt Agar
MoA	Ministry of Agriculture
MPN	Most Probable Number
PCA	Plate Count Agar
SCC	Somatic Cell Count
SNV	Netherland Development Organization
SOP	Standard operating Procedure
SPSS	Statistical Package for Social Science
STEC	Shiga toxin-producing Escherichia coli
TBC	Total Bacteria Count
XLD	Xylose Lysine Deoxycholate
YMC	Yeast and Mold Count
WHO	World Health Organization

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ABSTRACT

Raw cow milk serves as a good medium for microbial growth due to its complex biochemical composition and high water activity, Because of this character, the quality and shelf-life of milk is forced to be degraded. This study was conducted in Kolfekeranio and Lemikura sub-cities of Addis Ababa to evaluate milking practice, microbial quality and safety as well as antimicrobial susceptibility of bacteria in raw cow milk. Two districts were purposively selected from each sub-cities based on dairy potential. Small, medium and large-scale dairy farmers in the selected districts of study area owning dairy cattle for milk production were the targeted population. A total of 161 dairy producers were selected randomly based on proportion from each farm scale and interviewed individually using semi-structured questionnaire. For milk quality evaluation, 61 samples of raw cow milk were collected from producer's container and transported by ice box to the laboratory. The collected data were analyzed using SPSS software version 25.0. The result showed that the milking method practiced in the study area is hand milking. All the respondents wash their hands before milking but among them 27.3% of them washes with water only and 72.7% of them wash their hand with soap and water. The majority of the respondents use plastic materials for milking (83.9%), and milk storage (68.3%) respectively. The overall mean (±SD) of raw cow's milk for standard plate count (SPC), E. coli count (EC), Coliform Count (CC) and Total Yeast and Mold Count (TYMC) were 6.24±1.43, 2.44±0.56, 4.34 ±1.27 and 2.87±1.02 log10cfu/ml respectively. Salmonella was not detected in the collected milk sample and the prevalence of Staphylococcus aureus was 37%. The isolated S. aureus bacteria were 100% susceptible for antibiotic drugs like Amikacin, Gentamicin, Kanamycin, Erythromycin, Tylosin, Clindamycin and Florenicol. On the other hand S. aureus was 87% susceptible and 13% intermediate for Enfrofloxacin, 78.3% susceptible and 21.7% resistance for Tilcimosin, 60.9% susceptible and 39.1% resistance for Tetracycline, 95.7% susceptible and 4.3% intermediate for Trimethoprim-Sulfamethoxazole. Based on the result of the current study, milk samples contained higher microbial load than National and International standards and contains pathogenic bacteria which will result in public health risk to the consumer. All concerned bodies in dairy industry should get public education about hygienic milk production to reduce the risk of milk borne pathogens and losses due to rejection resulting from contamination.

Key words: *Antimicrobial susceptibility, Milk microbial quality, Milking Practice, Raw milk, Safety*

1. INTRODUCTION

Milk is a liquid secreted by the mammary glands of female mammals to feed their young for a time starting immediately after birth. The milk of domesticated animals is also an important source of food for humans, either processed into several dairy products or as a fresh fluid (Britannica, 2021). Milk has been defined as “the most perfect food” nutritionally. It is also an important part of daily food especially for the excited mothers and growing children (Solomon *et al.,* 2013).

In the next ten years world milk production is estimated to increase at 1.7% per year faster than most other major agricultural products (FAO, 2019). World per capita consumption of fresh dairy products is expected to increase by 1% annually in the coming years, somewhat faster than over the past ten years which is led by greater per-capita income progress (FAO, 2019).

Urban dairy production constituted an important sub-sector of the livestock production system in Ethiopia. Where consumption of milk and milk products is remarkably high this system is contributing hugely towards filling in the large demand-supply gap for milk and milk products in urban centers (As cited by Azage and Alemu, 1998 in Bruktawit 2016). To increase the supply of milk from smallholder farms both at rural and urban areas the government of Ethiopia has developed favorable policy environment on the development of dairying (Belay and Geert, 2015). At an average of 1.48 liter milk per cow per day over lactation period of 210 days approximately 7.1 billion liters of milk is produced in Ethiopia (CSA, 2020/21).

Poor herd management, mastitis, infectious pathogens in infected cow, poor hygiene milking practices and the occurrence of environmental pathogens by poor animal hygiene may be the expected sources of milk contamination. Microbial milk contamination, pathogens dissemination, and udder contamination may be occurred by poor hygiene practices and could occurs at milking time between cows, hands of milker man and milking machine from

others. An important factor to reduce contamination from production environment is good hygienic practices in the herd (Velázquez *et al.*, 2018).

Microorganisms in milk can be pathogenic and cause spoilage whereas some may play a double role. Pathogenic organisms are those which able to bring food poisoning, thus self-importance a risk to public health. Since the early days of dairy industry these pathogenic microbial contaminants in milk have been a key factor for public health concern. A constant challenge to those involved in milk production is that how to prevent or minimize the entry and subsequent growth of microorganisms in milk (Logan, 2012). The importance of producing milk of good hygienic quality is to provide a safe and wholesome food for the consumers. Milk from a healthy udder contains very few numbers of bacteria (4.47log10 cfu/ml) but during milking and milk handling it may become contaminated by microorganisms from the surrounding environment (Robinson, 2002). Dairy products quality defects have been recognized to poor microbiological quality of raw milk. The production of high-quality milk should be priority for good quality end products of long shelf life and for marketing of value-added products. However, in developing countries like Ethiopia this is very complicated to attain because of poor hygiene and cleanliness during milking and subsequent milk handling, contaminated water sources for cleaning milk utensils, lack of cooling facilities, high ambient temperature and insufficient infrastructures for milk transportation to the market (Alganesh, 2016).

1.1. Statement of the Problem

Dairy production is used as an initiative and economically feasible and greatly contributes to poverty reduction, food security, increased family nutrition and income and job opportunity creation (Niraji, 2014). Particularly in Ethiopia, dairy production used as both driving economic growth and profiting from it. However, due to a complex biochemical composition and high water activity raw cow milk serves as a good medium for microbial growth that degrades the milk quality and shelf-life (Tadele *et al*., 2016).

In developing countries like Ethiopia the risk of milk contamination with spoilage and pathogenic microorganisms is high for milk produced. The availability of documented information on the microbiological quality of raw milk across the milk supply chain is

critically important to protect from spoilage loss and consumers from milk born public health risk, these information may be essential for different organization to take on relevant development intervention on hygienic practices important for safe milk production and handling (Tadele *et al*., 2016).

Different scholars in the recent five years tried to assess the microbial quality of milk collected from different study areas such as: in Eastern Wellega zone (Adugna and Eshetu, 2021), Western Amhara region (Bekele *et al*., 2015), West Shewa zone of Oromia (Abdissa *et al*., 2020), Guraga zone (Kibebew *et al*., 2020) and in the selected sub city of Addis Ababa, Ethiopia (Abunna *et al*., 2019). Those studies focused on analysis of total bacteria count, total coliform count, and total yeast and mold counts. However, to design and implementation of safe milk production and handling practice in Ethiopia both bacteriological quality test of milk and presence and absence of major pathogens such as *Staphylococcus aurous* and *Salmonella Spp*., need to be included. Kolfekeranio and Lemikura sub-cities have a good dairy potential and more than 6.9 million liter of milk is produced annually but no research is conducted specifically in the area which is related to milk quality and safety. According to WHO (2014) report multi-drug-resistant bacterial strains cause life-threatening infections. Thus, to reduce the risk of public health as well as to improve the livelihood of dairy farmers, it is important to identify existing hygiene related problems and microbial quality, engaging the producers in quality milk production and handling of milk as well as detail investigation of sanitary condition.

To solve the above-mentioned problems, the scope of this study was rely on: assessing the milking and milk handling practices of farmers in Kolfekeranio and the newly established sub city Lemikura of Addis Ababa; testing microbial quality and major pathogens; and investigating the antimicrobial resistance of bacteria isolated from the raw milk by using a fully automated identification system.

1.2. Objective

1.2.1. General Objective

- To assess milking and milk handling practices, microbial quality, safety and antimicrobial susceptibility of bacteria in raw cow milk produced by dairy farmers in Lemikura and Kolfekeranio Sub-cities of Addis Ababa, Ethiopia.

1.2.2. Specific Objectives

1. To assess milking and raw milk handling practices of dairy farmers in Kolfekeranio and Lemikura sub-cities of Addis Ababa.
2. To examine the degree of microbial quality of the milk produced by dairy farmers living in Kolfekeranio and Lemikura sub-cities by using different growth media.
3. To identify potential food borne pathogens Salmonella and Staphylococcus aureus in raw cow milk produced by dairy farmers in Kolfekeranio and Lemikura sub-cities.
4. To investigate the antimicrobial resistance of bacteria isolated from raw milk by using a fully automated identification system (VITEK 2XL).

2. LITERATURE REVIEW

2.1. Peri- Urban and Urban Dairy Production Systems in Ethiopia

2.1.1. Peri-urban Dairy Production Systems

The peri-urban dairy cattle production systems have relatively well access to urban centers in which dairy cattle products are exceedingly required and are mainly located at the edge of the town areas (Yitay *et al*., 2007). Peri-urban dairy farms are growing nearby cities and towns because of gradually increasing demand in milk consumption. (Ulfina *et al*., 2013). Most of the dairy cattle producers be determined by on hybrid cows and they experienced supplementary concentrate feeding (Gebresellasie, 2019). It contributes only 2% of the total milk production of milk in Ethiopia and has animal types ranging from 50% crosses to high grade Friesian in small to large sized farms. Peri-urban dairy cattle production systems as allied to the rural dairy cattle production system, is typically found along roads within reasonable distance to urban centers and keepers are involved in fluid milk market (Nigatu *et al*., 2012).

2.1.2. Urban Dairy Production System

They are mainly concentrated in major cities of the country consists of dairy farms ranging from smallholder to specialized businessmen owned farms. There is no access for these dairy farms have to grazing lands. The main feed resources which are used in urban dairy cattle production system are concentrates, roughages and non-conventional feeds. They practice more intensive systems and have also access to animal health services (Yitay *et al.,* 2007). The urban dairy cattle production systems in most towns of Ethiopia are trained with little or no land resources for the production and sale of milk. It is the best market oriented dairy cattle production system correlated to other production systems. Under the use of intensive management system as compared to other dairy cattle production systems, urban dairy cattle production systems has better access to inputs and services providing by the public and private sectors (Gebresellasie, 2019).

Basic elements for developing applied, organized and viable breeding programs and categorizing of husbandry practices in low-input low-out put dairy production systems is

characterizing the existing of dairy production systems. Production systems of dairy farms were categorized into three major dairy production systems (Amare *et al.*, 2019). Large scale (>30 dairy cows), medium scale (>5≤30 dairy cows) and small scale (≤5 dairy cows).

2.2. Factors Affecting Milk Quality and Safety

Milk is basically sterile when secreted into the alveoli of the udder and is synthesized in dedicated cells of the mammary gland. Microbial contamination can generally occur from three main sources elsewhere this stage of milk production (Menane *et al*., 2007). These main sources are from inside the udder, from the outside of the udder and from the surface of milk handling and storage equipment. In influencing the level of contamination of raw milk, the health and hygiene of the cow, the environment in which the cow housing and milking is applied and the procedures used in cleaning and sanitizing the milk and storage equipment are all basic (Murphy *et al*., 2010). The temperature and length of storage, which allow microbial contaminants to reproduce and increase in numbers will influence standard plate count (SPC) (Murphy *et al*., 2010).

2.2.1. Dairy Cattle Health

Dairy cow health has sound public health implications in relation to its effect on milk quality and its relationship with cattle productivity. Among the disease, bovine tuberculosis and bovine brucellosis are the main one that can be tight through consumption of raw milk produced by infected animal (Acha and Szyfers, 2011). Therefore, the main indicator of the safety as well as the quality of milk produced is the health status of dairy herd. The health status of the lactating cow directly influences in general bacteriological quality of milk and particularly increases level of infection of udder (Alehegn, 2004).

Tuberculosis: is caused by bacteria called mycobacterium and an infectious, granulomatous disease of animals and man. The disease is spread by contact with infected domestic and wild animals and is contagious. Inhaling infected droplets which are expelled from the lungs by coughing is the usual route of infection. By ingesting raw milk from infected cows, calves and humans can also become infected. Taking months or years to kill an infected animal because the course of disease is slow and before it begins to manifest clinical signs, an animal can spread the disease to many other herd mates. So, the foremost ways of spreading the disease

are interaction with diseased wild animals and movement of undetected infected domestic animals (SNV, 2017).

The other major health problem is Mastitis**.** It is an inflammation of the udder/mammary gland almost always because of infection by bacterial pathogens. In the case of mastitis inflammation caused by bacteria damage the udder tissue. The Mastitis cause bacteria can live all over the place in the dairy farms like on the stable floor, in dung, on the skin of cattle and on the milker's hands. Environments with adequately of food and water and warm, wet and dirty are favorable conditions for multiplication and survival of these mastitis causing microorganisms (SNV, 2017). Mastitis is caused by microbes mostly bacteria that enter to the udder through the teat canal and it is of significant concern to the global dairy industry and is the most common disease of dairy cattle. Mastitis is prevalent disease, and it contributes for significant economic losses by which farms suffer (Asha and Heather, 2021). Prevalence of subclinical mastitis in Ethiopia and other African regions may impose significant costs due to indirect losses. In Ethiopia most dairy farmers normally do not recognize subclinical mastitis which incidentally occurs at a much higher frequency than clinical mastitis, while quite few ignore the disease. Subclinical mastitis is challenging to diagnose, persists longer in the herd, and is associated with higher losses compared to clinical mastitis (Ataro, 2020, Christine *et al.*, 2020).

2.2.2. Milking and Milk Handling Practices

The hygienic procedures followed during milking, storage and handover of milk into another container and dirtiness of milking and storage equipment are basic determining factor of milk quality (Melesse and Mustefa, 2019). The production of good quality milk is determined by the sanitary condition of the milking. One of the most important hygienic practices required to ensure clean milk production is cleaning the udder of cows before milking (Zelalem, 2010). Another important source of milk contamination can be the milker himself. Keeping good personal hygiene and assuring be in good health is very important during milking operation (Zelalem, 2010). Early cooling or chilling of milk at a temperature of 5°C or below is necessary to minimize microbial growth and prevent milk quality deterioration during handling, storing and transporting before the raw milk being processed. To facilitate bulking

of raw milk supply and transport the incoming milk refrigeration facilities should provide at points of collection and transport means to maintain the temperature as much as possible (Getachew *et al*., 2008). In the production of clean milk containers such as non-food grade plastic cans, buckets and jerry cans are not recommended. Aluminum containers are recommended because they don't have adhesive properties and easy to clean when compared with plastic containers (Kurwijila, 2006).

Good hygienic practices are the beginning point of quality milk production. An elevated bacterial level in the bulk tank is constituted by dirty cow, soiled equipment's, unhygienic parlors and dirty milker's hand. Several research results have shown that milk produced un hygienically can have bacterial load as large as millions and billons of bacteria per milliliters, but milk produced and handled under hygienic conditions can be expected to have colony count of less than $2*10^4$ /ml before pasteurization (Godefay and Mola, 2000, Alehegn, 2004; Chaye *et al*., 2004; Donkor *et al*., 2007).

According to Izabella (2021) milking procedure/step of milking is set orderly as follows. Wash hands and wearing gloves, pre-dipping (completely with a minimum of 30 seconds contact time), fore striping (check for milk aberrations stimulate the milk letdown reflex for 10 to 15 seconds), dry (apply with a downward twisting motion single use cloth or broadside towel), attach the milking unit, take away the milking unit and then post dipping. Another important point is milk cows in order and use properly cleaned and sanitized milking equipment (Izabella, 2021). Advance of hygiene and increase of income from milk and milk products comes from keeping all things clean which has contact with milk; i.e. following appropriate milking procedure and make hygienic the environment, milker, milk handling equipment, cow and all others which have touching base with milk (Gelane, 2018).

2.3. The Quality and Safety of Milk

2.3.1. Milk Quality

According to LAIA (2015) there are four quality categories (A, B, C and D): quality in terms of technological components (A), quality in terms of hygienic components (B), quality in terms of food safety (C) and quality in terms of sustainability (D). Every of these four categories have specific indicators to measure them. Thus, fat, protein and lactose which are

the main components of the milk are the indicators for quality in terms of technological components. These components can be affected by the nutrition and feeding practices, stage of lactation, cow's age, season, mechanical errors, such as cooling problems in the bulk tank and they are important. For quality in terms of hygienic components somatic cell count, antibiotic residues and bacteria are the indicators. There are regulatory standards in cow milk that help to measure whether the milk is appropriate or not for further processing either for indicators in category A or in category B. On the other hand, the indicators are organoleptic characteristics (auditors oversee evaluating whether the milk smells good or bad and the same for taste) for category C (milk in terms of food safety). The last category is quality in terms of sustainability. This category contains the newest quality perceptions, and its indicators are animal welfare, grazing practices, packaging and herd surveys which mean an evaluation of milking procedures, management, housing, equipment, and mastitis control according to certain standards (LAIA, 2015).

2.3.2. Microbial Quality of Milk

The amount and types of spoilage bacteria present in the milk describes the concept of milk quality. Among several levels at which spoilage is aimed to be reduced, one is by preventing contamination throughout the dairy chain to attempt to minimize the bacterial load. Actual temperature controller from the time of milking to storage in the raw milk bulk tank is important. Pasteurization is defined by FAO/WHO (2004) as "A microbiocidal heat treatment aimed at dropping the number of any pathogenic microorganisms in milk and liquid milk products, if present, to a level at which they do not constitute a significant health hazard. The primary choice to conduct enumeration of microbial quality indicator microorganisms is automated enumeration techniques (TEMPO®; a fully automated enumeration system that tests quality indicators in food products and environmental samples). It is an automated system based on conventional enumeration methods which design to replaces serial dilutions, media preparation and plate reading with a 1/10 dilution but enumerate based on the traditional and accepted MPN (Most Probable Number) method, miniaturized on a card format (Owen *et al*., 2010).

2.3.2.1. Standard Plate Count:

The most correct and useful method of testing bacteriological quality of milk is the standard plate (Godefay and Molla, 2000). Useful general information on the microbiological quality of milk can be provided by the total plate count of microbes in milk. The number of bacteria in aseptically drawn milk varies from different breasts of the same animal and from animal to animal. Aseptically drawn milk from healthy udders comprises average of between 500 and 1000 bacteria ml/l. The initial counts of 10^5 *bacteria mL-1* are evidence of poor production hygiene and Milk produced under hygienic conditions from healthy animals should not contain more than 5×10^5 bacteria per milliliter *(*ml*)* of milk (O'Connor, 1994). Milk harvested from uncontaminated, healthy cows normally contains SPC values of fewer than 1,000. If the value counted is higher it implies that contaminating bacteria are entering the milk from a possibly multiple sources. Counts of fewer than 5,000 or even 1000 are possible because it's difficult to remove all causes of contamination. Poor cleaning of the milking system is one of the most common causes of high SPC. Infectious bacteria that are shaded by mastitic cows can also contribute to higher count of SPC (Murphy and Boor, 2000).

2.3.2.2. Coliform Bacteria:

Coli forms are aerobic or facultative anaerobic, Gram-negative, non-spore forming rods when incubated on agar for 48 hours at 35°C that ferment lactose to produce gas (FAO, 1986). Coli forms are widely distributed in the farm environment, and they are dominant mastitis pathogens. The presence of coli form organisms in milk is a good indicator of unsanitary conditions of production, processing or storage. The products are potentially hazardous to the consumers' health when their existence is in large number in dairy products (Godefay and Molla, 2000). Coliform count delivers information about a warning of unsanitary production practices and/or mastitis infection. For milk intended to be pasteurized before consumption, a count less than 100 Colony Forming Units (Cfu)/ml are considered adequate Counts of 10 Cfu/ml or less are possible and required if raw milk will be consumed directly (Ruegg, 2003). Producing milk that is always free of coliforms is not practical and their presence in raw milk may be tolerated. But, if present in large numbers (over 100 coliform organisms per milliliter of raw milk) it indicates that the milk was produced under improper procedures (Walstra *et al.,*

2006). The products are potentially harmful to the consumers' health when their existence is in large number in dairy products (Godefay and Molla, 2000). Bacteria that are most commonly associated with manure or environmental contamination are detected by Coliform Count Procedure. There are strains that normally exist in the environment and yet coliforms are often used as indicators of fecal contamination. Milking soiled cows or dropping the milking cow into manure during milking may be the cause of entrance of coliforms. Commonly, counts >50 would show poor milking hygiene or other sources of contamination. Dirty equipment results higher coliform counts more and in few cases milking cows with environmental coliform mastitis can also be the cause (Murphy S. C. and Boor K. J., 2000).

In raw milk Existence of greater count of coliform could show the unsanitary status specifically the fecal of animal and environmental contamination (Robinson, 2002). Coliforms have similar biochemical characteristics and are not a single species of microorganism and they are group of Gram-negative rod-shaped bacteria. They are used to screen the quality of milk being able to ferment lactose with the production of acid and gas within 48 hr. Absence of coliforms in 1:10 dilution of pasteurized milk and 1:100 dilutions in raw milk is accepted as a satisfactory quality. A proof that contamination from excreta has occurred is the presence of *E. coli* (ILRI, 2008). But according to Ethiopian compulsory standard coliform should be 50,000 for milk that intended for processing and nil from 25ml. of milk for milk drink (CES 278: 2021, ES 6633:2021).

2.3.2.3. Yeast and Mold Counts;

Total Yeast and Mold Counts (TYMC) are used to distinguish and quantify the amount of fungal growth and let for identification of viable yeast and mold species present. The number of fungi is reported as the number of colony forming units (Cfu) (ILRI, 2008). The amount of Yeast and mold should not exceed Maximum of 10 Cfu/ml (CES 278: 2021). The amount of fungal growth is detected and measured by Total Yeast and Mold Counts (TYMC) and used for identification of viable yeast and mold species present. Additionally, the milk prepared for consumption should be free/nil from *Staphylococcus aureus* (per 25ml), *Salmonella* (per 25ml), *and E. coli* (per 25ml).

2.3.2.4. Staphylococcus Aureus

Staphylococcus aureus causes a wide variety of diseases in humans and animals and is an important foodborne pathogen. In dairy cattle, *S. aureus* may contaminate milk and other dairy products and is frequently associated with subclinical mastitis (Lowy, 1998). In human and animal highly pathogenic strains of *Staphylococcus aureus* can cause disease. In animal species, including ruminants, *S. aureus* may cause sub-clinical mastitis. Dairy animals with mastitis commonly shed *S. aureus* into the milk supply which can expose to food poisoning in humans (ILRI, 2008). Milk can be contaminated by *Staphylococcus aureus* when there is mammary gland infection. Additionally it can be contaminated during or after milking by improper washing of hands when milking and handling of milk storage materials, coughing and sneezing (Siingh and Prakash, 2010).

2.3.2.5. Salmonella Species

Natural infections of the udder are rare but due to infected persons, fecal contamination from the cow, and contamination of the environment raw milk and milk products can be contaminated with *Salmonella species*. Lack of hygiene in dairies, especially those from developing countries, has regularly been considered as one of the major causes for contamination of milk with both spoilage and pathogenic bacteria. Raw milk and milk products are progressively becoming key sources of human illness (ILRI, 2008).

2.3.3. Safety of Milk

The increasing number of incidences in which foodborne pathogens is detected in fluid milk and ready-to-eat dairy products clearly indicate that pasteurization is not the ultimate tool to control milk borne pathogens. It is likely that fecal and foodborne pathogen contamination occurs during the harvesting of raw milk (i.e., milking, collection, and storage) and the farm environment likely plays a major role in the presence of foodborne pathogens in bulk tank milk. Reducing the potential for contamination during harvesting of milk should result in a reduction of foodborne pathogens in raw milk (OLIVER *et al*., 2005).

Milk can be contaminated with potentially pathogenic bacteria even in controls where milk is of very good quality. The dairy industry has invested heavily in the implementation of food

safety management systems such as Hazard Analysis Critical Control Point (HACCP) to maintain the safety of the milk supply. HACCP introduction does not guarantee food safety and the system cannot stand alone even though it has been widely adopted (Goff1and Griffiths, 2006).

For many years Pasteurization has been a guarantee of safety of dairy products. But, even with the massive literature that indicates the usefulness of pasteurization in the minimization of milk borne illness, certain consumer groups continue to press for access to unpasteurized milk. Milk can be contaminated with potentially pathogenic bacteria even in controls where milk is of very good quality. Over the past decade it has been recognized that intervention strategies must be introduced at all points along the food chain to effectively manage food safety. While because of the difficulty in identifying meaningful critical control points that can be monitored (Goff1 and Griffiths, 2006). Milk and dairy products promote beneficial health outcomes and provide an excellent source of nutrition. People in both developed and developing countries use from dairy producers and manufacturers that keep a high standard of food safety certify a safe and wholesome product for consumers (Garciaa, *et al*., 2019).

2.4. Dairy Products and its Relation with Public Health

The amount and types of spoilage bacteria present in the milk describes the concept of milk quality. There are several levels at which spoilage is aimed to be reduced. One is by preventing contamination throughout the dairy chain to attempt to minimize the bacterial load. Actual temperature controller from the time of milking to storage in the raw milk bulk tank is important. FAO/WHO (2004) defined pasteurization as "A microbiocidal heat treatment aimed at reducing the number of any pathogenic microorganisms in milk and liquid milk products, if present, to a level at which they do not constitute a significant health hazard. *Mycobacterium tuberculosis* and *Coxiellaburnetii* are Organisms effectively to be destroyed by Pasteurization conditions" (FAO/WHO, 2004). *Mycobacterium tuberculosis* and *Coxiellaburnetii* are some of the more important pathogens. Furthermore, in recent years, raw milk marketing has increased (Fusco *et al.* 2020).

The dairy industry attempts to produce milk and dairy foods that seen to be healthful and wholesome and are safe and nutritious. There are several diseases of dairy cattle that are zoonotic and pathogens transmissible from dairy cattle. Adoption of pasteurization and eradication campaigns via the 20th century provided for nearly complete control of the potential for brucellosis, tuberculosis, and Q-fever from cow's milk. It means nothing that there have been very few major outbreaks of foodborne disease associated with dairy products more recently, and that many of the incidents that have occurred were associated with illegitimate consumption of raw milk. Due to *Salmonella, Listeria,* and *Campylobacter* milk is a potential source of zoonotic disease. Several public health issues challenge the dairy industry. For example, the potential relations of Johne's disease in cattle with Crohn's disease in people and bovine spongiform encephalopathy with variant Creuztfeld-Jacob disease, as well as the possible influence of antibiotic use in cattle to the development of antimicrobial resistance in human pathogens are important concerns (LeBlanc *et al*, 2006).

2.5. Pathogens Found in Milk

Numerous factors like farm extent, number of animals on the farm, sanitation, farm management practices, difference in sampling and kinds of samples measured, differences in detection methodologies used, geographical location, and season influences the prevalence of foodborne pathogens in milk (OLIVER *et al*., 2005). Dairy farms are a main tank of foodborne pathogens. Due to direct association to excretion from the udder of an infected animal and with contaminated sources in the dairy farm environment foodborne pathogens exists in milk. Dairy cattle are considered a major reservoir of *Salmonella, Campylobacter,* and *STEC* because of most foodborne pathogens live in the ruminant intestinal tract. Occurrence of foodborne pathogens in bulk tank milk give the impression to be directly linked to fecal contamination that occurs mostly during the harvesting of raw milk, nevertheless, in case the organism can be directly excreted into milk some foodborne pathogens can cause mastitis (OLIVER *et al.,* 2005).

2.6. Quality Parameters for Microbial Quality and Safety

Total yeast and mold are one of the parameters that indicate the amount of fungal growth and provide information for identification of viable yeast and mold species present (ILRI, 2008). Next to intra mammary infection the SCC in milk may be associated to the immune reaction. Subclinical mastitis is a condition in which without seeming visual changes in milk form leukocytes increase in milk, whereas there are apparent changes, sometimes in clinical mastitis in combination with local symptoms in the udder or systemic clinical signs in milk that can be recognized by the farmer (Hillerton and Berry, 2005). A large leukocyte level, measured as SCC, and in the production of enzymes that reduce milk components such as fats and proteins may result high TBC in milk (Li *et al*., 2014; Baur *et al*., 2015), thus lowering the quality of milk and milk products. This will affect the shelf life and decreases consumer acceptance of these products (Elmoslemany *et al*., 2009). Moreover, as they are zoonotic pathogens can contaminate bulk milk mastitis bacteria such as *Staphylococcus aureus* and be a public health concern (Zadoks *et al*., 2011; Bi *et al*., 2016). Numerous other zoonotic pathogens, including *Salmonella spp*., contaminate raw milk when milking techniques, hygiene, and handling during transportation are suboptimal at the farm or in the milk chain may be found in infected animals (Kamana *et al*., 2014).

On dairy value chain fungal contamination of milk and milk products can occur at different stages. It can occur from farm to table at dairy farms to dairy processing units and at homes of consumers. Raw milk generally contains between 3 to 5 log 10 $CFU \cdot mL^{-1}$ fungi with higher number of yeast cells than fungal spores independent of the animal species (Lavoie *et al*. 2012). The steady and milking parlor environments at the farm are key sources of fungi in the milk. In addition, an important yeast source is the teat surface (Lavoie, 2012). Because regulations that protect the health of consumers require adherence to key milk quality and safety guidelines. Increasing milk quality and safety around the world is highly significant. Fecal peeling of *Escherichia coli O157:H7*, which does not cause disease in cattle, but is possibly highly pathogenic to humans. There is indirect link between cattle and people through consideration of environmental or ecosystem health (LeBlanc *et.al*, 2006).

2.7. Microbial Possessions of Raw Cow Milk in Ethiopia.

Earlier researches conducted in different parts of the country shown that the microbial counts of milk produced and marketed are generally much higher than the acceptable limits (Zelalem, 2010). Research findings reported values of aerobic bacteria counts of milk sampled from udder, milking bucket, collection center, milk vending shops and cafeteria is range between 8.132 and 10.28 log10 cfu/ml (Haile *et al*., 2012, Melese *et al*., 2015, Tadele *et al*., 2016).

Increasing trend of counts as the milk passed through udder, milking bucket, collection centers and upon arrival at the processing plant in all cases. This could be due to improper milking and handling, storage and transport time after the milk is harvested from the udder and out from the dairy farms. Milk produced under sanitary environments from healthy cows should not have more than 4.69 log cfu/ml (O' Connor, 1994).The above shown count of milk samples collected from the country were reflected to be below the standard set for good quality milk. This indicates that the hygienic conditions in which milk has been produced and handled are poor exposing the product to microbial contagion and proliferation.

Coliforms are mainly of fecal origin and coliform count is especially associated with the level of hygiene during production and subsequent handling. It is not practical to produce milk that is always free of coliforms. Their presence in raw milk may then be tolerated. However, if present in large numbers means that the milk was produced under improper procedures. Therefore their existence in large number in dairy products is an indication that the products are potentially dangerous to the consumers' health (Godefay and Molla, 2000). Previous researchers informed values of coliform counts in raw cow milk experimented from different part of the country that range between 3.14 log cfu/ml to 6.57 log cfu/ml (Alganesh, 2002; Zelalem and Faye, 2006; Melesse and Mustefa, 2019; Adugna and Eshetu, 2021).

One of the most important sources of molds is airborne contamination that is usually occupied on walls and roofs of the dairy farms. Molds will enter in milk chain when the milk is exposed to open area. Growth of yeasts and molds is a usual cause of the spoilage of dairy products. The previous studies on yeast and mold count which are done at different parts of the country reports a result that ranges from 3.71±0.83log10 cfu/l to 3.77±0.47 log10 cfu/ml (Amistu K. *et al*., 2015; Adugna and Eshetu, 2021).

According to Ethiopian Standard Agency Raw whole cow milk shall be prepared under hygienic conditions in accordance with ES 577, ES 929 and ES ISO 22002-1. Raw whole cow milk shall be free from pathogenic microorganisms and shall comply with the microbiological limits indicated in Table 1 below (CES 278, 2021).

Table 1; Microbiological limits of raw whole cow milk

Characteristic	Maximum limit (cfu/ml)	Test Methods
Total viable bacterial count, incubated for 48 h at 32 ^{0} C, intended for processing	1,000,000	ES ISO 6610
Coliform count, incubated for 24 h at 37 ^{0} C, intended for processing	50,000	ES ISO 5541-1 ES ISO 5541-2

According to Ethiopian Standard milk drinks shall be free from pathogenic microorganism and shall comply with microbiological limits in Table 2 below (ES 6633:2021).

Table 2; Microbiological requirements for Milk drinks

Characteristics	Limit	Test method
Total colony count, per ml, Max.	10	ES ISO 6610
Total coliform, per 25ml	Nil	ES ISO 5541-1, ES ISO 5541-2
Staphylococcus aureus, per 25ml	Nil	ES ISO 6888-1, ES ISO 6888-2
Salmonella, per 25ml	Nil	ES ISO 6785
E. coli, per 25ml	Nil	ES ISO 11866-1, ES ISO 11866-2
Yeast and mold, cfu/ml, Max.	10	ES ISO 6611

2.8. Antimicrobial Resistance

Unconcerned and misuse of clinically applicable antibiotics in agriculture, veterinary and medical sectors have a contribution to the global widespread increase in antimicrobial resistance (Samreen *et al*., 2021). The increasing global prevalence of infectious diseases affecting the human population is that the antimicrobial resistance (AMR) crisis which are untreatable with any known antimicrobial agent. Both weakening and lethal diseases increase in frequency and scope and this crisis will have a devastating cost on human society (Michael *et al*., 2014). The major factors determine this crisis according to Michael *et al*., 2014 are; an evolutionary response to the widespread use of antimicrobials is the increasing frequency of AMR phenotypes among microbes; Pathogens in any environment access to all of humanity because of the large and globally connected human population; and the strong selective pressure that is driving the evolutionary response in the microbial world is provided by the extensive and often unnecessary use of antimicrobials by humanity (Michael *et al*., 2014). There are many contributing factors to the spread of antibiotic-resistant bacteria and their antibiotic resistance genes either right through antimicrobial drug use in health care or antibiotic residues released from various domestic settings (Samreen *et al*., 2021).

3. MATERIALS AND METHODS

3.1. Descriptions of the Study Area

The study was conducted at the capital city and administrative center for the Federal Democratic Republic of Ethiopia, Addis Ababa. The altitude of Addis Ababa ranges 2200 – 3000 m.a.s.l. and receives an annual rainfall of 1200 mm. The mean minimum and maximum annual temperature are 9^{o}C and 24.6^{o}C, respectively and the city occupies about 540sq.km (Unpublished data from Addis Ababa Farmers and urban Agriculture Commission 2019).

Kolfekeranio Sub-city;

Kolfekeranio sub city Bordered With; Sebeta in the South, Nifas silk lafto in the east, Addis ketema in the North and Oromiya special zone/Burayu in the west. Kolfekeranio sub city has good dairy potential and the sub city have a total of 10 Districts. There are 521 dairy farmers in the sub city holding a total of 3,752 cattle and among this cattle population 2,001 are dairy cows producing about 3.57 million litres of milk per year (Unpublished data Kolfekeranio sub-city farmers and Urban Agriculture office, 2021).

Lemikura Sub-city;

Lemikura sub city Bordered With; Kality in the South, Legetafo in North, Oromia special zone in East and Bole and Yeka in west. There are 175 dairy farmers in the sub city holding a total of 10,000 cattle and among this cattle population 2,000 are dairy cows producing about 3.36 million litres of milk per year (Unpublished data Lemikura sub-city farmers and Urban Agriculture office, 2021).

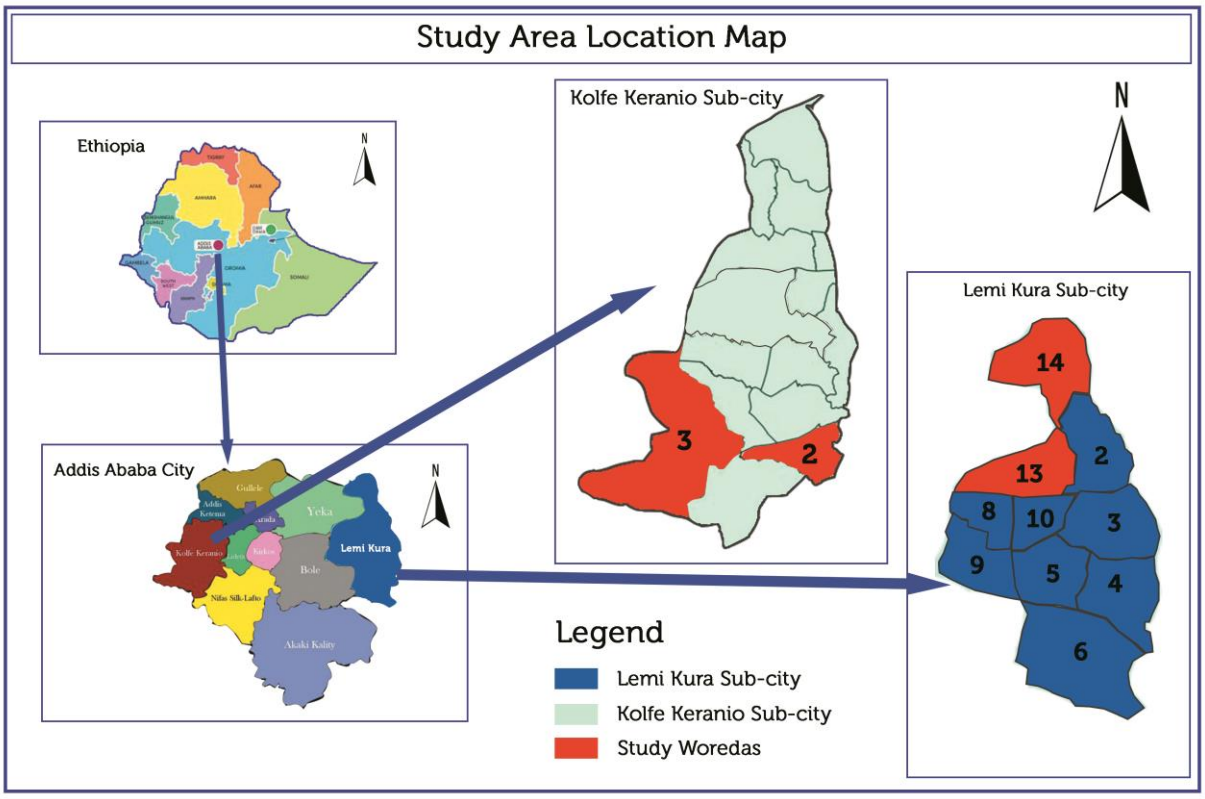

Figure 1; Location of the Study area

3.2. Study Design

A cross-sectional study including laboratory analysis of raw milk samples, farm inspection and questionnaire survey. In the laboratory test Bacteriological quality tests *i.e.* Standard Plate Count (SPC), Coliform Count (CC), Total Yeast and Mold Count (TYMC), *Escherichia Coli (EC)* count, *Staphylococcus aurous*, *Salmonella Spp*., test were performed by using ISO standard. A questionnaire-based survey was used to collect data needed for the assessment of dairy farmers milking, milk handling and farm hygiene practices in the study area. The study also involved field visits and observation and key informant interviews. Farms were categorized in to large scale (>30 dairy cows), medium scale (>5≤30 dairy cows) and small scale dairy farms (≤5 dairy cows) based on the number of their dairy cows (Amare *et al*., 2019). Type of housing and cleaning practices, hygiene of the milking area, hygienic condition of cows and milker, type of milking container and sanitary practices and source of

water used for cleaning were assessed carefully. Additionally, laboratory work was undertaken by following standard laboratory protocol on testing milk samples for quality and safety.

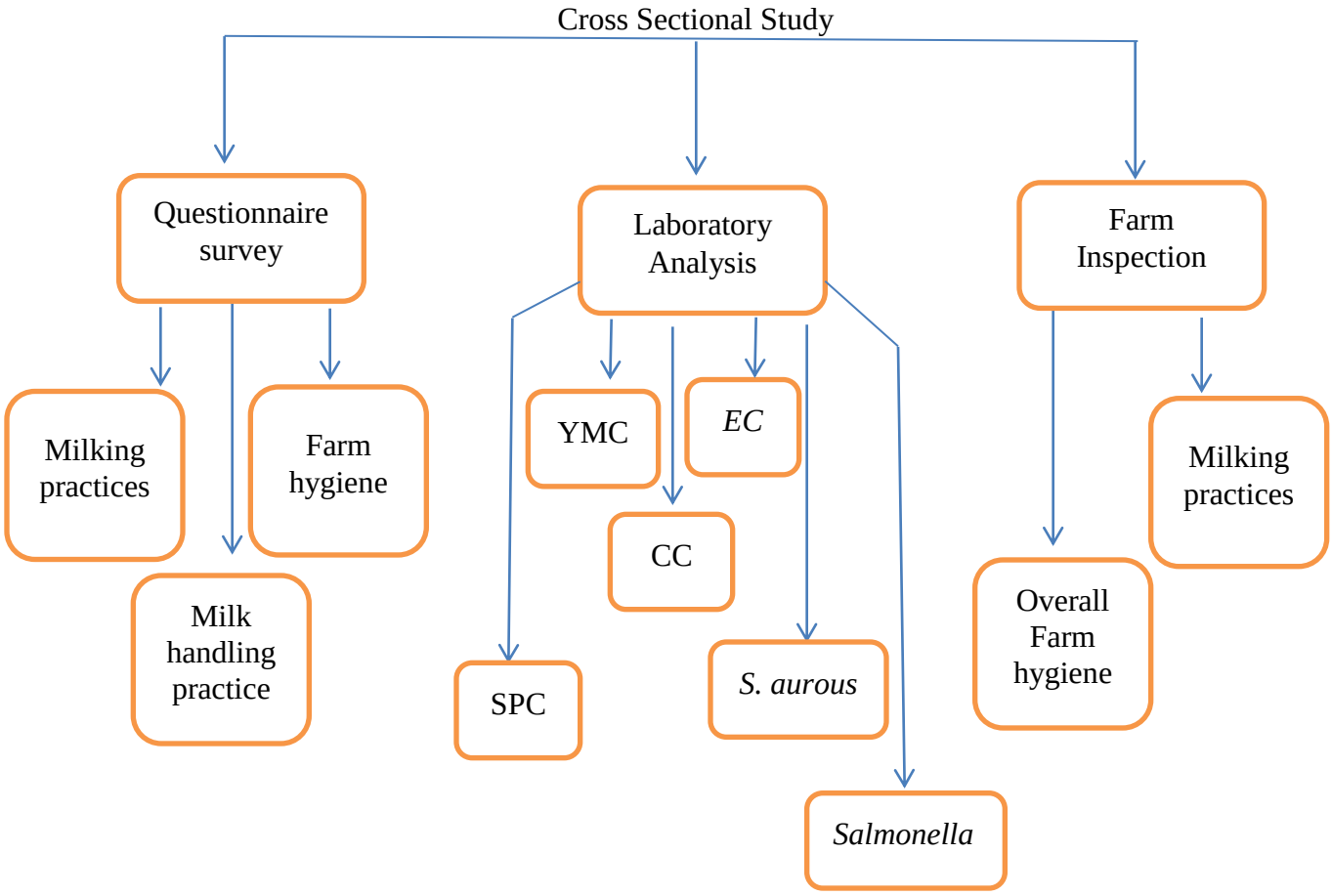

Figure 2; Framework of the study

3.3. Study Population

Small, medium and large-scale dairy farmers in Lemikura and Kolfekeranio Sub cities of Addis Ababa owning dairy cattle for milk production were included in the study population. Two top dairy potential districts were purposively selected from each sub city. From each selected districts, dairy producers were selected and interviewed.

3.4. Sources of Data

Primary data were collected through interviews of selected dairy farmers using semi-structured questionnaires. Results of key informant interview and field observation were also valuable first-hand data sources and it was used in this study. With close supervision by the researcher the questionnaires were administered to farmers by enumerators recruited and trained for the purpose. Based on the questionnaire, the perception of the dairy cattle owners about hygienic milk production was collected. Secondary data was also collected from literatures, documents and reports that have been written about the study area. Key informants' interview was also undertaken for the reasoning of the information collected from the household survey and for main issues which need more qualified and knowledgeable persons.

3.5. Determination of Sample Size

From Kolfekeranio sub city district 2 and 3 and from Lemikura sub city districts 13 and 14 were selected. The sample size was calculated according to the formula recommended by Cochran (1977) developed a formula to calculate a representative sample.

$$n_o = \frac{z^2 pq}{e^2}$$

Where, $n_0 =$ the sample size,

z = the selected critical value of desired confidence level,

p = the estimated proportion of an attribute that is present in the population,

q = p −1 and

e = the desired level of precision

Assuming the maximum variability, which is equal to 50% (p =0.5) and taking 95% confidence level with ±5% precision, the calculation for required sample size

p = 0.5 and hence q =1-0.5 = 0.5; e = 0.05; z =1.96

$$n0 = \frac{(1.96)2(0.5)(0.5)}{(0.05)\ 2} = \mathbf{384}$$

The sample size can be reduced slightly if the population is small. This is because a given sample size provides consistently more information for a small population than for a large population. The sample size (n_0) can be adjusted as

$$n = \frac{\text{no}}{[\![1+(\text{no}-1)/\text{N}]\!]}$$

Where n is the sample size and N is the population size (Nanjundes and Divakar 2021).

There are totally 278 dairy farmers in selected districts (Unpublished data from Addis Ababa Farmers and urban agriculture commission, 2021) and among them 161 farmers were selected for survey and 61 raw milk sample (500 ml. of milk from each farmer's container) was collected.

3.6. Sampling Techniques

The potential of the sub cities was identified to choose a representative sample. The number of districts in the sub city and their dairy potential was identified. From the list of these, districts were selected purposively. For this study, both purposive and systematic random sampling techniques were employed. The rationale for selecting these study areas includes they possess better number of livestock resources, and in the communities of the study area there are peoples who use dairy production as one of livelihood activities. The target sampling population was defined as all households in the purposively selected districts that own dairy farm and practicing dairy cattle production. To select household respondents, Systematic random sampling technique was employed. In this procedure dairy farmer who have cows and undertake milking and practicing dairy cattle production were identified and listed alphabetically in selected districts. Using the household list of the purposively sampled districts the sample respondent households were selected randomly based on proportional to the number of households owning dairy cow. Accordingly from Kolfekeranio District 3 milk sample collected was 24 and 63 farmers were used for questionnaire survey. From Kolfekeranio sub-city District 2 the number of milk sample collected was 15 and 40 farmers were used for questionnaire survey. From Lemikura sub-city District 13 the total milk sample collected was 8 and the numbers of farmers used for questionnaire survey were 21. Lastly from Lemikura sub-city District 14 the numbers of farmers used for questionnaire survey were

37 and the number of milk sample collected was 14. Totally 61 milk samples was collected and 161 dairy farmers were included in the survey. Quota sampling method was used to determine samples from different farm scales for both survey and milk sample collection.

3.7. Data Collection Methods

3.7.1. Qualitative Data

Different data collecting systems were applied to collect primary and secondary data which include individual interviews with the help of semi-structured questionnaires and a direct observation. The questionnaire that prepared and related type was pre-tested and administered. The questionnaire was focused on personal information of the study participants, dairy animal husbandry practices, milking procedures and milk handling practices. Farms were inspected, and activities observed during the farm visit were milking procedure, hygiene of milking parlor, kind and hygiene of utensils used, overall milking practices, milk handling system and feeding environment. Livestock experts and veterinarians at sub city and district level of urban agricultural office were participated on the study. Interviewing key informants; primarily, an overview of the area was apparent through discussion held with Urban Agricultural Livestock Officers and experts. Discussions were undertaken with key informants, Team Leaders of Livestock production, Animal health experts and Animal production experts, Agricultural extension experts, input and technology distribution experts were all included for key informant interview. And with the help of checklists for some qualitative data was collected and giving the freedom to raise new ideas and concerns not likely during the preparation of the questionnaire.

3.7.2. Milk Microbial Quality Test

Raw milk samples were collected from farms by following strict hygienic procedures. 500 ml of raw milk sample was collected from each of the farmers' containers using whirl-pack. Then raw milk samples were collected from producer's container and placed into whirl-packs. Therefore, samples were labeled and set in ice box (4°C) to limit microbial multiplication and transported as timely as possible to the Animal Product, Veterinary Drug and Feed Quality Assessment Center (APVDF, QAC)) laboratory for microbial quality analysis. The collected

samples were tested within 24 hours. Bacteriological quality tests: Total Yeast and Mold Counts (TYMC), Coliform counts (CC), *E. coli* count (EC) and Aerobic plate count (APC) were performed. Additionally, the identification of major pathogens *Staphylococcus aurous* and *Salmonella Spp*. and antimicrobial susceptibility were tested. The ratio of data collected (for questionnaire and milk sample collection) was 82.6% from small scale 13.1% from medium scale and 4.3% from large scale farms because the total dairy farms found in the selected four districts are present in this ratio.

3.7.3. Enumeration of Microbial Quality Indicators (APC, CC, EC and YMC)

The bacteriological medium used was prepared according to the manufacturer's recommendations and milk samples were subjected to bacterial culture and identification according to the standard operating procedures developed and validated in APVDF-QAC microbiological quality assessment laboratory against Ethiopian Standard (ES ISO 16654: 2001, ES ISO 6579-2: 2012 and ES ISO 6888-3:2003).

3.7.3.1. Determination Aerobic Plate Count (APC)

The total Aerobic Plate Counts (APC) was determined using sterile standard plate count agar (Himedia, India). Buffered Peptone Water (BPW) was used to prepare one to nine dilutions of the sample to get the required sample dilution. APC was determined using a conventional method which is standard plate count agar. One ml of milk sample was added into a sterile test tube containing 9 ml of sterile buffered peptone water. After thoroughly mixing, the suspension was serially diluted up to 10^{-7} and duplicate samples from the appropriate dilution (1 ml) was pour-plated using a 15-20 ml of cooled but still molten standard plate count agar solution and mixed thoroughly. The resulting plates were allowed to solidify and then incubated at 30ºC for 48 hours. The tests were conducted in triplicate for each dilution starting from 5^{th} dilution. The total bacteria on each plate were counted. APC was determined as the total number of CFU per milliliter of milk sample. The number of colony forming units (N) per milliliter of the milk sample was calculated using the formula as follows;

$$\mathrm{N} = \frac{\sum C}{V\left(n1 + n2\ (0.1)\right) * d}$$

Where; C = is the sum of colonies on all plates counted

V = is the volume applied to each plate

n_1 = is the number of plates at first dilution

n_2 = is the number of plates counted at second dilution

D = is the dilution from which first count was obtained

N = is average plate count

3.7.3.2. Determination of Coliform Count (CC), E. Coli Count (EC) and Yeast and Mold Count (YMC)

Yeast and mold count (YMC), Coliform count (CC) and *E. coli* count were conducted using TEMPO system. The TEMPO system (bioMérieux) consists of two parts: a Preparation Station and a Reading Station. The system comprises two single-use disposables: a vial containing dehydrated culture medium and an enumeration card that miniaturizes the Most Probable Number (MPN) method, using three series of 16 wells each. The culture medium, specific for the microbial group to be enumerated, is adapted to ensure rapid detection of microbial growth through the detection of changes in fluorescence. After inoculation with a dilution of the milk sample, the culture medium is automatically transferred into the card by the TEMPO Filler. The card is then incubated in a biological aerobic incubator. During incubation, microbial growth causes a modification of the substrate in the medium, revealed through a fluorescent signal that is automatically detected by the TEMPO Reader. Depending on the number of positive wells on the card, the system calculates the number of microorganisms present in the sample with a range of 1-9.8 x 10^7 CFU/g according to the dilution of the sample used. The TEMPO media were re-hydrated with sterile water and inoculated with an aliquot (part) of the homogenized sample. The TEMPO system was then used to perform the automated filling of the test card. The instrument then monitored the incubation time of the test cards and, following the appropriate incubation time, the cards were read using TEMPO reader station. The cards analyzed in the reader instrument and the final result was then automatically calculated. All media used in this study were prepared according to the instructions given by the respective manufacturers and sterilized by

autoclaving at 121ºC for 15 minutes. After sterilization, all media were cooled to 45-47^{o}C in a water bath before use. According to the manufacturer instruction for Aerobic plate Count Agar (APCA)**, t**he content of the bottle was melt in a water bath at 100°C until completely dissolved. Then the cap attached and checked the homogeneousness of the dissolved medium by turning the bottle upside down. Then, cooled at 45-50°C finally well mixed to avoid foam formation and aseptically distributed into Petri dishes. **RVS (**Selective Enrichment for *Salmonella)* (Himedia, India) 30ml (the equivalent weight of dehydrated medium per liter) added to 1 liter of distilled water. Then heated gently till completely dissolved. 10ml volumes of the solution dispensed into tubes and sterilized by autoclaving at 115°C for 15 minutes. Mannitol salt agar (MSA); 111g of the powder was suspended in 1 liter of distilled water. Then well mixed and heated for 1 minute to boil and then shook frequently until completely dissolved. Finally it was sterilized in autoclave at 121°C for 15 minutes. Xylose Lysine Deoxycholate (XLD) (Himedia, India); 56.7 g of dehydrated media was suspended in 1000ml of purified filtered water. Heated with frequent agitation and boiled for 1 (one) minute. Not sterilized but cooled to 45- 47°C. Mixed gently and dispensed in to sterile petri dishes. The prepared agar plates were taken to room temperature. Plates were incubated with sample and line for isolation and incubated at 35°C for 24-48 hours. Peptone water that was autoclaved at 121ºC for 15 minutes and cooled to 30ºC was used for serial dilution of the milk samples to determine APC, TCC (Biomerucs, France), EC (Biomerucs, France) and YMC (Biomerucs, France).

Ten (10) mL of milk sample was added into a sterile TEMPO bag containing 90 mL of sterile peptone water to prepare first dilution. After thoroughly mixing using Smasher, the suspension was serially diluted up to 10^{-4} and 0.1ml of each dilution was added in to the vial which contain proper nutrient and 3.9ml of BPW was added to the vial, after thoroughly mixing using Vortex meter the mixture was filled in to the Tempo Card using TEMPO filler Machine. The cards in which the sample was filled were incubated according to their incubation temperature, card incubated for CC at 35 ^{o} C for 22-27 hours, EC at 37^{o}C for 22-27 hours and YM at 25 ^{o} C for 72 – 76 hrs. After the incubation period was done the cards were read using the TEMPO reader Machine and the result was recorded.

3.7.4. Culture, Isolation and Identification of Potential Food-borne Pathogens (*Salmonella* and *S. aurous*)

a). Target Bacterial Culturing, Isolation and Identification Techniques

The techniques recommended by the International Organization for Standardization, ISO 6579:2002(E) and ES ISO 16654-2012 were employed for the sample preparation and morphological characterization of target bacteria colonies from the recommended selective media. Further confirmation of suspected colonies of the target bacterial isolation and identification was performed by a fully automated Vitek 2XL compact system following the manufacturer method.

b). Salmonella Species Identification

About 25 ml sample was pre-enriched in 225 ml of sterile buffered peptone water (BPW) to yield a 1/10 dilution and incubated at 37°C for 24 hr. Then after, 0.1ml of the incubated BPW culture was transferred into each of 10ml of Rappaport Vassiliadis Salmonella (RVS) broths as selective enrichment and incubated at 41.5°C for 24 hr. A Loop full from the inoculated RVS broth and streaked replicate onto selective media plates of Xylose Lysine Deoxycholate (XLD) agar and incubated at 37°C for 24-48 hr. On XLD expected salmonella colonies look reddish/pink colonies with or without black center confirmed salmonella colonies on XLD plates are sub-cultured on Nutrient Agar (NA), then incubated at 37°C for 24hr and confirmed by Vitek 2XL compact system using Gram Negative (GN) cards having 47 biochemical tests following the manufactuer method.

c). Staphylococcus Aureus Identification

About 10 ml sample was pre-enriched in 90 ml of sterile buffered peptone water (BPW) to yield a 1/10 dilution and incubated at 37°C for 24 hr. For *S.aureus* isolation, a loop full of suspension was taken from incubated BPW culture and sub-cultured on Mannitol Salt Agar (MSA) and then incubated at 37°C for 24 hour. Colonies showing small colonies surrounded by golden yellowish zones on Mannitol Salt Agar (MSA) again sub-cultured on nutrient agar, incubated at 37°C for 24hr and confirmed by automated Vitek 2XL compact system using Gram-Positive (GP) cards having 43 biochemical tests.

3.7.5. Confirmatory Identification of Target Bacteria Species by Vitek 2XL Compact System

VITEK® 2XL is fully automated identification system for most clinically significant fermenting and non-fermenting gram negative bacilli. It is working based on the established biochemical methods (47 tests for GN and 43 tests for GP) and newly developed substrates measuring carbon source utilization, enzymatic activities and resistance of the bacterium. From 20 types culture media recommended by the manufacturer, the laboratory used the available two media (Xylose lysine Desoxycolate /XLD) for Salmonella and Mannitol Salt Agar (MSA) for *S. aureus* (VITEK® 2GN and GP, 2019). The Vitek identification system automatically generated after 10 hours or less and displayed through the workstation and was printed by VITEK 2XL database, Version 9.0.

The Vitek ® 2 GN (Gram Negative) and Vitek ® 2 GP (Gram Positive) identification cards were used for confirmatory identification of Gram Negative *salmonella* and Gram Positive *S.aureus* bacteria respectively by Vitek 2XL compact system according to the manufacturer's recommendations. In brief, 2–3 pure fresh colonies of Salmonella and Staphylococcus aureus taken from incubated nutrient agar were suspended in 3 milliliters (ml) of sterilized saline (aqueous 0.45% to 0.50% NaCl, pH 4.5-7.0) and thoroughly mixed. The McFarland turbidity was adjusted in the range from 0.50 to 0.63 using calibrated Densi Chek Plus meter (turbidity meter). Gram negative and gram positive cards were selected and scanned by barcode scanner and the straw of the cards were inserted into the gram negative and gram positive bacteria suspected inoculated suspension tubes in the cassette, respectively. The cassettes loaded into the Vitek2XL compact system and the machine automatically proceeds to processing the cards once all the cards are loaded. The unknown organisms were compared to the reference strains stored in the Vitek 2XL compact system software for proper identification. The results were interpreted by the Vitek database at different confidence levels or probabilities as excellent (96-99%), very good (93-95%), good (89-92%), acceptable (85-88%), none or low reactive/discrimination bio pattern and unidentified microorganisms. The final identification results obtained automatically approximately 8 hrs. or less for Gram Positive (GP) bacteria and 10 hours or less for Gram Negative (GN) bacteria.

The same colonies of the bacteria incubated in to the suspension were sub-cultured to Nutrient agar and incubated at 37°C for 24hr. for Antimicrobial Susceptibility Testing (AST) of the isolated target bacteria (*S. aureus*).

3.7.6. Antimicrobial Susceptibility Test by using VITEK 2XL

AST by using VITEK system is determining the Minimum Inhibitory Concentration (MIC) of the drug. Both gram positive and negative AST cards contain a control well containing only microbiological culture medium and the remaining micro wells contain a premeasured amount of specific antimicrobials combined with culture medium. The organism suspension from nutrient agar (fresh colony 16-24 hrs. incubation) must be diluted to a standardized concentration in 0.45% saline before being used to rehydrate the antimicrobial medium within the card. The card was then filled, sealed and placed into the instrument incubator/reader automatically. The instrument was monitor the growth of each well in the card for about 18 hours and after the compilation of incubation cycle the Minimum Inhibitory Concentration (MIC) values were determined for each antimicrobial contained on the cards (GP AST and GN AST) (VITEK 2 AST-GP79, 2018).

The AST of target bacterial isolates *S. aureus* against selected antimicrobial agents coated on Vitek ® 2 AST-GN96 card and Vitek ® 2 AST-G79 cards conducted by fully automated Vitek 2XL compact system as recommended by the manufacturer. Eight antimicrobial classes containing a total of 23 antimicrobial agents coated with cards were used. The selection criteria of the antimicrobials depended on the microorganism-antimicrobial panel definition of our AMR surveillance plan and their use of the antimicrobials in the animal and human treatments.

The colonies of sub-cultured and incubated bacteria of the identified (positive) *S. aureus* were suspended in 3 milliliters (ml) of sterilized saline (aqueous 0.45% to 0.50% NaCl, pH 4.5-7.0) using different clear polystyrene tubes for each target bacteria and thoroughly mixed. The turbidity of the bacterial suspensions was adjusted with a DensiChekPlus meter (turbidity meter) to match that of a McFarland 0.5–0.63 standard. Then AST cards are selected (AST GP card for *S.aurues* and AST GN card for *Salmonella*), but **no** *Salmonella* ***was detected in the sample***, scanned by barcode scanner and the straw of the cards were inserted into the empty

tubes in the slot of cassette next to bacterial suspension. The antibiotic drugs used to test were Tetracycline, Trimethoprim-Sulfamethoxazole, Amikacin, Gentamicin, Kanamycin, Erythromycin, Tylosin, Clindamycin, Florenicol, Enfrofloxacin and Tilcimosin. All necessary information of the test sample entered into the Vitek PC through Vitek 2 FLEX prep data entry and the two priority bacteria identified were selected from the database. The cassette holding the suspension tubes with cards loaded into the Vitek XL compact system instrument at the cassette load station. Then the Vitek 2XL compact system automatically proceeds to processing the cards once all the cards are loaded. The results were interpreted by the Vitek database, and final results were obtained automatically. Susceptibility results of bacteria are available in less than 19 hrs.

The break point of antimicrobial is expressed numerically in µg/ml as minimum inhibitory Concentration (MIC) for AST/AMR interpretation. AST results interpretations were based on the guidelines of CLSI (Clinical and Laboratory Standards Institute), CA-SFM (Comite de l'antibiogramme de la SocieteFrancaise de Microbiologie), BTC (Breakpoints at Time of Clearance), BMX VET (BioMérieux Veterinary), EUCAST (European Committee on AST), ECOFF (Epidemiological Cut-Off value) and FDA (U.S. Food and Drug Administration). The results interpretations for AST/AMR given by Vitek 2XL compact system are Susceptible (S), Intermediate (I) and Resistant (R).

3.7.7. Inclusion and Exclusion Criteria

A) Inclusion Criteria

- Apparently milk from small, medium and large scale dairy farms at selected districts of Addis Ababa where farm owners/keepers/ administrators have consented and give their permission were included in the study.

B) Exclusion Criteria

- Personnel who refused to participate in the study or to give information about the dairy farm.
- Lactating cows who took antibiotics as a prophylactic or treatment purpose in the past two weeks and bulk tank milk including their milk were excluded.

3.8. Method of Data Management and Analysis

Statistical Package for Social Sciences software version 25 (SPSS) was used to conduct all the statistical analyses. The data collected was fed to Micro-soft-Excel database for handling the data and transformation of bacterial count to Log10, and then the data was analyzed using SPSS version 25. Means, standard deviation and frequency distribution and Percentage were used to explain the milking and milk handling practices. One-way ANOVA was used for the evaluation of the performance variation.

$Y_{ij} = \mu + s_i + e_{ij}$

Where: Y_{ij} = Individual observation for each test

μ = the overall mean

s_i = the i^{th} effect of differences among the three dairy farm scales.

e_{ij} = the error term

For all analysis, 95 % CI and P-value <0.05 was set for statistical significance of the calculation. The correlation statistical analysis was used to study the association among microbial quality of raw milk and farm scale. Quantitative and qualitative types of data were analyzed. Qualitative data resulting from direct observations and key informants were inspected and presented in the form of discussions. Descriptive statistics like mean, frequency distributions and percentages were working for quantitative analysis. The organized data was analyzed using SPSS software version 25; and the results are presented by means ± SD, tables and graphs.

4. RESULT AND DISCUSSION

4.1. Demographic Characteristics of the Respondents

The sex, age group, educational status and the training information of the respondents in the study area is summarized in Table 3. From a total of 161 respondents 70.8% were males and 29.2% were females. The highest age proportions of the respondents were ranged 30 - 50 years which accounts for about 48.4%, the next highest number of the respondents had the age ranges between 51 and 65 which account for about 29.8%, while the rest of the respondents were above 65 and less than 29 years which holds 14.3 % and 7.5% respectively. In respect to the educational level the majority 33.5 % of the respondents had an educational level of Secondary school (7-12) and then the rest of the respondents had an educational level of 1-6 (32.9%), read and write only 18% and respondents who attend college 15.5% which is less than the result of Belay and Geert (2015) who reports of the total households, 42.9% had college and university education in Jima. From the respondents, 49.1% of them had got training related to milk production and the rest 50.9% had not got any training related to hygienic milk production. This result is less than the result of Biruktawit (2016) which was 90% of the respondents had got training on milk production and handling practice in selected sub-cities of Addis Ababa.

Table 3*:* Demographic Characteristics of the Respondents

Variables		Small scale	Medium scale	large scale	Total
Sex of the respondent	Male	91(56.52%)	18 (11.2%)	5 (3.1%)	114 (70.8%)
	Female	42 (26.1%)	3 (1.8%)	2 (1.2%)	47 (29.2%)
Total		133 (82.6%)	21 (13%)	7 (4.35%)	161
Age of the respondent	29 and less	10 (6.2%)	2 (1.2%)	0	12 (7.45%)
	30 -50	67(41.65%)	8 (4.96%)	3 (1.86%)	78 (48.45%)
	51- 65	39 (24.2%)	7 (4.35%)	2 (1.24%)	48 (29.8%)
	>65	17 (10.6%)	4 (2.5)	2 (1.24%)	23 (14.3%)
Total		133 (82.65%)	21 (13%)	7 (4.35%)	161
Education Level	Read and write only	26 (16.1%)	3 (1.86%)	0	29 (18%)
	1-6	47 (29.2%)	5 (3.1%)	1 (0.62%)	53 (32.93%)
	7-12	41(25.5%)	11 (6.8%)	2 (1.24%)	54 (33.54%)
	Collage and above	19 (11.8%)	2 (1.24%)	4 (2.48%)	25 (15.53%)
Total		133 (82.7%)	21(13%)	7 (4.3%)	161
Training on milk production	YES	65 (40.45%)	11 (6.8%)	3 (1.86%)	79 (49.1%)
	NO	68 (42.2%)	10 (6.2%)	4 (2.48%)	82 (50.9%)
Total		133 (82.65%)	21(13%)	7(4.35%)	161

4.2. Respondents Knowledge and Practices on Milk Hygiene

All the respondents used hand milking and none of them had a milking machine. This result is in line with the result of the study conducted at Abuna Gindeberet district, west Shewa zone of Oromia region by Tadesse *et al*., (2015). The majority of the respondents (85.7%) had awareness of hygienic milking procedures and 14.3% didn't know about hygienic milk production (Table 4). All the respondents wash their hands before milking this result is greater than the report of Adugna and Eshetu (2021) at Wellega that was 22.5%, and the result is similar to the study report conducted around Addis Ababa by Melesse and Mustefa (2019). Of the respondents 72.7% of them wash their hands with soap and water and 27.3% of them with water only. Before milking the most important and the critical thing for hygienic practices of milk production is washing and cleaning the udder of the cows. Since the udder of the cow has direct contact with dirty materials like urine, dung, and feed refusal, so it is important to remove the dirty materials from the udder of the cows (Zelalem, 2010). In the present study 88.8 % of the respondents wash the teat and udder of cows and 11.2% of them milk without washing of the udder and teat. The result is greater than the report of Melesse and Mustefa (2019) around Addis Ababa which was 72% and the report of Adugna and Eshetu (2021) at Wolega which was 66.15%. About 58.4% of them use common towels 31.1% use individual towels to dry the teat and 10.6% didn't use towels to dry. The result is less than the report of Belay and Geert (2015) at Jimma, which was 61% use a common towel and 13% use an individual towel. In relation to the milking area 92.5% of the respondents milk their cows at the barn where the cows live and only 7.5% of the respondents milk their cows out of the barn or at separate milking area. The farmers prefer to milk in separate area but their raise place limitation as a challenge.

Table 4*:* Respondents Knowledge and Practices on Milk Hygiene

Having awareness about hygienic milking procedure	Yes 85.7%	No 14.3%		
Hand washing before milking	Yes 100%	No 0%		
How to wash hands	Water Only 27.3%	With Water and Soap 72.7%		
Washing of udder and Teat	Yes 88.8 %	No 11.2%		
Use of towel to dry the teat	Common towel 58.4%	Individual towel 31.1%		No towel used 10.5%
Cleaning of the milking area	Once per day 42.8%	Before every milking 26.7%	Twice per day 19.3%	Not daily 11.2%
Milking of mastitis cows	Last 68.9%	First 11.8%		No care 19.3%
Fore striping	Perform 54%	No 46%		
Complete milking	Perform 83.9	No care 16.1		

4.3. Milk Equipment and Hygienic Practices

Majority of the milk producers use plastic container for milking (83.9%) and the rest 14.3% uses stainless steel and 1.9% others. For milk storage 68.3% plastic, 23.6% stainless steel and 8.1% others (Table 5). The result is less than the report of Belay and Geert (2015) who reports 92.6% plastic materials. The farmers desire to use aluminum cans because of their durability and easy cleaning but they comment the availability and affordability as a reason for limitation to use. The serious concern that should not be forgotten is proper cleaning of milking and milk storage equipment, because proper cleaning of equipment is important to reduce microbial contamination of milk according to Adugna and Eshetu, 2021.

About 62.7% of the respondents wash milking and milk storage equipment before and after every milking and milk storage activities, 25.5% of them wash equipment before every milking and storage of milk and 11.8% of the respondents wash equipment after every milking and milk storage. About 90.3% of the respondent use tape water for cleaning of milking and milk storage equipment and 9.3% use burrow water. The majority of the respondents clean milking and milk storage equipment with warm water and soap (52.2%), and the rest with cold water and soap, warm water only and cold water only which indicate 23.6%, 19.2% and 5%, respectively (Table 5). Warm water can reduce bacterial multiplication and is better for cleaning of milking materials (Adugna and Eshetu, 2021). Majority of the respondents care about their personal hygiene but 12.4% of them had no care for their personal hygiene at the time of milking. Only 31.7% of the respondent cool milk before sell and the rest 68.3% of them sell milk freshly after milking. Majority of producers (62.1%) sell their milk for individual collectors, 18.6% for Café and hotels, 8.1% for processors and 11.2% for other users. Only 37.3% of the respondent’s know about the negative consequences of misuse of antibiotic drugs and the majority (62.7%) didn’t know (Table 5).

Table 5; Milk equipment and Hygienic practices

Kind of Milking material	Plastic 83.9%	Stainless Steel 14.3%	Others 1.8%	
Kind Of Milk Storage Material	Plastic 68.3%	Stainless Steel 23.6%	Others 8.1%	
	Before every milking and storage of milk	After every milking and storage of milk	Before and after every milking and milk storage	
When to clean milking and Storage material	25.5%	11.8%	62.7%	
	Tape water		Hole water	
Water source for cleaning of milk equipment	90.7%		9.3%	
	Yes		No	
Drying of milk equipment	85.7%		14.3%	
Care of health condition of the milker	57.1%		42.9%	
Care for personal hygiene while milking	87.6%		12.4%	
Awareness about the negative consequence of miss using of antibiotics	37.3%		62.7%	
	Individual collectors	Processors	Cafe and Hotels	Others
Where and for whom to sell the milk	62.1%	8.1%	18.6%	11.2%

4.4. Housing Type and Barn Hygiene

Type of Housing; among the respondents, 85.1% of the farmers use semi open houses and 14.9% of them use closed house for their dairy cattle. None of them had an open house. In relation to floor type 73.3% of the farms had concrete floors 9.3% raised wood, 5% earthen and 12.4% others like stone bedded. As visited during farm inspection most of the farmers clean the barn after milking in the morning and among the respondents 57.1% clean the barn once per day and the rest 42.9% clean the bar twice per day every morning and evening (Table 6). This result is somewhat in line with Sema *et al*., (2019) reports at Mukaturi and Sululta and is less than Melesse *et al*., (2015) about 74.5% of the respondents in and around Jigjiga who cleaned the barn once a day.

Table 6*;* Housing type and barn hygiene

Amount of milk produced	<10 liter	10- 20	21-50	51-100 liter	>100
	41.6%	17.4%	20.5%	12.4%	8.1%
Type of housing	Semi open			Closed	
	85.1%			14.9%	
Barn cleaning frequency	Twice per day			Once per day	
	43.5%			56.5%	
Washing of the floor	Once per week			No wash	
	73.3%			26.7%	
	Concrete		Earthen	Raised Wood	Others
Floor Type of the barn	73.3%		5%	9.3%	12.4%

4.5. Microbiological Quality

4.5.1. Aerobic plate count (APC):

Earlier researches conducted in different parts of the country has shown that the microbial counts of milk produced and marketed are generally much higher than the acceptable limits (Zelalem, 2010). In this study the mean of APC ± SD from small, medium and large scale farms were 6.42±1.35, 6.16±1.25 and 4.12±1.42 log10 cfu/ml respectively (Table 7). The mean APC of raw milk is 6.24±1.43 log10 cfu/ml. The result is greater than the national standard which sets the maximum limit of total viable bacteria should not exceed 6log10cfu/ml (CES, 2021). The European standard shows that raw milk intended for human consumption should contain plate count of 4.69log10cfu/ml (5×10^4 cfu/mL) and according to the Australian standard for raw milk APC should not exceed 4.39log10 cfu/ml (2.5×10^4 cfu/ml) (Institute of Medicine and National Research Council (US) (2003). The result in this study is high and it doesn't meet the national and international standard this might be because of unclean milking equipment and improper udder preparation. It is less than the previous research findings reported values of 8.2 log10cfu/ ml aerobic bacteria counts of milk sampled from study conducted at the Central Highlands of Ethiopia by Alganesh (2016), the result 6.46 log10cfu/ml by Zelalem (2010). The main reasons which affect the microbiological quality of raw milk may be inappropriate hygienic practices during milking, procedures used in cleaning and sanitizing the milking and storage equipment, poor storage temperature, health and hygiene of cows and milking personnel. In this study Statistical significant difference was observed between mean of Aerobic Plate Count (APC) of milk from small, medium and large scale farms (Table 7). This might be due to poor hygienic milking practices and lack of proper farm hygiene performed by smallholder milk producers.

Table 7*;* Microbiological quality of milk

Farm Scale	Milk samples examined	APC ± SD (log10cfu/ml)	*E. Coli* ± SD (log10cfu/ml)	TCC ± SD (log10cfu/ml)	YMC ± SD (log10cfuml)
Small Scale	49	6.42±1.35	2.45±0.57	4.41±1.32	2.99±1.08
Medium Scale	8	6.16±1.25	2.37±0.52	4.37±1.05	2.45±0.57
Large Scale	4	4.12±1.42	2.5±0.52	3.47±0.65	2.21±0.25
Over all Mean		6.24±1.43	2.44±0.56	4.34±1.27	2.87±1.02
Sig. level		.007	.919	.373	.150

4.5.2. *E. coli* Count (EC):

The mean of *E. coli* count of milk from small scale dairy farms was 2.45±0.57 log10cfu/ml, from Medium scale dairy farms was 2.37±0.52 log10cfu/ml and from large scale dairy was 2.5±0.52 log10cfu/ml (Table 7). There was no statistically significant difference between mean of *E. coli* count of milk samples from small, medium and large scale dairy farms. The mean *E. coli* of raw milk was 2.44±0.56 log10cfu/ml (Table 7). The large amount of *E. coli* count may be caused due to poor hygiene and sanitation, soil and fecal materials during milk production. The result is below the national standard which directs the raw cow milk should be free from E. coli (CES, 2021). The result obtained in this study is less than the result obtained from Sebeta (3.19±1.70 log10cfu/ml) and greater than the result from Holeta and Sululta that were 1.53±0.00 and 1.19±0.26 log10cfu/ml reported by Amistu *et al*., (2015). In milk and milk products the presence of *E. coli* organisms is a sign of poor sanitary production and/or wrong handling of either milk or milk equipment. The result is also less than the report of Teklemichael *et al*., (2013) who reported *E. coli* count of raw milk samples collected from dairy farms were; 3.64 ± 0.776 cfu/ml at Dire Dawa.

4.5.3. Total Coliform Count (TCC):

The mean of Total Coliform Count of milk from small scale dairy farms was 4.41±1.32log10cfu/ml, from Medium scale dairy farms was 4.37±1.05log10cfu/ml and from large scale dairy was 3.47±0.65log10cfu/ml. The total mean of TCC of raw milk samples was 4.34±1.27log10cfu/ml (Table 7). There was no statistically significant difference between mean of Total Coliform Count of milk samples from small, medium and large scale dairy farms. Coliforms are mainly of fecal origin and coliform count is especially associated with the level of hygiene during production and subsequent handling. It is not practical to produce milk that is always free of coliforms. However, if present in large numbers means that the milk was produced under improper procedures. Therefore their existence in large numbers in dairy products is an indication that the products are potentially unsafe to the consumers' health (Godefay and Molla, 2000). In the present study the total mean of CC of raw milk samples is 4.34±1.27log10cfu/ml. This result is in the acceptable maximum limit of the national standard which is 4.69log10cfu/ml (CES 278, 2021), and below the Australian standard which directs for unpasteurized milk 10^2 cfu/ml. This result is higher than the result reported by Adugna and Eshetu (2021) at the Eastern Wollega zone of Sibu Sire districts 3.14log10 cfu/ml, Melese and Mustefa (2019) study at Smallholder Dairy Farms around Addis Ababa 3.336log10cfu/ml. And the result in this study is less than the value obtained by Amistu K. *et al.*, (2015), at the farmers and retail shops of Sebeta, Holeta and Sululta 5.42±1.73 to5.78±0.95; 5.53±1.03 to 5.63±0.62 and 4.18±1.22 to 6.35±0.43 log cfu/ml. And less than the result 8.58 log 10 cfu/ ml which was obtained by Alganesh (2016) in the study conducted at the Central Highlands of Ethiopia. Finally the result is more near to the Coliform Count value of 4.84 log cfu/ml obtained by Haile (2015) at Adea Berga and Ejerie districts of West Shoa Zone.

4.5.4. Total Yeast and Mold Count;

The mean TYMC of milk from small scale dairy farms, medium scale and large scale dairy were 2.99±1.08log10cfu/ml, 2.45±0.57log10cfu/ml and 2.21±0.25log10cfu/ml respectively. The mean YMC of raw milk was 2.87±1.02 log10cfu/ml (Table 7). There was no statistically significant difference between mean Yeast and Mold Count (YMC) of milk samples from small, medium and large scale dairy farms. The high result of yeast and mold count in this

study might be because of poor air quality/ventilation and darker areas because of farm structure of the study area. The growth of yeasts and molds is a usual cause of the spoilage of dairy products. The mean of YMC in this study was 2.87±1.02 log cfu/ml and the result was less than research conducted in the country by Tadesse *et al*., (2015) (8.13±0.31 log10cfu/ml), Adugna and Eshetu (2021) (3.71±0.83log10 cfu/ml) the result acquired milk sample from Abuna Gindeberet district, west Shewa zone and Wolega of Oromia region respectively.

4.5.5. Isolation and Identification of Salmonella

When milking techniques, hygiene and handling during transportation are suboptimal at the farm or in the milk chain numerous zoonotic pathogens, including *Salmonella spp*., may be found in infected animals and contaminate raw milk (Kamana *et al*., 2014). In this study from 61 raw milk samples salmonella is not detected and all the milk samples that were collected from small scale, medium scale and large scale farms were free from salmonella pathogen.

4.5.6. Isolation and Identification of *S. aurous*

From the total samples tested, the prevalence of *Staphylococcus aureus* was 37.7%. Among 61 raw milk samples 23 milk samples were positive for *S. aureus* (Fig 2). About 19 of the positive samples were from small scale farms (38.8% of the samples from small scale farms). 2 of them from medium scale (25% of the samples from medium scale farms) and 2 *s. aureus* positive samples from large scale farms (50% of the samples from large scale). The findings of the present study is greater than that of Amistu *et al*, (2015) at different critical points of Oromia special zone surrounding Finfine that was isolated from 17(28.33%) of samples. The existence of high total *S. aureus* load in raw milk shows contamination probably from the udder or teat canal of lactating dairy cows. The results of the present study may be due to the contaminated hand of the milker, lack of proper washing of udder and teat before milking, manifestation of sub-clinical mastitis and poor general hygienic condition during milking and storage.

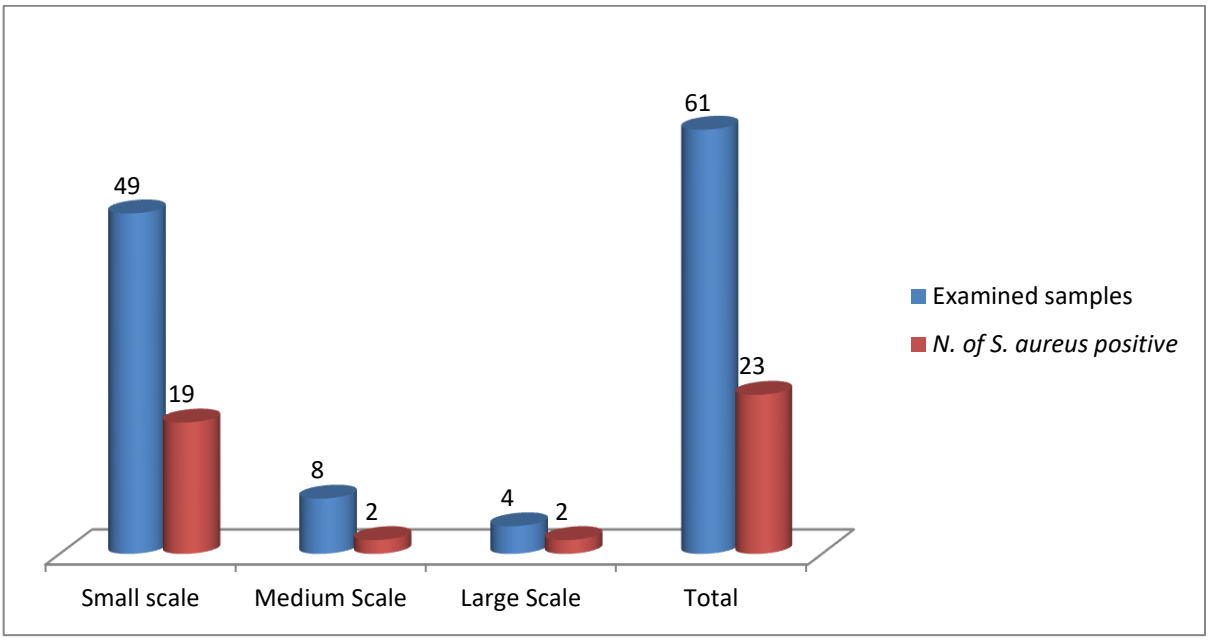

Figure 3; Isolation and Identification of S. aurous

4.6. Microbial Quality of Raw Milk in Two Sub-cities

The mean of Aerobic Plate Count (APC) of milk samples collected from Kolfekeranio sub-city was 6.3±1.42log10 cfu/ml while the mean of APC of milk samples collected from Lemikura sub-city was 6.13±1.47log10 cfu/ml and the result is some amount different but statistically it was not significant (Table 8).

The mean of *E. coli* count (EC) of the milk samples collected from Lemikura sub-city is lower than the result obtained from the samples that were collected from Kolfekeranio. The result was 2.01±0.08 log10cfu/ml *E. coli* count for Lemikura and was 2.68±.56log10cfu/ml for Kolfekeranio. There was statistical significant difference among the result of the two sub-cities (Table 8). The difference might be from variation of practices of farm hygiene and disparity of milking procedure.

The result of Coliform Count (CC) of milk samples collected from the two sub-cities was 4.27±1.2log10cfu/ml log10cfu/l for Kolfekeranio and 4.46±1.39log10cfu/ml for Lemikura. It is some amount higher in Lemikura than Kolfekeranio but statistically significant difference was not observed on the count of Coliform among the two sub-cities (Table 8). The result of Yeast and Mold Count (YMC) was some amount higher in Lemikura Sub-city than in

Kolfekeranio sub-city but statistically the difference was not significant. The result was 3.0±0.92log10cfu/l and 2.79±1.07log10cfu/ml for Lemikura and Kolfekeranio respectively (Table 8). The result might be different because of the difference in farm structure and farm hygiene.

The prevalence of *Staphylococcus aureus* on milk samples collected from two sub-cities is not that much different. It was 36.3% for Lemikura (8+ve among 22 milk samples) and 38.4% for Kolfekeranio (15+ve among 39 milk samples) (Table 9). The existence of this pathogen might be due to poor overall hygienic condition during milking and milk storage.

Table 8; Microbial quality of raw milk by sub-cities

Sub-city		APC **(log10cfu/ml)**	EC **(log10cfu/ml)**	YMC **(log10cfu/ml)**	CC **(log10cfu/ml)**
Kolfekeranio	Mean	6.3	2.68	4.27	2.79
	N	39	39	39	39
	Std. Deviation	1.42	.56	1.2	1.07
Lemikura	Mean	6.13	2.01	4.46	3.0
	N	22	22	22	22
	Std. Deviation	1.47	.068	1.39	.92
Total	Mean	6.24	2.44	4.34	2.87
	N	61	61	61	61
	Std. Deviation	1.43	.55	1.27	1.02
Sig. level		.662	.000	.589	.440

Table 9; prevalence of Staphylococcus aureus on two sub-cities

***Staphylococcus aureus* * Sub-city (Cross tabulation)**				
		Kolfekeranio	Lemikura	Total
Staphylococcus aureus	+ve	15	8	23
	-ve	24	14	38
	Total	39	22	61

4.7. Antimicrobial Resistance Level of *S. aureus*

The isolated *S. aureus* bacteria were 100% susceptible for antibiotic drugs like Amikacin, Gentamicin, Kanamycin, Erythromycin, Tylosin, Clindamycin and Florenicol. On the other hand *S. aureus* was 87% susceptible and 13% intermediate for Enfrofloxacin, 78.3% susceptible and 21.7% resistance for Tilcimosin, 60.9% susceptible and 39.1% resistance for Tetracycline, 95.7% susceptible and 4.3% intermediate for Trimethoprim-Sulfamethoxazole (Table 10). This result is far from the report of Umer *et al.,* 2022 at central Ethiopia which indicates 91% *S. aureus* were resistant to trimethoprim/sulfamethoxazole and 58% resistance to erythromycin. The misuse and overuse of antibiotics and poor method of infection control leads to antibiotic resistance.

Table 80; Antimicrobial susceptibility level of S. aureus.

Drug name	**Species of pathogen**	**Susceptible (S)**		**Intermediate (I)**		**Resistance (R)**	
		N	**%**	**N**	**%**	**N**	**%**
Amikacin	*S. aureus*	23	100				
Gentamicin	*S. aureus*	23	100				
Kanamycin	*S. aureus*	23	100				
Neomycin	*S. aureus*	23	100				
Enfrofloxacin	*S. aureus*	20	87	3	13		
Erythromycin	*S. aureus*	23	100				
Tilcimosin	*S. aureus*	18	78.3			5	21.7
Tylosin	*S. aureus*	23	100				
Clindamycin	*S. aureus*	23	100				
Tetracycline	*S. aureus*	14	60.9			9	39.1
Florfenicol	*S. aureus*	23	100				
Trimethoprim-Sulfamethoxazole	*S. aureus*	22	95.7			1	4.3

4.8. Correlation among Microbial Quality of Milk and Farm Scale

Aerobic plate count significantly had negative correlation with farm scale, (0-.353**). Coliform Count Yeast and Mold count *E. coli* count has no correlation the farm scale (Table 11).

Table 91*;* Correlation among microbial quality of milk with farm scale

		Farm scale
Farm scale	Pearson Correlation	1
	Sig. (2-tailed)	
Aerobic Plate Count	Pearson Correlation	-.353**
	Sig. (2-tailed)	.005
E. coli Count	Pearson Correlation	-.007
	Sig. (2-tailed)	.959
Coliform Count	Pearson Correlation	-.154
	Sig. (2-tailed)	.236
Yeast and mold Count	Pearson Correlation	-.248
	Sig. (2-tailed)	.054

N.B. **. Correlation is significant at the 0.01 level (2-tailed).

5. SUMMARY AND CONCLUSION

From the results of the study, it is concluded that there are poor milking and milk handling practices in Lemikura and Kolfekeranio sub-cities of Addis Ababa. Majority of the milk producers had awareness about hygienic milking procedure and all of them wash their hands before milking but still some of them didn't use detergents to wash hands before milking. Milk is harvested by hand milking method. Milking and milk handling practice is undertaken basically by using plastic utensils. The milking practice is undertaken inside the barn. These all may decreases quality and safety of milk and milk products and then reduces regular revenue of the producer and food safety for consumers. Generally, the microbiological quality of milk samples collected from the study area were not in the acceptable range of Ethiopian raw cow milk and European dairy standard it could be due to low hygienic practice and use of un cleaned milking and milk storage material. The poor bacteriological quality is possibly related to lack of good practices of milking and milk handling. In the current study the microbial qualities of the milk indicates poor, as understood from the high values of Aerobic plate count (APC) and yeast and mold count (YMC). This might be due to unhygienic condition of milking; unclean milking equipment and poor cleaning of milk utensils. This high bacterial content, the existence of pathogenic bacteria in numerous samples affects the raw milk quality and concerns a safety issue to the consumer. The *S. aureus* bacteria are to a certain degree resistant to some antibiotic drug and this might be due to misuse of the antibiotic drug. The mean Aerobic bacterial counts, *E. coli* counts and Yeast and Mold counts are higher than the national and International standards which indicate that the quality of milk produced in the study area had unacceptable levels of contamination with microorganisms that greatly increase across the milk collection points.

6. RECOMMENDATION

- Regular and sustainable training and extension service should be given for producers about hygienic milk production and the proper use of antibiotic drugs. National standard directs the raw milk should be free from pathogens including *S. aureus.* But in this study the pathogen is existed in the milk samples. The existence of *S. aureus* implies the poor hygienic practices during and after milking. So, hygienic milking procedures should be applied seriously, the health of the udder should be kept.
- Important inputs (Stainless steel milk cans) for milking and milk storage should be available and accessible for milk producers by appropriate price.
- It is better to use another antibiotic drug which substitutes tetracycline because it is somewhat resistance to *S. aureus*.
- Producers should get continuous follow up from animal health professionals and serious extension service should be provided for them. Veterinary service should be accessible nearly to eliminate inappropriate use of drugs.
- Consumption of unpasteurized milk and milk products should be avoided.

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8. APPENDICES

APPENDIX I; QUESTIONNAIRE

1. Owner name…… sex…… Age…….
2. Level of education;

   A. Read and write only B. Elementary C. High school D. College and above
3. Address; Sub city……. District …… Tell.no …….
4. Feed source: -

   a) Roughage: A. farm produced B. Bought C. Both

   b) Concentrate: A. farm produced B. Bought C. Both
5. From which source of water do you provide the cow for drink?

   A. River water B. Tape water C. wall water D. Stagnant water
6. Milk production per day……lit, amount sold…… lit/day, for home consumption ……. lit/day
7. Have you got any training related to dairy production? A. Yes B. No
8. If yes on what topic? A. On hygienic milk production B. Good dairy husbandry practices C. Others specify ……
9. What type of dairy house do you use?

   A. Open B. Semi open C. Closed
10. What type of floor the cow house has?

    A. Concrete B. Earthen C. Raised Wood D. Other
11. How many times do you clean the dairy cattle barn?

    A. Once per day B. Twice per day C. Others
12. When do you wash the cattle barn floor?

    A. Once per week B. Twice per week C. Others D. No I didn't wash the floor
13. Do you use bedding material for dairy cows? A. Yes B. No

14. If yes, what type of bedding materials you use?

A. Sawdust B. Grass C. Cow mat D. Other

15. Frequency of bedding material replacement.

A. Daily B. Once a week B. Twice a week C. other

16. Is there separate milking area? A. Yes B. No

17. Frequency of cleaning milking area whether it is separated or not.

A. Before every milking B. once per day C. twice per day

D. Once per week E. twice per week F. Three times per week

G. Other specify……..

18. Technique of milking; A. Hand milking B. Machine milking

19. Who milks the cow? A. Owner B. Worker C. Other family member

20. Do you know about hygienic milking procedures? A. Yes B. No

21. Do you wash your hands before milking? A. Yes B. No

22. If yes, A. with water only B. with water and soap

23. Do you wash the teat and udder of the cow? A. Yes B. No

24. If yes, when do you wash it?

A. Before milking only B. After milking only C. Before and after milking

25. If you wash the udder, what materials do you use for drying?

A. Collective towel B. Individual towel C. Just with hands

D. Others specify ……

26. About the towel that is used to dry;

A. Clean B. dirty C. No attention about the hygiene of the towel

27. Foremilk stripping; A. Yes B. No

28. Do you care about complete milking? A. Yes B. No

29. When do you milk mastitic cows?

A. First B. Last C. I do not care about the order

30. What type of equipment do you use for milking?

A. Plastic B. Stainless steel C. Other Specify……

31. What type of material do you use for milk storage?

A. Plastic B. Stainless steel C. Other Specify

32. How do you clean milk storage and milking equipment?

A. With warm water B. with Cold water C. Cold water and soap

D. Warm water and soap

33. Frequency of cleaning of milking and milk storage equipment

A. Before and after every milking and milk storage

B. Before every milking and milk storage

C. After every hand over milking and milk storage

D. No attention about it

34. Source of water used for washing milking utensil, A. River water B. Wall water C. Tape water D. Stagnant water

35. Have you let the equipment to dry after washing? A. Yes B. No care

36. If yes, how? .....

37. Have you check your health condition before undertaking the milking responsibility?

A. Yes B. No C. No care I can perform milking when I am sick

38. Do you care about your personal hygiene and wearing of clean cloth when you perform milking? A. Yes B. No

39. Do you cool the milk before sale? A. Yes B. No

40. If yes how? A. Refrigerator B. Traditional cooling system C. If others

41. Do you transport your products to market places? A. Yes B. No

42. If yes, what is the means of transportation?

A. On foot B. Public transport C. Private Car

43. Where do you sell your milk? A. Milk Processors B. Café, restaurant and hotels C. Individual collectors D. Other (specify) _____

44. Do the workers collecting the milk test the quality of milk before adding to the pool?

A. Yes B. No

45. If YES, indicate method of quality test and criteria use A. Alcohol test B. Density test C. Clot on boiling test D. Lacto scan E. Other (Specify)…….

46. Has your milk been rejected by the collectors? A. Yes B. No

E. If yes, why was it rejected? A. Dirt B. Abnormal color C. Failed Alcohol Test Low Density E. Abnormal smell

F. F. Other (Specify) ……

47. Do you use antibiotic for your milking cow? A. yes B. No

48. If yes, A. prescribed by veterinarian B. Randomly bought from market

49. If yes, do you take care of withdrawal period to use the milk? A. Yes B. No

50. Do you know the negative consequence of miss using of antibiotics?

A. Yes B. No

Any comment that you want to make regarding milk quality and safety -

_____

_____

A. Observation

All hygienic procedures followed during milking; Personal hygiene, milking equipment hygiene, milking area hygiene, washing of udder and teat, overall farm hygiene and other hygienic conditions were observed.

B. Discussions with livestock Experts

On Key informant interview and discussion issues like the availability of input (milking cans, stainless steel storage materials and milking machines), the extension service provided about quality milk production, the milk quality control mechanisms were discussed.

C. Farm inspection

1. Housing, closed type …… semi-open……... Open…….

2. Floor, concrete…… stone…… mud……. Other……

3. Roof, metal sheet……... Other (specific)…….

4. Drainage (slope), good……... Satisfactory……. Poor…….

5. Farm hygiene Excellent……. satisfactory……. poor…….

6. Separate milking area, yes……. No……...

6. If yes hygienic condition, excellent……...satisfactory……. poor…….

8. Milk storage area, Excellent……. satisfactory……. poor…….

9. Milking and milk handling equipment hygiene, Excellent… satisfactory… poor……

10. Overall milking practice, Excellent……. satisfactory……. poor……

11. Personal hygiene while milking, Excellent……. satisfactory……. poor……

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APPENDEX 2. LIST OF TABLES IN APPENDIX

Table in appendix 1; Data of Microbial quality of milk analyzed

Report					
Farm scale where milk sample is taken		Aerobic Plate Count	Ecoli Count	Coliform Count	Yeast and mold Count
Small scale	Mean	6.4233138967	2.4527233960	4.4095567935	2.9969231982
	N	49	49	49	49
	Std. Deviation	1.34676290733	.57292398804	1.32686474931	1.08051826334
Medium Scale	Mean	6.1657044201	2.3750000000	4.3679384312	2.4498351375
	N	8	8	8	8
	Std. Deviation	1.25235194718	.51754916951	1.05599010094	.57187141786
Large Scale	Mean	4.1256067800	2.5000000000	3.4746812955	2.2068423183
	N	4	4	4	4
	Std. Deviation	1.42173056121	.57735026919	.65129369958	.25024441110
Total	Mean	6.2388597282	2.4456302689	4.3427953363	2.8733653620
	N	61	61	61	61
	Std. Deviation	1.43571664041	.55804434817	1.27032582430	1.02044244282

Table in appendix 2; ANOVA between small, medium and large scale Farms

		Sum of Squares	Df	Mean Square	F	Sig.
Aerobic Plate Count	Between Groups	19.573	2	9.787	5.453	.007
	Within Groups	104.104	58	1.795		
	Total	123.677	60			
E. coli Count	Between Groups	.054	2	.027	.084	.919
	Within Groups	18.631	58	.321		
	Total	18.685	60			
Coliform Count	Between Groups	3.238	2	1.619	1.003	.373
	Within Groups	93.586	58	1.614		
	Total	96.824	60			
Yeast and mold Count	Between Groups	3.960	2	1.980	1.963	.150
	Within Groups	58.518	58	1.009		
	Total	62.478	60			

Table in appendix 3*;* One way ANOVA for APC, EC, CC and YMC by Sub-city

		Sum of Squares	Df	Mean Square	F	Sig.
Aerobic Plate Count	Between Groups	.404	1	.404	.193	.662
	Within Groups	123.273	59	2.089		
	Total	123.677	60			
E. coli Count	Between Groups	6.392	1	6.392	30.676	.000
	Within Groups	12.293	59	.208		
	Total	18.685	60			
Coliform Count	Between Groups	.483	1	.483	.296	.589
	Within Groups	96.341	59	1.633		
	Total	96.824	60			
Yeast and mold Count	Between Groups	.635	1	.635	.606	.440
	Within Groups	61.843	59	1.048		
	Total	62.478	60			