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Prevalence of Common Microbiological Pathogen Contamination in Processed Milk and Milk Products in Nairobi County, Kenya

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Milk has an outstanding nutritional quality but it is also an excellent medium for bacterial growth and an important source of bacterial infection when consumed without pasteurization. This study aimed at establishing the prevalence of *Total Viable Count* (TVC)*, Escherichia coli, Staphylococcus aureus* and *Listeria monocytogenes* contamination on processed milk and milk products. The study was carried out in Karen, Kibera and Langata Sub- Counties of Nairobi County which were purposively chosen because they have glaring contrasts in living standards. Samples of fresh milk, yoghurt cheese and ice creams were collected from supermarkets and prepared for analysis of microorganisms. All isolates were characterized and identified based on their morphological and cultural characteristics. TVC were detected in 100% of the samples collected and there was significant statistical variation ($P \leq 0.05$) in the contamination level among the products. Of the samples collected in Karen, ice cream had the highest contamination level (3.26 $\log_{10}$ CFU ml^{-1}). Ice

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cream samples from Langata had the highest TVC contamination levels at 4.35 log_{10} CFU ml^{-1}. The overall prevalence of *E. coli* in milk and milk products was 41.6% with a mean count of 0.34 log_{10} CFU ml^{-1} in Karen, 0.07 log $_{10}$ CFU ml $^{-1}$ in Kibera and 0.11 log $_{10}$ CFU ml $^{-1}$ in Langata while *Staphylococcus aureus* was detected in 33.3% of the milk and milk products. The occurrence and detection of *E. coli* and *S. aureus* foodborne pathogens in milk and milk products represent a health risk to consumers. Therefore, there is need to improve the microbial quality of milk and milk products by employing measures that will establish proper management practices to ensure improved hygiene, good manufacturing practices and food systems that will help to minimize microbial contamination.

Keywords: Pathogens; total viable count; Escherichia coli; Enterobacteriaceae; Staphylococcus aureus; Listeria monocytogenes.

1. INTRODUCTION

The demand for milk and milk products in Kenya is among the highest in the East African region and in the developing nations. According to FAO (2011), the annual per capita consumption of milk and dairy products is estimated at 19 kg in rural areas and 125 kg in urban areas. Milk is a healthy food product for humans, and it is obtained from a variety of animal sources, such as cows, goats, sheep, and buffaloes. Animal milk is processed into commercial products such as powdered, skimmed, condensed milk, yogurt and cheese or traditional products fermented milk and *warankasi* (cheese) and *nono* (Jans et al., 2017). In Kenya, a significant portion of milk is consumed locally with only a small fraction marketed commercially. This is because of inadequate infrastructure and skilled personnel required to commercially process milk. However, the safety of milk and milk products may be compromised by the presence of harmful microorganisms such as pathogenic bacteria and yeasts, parasites, and viruses (Azad & Ahmed, 2016)

A wide variety of dairy products such as butter, cheese, ice cream, yoghurt, paneer, etc. are manufactured mainly from the cow milk and also from the milk of other dairy animals such as buffaloes, goats, sheep and camels [1,2]. The unique composition of moisture and with an excellent richness of numerous nutrient that provide favorable environment for the growth and proliferation of microorganisms such as bacteria and fungi some of which are pathogenic to both human and animals. Milk-borne pathogenic bacteria constitute about 90% of all the dairy animals' related diseases (Ryser, 1998). The main microbiological hazards associated with consumption of raw milk include *Staphylococcus aureus, Listeria monocytogenes, Escherichia coli* O157:H7, *Campylobacter* and *Salmonella* spp. If milk and milk products are not prepared under strict hygienic and sanitary conditions, these microbes may gain access through different ways and cause spoilage resulting into economic loss to the dairy industry [3]. Again, it has been observed that if hygienic practices during milking, handling and storage of milk were substandard, the resulting milk products would be poor in quality (Merhawit *et al*., 2014). In many populations, milk processing is largely a household process and is characterized by poor hygiene practices such as inadequate washing of hands, improper use of sterilization equipment (Bereda et al. 2013); [4]. Some of the pathogens such as *Listeria monocytogenes*, *Salmonella* species, *Staphylococcus aureus*, have been implicated as food poisoning agents [5]. Depending on the concentration of these contaminants consumers of these products become exposed to disease such as listeriosis, shigellosis, hepatitis, compromised gut integrity etc.

It is difficult to estimate the incidence of foodborne infection by these contaminants because little is known about the magnitude of microbiological hazards associated with quality of raw bulk milk, especially with regard to contamination and the prevalence of foodborne pathogens. In 2005 close to 1.8 million children died because of diarrheal diseases because they are the most susceptible and are easily exposed due to their high consumption of dairy products either as cow's milk and related by-products in their diet. The microbiological safety is very important and plays a significant role in the quality control of milk and dairy products. This study focused establishing the prevalence of microbial contamination in fresh milk and milk products which are commonly consumed by majority of households in Kenya

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in Karen, Kibera and Langata Sub- Counties of Nairobi County. These locations purposively chosen because they have glaring contrast in living standards ranging from plush homes of Karen and Langata and sprawling slums of Kibera characterized poor living conditions. Karen, Langata and Kibera are located to the South west of Nairobi. Langata is predominantly mixed development with all categories of households from most affluent in Karen to the low-income groups spread across the wards while Kibera is characterized by an ethnic diverse community with high levels of poverty, crime and lack of common basic amenities (Ochungo et al., 2019).

2.2 Sample Size determination for Milk products

For each assessed milk processing company, random sampling technique was used in selecting their brands of milk and milk products at the point of sale in the 3 different study areas: Karen, Langata, and Kibera. A total of 36 samples of milk and milk products were picked from the 3 different study areas, 9 samples for each category namely, fresh milk, yoghurt, cheese and ice cream. The supermarkets or the selling points were randomly selected from Langata and Kibera sub- County in the areas where the consumers' samples were also picked.

2.3 Sampling

Samples of fresh milk, yoghurt cheese and ice cream were collected in 500ml packages at formal points of sell in Langata, Karen and Kibera and put in a cooler box with ice packs and transported to the laboratory. The samples were submitted to the lab on the same day of collection and stored 4 °C-6°C in a refrigerator until testing. Analysis commenced immediately under the guidance of the lab in charge.

2.4 Samples Preparation

Samples of fresh milk, yoghurt were vortexed in a vortexed mixture for 10 sec ensure they were homogenously mixed while the ice cream samples were prepared by first melting them in a fridge at 4°C-6°C. The melted ice cream was then blended at low speed for 1 minute to make a homogeneous mix. One ml was then pipetted and taken as the representative sample. Fifty gram of cheese samples was aseptically weighed and put into 450mL of the required diluent then blended at low speed for 2 minutes to make a homogeneous mixture after which a serial dilution was done with 50g:450ml taken as the primary dilution.

2.5 Making Dilutions

A bottle with the 9mL buffered peptone was labelled with sample lab reference number. The prepared sample were then aseptically opened near a Bunsen burner flame, a sterile 1ml Pipette was attached to the micropipette and one ml of the sample was drawn. The one ml of the sample was aseptically transferred to the 9ml peptone water and then mixed by gently inverting the bottle. This formed the primary dilution (10^{-1}). Following the same procedure above serial dilutions were then made by transferring 1ml of the primary dilution (10^{-1}) into another 9ml of buffered peptone water to make the second dilution (10^{-2}), subsequent serial dilutions were done up to the fourth dilution (10^{-4}), each time using a fresh sterile pipette. The prepared dilutions were then kept in refrigerated at 4°C-6°C.

2.6 Analytical Methods

2.6.1 Enumeration of total viable counts

The enumeration of the total viable count was done following the KS ISO 4833-1:2013 analysis method. Diluted samples of the fresh milk, yoghurt, cheese and ice-cream were tested and then inoculated by adding the samples into labelled sterile petri dish. Using a sterile pipette, 1 ml of the sample of the dilutions was then aseptically transferred into sterile petri dishes from the most dilute (10^{-4}). Approximately 15ml of Standard Plate Count Agar which had been tempered in a water bath at 47°C was aseptically added into each petri dish containing the sample. The contents of the petri dish were then mixed immediately by swirling gently the petri dishes repeatedly until the agar was properly mixed with the sample. This was done one plate at a time until all the samples were completed. The petri dishes were then left on a cool, flat surface to allow the mixture to solidify.

The Memmert incubator was set at 30℃, once it attained the set temperature and the agar poured in the petri dishes had completed solidified, the petri dishes were then inverted and placed in the

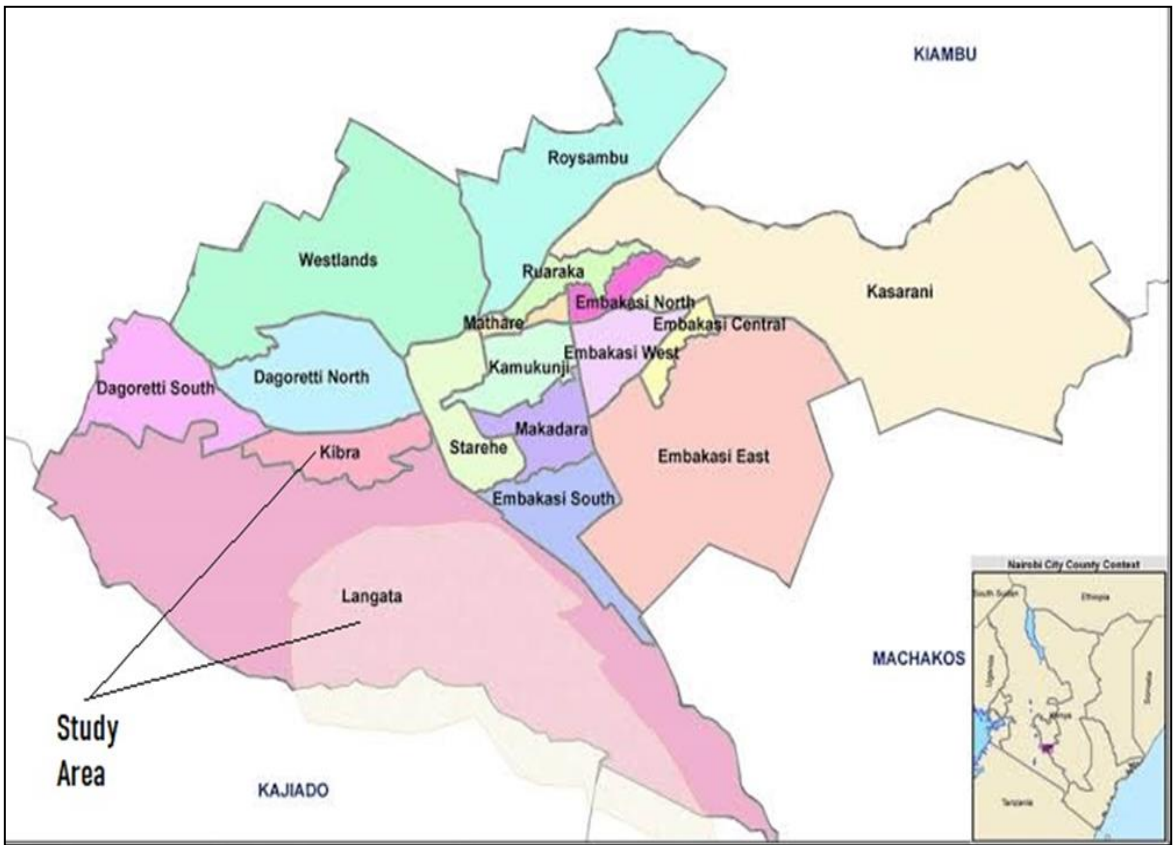

Fig. 1. Location of study areas in Nairobi County ©ResearchGate (2020)

incubator undisturbed for 72 h at 30°C. After the lapse of the 72 h, the petri dishes were removed from the incubator and colonies in each petri dish were examined under subdued light and counted using colony counting device. The results were then recorded for each plate examined and counted as colony forming units per ml or g (CFU ml^{-1} or CFU g^{-1}).

2.6.2 Detection of *Escherichia coli*

The enumeration of the *E. coli* was done following the KS ISO 7251:2005 horizontal method for the detection and enumeration of presumptive *E. coli*. Samples of fresh milk, yoghurt, cheese and ice-cream were analyzed. Sterile HiCrome chromogenic agar was added to dilutions of 10^{-2} to 10^{-4} of the samples which were pipetted on to sterile plates in duplicates, the plate was then gently rotated clockwise and anti-clockwise to mix and then left to dry. After drying, the plates were inverted and incubated at 37°C for 24 h. The plates were then examined for evidence of growth of blue/purple colonies which were interpreted as *E. coli* colonies. For the plates that had *E. coli* present, an indole test was done to further confirm the presence of *E. coli*. *E. coli* is able to break down the amino acid tryptophan into Indole and form a red ring, which is a property of *E. coli* to react with Kovac's (Indole) reagent to form a red ring. The results were either indicated as absent cfu/g or present cfu/g.

2.6.3 Enumeration of *Staphylococcus aureus*

Enumeration of *S. aureus* in all the samples was done as per the procedure laid down in KS ISO 6888-1:2021 method of analysis. The diluted samples starting by the highest dilutions were then inoculated by adding each of the samples into sterile petri dishes with Baird-Parker agar. The inoculum was then quickly and carefully spread over the surface of the agar plate using a sterile glass spreader. Care was taken not to touch the sides of the petri dish. The plates were then left to dry at room temperatures for about 15 minutes. Once the inoculated plates had dried, they were inverted and placed in the incubator set at 38℃ for 24 h and examined for any growth, and re-incubated for a total of 48 h. The plates were then removed and examined for evidence of growth of black-grey shinny colonies surrounded by thin white light borders an

indication characteristic of coagulase-positive *S. aureus* colonies on Baird Parker media. A coagulase test was then done on the colonies as a confirmatory test for *S. aureus*, three of the black-grey colonies observed were transferred using a sterile loop into a sterile test tube with 0.5ml reconstituted plasma into the test tube which were then incubated at 37°C for 4 h but observed at hourly intervals for any signs of clots formation which indicated positive results and vice versa.

2.6.4 Enumeration of *Listeria monocytogenes*

Enumeration of *Listeria monocytogenes* in all the samples was done as per the procedure laid down in KS ISO 11290-2:2017 method of analysis. Twenty-five ml of the 10^{-1} dilution prepared sample was placed into 225 ml of LEB (*Listeria* enrichment Broth) The solution was then uniformly mixed by slowly inverting the beaker and incubated at 30°C for 24-26 h. After the lapse of the time set, 0.1ml of the pre-enriched sample above was added to 10 ml of LEB (*Listeria* enrichment Broth) and incubated at 37ºC for 24 h for selective enrichment. A streak (0.5 ml) of the was then taken using a sterile loop wire and plated in a *Listeria* chromogenic agar, evenly distributing the inoculum throughout the surface of the plate using a sterile spreader while avoiding making contact with the plate's sides. This was then left to dry for 15 minutes and incubated for 24 h at 37ºC and a further 24 hours giving a total of 48 h. Observation was then done for any growth of blue or blue-green colonies surrounded by an opaque cycle that would have been an indication of *Listeria* spp. growth.

2.6.5 Statistical analyses

Data from microbiological analyses were entered into Excel and transformed to logarithm of colony forming units per milliliter of sample ($\log_{10}$ CFUml^{-1}) and the results were presented as mean of the three replicates. All the statistical analyses were performed by of Genstat version 15 software (England) and the difference were considered significant when $P \leq 0.05$. The bacterial contamination levels were compared with the Kenya standards relevant for each milk product (KEBs).

3. RESULTS

3.1 Total Viable Bacterial Counts from Fresh Milk and Other Milk Products

The prevalence of total viable count (TVC) isolated from milk and milk products is shown in Table 1. The TVC were enumerated and detected in 100% of the milk samples collected in all the sampling sites. A significant variation ($P \leq 0.05$) in the microbial load of TVC among the different milk products was observed in samples collected from Karen, Kibera and Langata. Of the samples collected in Karen, ice cream had the highest microbial load (3.26 $\log_{10}$ CFU ml^{1}) followed by fresh milk (2.79 $\log_{10}$ CFU ml^{-1}), while yoghurt samples collected from Kibera had the highest microbial concentration (3.04 $\log_{10}$ CFU ml-1) followed by yoghurt (2.77 $\log_{10}$ CFU ml^{-1}). Ice cream samples from Langata had the highest contamination levels at 4.35 $\log_{10}$ CFU ml^{-1} followed by yoghurt samples with contamination levels of 3.24 $\log_{10}$ CFU ml^{-1}. On average when comparing milk and milk product samples, ice cream samples were found to be contaminated (3.46 $\log_{10}$ CFU ml^{-1}) compared to other milk products. When compared to the acceptable limit of 6.0 $\log_{10}$ CFU ml^{-1} set up by the Kenyan Bureau of Standards (KEBS) [6], all investigated fresh milk and milk products samples were compliant.

Table 1. Microbial load of TVC ($\log_{10}$ CFU ml^{-1}) in fresh milk and other milk products collected from various sites in Nairobi County

	Location			
Milk and milk Products	**Karen**	**Kibera**	**Langata**	**Mean**
Fresh milk	2.79 ± 0.08^{ab}	2.74 ± 0.23^{ab}	2.25 ± 0.07^{b}	2.59 ± 0.30^{b}
Cheese	2.66 ± 0.13^{ab}	2.39 ± 0.12^{b}	2.41 ± 0.07^{b}	2.49 ± 0.15^{b}
Yoghurt	2.31 ± 0.10^{b}	3.04 ± 0.14^{a}	3.24 ± 1.3^{ab}	2.86 ± 0.49^{ab}
Ice cream	3.26 ± 0.08^{a}	2.77 ± 0.12^{ab}	4.35 ± 0.78^{a}	3.46 ± 0.81^{a}
Mean	2.75 ± 0.39	2.74 ± 0.27	3.06 ± 0.96	2.85 ± 0.46
LSD (P≤ 0.05)	0.62	0.42	1.53	0.69
CV (%)	22.8	22.8	0.92	0.18

Values followed by the same letter (s) are not significantly different at P ≤ 0.05 according to the Duncan's multiple range test

3.2 Prevalence of *Staphylococcus aureus* in Fresh Milk and Other Milk Products

Table 2 illustrates the prevalence of *S. aureus* investigated in the 36 samples examined. Overall, *S. aureus* was detected in 33.3% of the milk and milk products. However, in Karen, *S. aureus* was not detected in milk products such as cheese, yoghurt and ice cream. In Langata, *S. aureus* was only detected in ice cream samples.

Table 2. Prevalence of *Staphylococcus aureus* in fresh milk and other milk products collected from various sites in Nairobi County

	Location		
Milk and Milk Product	**Karen**	**Kibera**	**Langata**
Fresh milk	Detected	Detected	ND
Cheese	ND	ND	ND
Yoghurt	ND	Detected	ND
Ice cream	ND	ND	Detected

ND- Not detected

3.3 Prevalence and Microbial load of *E. coli* in Fresh Milk and Other Milk Products

The overall prevalence of *E. coli* in milk and milk products was 41.6% with a mean count of 0.34 $\log_{10}$ CFU ml^{-1} in Karen, 0.07 $\log_{10}$ CFU ml^{-1} in Kibera and 0.11 $\log_{10}$ CFU ml^{-1} in Langata. A significant difference ($P \leq 0.05$) in the occurrence of *E. coli* among the different products was observed in samples collected from Karen. However, there was no significant difference ($P \geq 0.05$) in the *E. coli* density between milk products collected from Kibera and Langata Wards. Of the samples collected in Karen, ice cream had the highest microbial load of *E. coli* in all the sites with an average microbial load of 0.48 $\log_{10}$ CFU ml^{-1}). No *E. coli* was detected in fresh milk, cheese and yoghurt samples from Kibera and Langata. However, in Karen, only yoghurt was positive for *E. coli* counting.

4. DISCUSSION

Food contaminants are important factors contributing to the high incidence of food borne diseases in developing countries. The Kenya standards for the milk products: KS EAS 69:2019 Pasteurized milk specification, KS EAS 33:2019 Yoghurt specification, KS EAS 70: 2019- Dairy ice cream specification, and KS EAS 28 1:2019- Cheese general requirements specification, indicate the acceptable limits for each of the products. The maximum allowable counts for TVC in any of the milk products, for ice cream it should not exceed 4×10^4 CFU/g and in pasteurized milk it's capped at 3×10^4 CFU/g respectively all the standards give the limit for *E.coli*, *Staphylococcus aureus* and *Listeria monocytogenes* are absent in 25g.

4.1 TVC

The current findings shows that the total viable count (TVC) were enumerated and detected in 100% of the milk product samples collected in all the sites. Even though the microbial load differed among the products and between the sites they were all within the set limits by the KEBS standards with regards to TVC. This outcome depicts those of Nur et al. [7] where all the pasteurized milk had high bacterial load ranging from 2.17×10^3 to 3.84×10^3 CFU ml^{-1}. The same results were reported by Hasan et al. [8] and Wanjala et al. [6] where various quantities of TVC were isolated from different sources. However, these results differ those reported by Wanjala et al. [6] where the average TVC in raw milk collected from rural, urban and slum areas of Nairobi were 7.57, 7.52 and 8.18 $\log_{10}$ CFU ml^{-1}. The same above-mentioned findings were reported by Bhatnagar et al. [9], Karthikeyan and Dhanalakshmi [10], Karthikeyan and Pandiyan [11], however the authors reported variations in number of total viable counts. According to Mendonca et al. (2020) though TVC is not a pathogen, their presence may increase the chances of the food having pathogenic microorganism because it raises doubts on the level of GMP implementation.

The occurrence of bacteria is of clinical significance and implies that these products can pose health risk to consumers. Milk processing handled in unhygienic conditions supports the growth of pathogenic microorganisms leading to contamination of milk products. Thus, this study indicates an improvement in the milk handling and hence improvement in milk quality. It is however important to note that the highest TVC contamination was observed in samples collected from Langata and not Kibera. However, there was not significant difference between the sites. The finding contradicts those of Wanjala et al. [6] where the highest TVC contamination was recorded slums while the lowest count was detected in urban Nairobi. This implies that milk contamination may be starting from the farms

Table 3. Prevalence and contamination levels of *E. coli* (log $_{10}$ CFU ml $^{-1}$) in fresh milk and other milk products collected from various sites in Nairobi County

	Location			
Milk and milk Products		**Kibera**	**Langata**	**Mean**
Fresh milk	0.46ab	0.00a	0.00a	0.15a
Cheese	0.16ab	0.00a	0.00a	0.05a
Yoghurt	0.00b	0.00a	0.00a	0.00a
Ice cream	0.73a	0.28a	0.43a	0.48a
Mean	0.34	0.07	0.11	0.17
LSD (*P < 0.05*)	0.58	0.58	0.58	0.58
CV (%)	200.70	200.70	200.70	200.70

Values followed by the same letter (s) are not significantly different at P ≤ 0.05 according to the Duncan's multiple range test

Table 4. Microbiological criteria for milk and milk products (Log$_{10}$CFU)

Microorganisms	**Mean (cfug^{-1})**			**Interpretation of microbiological quality** [a]
	Karen	**Kibera**	**Langata**	
TVC	2.75	2.74	3.06	Acceptable [b]
E. coli	0.34	0.07	0.11	Satisfactory
S. aureus	ND	ND	ND	Satisfactory

and collection centers and as such milk and milk products within the county may have contaminated. However, companies manufacturing milk products must adhere to stricter inspection and better management practices. Mehmeti et al. (2017) suggests a frequent microbial analyses and the findings shared with farmers so that they can improve on their hygiene practices.

4.2 *E. coli*

Findings from the current study show that the overall prevalence of *E. coli* in milk and milk products was 41.6% with a mean count of 0.34 $\log_{10}$ CFU ml^{-1} in Karen, 0.07 $\log_{10}$ CFU ml^{-1} in Kibera and 0.11 $\log_{10}$ CFU ml^{-1} in Langata. However, significant difference (*P ≤ 0.05*) in the occurrence of *E. coli* among the different products was only observed in samples collected in Karen. The results reported in this study are similar to those reported by Miranda et al. [12], Berhe et al. [3] and Tanih et al. [13] where *E. coli*, *S. aureus, L. monocytogenes* and *Salmonella* was detected. In their findings, Tanih et al. [13] reported *E. coli* as the most detected pathogen followed by *S. aureus*. Rai et al. (2020) working with milk samples from Kathmandu District, reported that nearly half of the samples showed the presence of *E. coli*. Pathogenic *E. coli* has been shown to be an important pathogen causing outbreaks of acute diarrhea especially in developing countries [14], Boisen et al. 2012; Rai et al., 2020) and thus their presence in the milk should not be overlooked. According to Kwenda [15], *E. coli* should not be present in a well-prepared milk product such as cheese as high acidity of the fermented product should restrict their survival. Therefore, the presence of *E. coli* and any other microorganisms suggest that slow acidity development may have allowed the build-up of *E. coli*. It is important to note that out of all the milk products, ice cream samples had high incidences of *E. coli* than any other. According to Verraes et al. [16] *L. monocytogenes, S. aureus* and *E. coli* are the main microbial hazards that are found in ice cream. The presence of *E. coli* and other microbes in ice creams indicates that the preparation process has not been done effectively or post process contamination might have occurred [17]. According to Osamwonyi *et al.* (2011) possible sources of these pathogenic microorganisms in ice cream include raw materials used for the composition of ice cream such as separated milk and milk powder, cream, flavoring, coloring substances, stabilizers.

4.3 *Staphylococcus aureus*

Overall, *Staphylococcus aureus* was detected in 33.3% of milk and milk products. However, the numbers could not be quantified. In Karen, *S. aureus* was not detected in milk products such as cheese, yoghurt and ice cream while in Kibera *S. aureus* was not detected in cheese and ice cream. These findings are similar to those reported by Latha et al. [18] and Dai et al. [19] where high prevalence of *S. aureus* was reported, however, the prevalence of *S. aureus* in this study is lower. This may be due to the fact

that the samples were branded samples sourced from various supermarkets. The results reported here concur with those of Rall et al. [20], Gundogan et al. (2006) and Holi et al. (2021) where various samples were found positive for *S. aureus*. *Staphylococcus aureus* is an environmental contaminant that is commonly found on surfaces, and it may result from poor hygiene practices such as inappropriate cleaning of surfaces or using contaminated water for cleaning [21,22]. *Staphylococcus aureus* is generally present in the skin and mucous membrane another pathogen that can be used to measure the sanitary conditions in which food is produced and handled [23,24].

5. CONCLUSION

The TVC counts isolated in all the samples were within the set limits by the KEBS standards. However, the microbial counts for *E. coli* were above the set standards by food safety regulatory bodies. Consequently, the consumption of microbiologically unsafe milk and milk products pose a significant health risk to consumers due to their potential to cause illnesses. With a projected increase in the production and consumption of dairy products in Kenya and the whole of Africa, production and handling practices are likely to play a key role in the safety of these products. Detection of *E. coli* and *S. aureus* foodborne pathogens in milk and milk products, even if in few samples indicate possible lapses in industrial implementation of food safety management systems.

6. RECOMMENDATIONS

There is need to improve the microbial quality of milk and milk products by employing measures that will establish proper management practices to ensure improved hygiene, good manufacturing practices and food systems that will help to minimize microbial contamination. The processing plants need to improve on the implemented food safety management systems to ensure that the products processed are of the highest microbiological quality. Additionally, regulators need to intensify on market surveillance and product testing to protect the consumers from getting contaminated products.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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