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Occurrence and Survival Dynamics of Selected Foodborne Pathogens in Naturally Fermented Milk from Informal Markets in Zimbabwe

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Abstract

Informal markets are important in the supply chain of dairy products in Zimbabwe. The objective of this study was to determine the prevalence of *S. aureus*, *Salmonella* spp and *E. coli* in naturally fermented milk and its survival during controlled milk fermentation. Thirty samples of naturally fermented milk were collected from milk vendors in Harare and Gweru central business districts together with 15 raw milk samples from five dairy farms around two towns. All samples were analysed for pH while naturally fermented milk samples were further analysed for total bacterial count, total coliform, lactic acid bacteria, *Salmonella* spp., *E. coli* and *S. aureus* at 0 h while raw milk samples were analysed for the same parameters at 0, 12 and 24 h during natural fermentation. Four samples of commercial UHT milk were obtained, 2 were inoculated with a starter culture together with *S. aureus* and *E. coli* respectively while the other 2 were inoculated with starter culture, allowed to ferment and then inoculated with both *E. coli* and *S. aureus* respectively when the milk had set. Samples were tested for pH, *E. coli* and *S. aureus* during fermentation at 0, 6, 12, 18 and 24 h. Total bacteria counts of samples from vendors ranged between 6.55±0.31 to 9.00±0.04 $\log_{10}$ CFU/mL while total coliform ranged from 2.06±0.14 to 6.70±0.10 $\log_{10}$CFU/mL. *E. coli* were enumerated in all samples in the range of 1.33±0.15 to 5.83±0.10 $\log_{10}$CFU/mL. *S. aureus* was enumerated in 5 of the 10 samples where it ranged between 5.00±0.20 to 6.07±0.25 $\log_{10}$CFU/mL. Both *S. aureus* and *E. coli* survived acidic conditions during fermentation. The occurrence and survival of these pathogens raise compliance concerns with Zimbabwean food safety legislation. It is therefore recommended that food business operators producing and or selling naturally fermented milk must adhere to strict hygienic practices. In addition, strengthening enforcement of existing food legislation in Zimbabwe and improving the capacity of national food control systems; particularly for informal and small-scale dairy processors; remain critical priorities, as widely recognised across developing countries.

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Introduction

For decades, fermentation has been used as a technique of food preservation and fermented food has long been a vital part of the human diet on a global scale. The significance of fermented foods includes enhanced organoleptic properties, improved nutritional quality, boosting of the immune system, improved digestibility and contribution to food security as well as cultural importance (Hasan et al., 2014; Tamang and Thapa 2022). Naturally fermented milk is traditionally a part of the African diet, including Zimbabwe especially among rural and peri-urban communities whose lives rely on livestock farming where fermented milk is mainly produced for household consumption (Bell et al., 2023). Naturally fermented milk is produced through spontaneous fermentation or back slope method utilising the previous batch as the source of the starter culture. However, the quality of the final product is usually unpredictable due to reliance on uncontrolled fermentation conditions.

In Zimbabwe, the production, handling and sale of milk and milk products are regulated under the Food and Food Standards Act [Chapter 15:04] and the Public Health Act [Chapter 15:17], which require that food offered for sale must be safe, wholesome and must be prepared under hygienic conditions (ISO4833: 2013; ISO 4832:2006). However, most informal producers of fermented milk are not aware of the legislation governing the production, and handling of dairy products. Additionally, lack of strict enforcement in such communities has been reported (Mugadza et al., 2025). Due to the faltering economy of Zimbabwe, some dairy farmers have been downsizing operations and ultimately prefer to sell their milk product directly to consumers as raw or naturally fermented (Chimuti et al., 2016). Consequently, there has been an increased consumption of naturally fermented milk from the informal sector (Gweshe 2018). This growing reliance on informal dairy markets presents a major challenge to the national food control system, since small-scale and traditional processors often operate outside routine inspection and licensing structures in Zimbabwe and other low- and middle-income countries (Ashraf and Smith 2015).

Despite the probable benefits of naturally fermented milk, its microbial safety is rarely considered due to its presumed low pH, which would naturally inhibit the growth of bacterial pathogens (All and Dardir 2009; Saviano and Hutkins 2021; Yerlikaya 2023). While previous studies have reported substantial reductions or inactivation of *E. coli* and *S. aureus* during milk fermentation as pH approaches 5.0, more recent evidence indicates that acid-adapted strains may survive and, under certain conditions, persist at even lower pH values (Medveďová and Valík 2012; Medved'ova et al., 2018; Chimuti et al., 2016). The presence of *S. aureus* and *E. coli* in fermented milk could be a direct result of milk contamination along the food chain due to poor hygiene practices by the milk handlers (Chimuti et al., 2016).

Previous studies have also indicated the emergences of acid-resistant *E. coli*, which has been attributed to the presence of acid adapting genes (Dlamini and Buys 2009; Fayemi and Buys 2017). Similarly, the presence of *S. aureus* in naturally fermented milk maybe a result of its acid tolerant nature (Bore et al., 2007). At the international level, the Codex Alimentarius Commission provides specific guidance on the hygienic production of milk and fermented milk products, which is intended to support national food control authorities in developing countries in managing microbiological hazards in traditional dairy foods (Aijuka et al., 2015). The objective of this study was to determine the prevalence of *S. aureus, Salmonella* spp and *E. coli* in naturally fermented milk and its survival during controlled milk fermentation. There are usually poor hygienic standards in the traditional milking process where milking is done by hands and the udder is usually not cleaned, if the udder is cleaned at times the sources of water will be poor (Angelidis 2015; Aliyo and Teklemariam 2022; Yambayamba and Zulu 2011). These factors usually contaminate milk and makes it unsafe for consumption. It is important to understand how microorganisms such as *E. coli* and *S. aureus* behave in fermented milk since they are usually present in raw milk used for production of fermented milk due to the unhygienic conditions as well as contamination from the udder with mastitis. Evidence generated from studies of this nature is essential to inform risk-based food control interventions and policy decisions aimed at improving the safety of traditionally fermented milk in Zimbabwe and other low- and middle-income countries (Ashraf and Smith 2015; Aijuka et al., 2015).

Methods

Milk sampling

A total of 30X500 mL of naturally fermented milk samples were collected from 10 milk vendors (VA–VJ) located in the central business districts of Harare and Gweru. Each vendor was sampled on three separate occasions over a six-month period (September - March), resulting in three replicate samples per vendor. In addition, 15X500 mL raw milk samples were collected from five dairy farms (A–E), with each farm sampled three times during the same period. The sample size was considered adequate to provide representative microbiological data from informal fermented milk markets and dairy farms supplying milk to these urban centres while remaining manageable within available laboratory resources. Only naturally fermented milk actively offered for sale and fresh raw cow milk intended for human consumption were included in the study. Samples exhibiting spoilage, leakage, damaged packaging, or insufficient volume for analysis were excluded. Naturally fermented milk samples were collected in the same packaging they are sold in during three visits done over a period of six months while raw milk samples were collected in 500 mL sterile Duran bottles. Samples were transported to the Department of Food Science and Nutrition, Midlands State University, ensuring that the cold chain was maintained. Samples were analysed for pH using a bench top pH meter. Naturally fermented milk samples were further analysed for total bacterial count, total coliform, lactic acid

Table 1. Methodology for enumeration of bacterial organisms in milk samples

Organism	Media and Manufacturer	Temperature (°C)	Incubation time (h)	Reference
Total bacterial count	Nutrient Agar (High media)	37	18-24	ISO4833-1:2013
Total coliform count	McConkey (High media)	37	18-24	ISO4832:2006
Lactic acid bacteria	MRS, (Oxoid Basingstoke Hampshire, England)	37	24-48	Ashraf and Smith (2015)
E. coli	Tryptone Bile X-glucuronide (TBX) (Oxoid Basingstoke Hampshire, England)	44	18-24	Ajuk et al., (2015)
S. aureus	Baird-Parker, (Biolab, South Africa)	37	18-24	Ajuk et al.,(2015)
Salmonella	Xylose-Lysine-Desoxycholate (XLD) Agar (High media)	37	18-24	Omar et al., (2018)

bacteria*,* *Salmonella* spp., *E. coli* and *S. aureus* as described in Table 1, within 4 h of collection. Raw milk samples were used for natural fermentation.

Natural fermentation

Raw milk samples were placed in sterile plastic (polyethylene terephthalate) containers (mimicking the general containers used by farmers and vendors during fermentation and storage) covered with aluminum foil as lids and allowed to ferment naturally at a temperature of 22 – 28 °C. Addition of aluminum foil helps to prevent air from entering into the sample as this might affect the development and multiplication of Lactic acid bacteria. Samples were tested for pH throughout fermentation at 0, 12 and 24 h. In addition, they were also analysed for total bacterial count, total coliform, lactic acid bacteria, *E. coli* and *S. aureus* as described in Table 1 at 0, 12 and 24 h of fermentation.

Growth of *E. coli* and *S. aureus* during controlled fermentation of milk

4X500 mL of commercial UHT milk samples was obtained and inoculated with 1 g of FD-DVS CHN22 a mesophilic aromatic culture made up of the homofermentative microbes *Lactococcus lactis subsp cremoris*, *Lactococcus lactis subsp. lactis*, *Lactococcus lactis subsp.lactic biovar diacetylactis* and the heterofermentative *Leuconostoc mesenteroides subsp* (Chr-Hansen). Two bacteria isolates obtained from naturally fermented milk and identified as *E. coli* and *S. aureus* were regrown. The isolates were identified using colony morphology on selective media, Gram staining and standard biochemical tests. The presumptive isolates were then identified using the VITEK® 2 compact system (bioM´erieux, Marcy L'Etoile, France) according to manufacturer's instructions. Eighteen-hour-old cultures were adjusted to a 0.5 McFarland standard (approximately 1.5 × 10^8 CFU/mL), serially diluted in buffered peptone water and inoculated into milk to achieve an initial concentration of 3.0–3.5 log$_{10}$CFU/mL.

The two isolates were diluted in buffered peptone water and individually inoculated into separate 500 ml portions of commercially sterile UHT milk previously inoculated with starter culture to achieve an initial concentration of approximately 3.0–3.5 log$_{10}$CFU/mL. In addition, a 500 ml negative control consisting of commercial UHT milk without starter culture or pathogen inoculation was prepared. The inoculated milk samples were aseptically sealed and fermented at 22–28°C. To evaluate the behaviour of the pathogens when present at different stages of the fermentation process, a second set of two 500 ml UHT milk samples was first inoculated with starter culture and allowed to ferment until coagulation (set milk) had occurred. Thereafter, *E. coli* and *S. aureus* were separately inoculated into the fermented milk. This experimental design enabled the assessment of pathogen survival and growth under two scenarios: (i) when contamination occurs before or at the initiation of fermentation, and (ii) when contamination occurs after fermentation has been completed, thereby simulating post-fermentation contamination during handling, storage, or marketing. The two sets of samples were analysed for pH, *E. coli*, and *S. aureus* counts at 0, 6, 12, 18, and 24 h of fermentation and post fermentation respectively. This experiment was repeated again using the same bacteria isolates**.**

Statistical Analysis

One-way ANOVA (p ≤ 0.05) with multiple comparisons (Tukey's HSD test) was used to compare the log counts for the different groups of organisms in different milk samples as well as pH of the different milk samples. Analysis was done using IBM SPSS version 16.0 (IBM Cooperation, Chicago).

Results and Discussion

The results in Table 2 show that there were high counts of total bacteria, ranging from 6.55±0.31 - 9.00±0.04 log$_{10}$CFU/mL. There were significant differences (P ≤ 0.05) in total bacteria counts among vendors. Generally, for total bacterial count (TBC), *E. coli*, *S. aureus*, lactic acid bacteria (LAB) and Coliforms there was an increase in counts between 0 to 24 h for raw milk obtained from farms (Fig 1). The general trend of total bacterial count in all farms was that the counts increased between 0 and 12 h and the count was maintained at 24 h. However, in samples from farm E an opposite trend was observed whereby there was a drop in counts between 0 and 12 h and a rise after 12 h.

Table 2. Microbiological counts and pH of naturally fermented milk samples from various milk vendors

Vendor	Total bacterial count (Log$_{10}$CFU/mL)	Total coliform count (Log$_{10}$CFU/mL)	Lactic acid bacteria (Log$_{10}$CFU/mL)	*E. coli* (Log$_{10}$CFU/mL)	*S. aureus* (Log$_{10}$CFU/mL)	pH
VA	8.43±0.21^{a}	2.14±0.21^{a}	7.98±0.14^{a}	1.87± 0.12^{a}	nd	4.59±0.01^{a}
VB	8.23±0.35^{a}	2.06±0.14^{a}	8.14±0.05^{a}	1.50±0.30^{a}	nd	4.10±0.03^{a}
VC	9.00±0.04^{ab}	2.11±0.10^{a}	8.82±0.24^{ab}	1.17±0.61^{a}	nd	5.10±0.26^{ab}
VD	8.37±0.25^{a}	2.37±0.24^{a}	8.17±0.04^{a}	1.75±0.04^{a}	nd	4.48±0.02^{a}
VE	8.20±0.66^{a}	2.43±0.11^{a}	7.79±0.11^{a}	1.33±0.15^{a}	nd	4.27±0.08^{a}
VF	6.59±0.12^{c}	6.38±0.02^{b}	5.99±0.09^{c}	5.40±0.3^{b}	5.00±0.20^{a}	4.37±0.04^{a}
VG	6.88±0.08^{c}	6.70±0.10^{b}	5.88±0.45^{c}	5.76±0.07^{b}	5.87±0.15^{ab}	4.39±0.16^{a}
VH	6.55±0.31^{c}	6.34±0.06^{b}	5.37±0.31^{c}	4.83±0.57^{bc}	5.57±0.12^{a}	4.32±0.05^{a}
VI	6.62±0.03^{c}	6.46±0.12^{b}	6.00±0.30^{c}	5.42±0.40^{b}	5.00±0.40^{a}	4.22±0.02^{a}
VJ	6.70±0.17^{c}	6.49±0.25^{b}	5.59±0.08^{c}	5.83±0.10^{bc}	6.07±0.25^{ab}	4.59±0.03^{a}

[*] nd means not detected; Counts recorded as Mean Log$_{10}$CFU/mL ± Standard deviation; Means in the same column with a different superscript are significantly different (P ≤ 0.05) (n = 10).

[*] No *Salmonella* spp. was detected in any of the samples

The general trend of *S. aureus* in all farms was that the counts increased significantly between 0 and 12 h and the count dropped at 24 h. However, in samples from farm E an opposite trend was observed whereby there was a slight increase in counts between 0 to 24 h.

The increase in the microorganisms over the storage period was generally expected since milk is a nutrition medium that supports their growth. The naturally fermented milk samples from the different vendors showed a very high TBC which was expected due to the presence of starter cultures which are mainly composed of LAB. . Fermented milk is normally required to have a minimum of 10^6-10^7 CFU/mL when the product is stored under proper storage conditions (Codex Alimentarius 2003). In this study, the naturally fermented milk sampled from the different vendors was considered to be fairly good quality since the initial bacteria load was within the acceptable it, with the highest count being 9 $\log_{10}$CFU/mL. The differences observed in the TBC among the fermented milk samples could be due to production conditions such as different fermentation temperatures as well as the amount of initial culture. Furthermore, higher contamination could be due to poor hygienic practices and improper sanitation during/after the manufacturing process (Ahmed et al., 2022). From a food control perspective, the high total bacterial and coliform counts observed in some fermented milk samples indicate gaps in the application of basic hygiene and process control requirements stipulated in national food legislation and good manufacturing practices in Zimbabwe (Food and Food Standard Act 15:04). Comparable constraints in food safety governance, inspection coverage and laboratory surveillance have also been reported across developing countries, where informal and small-scale dairy processing remains largely outside effective regulatory oversight (Grace 2015; Mugadza et al., 2025). Total coliform ranged from 2.06 ± 0.14 - 6.70 ± 0.10 $\log_{10}$CFU/mL among the samples.

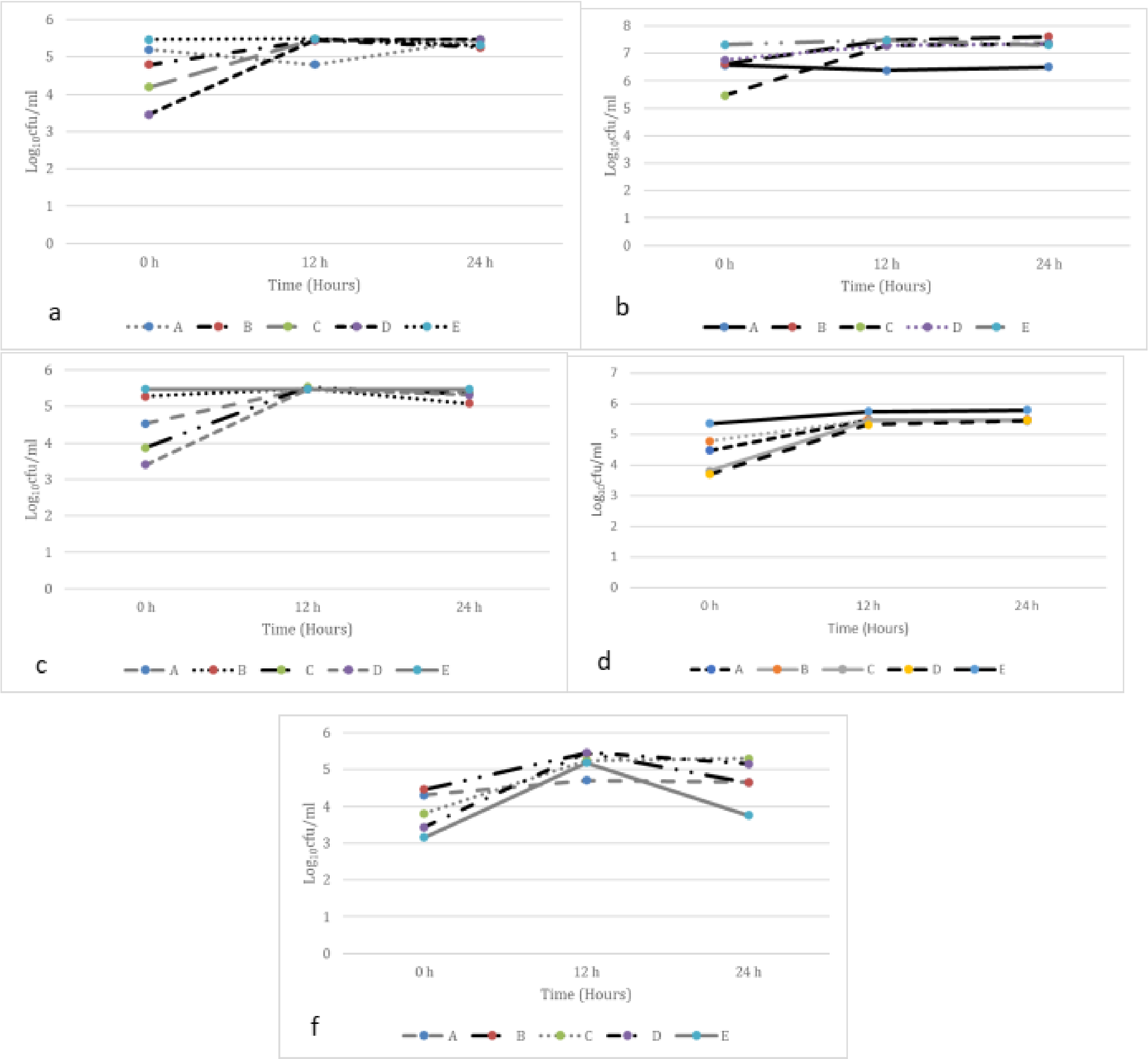

Fig 1. Microorganisms enumerated in raw milk samples collected from various farms (A-E) during natural fermentation over a 24 h period. *a – *E. coli*; b – Lactic acid bacteria; c – Coliforms; d – Total bacteria count; f – *S. aureus.* *Counts recorded as mean $\log_{10}$CFU/ml ± standard deviation

There was also a significant difference ($P<0.05$) in total bacteria count, there was a significant difference ($P \leq 0.05$) among samples. The presence of coliform counts in all the fermented milk samples could be used as an indicator of poor sanitary practices, inadequate processing or post-pasteurisation contamination. Furthermore, the microorganisms are used as indicator organisms for bacteriological quality of milk and it products. Interestingly, 50% of the fermented milk samples from vendors VA-VE had very low (all under 2.45 $\log_{10}$CFU/mL) coliform counts whilst the other half (from vendor VF-VJ) had coliform counts within the range 6 $\log_{10}$CFU/mL. Findings from vendor VF-VJ are similar to those reported in a study by Mhone et al., (2011) whereby the coliform counts of fermented milk from selected dairy farms in Zimbabwe ranged from 6.3-6.4 $\log_{10}$CFU/mL. The significant difference in coliform counts may suggest that vendors VA-VE had better food hygiene practices compared to those of vendors, VF-VJ. Furthermore, the differences in the coliform counts could be an indicator of inadequate processing or lack of a pasteurization step in the naturally fermented milk production process for milk from vendors, VF-VJ. This variability in microbiological quality among vendors further highlights the need for strengthened implementation of official food inspection and vendor registration systems as provided for under Zimbabwe's public health and food safety legislation (Public Health Act 15:17).

Lactic acid bacteria also had high counts ranging from 5.37 ± 0.31 - 8.82 ± 0.24 $\log_{10}$CFU/mL. In addition, the means counts were significantly different ($P \leq 0.05$). *E. coli* was low in some samples recording 1.17 ± 0.61 $\log_{10}$CFU/mL as lowest while in other samples the counts were high recording $5.83+0.10$ $\log_{10}$CFU/mL as highest count. *S. aureus* was not detected in 5 of the vendors while the other 5 samples had counts ranging from 5.00 ± 0.20 - 6.07 ± 0.25 $\log_{10}$CFU/mL exhibiting a significant difference ($P \leq 0.05$) among samples. Milk samples had pH range of 4.10 ± 0.03 – 4.59 ± 0.03 and they significantly differed ($P \leq 0.05$). Lactic acid bacteria count enumerated from the naturally fermented milk sampled from the vendors was as high as 8.8 $\log_{10}$CFU/mL. These results are in accordance with previous studies by Sohanang et al., (2021) which also reported LAB counts as high as 8.9 $\log_{10}$CFU/mL in traditional fermented milk. Lactic acid bacteria are one of the most extensively researched bacteria in fermented milk and its species have been reported to be well adapted in the milk environment, producing lactic and/or acetic acid as well as bacteriocins which may exhibit antimicrobial properties. The presence of LAB in milk can be either a spontaneous or inoculated starter culture. In addition, these microorganisms are ubiquitous and can be found in the mucosa of mammals. The low pH detected from the sampled naturally fermented milk was in accordance with studies by Nahidul-Islam et al., (2018) which reported a final pH range of 3.9- 4.0 in traditional fermented milk. Previous studies associated the low pH in fermented milk with the presence of lactic acid produced by the fermenting Lactic Acid Bacteria (Obioha et al., 2021). A recent study by Falfan-cortes et al., (2022) stated that LAB has an antimicrobial effect through reducing pH by producing organic acids which alter the redox potential of the milk environment, hydrogen peroxide, bacteriocins as well as other inhibitory substances. Internationally, the Codex Alimentarius Commission provides guidance for fermented milk products with respect to hygienic production, starter culture control and process hygiene, which are intended to support national food control systems, particularly in developing countries where traditional fermentation is widely practised (Codex Alimentarius Commission 2004). Alignment of local production practices with Codex-based standards is therefore essential for improving the safety of traditionally fermented milk in Zimbabwe and similar settings (FAO and WHO 2006; Codex Alimentarius Commission 2004).

Two major trends were noted with LABs counts, (i) samples from farms A, B and C had LAB increasing from 0 to 12 h. After 12 h, they remained steady with neither growth nor decrease of counts, (ii) samples from farms D and F showed a continuous growth from 0 h – 24 h. The pH of all samples gradually decreased from 6.7 (maximum) to 4.3 (minimum). The general trend of coliforms in all farms was that the counts increased between 0 and 12 h and the counts dropped at 24 h. However, in samples from farm F an opposite trend was observed whereby there was a drop in counts between 0 and 12 h and a rise after 12 h. The continuous presence of pathogenic microbes during fermentation may be attributed to less effective bacteriocin production and action. This is in agreement with studies by Maoloni et al., (2020) which reported the growth of spoilage and pathogenic microorganisms despite the low pH values associated with lactic acid bacteria secondary metabolites. It is noteworthy in regards to the above-mentioned study by Maoloni et al., (2020) that the relatively low (2.9- 5.8 $\log_{10}$CFU/mL) counts of lactic acid bacteria reported did not match the low (3.8-4.3) pH values detected at the end of milk fermentation suggestion that strong acidifiers were present though in low cell numbers. Contrary to milk samples from farms A, B and C, milk from farms D and E showed a continuous growth of LAB up to 24 of fermentation, suggesting that growth conditions for LAB were maintained throughout the fermentation process in farms D and E.

In the Zimbabwean context, the observed microbial loads in raw and fermented milk raise direct food safety and regulatory concerns, as the production, handling and sale of milk and milk products are regulated under the Food and Food Standards Act [Chapter 15:04] and the Public Health Act [Chapter 15:17], which require food offered for sale to be safe, wholesome and free from contamination that may be injurious to health. Similar regulatory challenges related to enforcement capacity and informal dairy markets have been widely reported in low- and middle-income countries, where weak food control systems contribute to persistent exposure to microbiological hazards in traditionally processed foods (Grace 2015; FAO and WHO 2006). The general trend of *E. coli* in all farms was that the counts increased between 0 and 12 h and the count drop at 24 h (Fig 2). However, in samples from the farms, an opposite trend was observed whereby there was a drop in microbial counts between 0 and 12 h and a rise after 12 h. Both *E.*

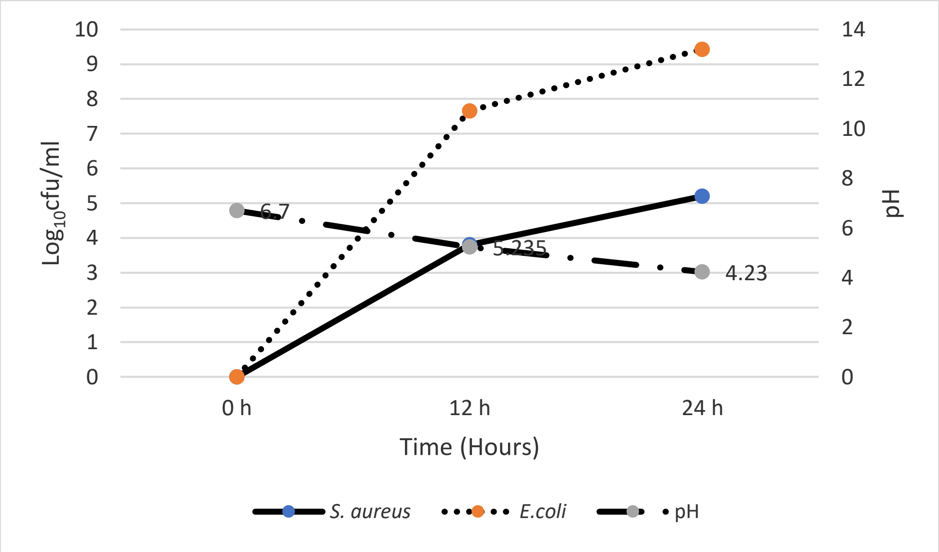

Fig 2. pH, counts of *E. coli* and *S. aureus* inoculated in sterilized milk during controlled fermentation over a 24 h period. *Microbial counts recorded as mean log$_{10}$CFU/mL + standard deviation.

coli and *S. aureus* that was inoculated in commercially sterile raw milk increased from 0 log$_{10}$CFU/mL at 0 h to 5.43 and 5.2 log$_{10}$CFU/mL at 24 h respectively. Further, pH decreased from 6.7 at 0 h to 4.2 at 24 h. Both *E. coli* and *S. aureus* that was inoculated in commercially sterile raw milk during fermentation increased from 0 log$_{10}$CFU/mL at 0 h to 5.19 and 5.06 log$_{10}$CFU/mL at 18 h respectively. Further, pH decreased from 4.48 at 0 h to 3.89 at 18 h.

The presence and transmission of *E. coli* and other pathogens such as *S. aureus* in raw milk have been reported in previous studies (Dai et al., 2019). *E. coli* and *S. aureus* have been shown to survive and grow under cold storage conditions (Hill and Kethireddipalli 2013). *S aureus* is a common contaminant of milk and dairy products. Furthermore, it is considered a pathogenic microorganism that is responsible for food poisoning due to its ability to produce an enterotoxin (Argudin et al., 2010; Grispoldi et al., 2021). The presence of coliforms in milk at the farm level is an indicator of faecal contamination associated with soiled udders and teats. If the coliform count is greater than 10^3 CFU/mL in raw milk this may indicate poor environmental hygiene, poor hygienic milking practices and further handling, improperly cleaned milk equipment, contaminated water, inadequate refrigeration, or the presence of coliform mastitis (Pantoja et al., 2009; Martin et al., 2016).

Fermented milk from all the vendors indicated the presence of *E coli* in the samples. However, samples VA to VE had a relatively lower count compared to vendors, VF to VJ. The significantly higher count could be attributed to a lack of pasteurisation of milk used in fermented milk production for vendors, VF to VJ. The higher *E coli* count also potentially means increased risk to the safety of consumers. The presence of *E coli* especially at relatively high counts in fermented milk is of concern considering the possibility of the presence of *E. coli* O157:H7 which is considered an important human pathogen. The mean *E. coli* count of as high as 5 log$_{10}$CFU/mL, observed in this study is therefore of concern since, in the presence of pathogenic species enough toxins may be produced to cause illness to consumers. Since the *E coli* and *S aureus* were predominantly found only in the fermented milk obtained from vendors, VF-VJ. This result supports the earlier assertion proposed that there were poor sanitary practices in milk obtained from those vendors according to their corresponding coliform count and *E coli* count. The presence of *S aureus* in the milk samples is a public health concern given the ability of the pathogen to produce enterotoxins that cause food poisoning. Previous studies have shown that the enterotoxin may be produced when *S aureus* counts exceed 10^5 CFU/mL (Whiting et al., 1996). This is of great concern since the fermented milk from the vendors in this current study had *S aureus* counts which ranged from 5-6 log$_{10}$CFU/mL. The detection of potentially pathogenic bacteria in fermented milk sold to the public is inconsistent with the legal requirement under Zimbabwean food legislation that prohibits the sale of food that is contaminated, unfit for human consumption or prepared under unhygienic conditions (Food and Food Standards Act 15:04; Public Health Act 15:17). In many developing countries, including Zimbabwe, inadequate routine microbiological surveillance of informal food markets continues to undermine effective enforcement of these legal provisions (FAO and WHO 2006; Grace 2015).

S. aureus and *E. coli* inoculated in commercially sterile milk increased in number as pH dropped during the fermentation. An increase in concentration of these pathogens shows that they could survive and grow despite the drop in pH. Since the microbes were introduced at a low concentration, the results of *E. coli* and *S. aureus* multiplying from a small minute concentration to very high amounts despite low pH conditions leads to the conclusion that these microbes are resistant to adverse conditions of low pH. After inoculating *E. coli* and *S. aureus* in already set fermented milk continued to survive and multiply at pH as low as 3.89 with a final colony count of 5.19 and 5.06 log$_{10}$CFU/mL at 18 h, respectively (Fig 3). These findings are similar to those reported by Schoustra et al. (2022) which noted a pH decrease during fermentation to 3.8 whilst *S. aureus* counts continued to increase with 24 h

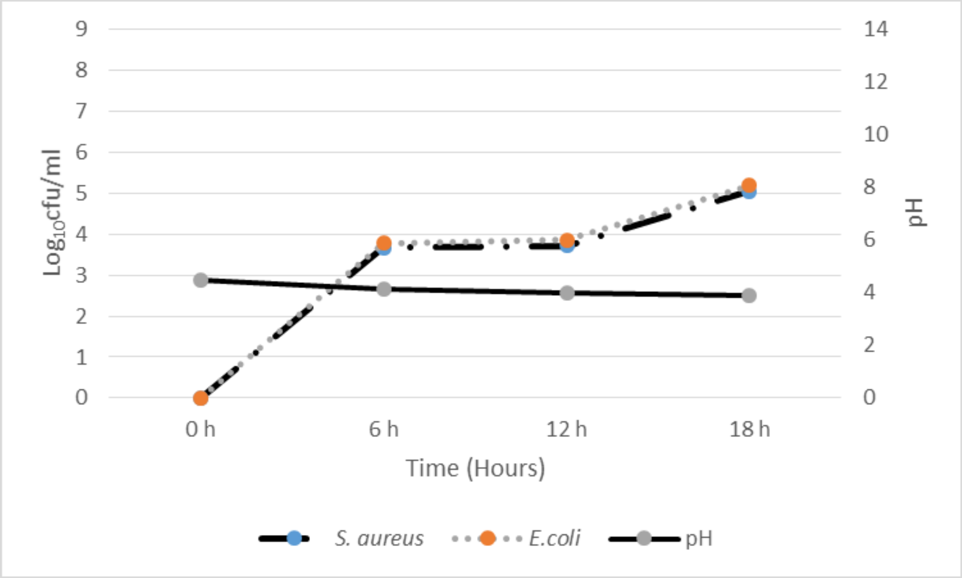

Fig 3. pH, counts of *E. coli* and *S. aureus* inoculated in fermented sterilized milk post controlled fermentation (after milk coagulation) over an 18 h period. *Microbial counts recorded as mean log$_{10}$CFU/mL + standard deviation.

and became sable at 48 h at a final count of 4.2 log$_{10}$CFU/mL. Low pH conditions proved not to affect the growth and survival of *E. coli* and *S. aureus* which is contrary to previous studies by Medvedova et al. (2020), which suggested that inactivation of *S. aureus* is achieved at around pH 5 and the existence of bacteriocins in naturally fermented milk which are bactericidal as stated by Darbandi et al., (2022). These findings reinforce the importance of regulatory emphasis on preventive food safety controls, including heat treatment and hygienic fermentation practices, as promoted under national food control systems and Codex-based risk management approaches for traditionally processed foods in developing countries (FAO and WHO 2006; Codex Alimentarius Commission 2004).

Conclusions

This study demonstrated that naturally fermented milk sold in informal markets in Zimbabwe contained high microbial loads, including the foodborne pathogens *E. coli* and *S. aureus*. Although no *Salmonella* spp. was detected, the presence of *E. coli* in all fermented milk samples, and *S. aureus* was in 50% of the samples is a cause for food safety concern. Furthermore, controlled fermentation experiments showed that both *E. coli* and *S. aureus* were able to survive and proliferate under acidic conditions, indicating that low pH alone may not be sufficient to ensure the microbiological safety of naturally fermented milk.

These findings highlight significant food safety concerns associated with the production and sale of naturally fermented milk in informal markets and suggest potential non-compliance with existing food safety requirements in Zimbabwe. The presence and survival of these pathogens underscore the importance of implementing effective hygienic practices throughout the dairy value chain, including the use of heat-treated milk, improved sanitation during production and handling, and appropriate fermentation controls. Strengthening food safety awareness among producers and vendors, together with enhanced enforcement of existing food legislation and support for small-scale dairy processors, is essential to reduce microbiological risks and improve consumer protection. Further, the findings of the study can be used as a baseline for risk analysis and development of science-based food safety policies and legislation.

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