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Determination of Microbial Contamination in Raw Milk, Processed Milk, and Yoghurt Consumed in Mbarara City, Western Uganda

Akambamu Brian and Ampaire Wycliffe

Department of Physical Sciences, School of Natural and Applied Sciences, Kampala International University, Uganda

ABSTRACT

Milk, a staple in diets worldwide, is rich in essential nutrients like minerals, vitamins, proteins, and fats. However, its nutrient content makes it difficult to avoid the presence of microorganisms, which can affect its quality. This research aimed to assess the bacterial contamination content in raw milk, UHT, and yogurt, gathered from various retailers, markets, and supermarkets across Mbarara City. The study involved 36 samples of milk and milk products, mainly raw milk, UHT, or processed milk and yogurt. The tests done in the lab included finding *Escherichia coli* (*E. coli)*, staphylococcus, salmonella, and coliform, as well as the Total Plate Count (TPC). Biochemical tests, including gramme staining, were employed to isolate and identify bacteria in the samples. The findings indicated the presence of Staphylococcus spp. (25%), Salmonella spp. (33.3%), YM (41.6%), TPC (50%), coliforms (33.3%), and E. coli (25%). UHT milk had a percentage of *Staphylococcus spp.* (0%), *Salmonella spp.* (16.6%), YM (8.3%), TPC (16.6%), coliforms (16.6%), and E. coli (8.3%). Lastly, yogurt had *Staphylococcus spp.* (8.3%), *Salmonella spp.* (8.3%), YM (16.6%), TPC (25%), coliforms (16.6%), and E. coli (0%). In conclusion, detecting microbial contamination in raw milk and milk products suggests inadequate sanitary practices and poor storage practices. Consuming products contaminated by these toxic metabolites may result in food poisoning, and proper awareness among stakeholders and consumers is necessary.

Keywords: Microbial Contamination; Raw Milk; Processed Milk; Yoghurt; Mbarara City

INTRODUCTION

Globally, microbial contamination in dairy products poses significant risks to consumers, leading to foodborne illnesses and economic losses [1, 2]. The World Health Organization (WHO) and other international organizations emphasize the importance of ensuring the microbiological quality of dairy products to protect public health [3, 4]. At the regional level, in Africa, including Uganda, dairy farming and consumption are integral parts of the agricultural and dietary landscape. However, inadequate hygiene practices along the dairy production chain can result in microbial contamination of dairy products, leading to food safety and health concerns [5]. Studies across the African continent have highlighted the prevalence of microbial pathogens in dairy products and the need for effective control measures [6–8]. In Uganda, a country with a growing dairy industry, ensuring the safety of dairy products is a priority. The government, in collaboration with international agencies and research institutions, conducts surveillance and research to assess the microbial quality of dairy products consumed by the population. Uganda's National Bureau of Standards (UNBS) establishes regulations and standards for dairy product safety, including microbial criteria for raw and processed milk [9]. Within Uganda, Mbarara City in the western region is a significant hub for dairy production and consumption. The city's proximity to major dairy farming areas makes it a focal point for studying the microbial contamination of dairy products. Uganda has a deep-rooted culture of cattle husbandry, particularly prevalent in the southwestern region, where expansive herds of Ankole cows with long horns graze across the grassland. Milk and yogurt are essential components of the daily diet across the world, providing valuable nutrients such as proteins, vitamins, and minerals [10, 11]. In Mbarara City, located in the Western part of Uganda, these dairy products form a significant part of the local diet, contributing to the nutritional well-being of the population. Foodborne illnesses pose a significant threat to the population in Africa, contributing to 33-90% of child mortality cases [12–14]. While foods derived from animals may constitute a minor portion of most diets, they account for the majority of incidents related to

foodborne diseases, with dairy products being a common culprit [15]. Despite the nutritional balance provided by milk, it is widely recognized as a conducive medium for the growth of various microorganisms. Pathogenic bacteria present in milk are responsible for up to 90% of all diseases associated with dairy consumption [16, 17]. The production, processing, and distribution of dairy products are intricate processes that involve various stages, each susceptible to microbial contamination. Microorganisms such as bacteria, yeasts, and molds can compromise the quality and safety of dairy products, leading to adverse health effects when consumed [2] The western region of Uganda, including Mbarara City, is characterized by a growing urban population with an increasing demand for processed dairy products. As urbanization progresses, the risk of microbial contamination in dairy products may escalate, necessitating a comprehensive investigation to ensure the safety of these essential food items [2].

This research seeks to assess the microbial quality of processed milk and yogurt available in Mbarara City. By identifying and quantifying microbial contaminants, we aim to understand the potential health risks associated with the consumption of these dairy products. Additionally, the study intends to evaluate the adherence of local dairy processing facilities to recommended hygiene and safety standards, contributing valuable insights to the improvement of food safety regulations and practices in the region. The outcomes of this research will not only provide crucial information about the microbial safety of processed milk and yogurt in Mbarara City but also form a basis for targeted interventions to enhance the quality control measures within the dairy industry. Ultimately, ensuring the safety of dairy products aligns with broader public health goals and contributes to the overall well-being of the community.

MATERIALS AND METHODS

Materials and Equipment Used

The raw materials used were raw milk UHT milk and yoghurt, staining reagents (crystal violet, iodine, ethanol, safranin), 95% alcohol, peptone water, distilled water, equipment used include Petri dishes, micro pipets, weighing scale, spatula, test tubes, microscopes, incubators, autoclaves, centrifuges, pipettes, Bunsen burners, microbiological loops, safety cabinets, refrigerators, glass slides, sink for washing and heat fixation, cotton, hot air oven, air blower, thermometer, pair of scissors.

Description of the Area under the Study

Mbarara is a municipality nestled in the southwest of Uganda, approximately 167 miles (270 km) to the southwest of Kampala, with an elevation of around 4,850 feet (1,480 meters). It is connected by road to nearby areas such as Kikagati, Bushenyi, and Masaka. Mbarara City holds the distinction of being the second largest city in the country, following Kampala. The city comprises six divisions: Kakoba Division, Kamukuzi Division, Nyamitanga Division, Biharwe Division, Kakiika Division, and Nyakayojo Division. Serving as the primary commercial hub for numerous southwestern districts of Uganda, Mbarara also serves as the administrative center for the district. In a significant development, the city was granted official city status by Uganda's cabinet in May 2019, a status that officially commenced on July 1, 2020. According to the 2014 national census, the population of Mbarara City was 195,013. Mbarara City plays a significant role in the country's dairy industry. The city serves as a major hub for dairy processing and production, contributing significantly to Uganda's economy. Several factors contribute to Mbarara's prominence in the dairy sector: Mbarara is home to several dairy processing plants and factories that specialize in the production of various dairy products, including milk, yogurt, cheese, and butter. These facilities utilize modern processing techniques and equipment to ensure product quality and safety.

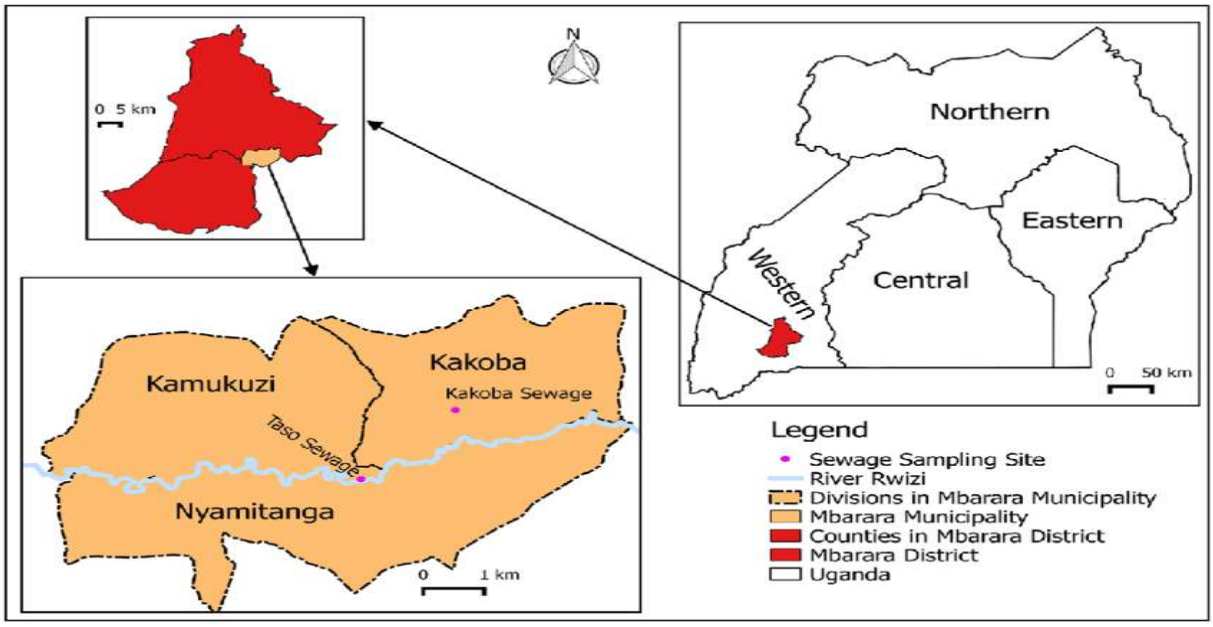

Figure 1. Showing Mbarara city and its divisions (source research gate)

Sample Collection

Selection of Sampling Sites

Sampling sites were within Mbarara city, located in the western part of Uganda and they were selected based on geographical distribution, including dairy processing facilities, retail outlets, and supermarkets where raw milk, processed milk, and yogurt that are sold in six divisions of Mbarara city.

Sampling Procedure

Six samples of two raw milk, two processed milk, and two of yogurt were bought from each of the selected divisions and 250g of each sample was bought Sampling was conducted at different times to ensure representative sampling, covering various batches and production periods and then they were kept under refrigeration before analysis.

Sample Preparation

Mixing

Upon collection, samples were thoroughly mixed to ensure homogeneity, especially for composite samples collected from multiple sources like milk and yogurt. For yogurt samples, mixing was carefully conducted to avoid damaging the product's texture and consistency.

Milk Preparation

Milk was first diluted before inoculation in microbiological testing to reduce the microbial load, facilitate accurate colony counting, and prevent overgrowth of fast-growing microorganisms. Five tubes were filled with 9 ml of sterilized normal saline solution. The milk sample underwent tenfold serial dilution, using a sterile normal saline solution. Initially, 1 ml of the raw milk sample was added to the first tube containing 9 ml of normal saline. Subsequently, 1 ml of this mixture was transferred to a second tube containing 9 ml of normal saline and this process was repeated for subsequent dilutions as shown below. The method used was the serial dilution method described by Pasteur, L. (19th century).

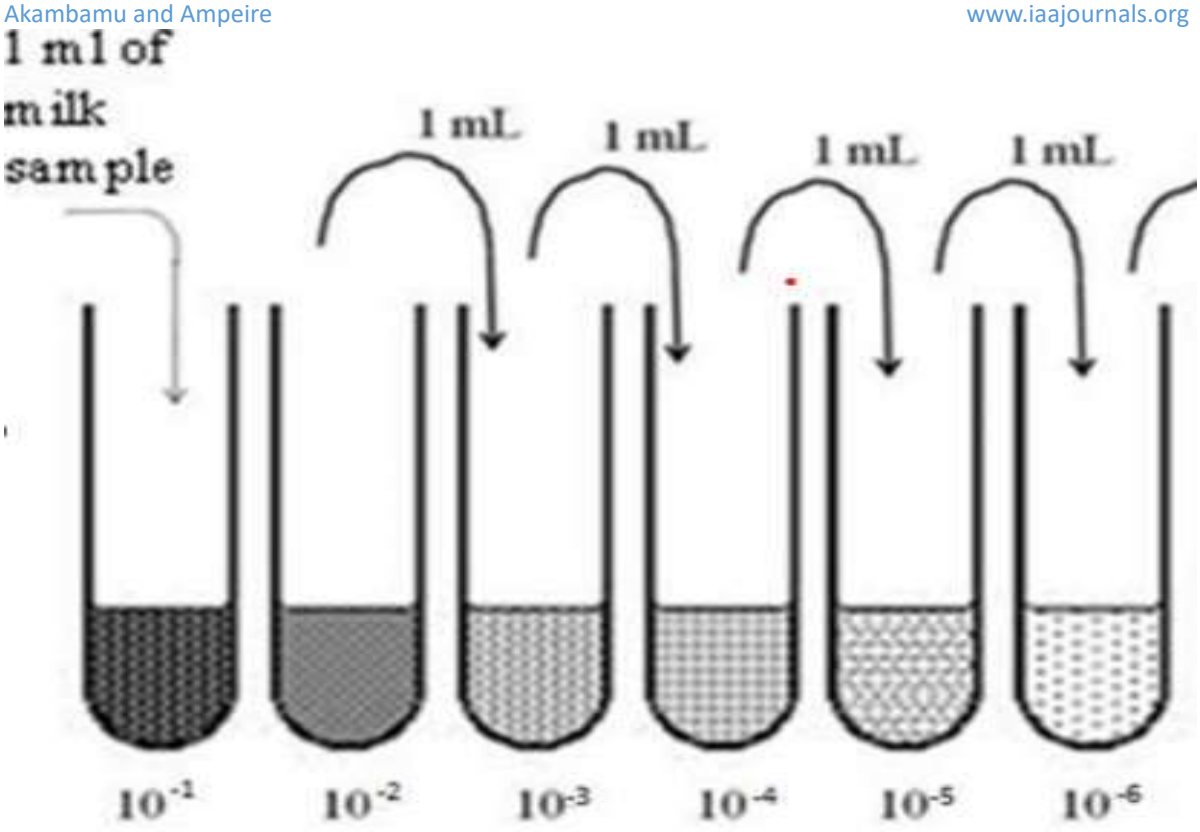

Figure 2. Serial dilutions of milk samples in sterile normal saline before inoculation (source research gate)

Microbiological Analysis Procedure

Media Preparation and Procedure

Different microbiological media to be used were prepared in liquid form because most of them are in powder form. These were converted to liquid form for easier handling, sterilization, and uniform nutrient distribution to support microbial growth, and these include;

Plate Count Agar (PCA)

The preparation of Plate Count Agar (PCA) involves several steps to ensure the growth of a wide range of microorganisms, making it suitable for total plate count analysis [18].

Procedure

23.5 grams of the PCA powder were dissolved in 1 litre of distilled water and mixed thoroughly. The mixture was heated gently to dissolve the components completely, the dissolved solution was sterilized by autoclaving it at high pressure and temperature to eliminate any potential contaminants. After sterilization, the agar was poured into sterile petri dishes and then mixed with the sample to form a uniform solution, the plates were then left to cool and solidify under lamina flow before being stored in the incubator. It was essential to maintain sterile conditions throughout the preparation process to prevent contamination, which could interfere with the microbial results.

Chloramphenicol Yeast Glucose Agar

Chloramphenicol yeast glucose agar (CYGA) is a type of agar medium used in microbiology for the selective isolation and cultivation of yeasts and molds. The addition of chloramphenicol, an antibiotic, to the medium helps inhibit the growth of bacteria, allowing for the selective growth of fungi.

Procedure

40.1 grams of the substance were dissolved in 1 litre of distilled water and heated the mixture ensuring complete dissolution of the solution after, sterilized it by autoclaving at a pressure of 15 pounds per square inch (121°C) for 15 minutes. After sterilizing, the agar was left to cool to approximately 45-50°C. Then, poured the agar into sterile Petri dishes mixed with a sample, and allowed it to solidify before storing it in an incubator.

Xylose-Lysine Deoxycholate Agar (XLD)

(XLD) is a specialized medium designed for the isolation and quantification of Salmonella Typhi and other Salmonella strains

Procedure

56.6 grams of the medium were dissolved in 1 litre of distilled water and applied heat to the mixture while stirring regularly until it dissolved, the dissolved solution was sterilized by autoclaving it at high pressure and temperature to eliminate any potential contaminants. After sterilization, the agar was poured into sterile petri dishes and then mixed with the sample to form a uniform solution, the plates were then left to cool and solidify under lamina flow before being stored in the incubator.

Violet Red Bile Agar (VRBA)

Violet Red Bile Agar is a specialized medium employed for isolating, identifying, and quantifying coli-aerogenes bacteria present in water, milk, various dairy products, and clinical specimens.

Procedure

41.53 grams of the medium were dissolved in 1000 milliliters of distilled water and applied heat and stirring until the medium was fully dissolved, the dissolved solution was sterilized by autoclaving it at high pressure and temperature to eliminate any potential contaminants. After sterilization, the agar was poured into sterile petri dishes and then mixed with the sample to form a uniform solution, the plates were then left to cool and solidify under lamina flow before being stored in the incubator.

Baird-Parker Agar

Baird-Parker agar is a selective and differential medium used for the isolation and enumeration of Staphylococcus aureus from food and clinical samples. It contains ingredients that inhibit the growth of most bacteria, allowing for the selective isolation of Staphylococcus species. Additionally, the medium contains egg yolk tellurite emulsion, which is essential for the detection of coagulase-positive Staphylococci, particularly Staphylococcus aureus.

Procedure

63 grams of Baird-Parker Agar were dissolved it in 1000 milliliters of distilled water by boiling. Sterilized the solution by autoclaving at 121°C for 15 minutes. After it was cooled to 47°C, and introduced 50 milliliters of Egg Yolk Tellurite Emulsion (SR0054) and thoroughly mixed. Then poured the mixture into sterile Petri dishes.

MacConkey Agar

MacConkey Agar is a selective and differential culture medium commonly used in microbiology to isolate and differentiate lactose-fermenting Gram-negative bacteria, especially members of the Enterobacteriaceae family.

Procedure

To prepare MacConkey Agar, I dissolved 49.53 grams of the medium and dissolved it in 1000 ml of distilled water and heated it to ensure complete dissolution, followed by sterilization through autoclaving at 15 lbs pressure (121°C) for 15 minutes. After sterilization, then allowed the solution to cool to a temperature between 45°C and 50°C, then thoroughly mixed it by shaking it before pouring it into sterile Petri plates and then mixed it with the sample to form a uniform solution, the plates were then left to cool and solidify under lamina flow before being stored in the incubator.

Simmons Citrate Agar

24.28 grams of Simmons Citrate Agar were dissolved in 1000 milliliters of distilled water then heated until the medium had fully dissolved, then thoroughly mixed and distributed the solution into a flask. Then sterilized the prepared solution by autoclaving it at 15 lbs pressure (121°C) for 15 minutes. After sterilization, the agar was poured into sterile petri dishes and then allowed to solidify, mixed with the sample by inoculum of colonies using the streak plate method, the plates were then left to cool and solidify under lamina flow before being stored in the incubator.

General Procedure

The media was first dissolved and autoclaved to kill any microbial organisms that could interfere with the results, the Petri dishes were washed and sterilized by use of a hot air oven, and after 1ml of the sample was pipetted on the petri dish in the air blower to prevent contamination, followed by pouring of the media and then mixed thoroughly to form a uniform solution and left for five minutes to solidify after they had solidified they were put in the incubators of different temperatures for two to three days, after which they were removed and observed for analysis, by use of colony counter to count the colonies on plates that had many colonies and then a sample was picked from one of the colonies to do gram staining on it. The methods used include;

Serial Dilution: A technique used to reduce the concentration of a sample systematically by

repeatedly diluting it with a known diluent, where five dilutions were done.

Streak Plate Method: A method used to isolate pure microbial colonies by spreading an inoculum across the surface of an agar plate in a pattern of streaks with a sterile spreader.

Pour Plate Method: Involves mixing a diluted microbial sample with liquid agar and pouring the mixture into a sterile plate, allowing the microbes to grow within the agar medium.

Enrichment Culture: Involves providing conditions favoring the growth of specific microorganisms within a sample by adding selective nutrients or adjusting environmental parameters.

Gram Staining: A differential staining technique used to classify bacteria into Gram-positive and Gram-negative based on differences in cell wall composition.

Gram Staining

Gram staining is a fundamental technique used in microbiology to differentiate bacteria into two major groups based on differences in their cell wall structure: Gram-positive and Gram-negative.

Gram-positive

Gram-positive bacteria retain the primary stain, the crystal violet-iodine complex, and appear purple or blue under the microscope. Some notable examples of Gram-positive bacteria include Staphylococcus aureus, Streptococcus pyogenes, and Bacillus subtilis.

Gram-negative

Gram-negative bacteria will lose the crystal violet-iodine complex during decolorization and take up the safranin counterstain, appearing pink or red under the microscope, examples of Gram-negative bacteria include Escherichia coli, Pseudomonas aeruginosa, and Salmonella enterica.

Procedure for Gram Staining

Materials Used

Glass slide, Crystal violet (primary stain), Gram's iodine (mordant), Gram's decolourizer, Safranin (counterstain), Distilled water, alcohol lamp, Microscope, Immersion oil, and dropper.

Steps Carried Out

A small amount of bacterial culture was picked and spread thinly onto a clean glass slide and the smear was air-dried, after it was gently heat-fixed by passing the slide through the flame of an alcohol lamp. Then flooded the heat-fixed smear with crystal violet (primary stain) and left it to stand for one minute. After, rinsed the slide gently with distilled water to remove excess crystal violet, then flooded the smear with Gram's iodine solution (mordant) and left it to stand for one minute, and rinsed the slide again with distilled water. Then tilted the slide at a 45-degree angle and gently applied the Gram's decolourizer drop by drop until no more color ran off. Then rinsed the slide immediately with distilled water. After the smear was flooded with safranin (counterstain) and left it to stand for one minute, and after rinsed the slide with distilled water. Then, left the slide to dry by air, after the stained smear was observed directly under a microscope.

RESULTS

The observed colony-forming units on the media plate were counted under the colony counter and recorded in a suitable form. The number of colonies presented were calculated for 10 ml of sample. The colony-forming units were counted by utilizing a bacterial colony counter. The number of counted bacteria was represented as colony-forming units per milliliter using the subsequent equation.

$$\text{Number of bacteria} = \frac{\text{Number of colony-forming units (CFU)}}{\text{Volume plated (ml)} \times \text{total dilution factor}}$$

Table 1: The frequency distribution of the microbial organism's contamination in 12 raw milk, 12 pasteurized milk and 12 yoghurt samples of Mbarara city in 2024

Type of sample	No. of samples	Salmonella	YM	E coli	TPC	Coliforms	Staph
Raw milk	12	4	5	3	6	4	3
Pasteurized milk	12	2	1	1	2	2	0
Yoghurt	12	1	2	0	3	2	1

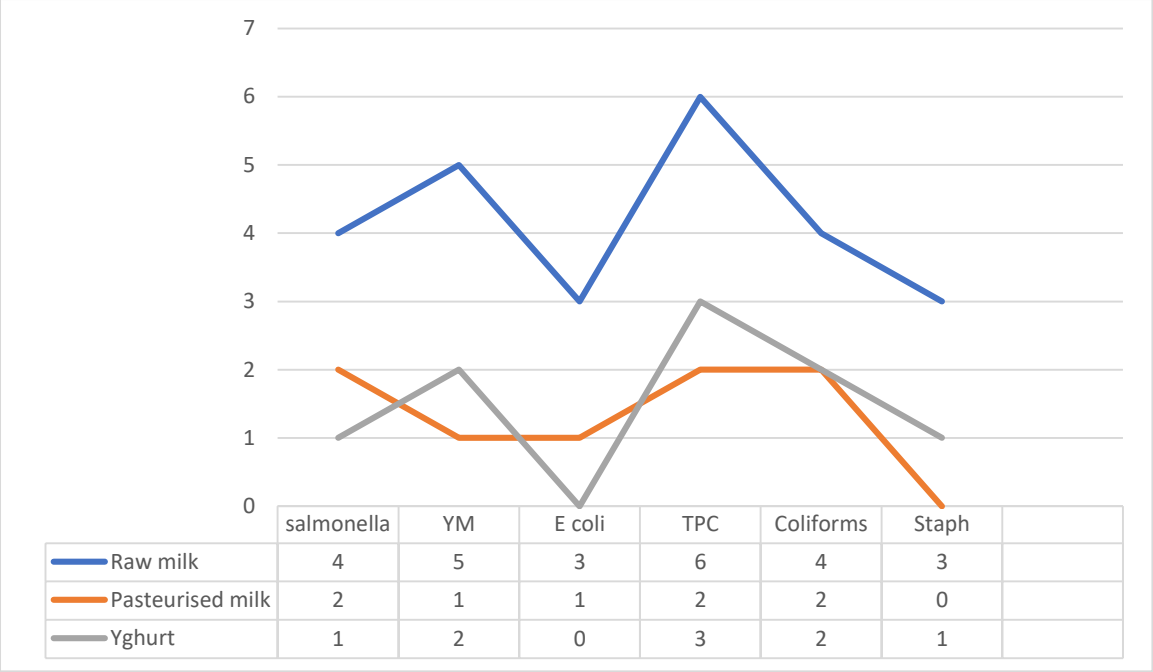

Figure 1. A graph showing the microbial organism's contamination in raw milk, pasteurized milk, and yoghurt samples

Table 2. The average frequency distribution of the microbial organism's contamination in 12 raw milk samples sold in different divisions of Mbarara city.

	Divisions	Salmonella (cfu/ml)	YM (cfu)	E coli (cfu/ml)	TPC (cfu/ml)	Coliforms (cfu/ml)	Staphylococcus (cfu/ml)
Raw milk	Nyamitanga	00	$124x10^{-4}$	00	$120x10^{-5}$	$32x10^{-4}$	00
	Kakiika	$10x10^{-4}$	00	$60x10^{-4}$	$84x10^{-5}$	$28x10^{-4}$	$09x10^{-4}$
	Kamukuzi	$08x10^{-4}$	$96x10^{-4}$	$28x10^{-4}$	$12x10^{-5}$	00	00
	Nyakayojo	00	$128x10^{-4}$	$03x10^{-4}$	$12x10^{-5}$	$52x10^{-4}$	$O6x10^{-4}$
	Biharwe	$05x10^{-4}$	$100x10^{-4}$	00	60x10-5	$16x10^{-4}$	00
	Kakooba	$02x10^{-4}$	$160x10^{-4}$	00	$06x10^{-5}$	00	$02x10^{-4}$
	UNBS Standards	Absent	$<10^{4}$	Absent	$<10^{5}$	$<10^{4}$	Absent

Table 3. The average frequency distribution of the microbial organism's contamination in 12 pasteurized milk samples sold in different divisions of Mbarara city.

	Divisions	Salmonella (cfu)	YM (cfu)	E coli (cfu)	TPC (cfu)	Coliforms (cfu)	Staphylococcus (cfu)
Pasteurised milk	Nyamitanga	00	00	00	00	00	00
	Kakiika	00	00	00	00	02	00
	Kamukuzi	00	00	00	04	00	00
	Nyakayojo	02	00	02	00	01	00
	Biharwe	00	00	00	03	00	00
	Kakooba	01	07	00	00	00	00

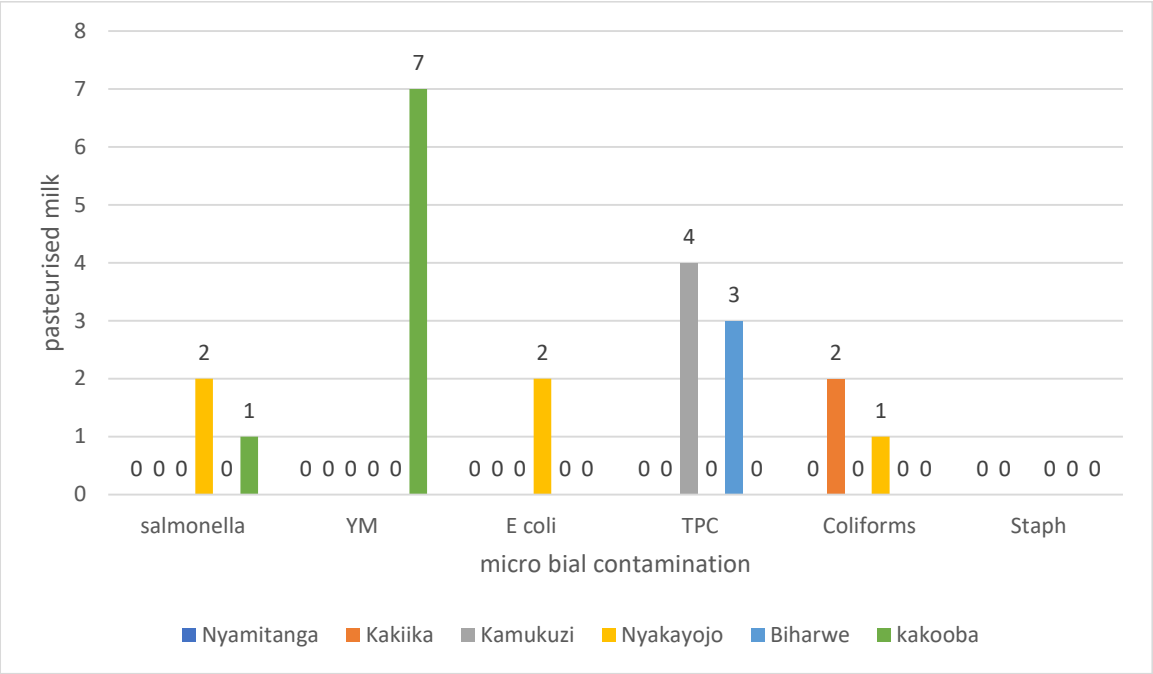

Figure 2. A graph showing the frequency distribution of the microbial organism's contamination in pasteurised milk samples sold in different divisions of Mbarara city.

Table 4. The frequency distribution of the microbial organism's contamination in 12 yoghurt samples sold in different divisions of Mbarara city.

	Divisions	Salmonella (cfu)	YM (cfu)	E coli (cfu)	TPC (cfu)	Coliforms (cfu)	Staphylococcus (cfu)
Yoghurt	Nyamitanga	00	00	00	00	$2x10^{-1}$	00
	Kakiika	00	$07x10^{-1}$	00	$08x10^{-1}$	00	00
	Kamukuzi	00	00	00	$04x10^{-1}$	00	$02x10^{-1}$
	Nyakayojo	00	00	00	00	$03x10^{-1}$	00
	Biharwe	$09x10^{-1}$	00	00	$1x10^{-1}$	00	00
	Nyakayojo	00	$05x10^{-1}$	00	00	00	00
	UNBS Standards	Absent	$<10^{1}$	Absent	$<10^{1}$	$<10^{1}$	Absent

Table 5. The percentage distribution of the microbial organism's contamination in 12 raw milk, 12 pasteurized milk and 12 yoghurt samples of Mbarara city in 2024

Type of sample	Salmonella	YM	E coli	TPC	Coliforms	Staph
Raw milk	33.3%	41.6%	25%	50%	33.3%	25%
Pasteurised milk	16.6%	8.3%	8.3%	16.6%	16.6%	0
Yoghurt	8.3%	16.6%	0	25%	16.6%	8.3%

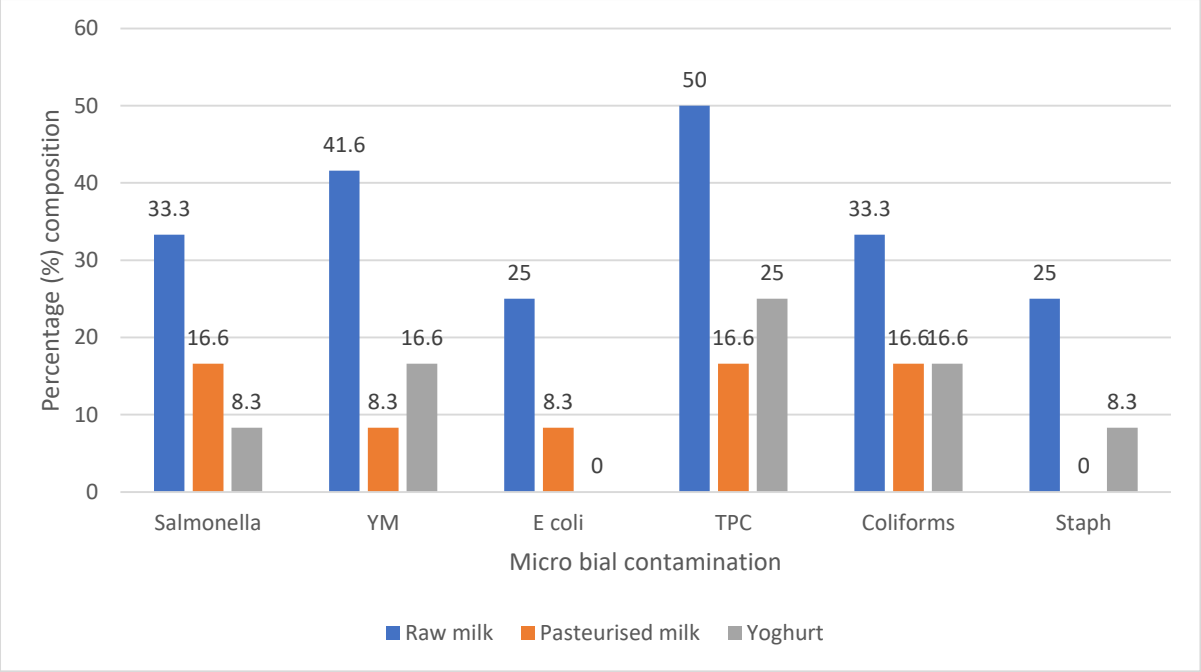

Figure 3. The percentage frequency distribution of the microbial organism's contamination in 12 raw milk, 12 pasteurized milk, and 12 yoghurt samples of Mbarara City in 2024

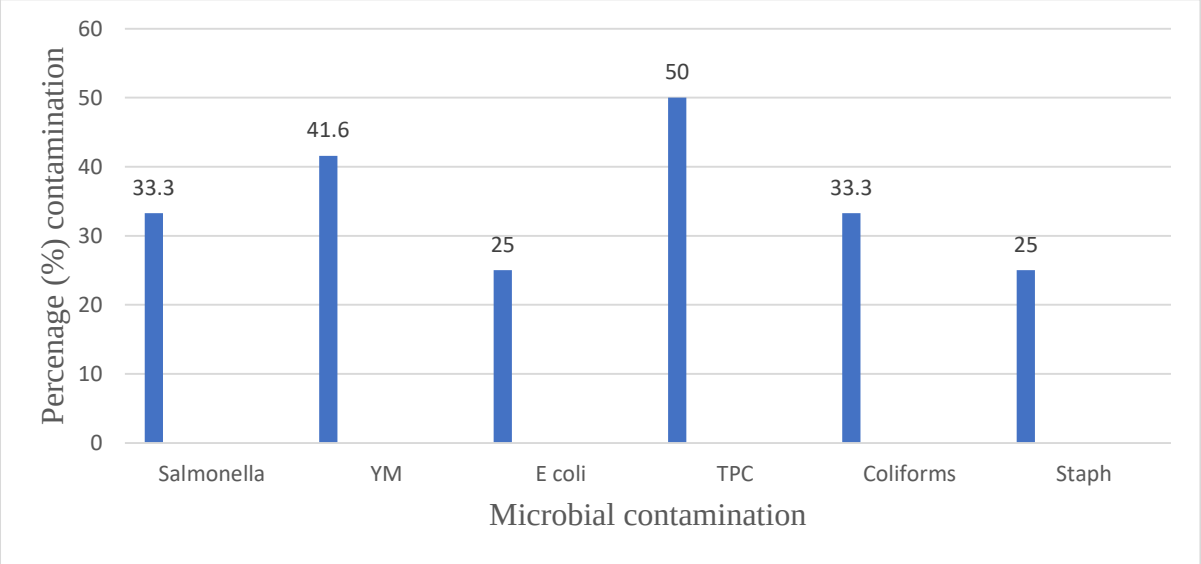

Figure 4. The percentage frequency distribution of the microbial organism's contamination in raw milk, samples of Mbarara city in 2024

DISCUSSION

Over the last twenty years, dairy farming in urban areas has played a significant role in addressing the considerable demand for milk and its products in cities, where consumption is notably high. In this study, the results revealed more presence of contamination of various microorganisms compared to UNBS standards, in raw milk, including bacteria such as Escherichia coli, Salmonella spp., Staphylococcus aureus, Yeasts and molds, TPC, and coliforms. These pathogens may contaminate milk during milking, handling, storage or processing stages, posing risks of foodborne illnesses if consumed without adequate treatment, a total of 36 milk samples were gathered, comprising of 12 pasteurized milk, 12 yoghurt and 12 raw milk samples. Among the pasteurized milk samples, percentage of Salmonella, YM, E. coli, TPC, coliforms, and Staph. aureus was detected in 16.6%, 8.3%, 8.3%, 16.6%, and 16.6% respectively. For Yoghurt it was 8.3%, 16.6%, 25%, 16.6% and 8.3% respectively. In contrast, raw milk samples, the prevalence was notably higher, with 33.3%, 41.6%, 25%, 50%, 33.3%, and 25% respectively. Raw cow's milk samples were contaminated with E. coli. Fulya's study revealed a lower contamination rate, with 10% of the raw milk samples showing E. coli

presence. [19, 20]. conducted a study on 216 raw milk samples, reporting a contamination rate of 13% with E. coli. The contamination of milk storage tankers with E. coli was observed in 1.46% of samples, attributed to contamination from animal faeces. A higher Coliform count in raw milk samples was possibly due to initial contamination from various sources including cows, milkers, containers, and the milking environment. [21]. suggested that lower bacteria counts could be attributed to effective cleaning systems and proper handling during transportation. The higher prevalence of E. coli in raw milk samples could be due to favourable growth conditions or the absence of cooling systems. The detection of Coliform and other food pathogens in dairy products poses significant food safety concerns. The presence of Staph. aureus in milk may originate from mastitis animals or human sources, indicating spoilage. In Sombie et al.[22] study, Staph. aureus was detected in 75% of raw cow's milk samples, while no E. coli was isolated. Their findings also showed contamination rates of 38% for raw milk and 11% for pasteurized milk with Staph. aureus. Drawing from the results of this investigation and analogous studies, it can be inferred that although the detection of coliform and other bacteria may not necessarily signify direct fecal contamination of milk, it does serve as a dependable indicator of poor hygiene during handling and milking processes and storage by different producers, processors and suppliers. Identifying the origin of the contamination becomes paramount in such scenarios. Considering the notable occurrence of TPC, YM, coliform and E. coli contamination in raw milk within Mbarara city, it is recommended to uphold stringent hygiene standards and implement oversight measures during milk processing, transportation, and storage.

CONCLUSION

The elevated bacterial count observed in this study suggests unsatisfactory sanitary conditions. Additionally, the milking equipment, individuals involved in milking, and the overall milking environment and storage serve as significant sources of milk contamination. It was observed that some small retailers of milk and its products in Mbarara city use poor storage means where some people swift on the fridge for a few hours and then turn it off for more hours trying to save money for electricity leading to temperatures of milk and its products to increase, this was observed while collecting raw milk samples and milk products from some different retailers whereby the temperatures were high and this could result in the microbial growth of raw milk and its products like yoghurt leading to the contamination and easily spoilage before the shelf life and if these are consumed they pose a considerable risk to consumers.

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