BUSITEMA UNIVERSITY
Pursuing Excellence

P.O. Box 236, Tororo, Uganda
Gen: +256 - 45 444 8838
Fax: +256 - 45 4436517
Email: info@adm.busitema.ac.ug
www.busitema.ac.ug

A STUDY OF THE MICROBIAL DIVERSITY OF MILK PRODUCTS OBTAINED FRESHLY FROM FARMERS AND IN COMPARISON, WITH URBAN DAIRIES OF TORORO MUNICIPALITY.

BY

WANZUSU EZEKIEL

BU/UP/2020/0683

DEPARTMENT OF BIOLOGY

A RESEARCH REPORT SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A BACHELOR'S DEGREE OF SCIENCE IN THE DEPARTMENT OF BIOLOGY OF BUSITEMA UNIVERSITY.

2023

DECLARATION.

I **Wanzusu Ezekiel,** do hereby declare to the department of Biology at Faculty of Science Education of Busitema University that this thesis/dissertation is my original work and has not been submitted for the award of a degree or diploma in any other University or college.

Wanzusu Ezekiel

BU/UP/2020/0683

Signature ..... Date 4th/DEC/2023

THE SUPERVISOR

I, the undesigned, confirm that this thesis has been submitted for examination with my approval as University Supervisor.

1. Mrs. Natukunda Flavia Bwonya.
   Lecturer, Department of Biology, Faculty of Science Education, Busitema University.

Signature ..... Date 04/12/2023

i

ACKNOWLEDGEMENT.

In the first place, I would like to give great thanks to our Almighty God, the creator of the universe for the immense love and care He has always rendered to me. It has been through His grace and mercy that now I boldly come up with such a write up which in the beginning of it all seemed impossible. Glory be to His Name.

Before I forget, I also would like in a special way to thank my supervisor, Mrs. Natukunda Flavia Bwonya for her tireless efforts in guiding me so that I would come up with this document, sincere thanks still to to Mrs. Namusana Hellen, a mother who has always kept on reminding me to work harder, despite the various challenges I encountered. I greatly thank them and only pray that God the Almighty gives them a long life.

Still in the same vein, I also extend my sincere appreciation to Mr. Kato Moses, Mr. Oketch Benard, a loving Uncle, Pastor Moses Abraham Mairah of the Busiu End Time Message church together with all stakeholders who offered support to me during this tenure whose names have not been listed. I thank you all for loving and taking care of me, that you wished I become a noble citizen in this country. May God bless you all so richly.

Lastly, my sincere appreciation goes to my Family members, the Family of the Late Mr. Jimmy Watsusi and Mama Juliet Akware Watsusi of Waninda A, village, Lukhonge Sub-County, Mbale District. Thanks for the love and prayers dear family. To my siblings, Joan, Joshua, Ezra, Caleb, Joy and Shalom, thank you for loving your big brother, and thanks for your blessings for success, I must say God is with us all and He is indeed a loving God.

To my beloved Aunties, (Rita, Gorreti, Salume, Agnes, Sharon, Florence, Catherine, Consatance and immaculate), thank you all for the spiritual and material support you offered to me during this journey, I am pleased to inform you that you are soon reaping what you sowed, you did not sow on bear ground but fertile ground, bravo to you all. To my best friends, I must also thank you for your advice whenever the going was tough, you always encouraged me not to give up. Thank you all for the love and support. Good luck.

DEDICATION.

This work is a special dedication to my family, headed by Mrs. Juliet Akware Watsusi, our beloved mother and to my dear pastor, Moses Abraham Mairah. May you all live to enjoy the paramount delights and desires of this work for which you bore little distress.

ABSTRACT

Microorganisms play a crucial role in various aspects of our lives, including food production and safety. One such area where microorganisms are of significant importance is in the production of milk. Milk is a highly nutritious and widely consumed beverage that serves as a source of essential nutrients for humans. However, it's also an ideal medium for the growth and proliferation of microorganisms, which can have both positive and negative effects on its quality. This research study aimed at understanding the bacterial spp. composition in fresh milk obtained from two different sources for example that obtained from local milk vendors and that from urban dairies located within Tororo Municipality. The methods that were employed in this study involved an experimental research design in which microbes were cultured in the Busitema University Biology laboratory using different milk samples collected aseptically from Tororo town using an ice box with ice parks to prevent further contamination during transportation. The collection of milk samples involved a random selection of local milk vendors and Diary outlets in which a single sample of 100ml of fresh milk was collected from 3 local milk vendors and 3 dairy outlets within Tororo Municipality plus one milk sample from UV-treated milk from **Lato Milk Company**. This gave a total of n=7 samples at each time of sampling. Each sample was diluted to make 3 more replicates. 21 petri dishes of Nutrient agar (NA) were each inoculated with microbes from milk samples from all the **three** sources. Pure cultures of the bacterial colonies made on TSA (Tryptic Soy Agar) were Gram stained before microscopy. Gram staining helped in classifying the bacteria into two groups of Gram positive and Gram-negative bacteria based on their ability to retain crystal violet, the primary stain. The petri plates were incubated at 37°C for 24-48 hours. Colonies on the petri dishes were counted using the total plate count technique and the plates having colonies were recorded. Data collection involved the physical counting of bacterial colonies based on colony color, colony characterization based on phenotypic characteristics such as colony morphology, elevation and shape/form using the Total plate count technique so as to obtain numerical data that was tabulated in statistical tables and graphs. This data was used to calculate the microbial load in all the milk samples. Data was analyzed using Microsoft Excel software where numerical data was entered and different graphs and pie-charts were drawn and used to describe various relationships between variables. It was discovered that milk from local vendors had the greatest microbial diversity of 96.1 CFUs/100ml of sample, milk samples from dairy outlets constituted 56.3

CFUs/100 ml of sample meanwhile that from UV-treated milk gave the lowest bacterial load of 15.5 CFUs/100 ml. In conclusion, all stakeholders involved in milk handling and processing were advised to implement proper milk handling practices such as use of clean milking, storage and handling equipment, ensuring proper housing of the animals and regular inspections and education of the people involved in milk production.

LIST OF ACRONYMS AND ABBREVIATIONS.

ACRONYMS

Probiotic bacteria. These are harmful bacterial species.

Proteolytic bacteria. These are bacteria that cause the process by which amino acids are converted into flavor complexes.

Psychrotrohic bacteria. These are bacteria that are able to withstand very low temperatures.

ABBREVIATIONS

FAO – Food and Agriculture Organization.

LAB – Lactic Acid Bacteria.

ANOVA – Analysis of Variance.

UN – United Nations.

I.D – Identification Number.

CFUs – Colony Forming Units.

ml - milliters.

NA – Nutrient Agar.

PDA – Potato Dextrose Agar.

TSA – Tryptic Soy Agar.

TABLE OF CONTENTS.

LIST OF TABLES.

LIST OF FIGURES.

LIST OF PLATES.

1 CHAPTER 1. INTRODUCTION

1.1 Background

Microorganisms play a crucial role in various aspects of our lives, including food production and safety(Caplice and Fitzgerald 1999). One such area where microorganisms are of significant importance is in the production of milk. Milk is a highly nutritious and widely consumed beverage that serves as a source of essential nutrients for humans. However, it's also an ideal medium for the growth and proliferation of microorganisms including bacteria and fungi (yeasts and molds), which can have both positive and negative effects on its quality.

Fresh milk contains various types of fungi including; **Aspergillus**, a genus that includes several species such as *A. flavus, A. niger,* and *A. versicolor* which produce mycotoxins, which are toxic compounds that can be harmful to human health. **Penicllium:** *P. chrysogenum* a species that can grow in fresh milk and produce penicillin, a widely used antibiotic. However, other species of penicillin such as *P. roqueforti* and *P. viridicatum,* can grow in fresh milk and produce mycotoxins. **Mucor:** *M. cacinelloides* is a species of Mucor that can grow in fresh milk and produce mycotoxins. **Rhizopus:** *R. stolonifer* is a species of Rhizopus that can grow in fresh milk and produce mycotoxins. It is worth noting that the presence of these fungal species in fresh milk is not always a cause of concern, as many of them can be present in low levels without causing any issues. However, if levels of these fungi become too high, it can lead to spoilage and potentially harm human health. Therefore, it's important to ensure that milk is stored and handled properly to prevent the growth of these fungi.

The diversity of microorganisms present in milk can vary depending on various factors, including the source of the milk (Kim and Yi 2020). In this study therefore, insight will be gained into how different production methods and environments influence the microbial composition of milk. Fresh milk from farms refers to milk that is obtained directly from cows and other animals on the farm. This type of milk is typically produced using traditional farming practices, where animals are raised in open pastures and fed natural diets (Heckman 2019).

On the other hand, milk in dairies refers to milk that is collected from multiple farms and processed in a centralized facility. Dairy farms often employ modern production techniques, including automated milking systems and controlled feeding regimes (Clark, Caradus et al. 2007).

Understanding the diversity of microorganisms in fresh farm milk compared to dairy milk is important for several reasons.

Firstly, it can provide valuable insights into potential risks associated with consuming raw or minimally processed milk. Raw milk has been linked to outbreaks of foodborne illnesses caused by pathogenic bacteria such as *Salmonella, Escherichia coli* (E. coli) and *Listeria monocytogenes* (Jayarao and Henning 2001). Therefore, by comparing bacterial profiles of fresh farm milk and dairy milk, potential differences in contamination risks can be assessed.

Secondly, this study will help to shed light on the impact of different production practices on milk quality. The presence of certain microorganisms in milk can affect its taste, texture, and shelf-life (Singh and Cadwallader 2004). For instance, the growth of spoilage bacteria can lead to the off-flavors and reduced shelf-life, while the presence of beneficial bacteria can contribute to the development of desirable flavors and enhance the nutritional value of milk.

1.2 Problem statement.

There was need to understand the microbial composition in fresh milk obtained from different sources for example that obtained from local milk vendors and that collected from urban dairy outlets located within Tororo Municipality so that studies could be conducted to investigate their microbial diversity and also provide valuable insights into potential variations that may exist and their implications to milk quality.

1.3 Justification

Milk is a highly perishable product that can serve as a medium for the growth of microorganisms, including bacteria, yeasts, and molds. Some of these microorganisms can be harmful to human health, causing foodborne illnesses such as salmonellosis or listeriosis (Gourama 2020). Therefore, by comparing the microbial diversity between farm – produced milk and dairy – processed milk, there is need to identify potential sources of contamination and develop strategies to mitigate the risks associated with the microorganisms.

Also, since farms and dairies employ different practices in milk production, such as feeding regimes, milking techniques, and storage conditions (O'Mahony 1988). These variations can influence the microbial composition of fresh milk. So, by conducting this study, there is need to determine whether certain production methods favor the growth of specific microorganisms or

affect overall milk quality. This knowledge will help optimize production processes to ensure safer and higher quality milk.

1.4 Scope of the study.

This study was conducted in a period of 2-3 months based on its protocol in order to be able to obtain desirable results. This time also catered for the irregularities and in conveniences that came under way.

1.5 Research questions.

1. What is the microbial diversity in fresh milk obtained directly from farms and Dairies?
2. How does the microbial diversity of milk obtained directly from farms compare to that in milk processed in dairies?
3. What factors contribute to variation in microbial diversity between fresh farm milk and milk obtained from Dairies?

1.6 Hypotheses

1. Null hypothesis.

   There is no significant difference in the diversity of microbes between fresh milk from local milk vendors and milk obtained from urban dairy outlets in Tororo Municipality.

2. Alternative hypothesis.

   There is a significant difference in the diversity of microbes between fresh milk from local milk vendors and milk obtained from urban dairy outlets in Tororo Municipality.

1.7 Objectives of the study

1.7.1 General objective

To investigate microbial load in milk products obtained freshly from farmers and from urban dairies of Tororo Municipality.

1.7.2 Specific objectives.

1. To characterize microbes, present in fresh milk from farmlands (including bacteria and fungi).
2. To characterize microbes, present in fresh milk obtained from urban Dairies of Tororo Municipality.
3. To identify and document management processes in handling milk products.

1.8 Significance of the study.

This research study will allow for a comprehensive understanding of the microbial diversity in milk, which has implications for food safety, quality control, and public health. In relation to food safety, understanding the diversity of microbes in fresh farm milk and that processed in dairies will

help to identify potential pathogens and develop appropriate control measures to ensure safety of milk.

Furthermore, comparing the microbial diversity in fresh milk will contribute to quality control in the dairy industry. The presence of microbes can affect the sensory attributes, shelf life, and nutritional value of milk. Therefore, this knowledge can be used to implement measures to maintain or improve the overall quality of dairy products.

In addition to food safety and quality control, this study will be significant as it will have implications for public health. Milk is a staple food consumed by people worldwide, and understanding its microbial composition is crucial for assessing potential health risks associated with its consumption. Therefore, by gaining insight into the prevalence of beneficial microbes such as **probiotics** or potentially harmful ones such as anti-biotic resistant bacteria, strategies may be developed to ensure the safety and quality of dairy products, improve production methods, and protect public health.

2 CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

According to this chapter, it provides insight into the microbial composition in milk, the possible causes of contamination of milk and the gaps in the existing literature that will call for a more detailed analysis through conducting studies.

2.2 Microbial composition in fresh milk

According to a report by the Food and Agriculture Organization (FAO) of the United Nations, more than 150 million house-holds globally are involved in milk production. According to FAO statistics, global milk production rose by over 200 million tones between 1983 and 2013 (Issa and Tahergorabi 2019). Although the larger percentage of commercially available milk is from cows, there are a number of other mammals that are reared for their milk. Some of these animals include buffalos, goats and sheep. Camel milk is also popular in certain African and Arabic regions (Faccia, D'Alessandro et al. 2020). It's important to note that milk and other dairy products are important economic activities for millions around the globe (Staal, Delgado et al. 1997). As such, understanding the microbial composition of milk is essential, both from a food safety and spoilage perspective, and is indispensable in the dairy industry.

Raw milk is highly nutritious and provides an ideal medium for proliferation of a diverse population of microorganisms (Issa and Tahergorabi 2019). Many of these microbes are technologically relevant, particularly in the dairy industry. Nevertheless, the regulations governing the dairy sector in many countries require dairy products to be processed from pasteurized milk (Douphrate, Hagevoort et al. 2013). In essence, such guidelines signify that certain microbes that promote natural fermentation and those that influence the taste and texture of the intended end product have to be reintroduced into the treated milk as staters.

Some of the relevant genera of bacteria (health-promoting) that can be isolated from raw milk. These classes of bacteria, commonly known as probiotics, are live bacteria, which, when in sufficient quantities, present health benefits to their hosts (Quigley, O'Sullivan et al. 2013).

Lactoccocus which consists of the several genera forming the Lactic Acid Bacteria (LAB family). This genus is a homofermenter that ferments sugars, releasing only one bi-product, lactic acid (Hart 2005). Although these microbes are present in raw milk, they are reintroduced in pasteurized milk because the milk sterilization process significantly reduces the *Lactococci* population in raw

milk. In addition, the *Lactococcus* genus is responsible for proteolysis, a process in which amino acids are converted into flavor complexes.

The *Bifidobacterium* genus is an essential probiotic group in dairy products. Probiotics produce bacteriocins which are bioactive peptides that display antimicrobial characteristics and thus help in the preservation of fermented dairy products (Fernández, Hudson et al. 2015).

Streptoccoccus genus consists of more than 90 species, the majority of which are pathogenic. Nevertheless, the *S. thermophilus* is of technological relevance in the dairy industry. This Streptococcus strain is defined as a thermophilic LAB and a vital starter in the adjunct in the fermentation of milk. Additionally, this *Streptococci* species releases exopolysaccharide, which aids in attaining desired viscosity and texture of yoghurts.

Lactobacillus genus extremely is diverse, with over 170 species and 27 subspecies documented. *Lactobacilli* are prevalent in environments rich in carbohydrates such as milk. These microbes are extensively employed in the dairy sector as starter adjuncts to pasteurized milk to facilitate fermentation and produce aroma.

Propionibacterium. It's important to distinguish between two major groups of this genus. *Propionibacterium* is differentiated in relation to where they are found. The first group of propionibacteria colonizes the human skin. The second strain, often taken as *classic* propionibacteria is found in raw milk. It is used in the making of cheese because it ferments lactose to acetate, propionate, and carbon dioxide and at the same time enhances flavors through lipolysis of fatty acids.

There pathogenic bacteria found in milk that may lead to health complications on consumption of unpasteurized milk. Milk borne illnesses range from slight gastrointestinal disturbances to serious and life- threatening conditions.

Coliform bacteria are a group of bacteria that are commonly used as indicators of sanitation and hygiene practices during milk production. *Escherichia coli* (E. coli) is an example of the coliform bacteria that can be found in raw milk and may cause food borne diseases through contamination from infected udders or from human handlers (Chambers 2002).

Psychrotrophic bacteria are a group of microorganisms that are capable of withstanding and proliferating under extremely low temperatures (Issa and Tahergorabi 2019). This unique property is a major cause of concern with regard to spoilage. In milk, the major psychrotrophic populations is of the genus *Pseudomonas,* specifically *Pseudomonas fluorescens. P. fluorescens* is a constant headache in the dairy industry as it employs the **biofilms**, which aid in their persistence. The ability to alter the phenotype of their biofilm allows psychrotrophs to withstand environmental stresses such as reduced temperatures.

Mycobacterium bovis is the bacterium responsible for **bovine tuberculosis**. While rare, it can present in raw milk from infected cows. Consumption of raw milk contaminated with this bacterium can lead to tuberculosis in humans.

Listeria monocytogenes is a pathogenic bacterium that can survive and grow in refrigerated environments, making it a concern for raw milk consumers. it can cause **listeriosis,** a serious infection that primarily affects pregnant women, newborns, the elderly, and individuals with weakened immune systems.

Salmonella is another pathogenic bacterium that can be present in raw milk. It is a leading cause of foodborne illnesses worldwide and can cause symptoms such as fever, abdominal pain, and diarrhea. *Salmonella* contamination of milk can occur through contact with infected animals or contaminated equipment.

Staphylococcus aureus is a pathogenic bacterium that can be found in raw milk. Its responsible for causing **staphylococcal** food poisoning, which is characterized by symptoms such as nausea, vomiting and diarrhea. This bacterium can enter raw milk through contamination from infected udder. This bacterium also causes **bovine mastitis** (Bogni, Odierno et al. 2011)**.**

Campylobacter is a genus of bacteria that can be found in raw milk and is a common cause of bacterial **gastroenteritis.** Ingestion of milk containing this bacterium can lead to symptoms such as diarrhea, abdominal pain, and fever.

Brucella. **Brucellosis** is a milk borne disease that can be transmitted to humans through direct contact with an infected animal or consumption of milk contaminated by the *Brucella spp.* Brucellosis may result in acute fever, which might develop into a chronic and incapacitating complication.

2.3 Microbial contamination sources (sources of milk organisms).

Milk offers a conducive milieu for microbial activity. Nevertheless, there is a general acceptance that milk production in healthy mammary glands is sterile and only becomes contaminated once it comes into contact with the external environment (Issa and Tahergorabi 2019).

1. Contamination in the mammary glands. Milk contamination can occur both in the udder and outside. Although milk from a healthier udder is thought to be sterile, it has a low bacterium count of not more than 1000 microorganisms in a milliliter of milk (Wallace 2009).
2. Contamination sources in the external environment. In the exterior of the mammary glands, microbial contamination of milk is through a number of sources. To begin with, milk can be contaminated by bacteria associated with teats (Bonfoh, Wasem et al. 2003). In the case of the cow, the udder and teats are colonized by bacteria that are either associated with the animal's skin or present in the environment in which the animal is kept while milking.
3. Contamination from handling and storage equipment. Milk handling equipment is an ideal environment for the growth of bacteria and if not properly cleaned may contaminate raw milk from healthy organisms. Those microorganisms present in the water used for cleaning milk handling equipment may also colonize the equipment and end up contaminating milk.

2.4 Gaps in knowledge

From the reviewed literature, studies have been conducted by members of the science community about the various microorganisms that affect milk leading to its deterioration in terms of quality and shelf-life, their composition, the different illnesses that they cause to human health when contaminated milk is consumed by the people and then the possible methods of milk preservation (Li, Wang et al. 2018).

However, little interest had been put in trying to compare the diversity of some of these microbes in milk from different sources where consumers can easily access it. Therefore, there was need to conduct a comparative study to identify microbial load in milk obtained from different areas of production so that this information obtained will help future researchers to improve on milk quality by designing better milk production and handling techniques.

3 CHAPTER THREE: METHODOLOGY OR MATERIALS AND METHODS.

3.1 Study site.

This study was conducted in Tororo Municipality with coordinates 00 41 34N, 34 10 52E which is located in Eastern Uganda and only 10 km from Malaba border with Kenya. The town is about 230 Km from Kampala Capital city of Uganda. This study was focused in this area because it is the one where milk production and supply is common. This is evidenced by the large number of cattle farmers within the area.

3.2 Sample collection.

The milk samples were collected aseptically **three** times into sterile Duran bottles for 3 consecutive weeks. Samples were stored in a freezer with ice packs or an ice box and transported to Busitema University Biology Laboratory for analysis within 24 hours. A total of **6** milk samples were collected at each time of sampling (i.e 100ml per sample for each sampling), from **two** different milk sources of local milk vendors and that from selected Dairy Outlets located within Tororo Municipality. Therefore, **3** milk samples were taken from three different local milk vendors and **3** milk samples were taken from three different selected urban Dairies plus **1** other sample from UV -treated milk of **Lato Milk Company** which was treated as a control sample for the study. The total number of samples per sampling was; n = 7 at each time of sampling.

3.3 Sample size and determination

The sample size determination involved random selection of local milk vendors and Dairy Outlets that constituted the study within the study area. A total of **7** milk samples were collected from both the local milk vendors and Dairy Outlets. Those milk samples from the local milk vendors were assigned an identification number (I.D) of A_1, A_2, and A_3. While those milk samples that were collected from Dairy outlets were assigned an I.D number of B_1, B_2, and B_3, respectively. The control milk sample C was also replicated to give C_1, C_2 and C_3 respectively. Samples A and B were further replicated through serial dilution to give replicates R_1, R_2 and R_3 respectively giving a total sample space of n =21 samples (petri dishes). Each milk sample was replicated in order to reduce bias and cater for any possible errors during the experiment.

3.4 Study design.

This study was an experimental research design where milk samples collected from the two different milk sources were clustered into pairs and then analyzed in the lab for microbial activity in milk through following the protocol of culturing microbes from each of the samples and then

comparing their composition by basing on phenotypic traits or colony morphological characteristics such as colony color, form, margin, and elevation on the agar plates.

3.5 Laboratory procedures

The experimental procedure involved culturing bacteria in the lab typically follows a series of steps to ensure the growth and maintenance of bacterial cultures. These steps include;

- **Sterilization**. In order to prevent further contamination, all equipment were sterilized occasionally by physical heating in an oven at 110 °C for glassware for a period of about 16 minutes, 15Ibs pressure and high temperature of 120 °C using an Autoclave for 15 minutes. This was for sterilization of glassware such as petri dishes or agar plates, Mc Cartiney bottles, Duran Bottles etc. Sterilization by chemical means involved using 70% ethanol.
- **Preparation of culture media**: culture media used for culturing microbes involved Nutrient Agar (NA) a general growth medium and Tryptic Soy Agar (TSA) a pure culture medium used to support the growth of certain strains of microbes.

  23 g of NA were suspended in 1 liter of distilled water, mixed well to dissolve at each time of preparation and then boiled in a sauce pan while stirring to completely dissolve the mixture. The solution was dispensed in two Duran bottles and sterilized in an Autoclave with their stoppers loosened to allow for expansion at 121°C for 15 minutes. After sterilization, the medium was allowed to cool to a temperature of about 50°C. After cooling to appropriate temperature, the medium was then poured on petri plates and then it was allowed to solidify.

Plate 1: Equipment used for preparation of culture media.

- **Inoculation**. Serial dilutions of the milk samples were prepared by mixing stock solution (milk samples) in diluent like buffered peptone water or sterile saline to make 100 ml of the sample for all samples. During dilution, 10 ml of sample were transferred from the diluting tubes until the concentration of microbes lowered and this was done three times for each sample to make three different replicates.

  Microbes were then introduced onto the culture medium by streaking. In streaking, a sterile inoculation loop was used to streak the surface of an agar plate with the bacterial sample back and forth all over the agar surfaces and each petri dish was sealed with parafilm and labelled appropriately.

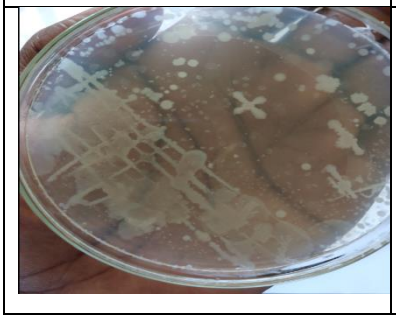
Streaked petri dish	Sealed petri dishes	Serial dilution tubes
		

Plate 2: Steps involved in introduction of microbes into culture media.

- **Incubation**. After inoculation, the culture plates were placed in an incubator set at an optimal temperature for microbial growth of about 37°C. This was done for a period of 24 – 48 hours to allow the growth and multiplication of microbes.

  After incubation, the petri dishes were removed and then each was analyzed separately for microbial growth through observation of the different colonies. The microbes were characterized and quantified based on colony morphological characteristics such as colony color, margin, elevation form and shape. The results obtained were tabulated in tables.

Plate 3: Incubation of microbes to allow for their growth after inoculation.

- **Sub culturing/ preparation of pure culture**. This involved transferring a small number of bacteria from Nutrient Agar medium to a fresh culture medium, TSA. TSA was prepared by weighing 20g of medium and transferring 1000ml of distilled water and the mixture was boiled in a hot sauce pan until the broth was completely dissolved. The solution was allowed to cool to around 45 – 50°C and then poured in Duran bottles and then sterilized in an Autoclave for 15 minutes. The broth was then removed and allowed to solidify. Pure strains of microbes were

then transferred from the Nutrient Agar plates onto TSA plates using sterile inoculating loops, stoppered tightly and then incubated for a period of 24 -48 hours. Pure strains were prepared from a single replicate of every sample for example sample $A_1(R_2)$.

After incubation, the pure cultures were then removed and pure bacterial strains were Gram stained for identification of the different bacterial shapes under the microscope. The results of the bacterial shapes and the different bacterial strains either Gram positive or Gram Negative were recorded.

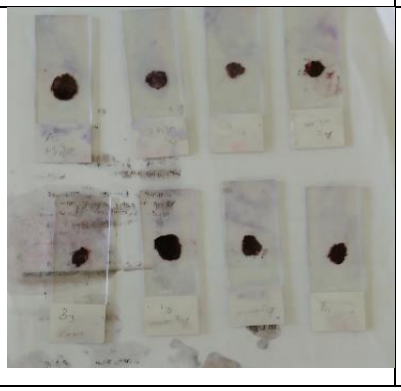
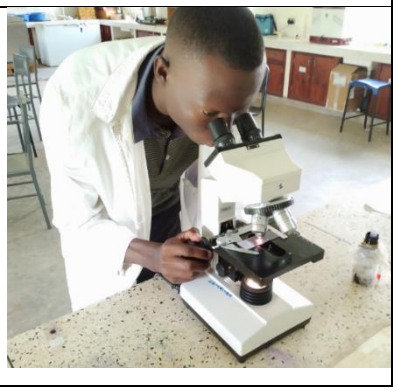
Preparation of pure cultures	Preparation of smears/slides	Microscopic observation.
		

Plate 4: Preparation of bacterial smears to be viewed under the microscope.

Gram staining of the bacteria isolated from different colonies.

Protocol.

- Bacterial smears of the different bacterial colonies were prepared on microscopic slides by picking bacterial strains from the Duran bottles using a sterile inoculation loop and spreading them on a microscopic slide. The slides were then heat-fixed for 1 minute by passing them over a heat source after which they were air – dried for 3 minutes.
- The air-dried, heat fixed smear of cells were flooded for 1 minute with crystal violet staining reagent. The slides were then washed in a gentle and indirect stream of tap water for 2 seconds and after wards slides were flooded with the mordant; Gram's iodine for 2 seconds.
- The slides were flooded with decolorizing reagent, ethanol for 15 seconds drop by drop until decolorizing agent running from the slide became clear. The slides were immediately flooded with counter stain; safranin for 1 minute after which they were washed in a gentle

and indirect stream of tap water until no colors appeared in the effluent and then blot dried with absorbent paper.

- The results of the staining procedure were observed under an oil immersion using a bright field microscope. At the end of the Gram staining, bacteria were stained pink/red. Gram positive bacteria retained crystal violet stain in the gram staining technique and they appeared **purple or blue** and they include members of the LAB family whose role is to preserve milk and give it a sour taste such as *Lactococcus lactis, Streptococcus thermophilus* and *Lactobacillus spp.* Gram negative bacteria did not retain the crystal violet stain during decolorization step and took up the red safranin counterstain and appeared **red or pink** after counterstaining with safranin. Examples include; *E. coli, Salmonella spp* and *Pseudomonas spp.*

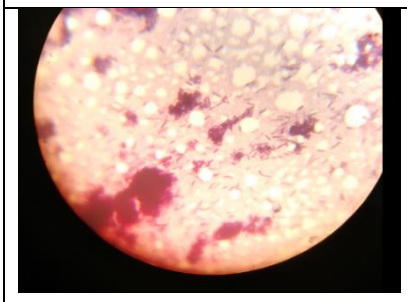
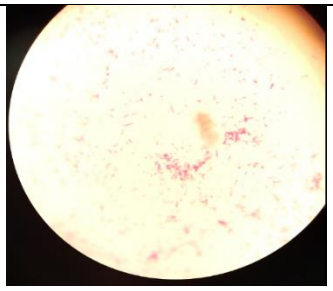
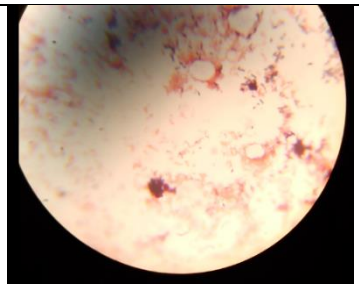
Spherical-shaped (cocci) (Gram positive bacteria)	Rod-shaped (bailli) (Gram negative bacteria)	Spherical cells arranged in chains (streptococci) (Gram negative bacteria)
		

Plate 5: Different colours of bacterial colonies after Gram staining as observed under the microscope.

3.6 Data collection.

The quantitative data was collected by counting the number of colonies using a colony counter technique or the total plate count method (standard plate method) in which the total number of viable microorganisms in a sample can be estimated. This method is used in calculating the total microbial load in Colony Forming Units per ml of sample.

The total number of bacteria cultured was determined by counting different bacteria colonies on agar plates which were distinguished by different colors which were as a result of production of pigments by microbes due to their metabolic activity for example *micrococcus luteus* and *Staphylococcus aureus* produce yellow pigments forming yellow colonies while most bacterial species do not form colonies on agar plates and these include; *Escherichia Coli and Staphylococcus.* The total number of microbes counted were tabulated in appropriate tables.

The results obtained after Gram staining showed the possible shapes of the pure bacterial strains and their ability to retain crystal violet stain. This staining technique was used to identify the Gram-positive and Gram-Negative bacteria.

3.7 Data presentation

The data was entered into graphical software majorly Microsoft Excel to be able to create various types of graphs, including bar charts, stacked bar graphs, pie charts that were used to identify the patterns, trends and relationships between variables. The total plate count of bacterial colonies was made on each agar plate and then tabulated

The total bacterial load (bacterial species abundance/ diversity) in Colony Forming Units (CFUs) per 100ml of bacterial samples was determined using the formula;

$$\boldsymbol{microbial\ load} = \frac{\boldsymbol{total\ number\ of\ colonies\ counted}}{\boldsymbol{dilution\ factor}} \times \frac{\mathbf{1}}{\boldsymbol{volume\ plated}}$$

Or

$$\boldsymbol{microbial\ load} = \boldsymbol{total\ number\ of\ colonies\ counted} \times \boldsymbol{dilution\ factor}$$

Where; - Number of colonies counted refers to the total count of visible colonies on the agar plates.

- **Dilution factor** refers to any dilutions made during sample preparation.
- **Volume plated** refers to the volume of diluted sample plated onto the agar plate.

But:

$$dilution\ factor = \frac{volume\ of\ stock\ solution}{total\ volume(volume\ plated)}$$

$$= \frac{10ml}{100ml}$$

$$= 0.1$$

4 CHAPTER FOUR: RESULTS.

4.1 Data presentation.

A – Milk from local Dairy vendors.

B - Milk from Urban Dairy outlets.

C – UV – treated milk as a control.

4.1.1 QUANTIFICATION OF COLONIES:

Table 4. 1: Quantifications of bacterial colonies from all Milk samples after 1st sampling.

Colour of bacterial colonies.	Petri - dishes /Samples									**Total bacterial load.**
	A_1	A_2	A_3	B_1	B_2	B_3	C_1	C_2	C_3	
cream	34	13	30	22	22	34	-	-	-	155
white	49	61	120	45	55	41	-	07	04	382
yellow	-	-	-	-	-	-	-	-	-	-
Total bacterial load per sample	**307**			**219**			**11**			**537 Colonies**

Table 4. 2: Quantifications of bacterial colonies from all Milk samples after 2nd sampling.

Colour of bacterial colonies.	Petri - dishes /Samples									**Total bacterial load.**
	A_1	A_2	A_3	B_1	B_2	B_3	C_1	C_2	C_3	
cream	14	19	63	08	35	11	02	-	-	152
white	105	90	17	35	37	50	20	30	27	411
yellow	-	-	-	-	-	-	-	-	-	-
Total bacterial load per sample	**308**			**176**			**79**			**563 Colonies**

Table 4. 3: Quantifications of bacterial colonies from all Milk samples after 3rd sampling.

Colour of bacterial colonies.	Petri - dishes /Samples									Total bacterial load.
	A_1	A_2	A_3	B_1	B_2	B_3	C_1	C_2	C_3	
cream	28	36	34	32	11	24	-	-	-	165
white	98	97	53	32	32	37	20	15	30	414
yellow	-	-	-	-	-	-	-	-	-	-
Total bacterial load per sample	**346**			**168**			**65**			**579 Colonies**

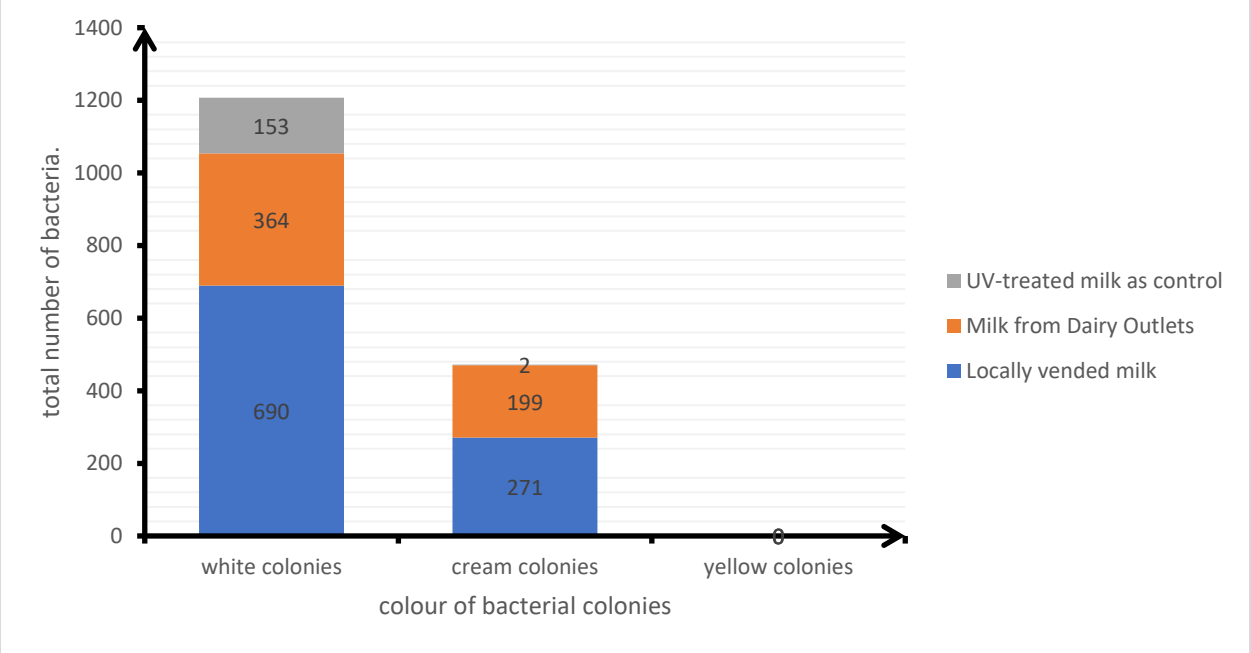


Figure 4. 1: A Stacked bar chart showing the number of colonies of different colors of all milk samples

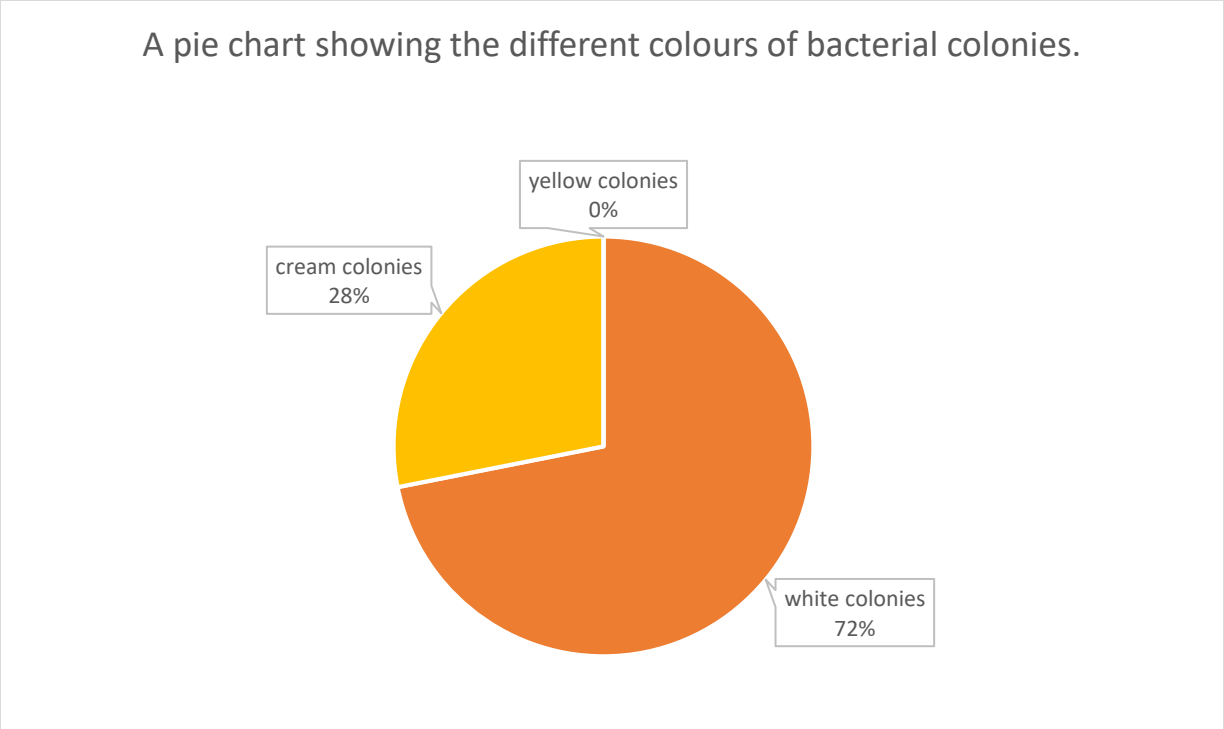


Figure 4. 2: A pie chart showing the different colors of bacterial colonies.

4.1.2 SUMMARY OF THE OVERALL BACTERIAL LOAD FOR ALL SAMPLES:

Table 4. 4: Overall number of bacteria counted for all the three samples**.**

Sample	**Total (overall) number of bacteria in all samplings.**			**Total bacterial colonies.**
	First sampling	Second sampling	Third sampling	
A	307	308	346	961
B	219	176	168	563
C	11	79	65	155
TOTAL.	**537**	**563**	**579**	**1,679 Colonies**

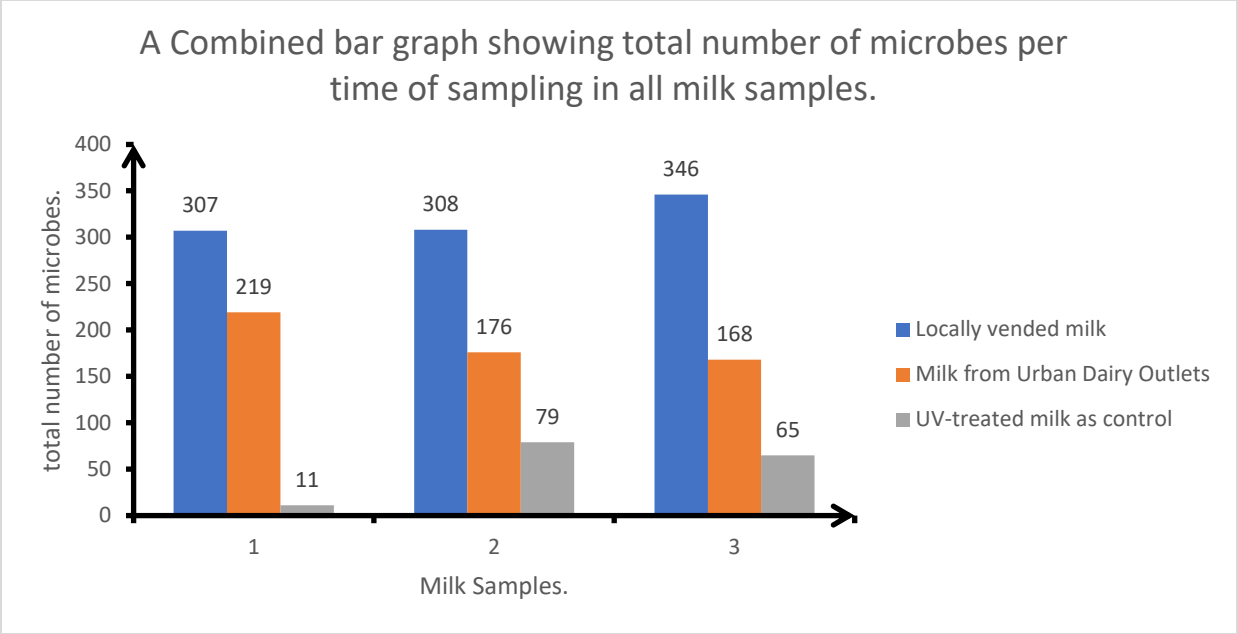


Figure 4. 3: A combined bar graph showing total number of microbes per time of sampling in all milk samples.

Using the formula for total microbial load;

$$\boldsymbol{microbial\ load = \frac{total\ number\ of\ colonies\ counted}{dilution\ factor} \times \frac{1}{volume\ plated}}$$

For milk sample A (locally vended milk)

$$= \frac{961}{0.1} \times \frac{1}{100ml}$$

$$= 96.1\ CFUs/100ml$$

For milk sample B (milk from urban dairy outlets)

$$= \frac{563}{0.1} \times \frac{1}{100ml}$$

$$= 56.3\ CFUs/100ml$$

For milk sample C (UV-treated milk as control)

$$= \frac{155}{0.1} \times \frac{1}{100ml}$$

$$= 15.5\ CFUs/100ml$$

Table 4. 5: showing the overall bacterial load in all milk samples.

Sample	Total(overall) bacterial load in all samplings.			Total bacterial load.
	1st Sampling	2nd Sampling	3rd Sampling	
A	30.7	30.8	34.6	96.1
B	21.9	17.6	16.8	56.3
C	11.0	79.0	65.0	15.5
TOTAL.	**53.7**	**56.3**	**57.9**	**167.9 CFUs/100ml**

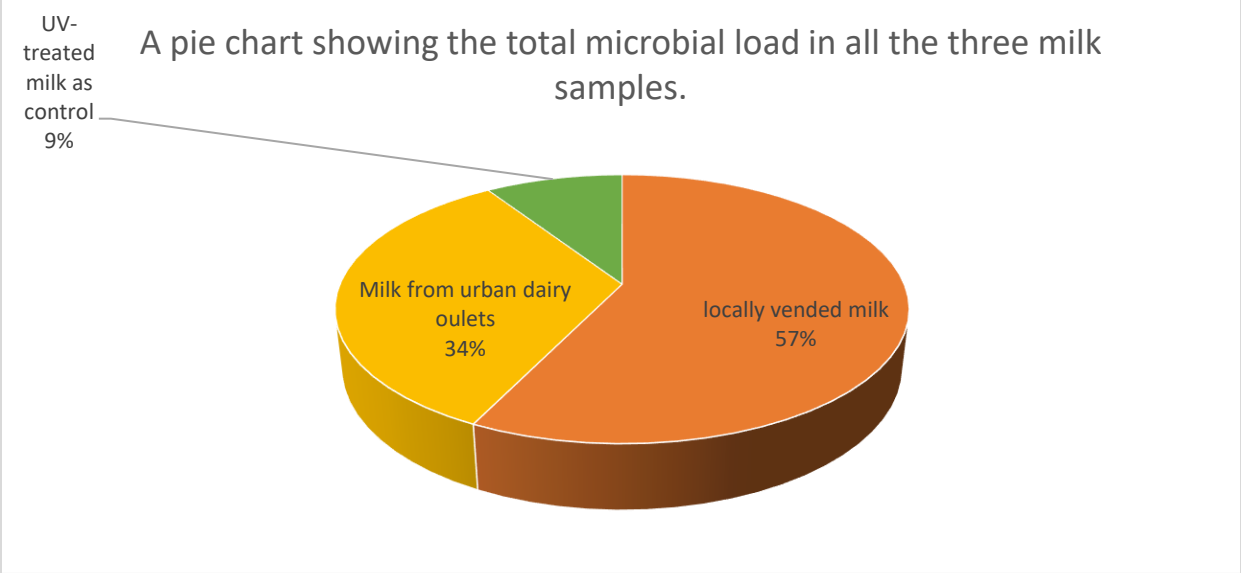


Figure 4. 4: A pie chart showing the total microbial load in all the milk samples.

4.1.3 AVERAGE BACTERIAL LOAD FOR ALL SAMPLES:

$$= \frac{53.7+56.3+57.9}{3}$$

$$= \frac{167.9}{3}$$

$$= \frac{168}{3}$$

= 56 CFUs/100 ml

4.1.4 RESULTS AFTER GRAM STAINING:

Table 4. 6: Results for Gram staining after first sampling:

SLIDE	Bacterial nomenclature (possible strains)	Gram positive	Gram negative	Morphology
A_1 *(white)*	*Pseudomonas aeruginosa* *Lactobacillus acidophilus* *Enterococcus faecalis*	× √ √	√ × ×	Rod – shaped cells with square ends and occurring as single cells or in colonies.
A_1 *(cream)*	*Staphylococcus aureus* *Escherichia coli* *Klebsiella pneumoniae*	√ × ×	× √ √	Circular (spherical or oval in shape, smooth colonies with distinct edges.

Table 4. 7: Results for Gram staining after second sampling:

SLIDE	Bacterial nomenclature (possible strains)	Gram positive	Gram negative	Morphology
B_3 *(white*)	*Salmonella spp* *Streptococcus spp for example streptococcus thermophilus.*	× √	√ ×	Cells are spherical in shape and occur as single or multiple cells in colonies arranged in chains.
B_3 *(cream)*	*Serratia marcescens*	×	√	The cells are straight or curved which occur as single cells or in stacks.

Table 4. 8: Results for Gram staining after third sampling:

SLIDE	Bacterial nomenclature (possible strains)	Gram positive	Gram negative	Morphology
C_3 *(white)*	*Streptococcus thermophilus* *Lactococcus lactis.*	√ √	× ×	The bacterial cells are circular or oval in shape. They occur in pairs(diplococci) or in multiple colonies in stacks or chains.
C_3 *(cream)*	*Micrococcus luteus*	√	×	Slightly elongated and spherical bacteria that occur in tetrads or irregular clusters.

NOTE:

1. $R_{1, 2, 3}$ and $C_{1, 2, 3}$ are replicates of samples $A_{1, 2, 3}$, $B_{1, 2, 3}$, and C.
2. $A_{1, 2, 3}$, $B_{1, 2, 3}$ and $C_{1, 2, 3}$ are replicates of samples A, B and C

5 CHAPTER FIVE: DATA ANALYSIS AND DISCUSSION OF RESULTS:

White and cream colonies were dominant on the Nutrient agar plates than yellow colonies. White colonies constituted the highest percentage of 72%, followed by cream colonies with 28% while yellow colonies constituted 0%. White and cream colonies were dominant because most bacterial species are lactose fermenters. These bacteria have the ability to ferment lactose in milk resulting in the formation of lactic acid and other compounds. The resulting colonies are white or cream-colored colonies depending on the species present. Bacteria of the **LAB** family such as *Lactococcus, Lactobacilli, Staphylococci, and Streptococci* are examples.

Yellow colonies were not evident on agar plates because few bacterial species produce colonies on Nutrient agar. Most of them produce pigments on selective media as a result of their metabolic activity. Only species like *Staphylococcus aureus* and *Micrococcus luteus* can produce yellow colonies on Nutrient Agar but these species are always rare in fresh milk since they are part of the natural microbial flora present in the environment and can only be found in low numbers even in properly produced and handled milk. Other colors are only produced by microbes when introduced on selective media with specific growth requirements of the species in question.

The total calculated microbial load in milk collected from local vendors was highest at 96.1 CFUs/100 ml of sample. This contributed 57% microbial load in all samples. This was then followed by milk collected from urban Dairy Outlets within Tororo Municipality having an overall microbial load of 56.3 CFUs/100 ml with a 34% contribution of microbes. Lastly, the microbial load in the UV-treated sample had the lowest total microbial load of 15.5 CFUs/100 ml of milk sample which contributed 9% microbes throughout the investigation.

Locally vended milk had the greatest number of microbes possibly because of the following reasons; many of the local dairy farmers and milk vendors within Tororo Municipality do not follow proper sanitation and hygiene practices. Most of them do not sterilize and disinfect their hands while handling milking equipment. Other local dairy farmers rarely clean the teats and udder of the milk animals before milking which provides a gateway for the microbes to inhabit the milk. The local milk vendors lack appropriate storage facilities for their milk before transportation to the consumers. Lack of proper refrigeration for milk renders it to microbial attack which may cause its spoilage. The various equipment used by these vendors are sometimes washed using contaminated water that may contain microbes like *E. coli, Salmonella* and many other microbes

that may easily contaminate the milk during milking. Other vendors dilute the milk with dirty water in order to increase on its quantity for them to be able to maximize profit. Lastly the dairy animals are exposed to microbes within their surroundings such as soil, beddings, water, and air coupled by their feeding regimes, may act as potential sources of contamination.

Milk collected from urban Diary outlets had the next highest bacterial load (mild load) because of the following reasons: During transportation and storage, milk may come into contact with additional microorganisms. Some of the Diary outlets do not maintain proper refrigeration temperature which encourages the rapid multiplication hence an increase in microbial load. Some dairy dealers collect their milk from local milk farmers whose hygiene and sanitation practices are un known, which makes it a possible cause for microbial presence in Dairy milk. Despite proper cleanliness and disinfection of the udder and teats of the dairy animals before milking, microbes are natural inhabitants of the teat canals and skin of these animals and therefore may not be completely eliminated.

UV- treatment is a technique that significantly reduces the microbial load in milk because it has a specific wavelength range known as the germicidal range of 200-280nm. This damages their DNA or RNA preventing them from reproducing and rendering them inactive or dead because it targets both vegetative cells and spores. UV-treatment does not completely eliminate all microbes because some have highly resistant spores and survive UV-treatment and remain after treatment. This is the reason for the presence of some microbes within the control sample though it contributed the lowest microbial load on computation.

Plate 6: Sachet of UV-treated milk used as control.

The results of the Gram staining technique that there was a variety of bacterial shapes within the milk samples ranging from single rod-shaped cells to many cells occurring as linear chains and stacks of cells while others were oval-circular(spherical) cells which occurred in large numbers as observed under the light microscope. Among these cells, there were those that appeared red/pink in the background while others appeared purple. Those that appeared purple were identified as pure strains of Gram-positive bacteria while those that appeared pink/red were identified as gram-negative bacteria.

5.1 Recommendations:

The following recommendations can be considered by all individuals in the milk production chain within Tororo Municipality from milk production to milk processing including the milking personnel, milk vendors, Dairy processors and the consumers including the Food Safety Regulatory Authorities in order to safety and quality of milk.

Good hygienic practices should be implemented. Implementing good hygienic practices will be crucial in preventing milk contamination with microbes. This includes proper personal hygiene for individuals involved in milk production and processing, such as wearing clean clothing, washing hands thoroughly before handling milk and maintaining overall cleanliness.

Regular cleaning and sanitization of milking equipment, storage containers and processing facilities will be essential in preventing the growth and spread of harmful microorganisms.

Proper milking techniques should be adhered to since they are vital to minimizing microbial contamination in milk. They involve ensuring that cows or other dairy animals are clean before milking as dirt of fecal matter can introduce harmful bacteria into the milk.

Milking equipment should be properly cleaned and sanitized before use, and single-disposable gloves should be worn during the milking process to prevent cross-contamination.

Adequate cooling and storage. Proper cooling and storage conditions are critical to inhibit microbial growth in milk. Milk should rapidly be cooled after milking to a temperature below 4°C to slow down bacterial growth. It is recommended to use dedicated refrigerated bulk tanks or cooling systems specifically designed for milk storage. These systems should be regularly cleaned and maintained at the appropriate temperature to ensure the freshness and safety of milk.

Regular testing procedures and quality control measures should be implemented to help identify any potential sources of microbial contamination in milk. This includes testing for the presence of pathogens and indicator organisms such as total bacterial counts.

Comprehensive training and education should be provided to all individuals involved in milk production, processing, and handling. This ensures that everyone understands the importance of preventing microbial contamination and follows proper protocols to maintain milk safety and quality.

Appropriate storage and packaging materials that are resistant to microbial contamination should be used. Dairy outlets can adopt pasteurization where milk is boiled to a certain temperature that kills the microbes hence reducing harm to the milk consumers.

Proper storage conditions should be maintained during transportation and distribution to minimize the risk of microbial growth such as use of mini freezers to store milk during transportation.

Traceability systems should be implemented to help identify the source of any potential microbial in milk. This will allow for quick identification and recall of contaminated products, minimizing the risk to consumers.

Regulatory authorities should carry out regular inspections to help ensure compliance with hygiene and safety standards by most of the milk dealers within the Municipality.

5.2 Challenges.

During this investigation, a number of challenges were encountered, some of which are listed below which the next investigators should be cautious of;

There was a challenge identifying some bacterial colonies on the agar plates due to over growth. Therefore, for better and consistent results, characterization and quantification of colonies should be made in time to avoid crowding of colonies on the agar plates.

Secondly, proper dilutions of the stock solution should always be made prior to inoculation to avoid the challenge of crowding of colonies as dilution reduces on the concentration of microbes within the stock solution.

There was also a challenge of identification of bacterial shapes after Gram staining. This is because of failure of spreading of bacterial strains on the microscopic slide. Therefore, there is need to properly spread the bacteria strains over the microscopic slide to ensure a uniform distribution of microbes over the slide. This eases visibility under the microscope.

Another challenge was encountered with the period of collection of samples. This should be done on the day of inoculation to avoid secondary contamination. During sample collection, they were collected a night before the inoculation which brought about inconsistence in the results.

Lastly, it was difficult convincing the Dairy milk dealers and the local milk vendors as they requested for anonymity for purposes of safety and not to spoil their customer base. So, they all require strict and tight guidelines for collecting such samples. This made the study costly because most of the milk dealers requested for money for 100ml of milk sample.

5.3 Conclusion.

In conclusion, milk locally vended, showed the highest microbial load, which was followed by a mild microbial load in milk collected from urban outlets within the Municipality. The control sample have the lowest microbial load.

REFERENCES.

1. Bogni, C., et al. (2011). "War against mastitis: Current concepts on controlling bovine mastitis pathogens." Science against microbial pathogens: Communicafing current research and technological advances: 483-494.

2. Bonfoh, B., et al. (2003). "Microbiological quality of cows' milk taken at different intervals from the udder to the selling point in Bamako (Mali)." Food control **14**(7): 495-500.

3. Caplice, E. and G. F. Fitzgerald (1999). "Food fermentations: role of microorganisms in food production and preservation." International journal of food microbiology **50**(1-2): 131-149.

4. Chambers, J. V. (2002). "The microbiology of raw milk." Dairy microbiology handbook: 39-89.

5. Clark, D., et al. (2007). "Issues and options for future dairy farming in New Zealand." New Zealand journal of agricultural research **50**(2): 203-221.

6. Douphrate, D. I., et al. (2013). "The dairy industry: a brief description of production practices, trends, and farm characteristics around the world." Journal of agromedicine **18**(3): 187-197.

7. Faccia, M., et al. (2020). "Milk products from minor dairy species: A review." Animals **10**(8): 1260.

8. Fernández, M., et al. (2015). "Impact on human health of microorganisms present in fermented dairy products: an overview." BioMed research international **2015**.

9. Gourama, H. (2020). "Foodborne pathogens." Food safety engineering: 25-49.

10. Hart, K. J. (2005). Evaluation of ensiled whole-crop legumes for ruminants, Open University (United Kingdom).

11. Heckman, J. R. (2019). "Securing fresh food from fertile soil, challenges to the organic and raw milk movements." Renewable Agriculture and Food Systems **34**(5): 472-485.

12. Issa, A. T. and R. Tahergorabi (2019). "Milk bacteria and gastrointestinal tract: Microbial composition of milk." Dietary Interventions in Gastrointestinal Diseases: 265-275.

13. Jayarao, B. and D. Henning (2001). "Prevalence of foodborne pathogens in bulk tank milk." Journal of Dairy Science **84**(10): 2157-2162.

14. Kim, S. Y. and D. Y. Yi (2020). "Components of human breast milk: From macronutrient to microbiome and microRNA." Clinical and experimental pediatrics **63**(8): 301.

15. Li, N., et al. (2018). "Variation in raw milk microbiota throughout 12 months and the impact of weather conditions." Scientific reports **8**(1): 2371.

16. O'Mahony, F. (1988). Rural dairy technology: Experiences in Ethiopia, ILRI (aka ILCA and ILRAD).

17. Quigley, L., et al. (2013). "The complex microbiota of raw milk." FEMS microbiology reviews **37**(5): 664-698.

18. Singh, T. and K. R. Cadwallader (2004). "Ways of measuring shelf-life and spoilage." Understanding and Measuring the Shelf-life of Food: 165-183.

19. Staal, S., et al. (1997). "Smallholder dairying under transactions costs in East Africa." World development **25**(5): 779-794.

20. Wallace, R. L. (2009). "Bacteria counts in raw milk." Dairy Cattle. Management: 1-4.