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Determination of microbial contamination in raw milk, processed milk and yoghurt consumed in Mbarara city, western part of Uganda

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ABSTRACT

The microbial composition of milk serves as a key indicator of its quality. While its nutritional value is significant, the inherent nature of milk also makes it an ideal environment for the proliferation of microorganisms, some of which lead to human illnesses, while others contribute to the spoilage of milk, making it unsafe for consumption. This research therefore aimed to assess the bacterial contamination content in raw milk, UHT and yoghurt, a total of 36 samples were gathered from various retailers, markets and supermarkets across Mbarara city. Contaminated milk and its products are accountable for human dairy-related illnesses. This cross-sectional study was conducted to assess micro bacterial contamination and the presence of selected milk-borne zoonotic pathogens. The study involved 36 samples of milk and milk products mainly raw milk, UHT, or processed milk and yoghurt. Laboratory analysis conducted included Total Plate Count (TPC), Yeasts and molds (YM), staphylococcus, salmonella and coliform, as well as detection of Escherichia coli (E coli), biochemical tests including gram staining were employed to isolate and identify bacteria in these samples. The findings indicated the presence of these types of bacteria in raw milk, Staphylococcus spp (25%), Salmonella spp (33.3%), YM (41.6%), TPC (50%), coliforms (33.3%) and E.coli (25%), for UHT milk the percentage was Staphylococcus spp (0%), Salmonella spp (16.6%), YM (8.3%), TPC(16.6%), coliforms (16.6%) and E.coli (8.3%), finally for yoghurt the results were Staphylococcus spp (8.3%), Salmonella spp (8.3%), YM (16.6%), TPC (25%), coliforms (16.6%) and E.coli (0%). In conclusion, the detection of microbial contamination in raw milk and milk products suggests inadequate sanitary practices and poor storage practices. Products that are contaminated by these pathogenic organisms when consumed may result in food poisoning and a proper awareness among the stakeholders and consumers regarding possible outcomes of consuming contaminated food items is necessary.

keywords: Milk, Yoghurt, Food poisoning, Microorganisms, Contamination

INTRODUCTION

Globally, microbial contamination in dairy products poses significant risks to consumers, leading to food borne illnesses and economic losses [1] The World Health Organization (WHO) and other international organizations emphasize the importance of ensuring the microbiological quality of dairy products to protect public health [2] At the regional level, in Africa, including Uganda, dairy farming and consumption are integral parts of the agricultural and dietary landscape [3] However, inadequate hygiene practices along the dairy production chain can result in microbial contamination of dairy products, leading to food safety and health concerns (4). Studies across the African continent have highlighted the prevalence of microbial pathogens in dairy products and the need for effective control measures (5).

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 establishes regulations and standards for dairy product safety, including microbial criteria for raw and processed milk (6). 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 [7].

Over the past ten years, the per capita consumption of milk in Uganda has risen, reaching approximately 50 liters annually. In terms of utilization, around 30% of the milk is

consumed within the households engaged in its production, while 70% is marketed. Additionally, between 10% and 20% of the marketed milk undergoes processing to yield various dairy products [8]. Despite the integral role of raw milk, processed milk, and yogurt in the daily diet of Mbarara City residents, there exists a critical concern regarding the microbial safety of these dairy products. Milk is rich in nutrients and stands out as one of the rare foods capable of sustaining the growth of young individuals, whether they are animals or humans [9], without the need for additional supplementation. While its nutritional value is significant, the inherent nature of milk also makes it an ideal environment for the proliferation of microorganisms, some of which lead to human illnesses, while others contribute to the spoilage of milk, making it unsafe for consumption [10].

This spoilage of milk and yogurt also leads to economic losses. The risk of microbial contamination poses a potential threat to public health, as evidenced by the prevalence of food borne diseases globally [11]. While dairy products, particularly milk, are known for their nutritional value, they also serve as favorable environments for the proliferation of various microorganisms [12]. The lack of comprehensive studies on the microbial quality of raw milk, processed milk, and yogurt consumed in Mbarara City leaves a gap in understanding the extent of potential health risks associated with their consumption. This research aimed to address this gap by conducting a systematic investigation into the microbial contamination levels in processed milk and yogurt, identifying specific pathogens, and assessing the adherence of local processing facilities to recommended hygiene and safety standards [13,14] By doing so, it is intended to contribute to the enhancement of food safety practices and regulations in the region, ultimately safeguarding the health and well-being of the community and reducing economic losses associated with the spoilage of milk and yogurt.

METHODOLOGY

Materials 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, equipments used include Petri dishes, micro-pippets, 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).

Mbarara is the second largest city in the country, following Kampala. Serving as the primary commercial hub for numerous southwestern districts of Uganda 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

Sample Collection

Selection of Sampling Sites

Sampling sites were within Mbarara city

Sampling Procedure

Six samples of two raw milk, two processed milk, and two of yogurt were bought 250g from each of the selected divisions

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.

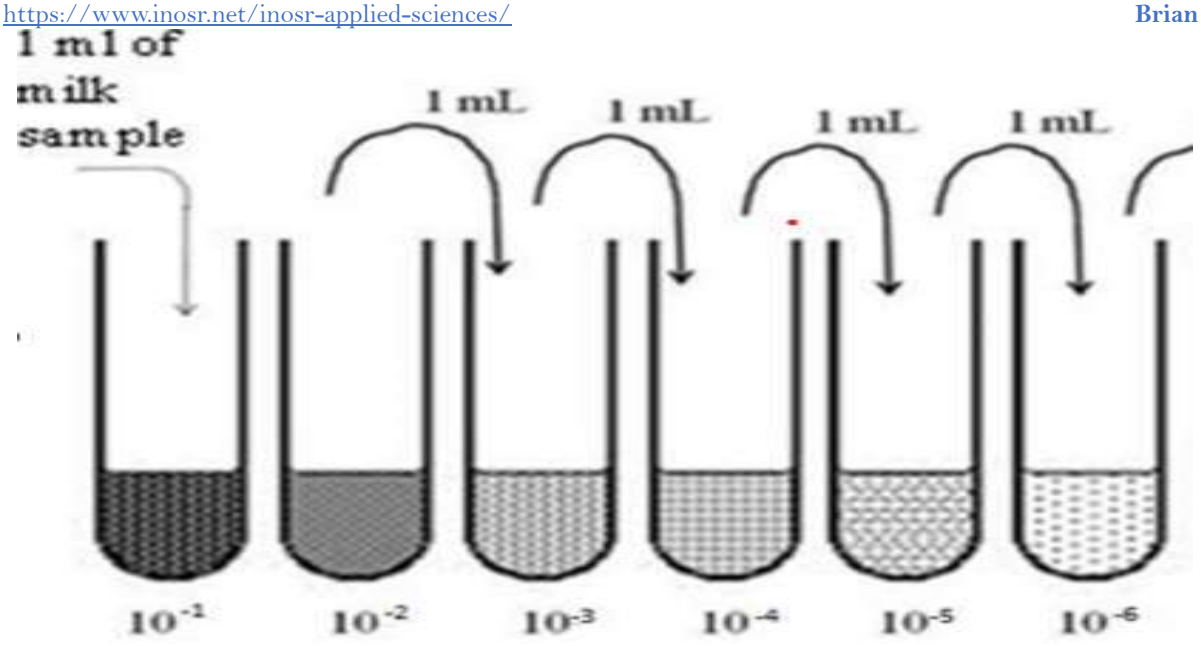

Figure 1. Serial dilutions of milk samples in sterile normal saline before inoculation (source research gate)

Microbiological Analysis Procedure

Media preparation and procedure

Different microbiological media to be used were prepared into liquid form for easier handling, sterilization, and uniform nutrient distribution to support microbial growth, and these include; Plate Count Agar (PCA), Chloramphenicol Yeast Glucose Agar, Xylose-Lysine Deoxycholate Agar (XLD), Violet Red Bile Agar (VRBA), Baird-Parker Agar, MacConkey Agar and Simmons Citrate Agar

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.

RESULTS

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

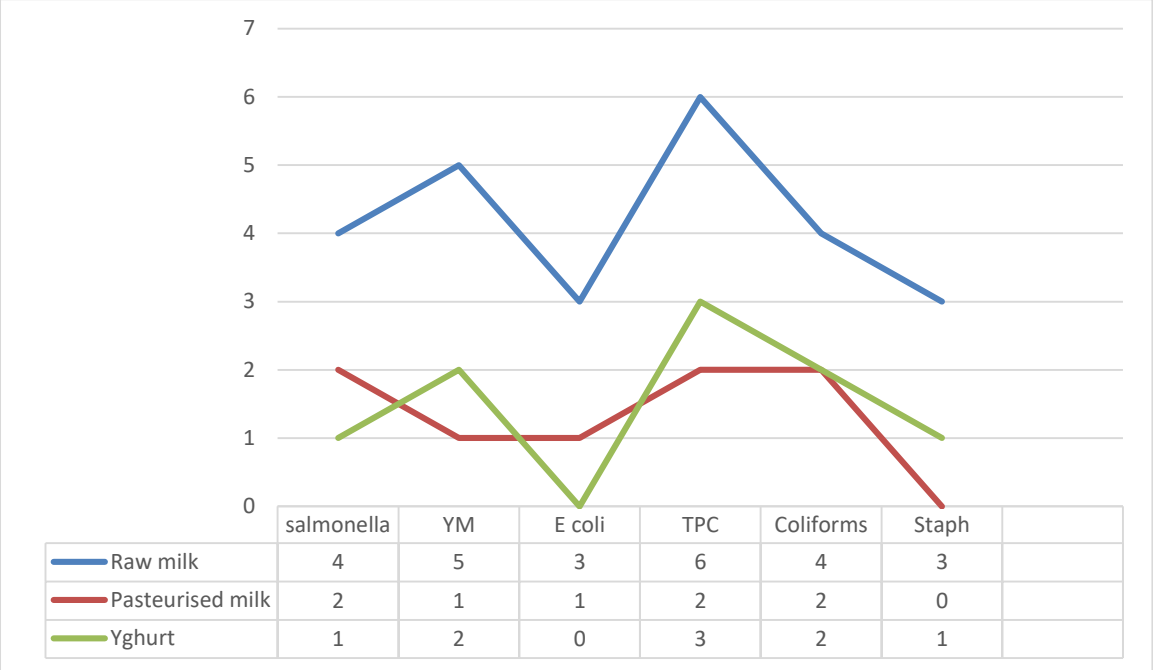

Figure 2. A graph showing the microbial organism's contamination in raw milk, pasteurized milk and yoghurt samples

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

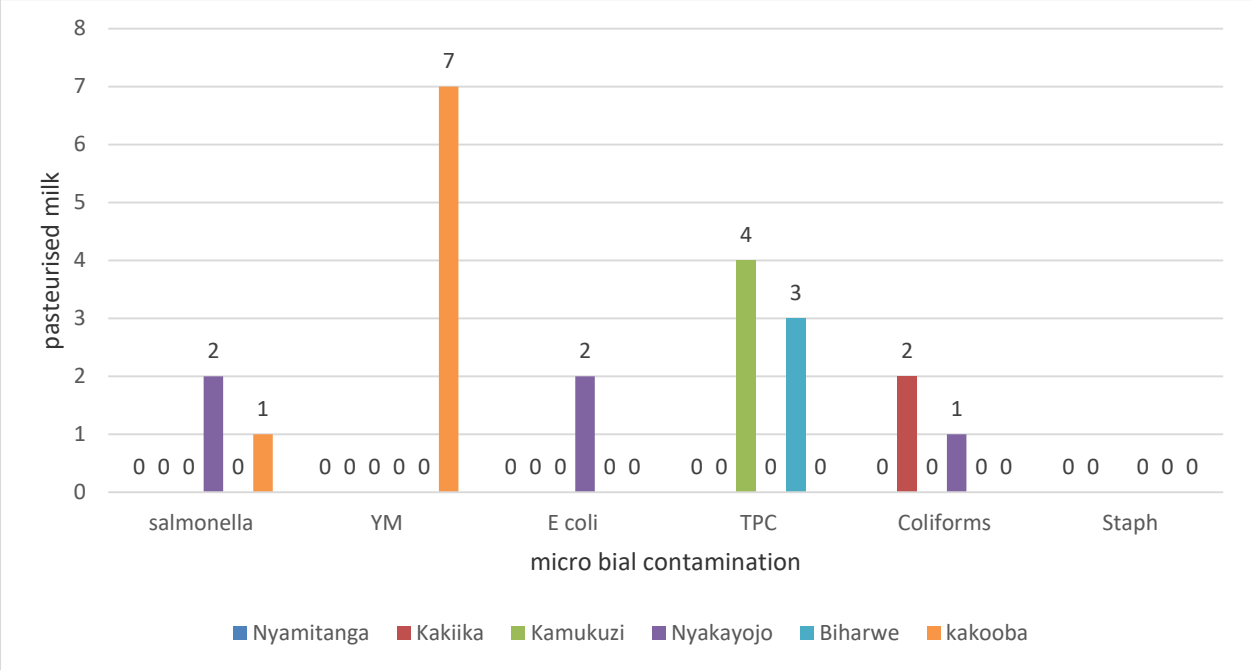

Figure 3. A graph showing the frequency distribution of the microbial organism's contamination in pasteurised milk samples sold in different divisions of Mbarara city.

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%

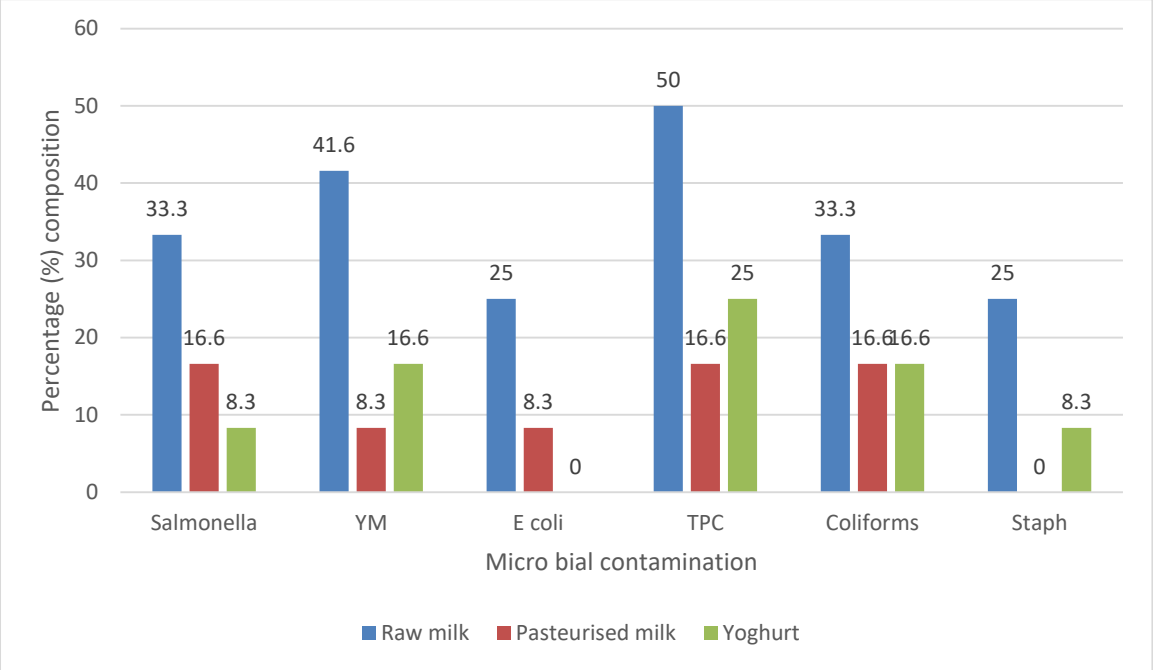

Figure 4. The percentage frequency distribution of the microbial organism's contamination in 12 raw milk, 12 pasteurized milk and 12 yoghurt samples of Mbarara city in 2024

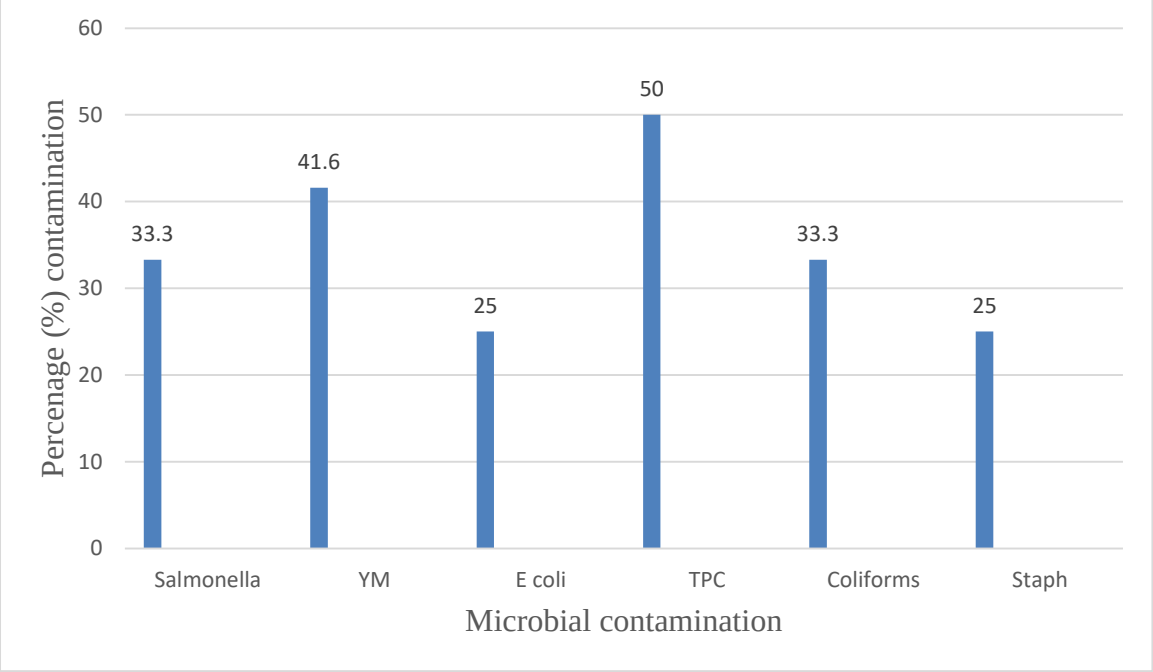

Figure 5. The percentage frequency distribution of the microbial organism's contamination in raw milk, samples of Mbarara city in 2024

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 [15] 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 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. [16]. 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. [17] Ndahetuye et al., [18] 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. Sombie et al., et al., found out 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 easy spoilage before the shelf life and if these are consumed they pose a considerable risk to consumers.

Recommendations

Based on the findings of this study, it is crucial to offer dairy farmers sufficient extension services and training to enhance their understanding of milk hygiene, livestock health, and methods for controlling milk-borne diseases. This approach aims to mitigate public health hazards.

Retailers of milk and its products should be educated on the benefits of proper means of storage of milk and milk products

During milking, it should be important to use clean and sanitized milking equipment to avoid introducing contaminants into the milk. Regular maintenance and cleaning of milking machines, buckets, and other utensils are necessary to prevent the build up of bacteria. After milking, proper hygiene measures include promptly cooling the milk to the appropriate temperature to slow down bacterial growth. Additionally, the milking area should be cleaned and sanitized to eliminate any potential sources of contamination for the next milking session. Further research should be done to find out the possible sources of microbial contamination in the milk and milk products.

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