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Microbiological Quality of Typical Traditional Fermented Milk from Northern Uganda and Western Kenya

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Abstract: In this study, the microbiological quality of traditionally fermented milk from Northern Uganda and Western Kenya was analysed. Six samples of typical traditionally fermented milk were collected randomly from traditional cattle keepers in Karamojong (UG 1) and Acholi (UG 2) in Northern Uganda and Kalenjin in Western Kenya (KE). The microbial quality of the collected samples was assessed through the use of conventional methods for total aerobic mesophilic bacteria, total coliform, lactic acid bacteria, *Staphylococcus aureus*, *Listeria monocytogenes*, yeasts, and mould counts. The mean aerobic mesophilic bacterial counts were 5.14×10^9 coliform forming units (cfu)/mL. The mean counts for mesophilic lactobacilli ranged from 10^6 to 10^8 cfu/mL. The mean thermophilic lactobacilli count ranged from 10^7 to 10^9 cfu/mL, while the mean thermophilic lactococci counts ranged from 10^6 to 10^9 cfu/mL. On the other hand, the Streptococci counts were between 10^6 and 10^8 cfu/mL. The mean count for the non-sorbitol *E. coli* was 3.87×10^3 cfu/mL. These results suggest that although the pH of the traditional yoghurt in this study was low, the acidity was not sufficient to inhibit growth of microorganisms in the product. Although it is difficult to avoid the microbial contamination of milk during milking, it is of the utmost importance to maintain a very high level of hygiene in dairy farming practices, and the correct cleaning of teats during milking is very important for good udder health and optimum milk quality, and to ensure safety. The results regarding microbial contamination pose public health concerns, and therefore the appropriate government agencies must pay attention to ensure that the environment in which yoghurt is produced is in the best condition to reduce contamination.

Keywords: microbiological quality; traditional African fermented milk; public health concern

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1. Introduction

Dairy products, especially traditionally fermented milk, are important foods among cattle keepers in Africa. They are integral to rural families' diets and play a central role in the enhancement of food security and income generation [1]. Traditional African herders ferment raw milk to preserve it as they lack refrigeration facilities, but also because fermented or acidified milk products are used in weaning and as a drink or complementary food, especially among nomads.

In the traditional sector, milking is performed by hand in the open air or in a shelter, generally under poor hygiene conditions [2]. The udder is rarely washed before milking; if this is performed, the water is derived from various sources other than tap water, which contributes to the contamination of the raw milk [3], as do other sources of contamination during milking [4]. Hygiene practices regarding the hands of the milker or the udder, the milking equipment and the surrounding environment are inadequate [5]. Traditional farmers mitigate the impact of a lack of refrigeration, poor handling, and environmental contamination by fermenting the milk into several products. The microbiological aim of

fermentation is to achieve a pH fall that prevents or reduces the growth of pathogens. Milk is fermented at household/village level through the application of traditional fermentation technology. The milk ferments spontaneously due to acidification of the raw milk by indigenous microflora in the milk [6]. Some of these products have demonstrated therapeutic and probiotic effects; although they show inconsistent product quality and limited shelf life.

The fermentation period varies from community to community. The main process parameters are fermentation time and temperature, type of fermentation container, the presence/absence of back slopping, removal of whey, and addition of raw milk. In Ghana, *Mashini ghakushika*, a traditional fermented milk, is fermented for three to four days at ambient temperatures before consumption. *Mabisi* is fermented for about 48 h, after which the product is stirred and ready for consumption. *Âudaï* is left to stand overnight to allow fermentation at room temperature [7]. The fermented milk is consumed the following day. In Southern Africa, milk is fermented for between 24 h and 48 h before it is ready for consumption [8]. *Kule naoto* of Kenya is fermented for two to three days before consumption [9]. In Uganda, among the Karamojong and Acholi, milk is fermented for 2448 h in a warm part of the house, after which time it is ready for consumption. The practice of boiling milk before use is increasingly used in Africa; however, people still more frequently consume and even prefer raw milk and/or products derived from unpasteurised milk.

From a food safety perspective, the important pathogens that are related to raw milk and fermented milk products are bacteria such as *Staphylococcus aureus*, *Salmonella* spp. *Escherichia coli*, *Listeria monocytogenes*, and *Bacillus cereus*.

Salmonella belongs to the Enterobacteriaceae family. It is a rod-shaped, Gram-negative, facultative anaerobic bacterium that can use glucose for fermentation. It causes enteric (typhoid) fever, gastroenteritis, and a localised type of disease with loci in one or more organs and which is accompanied by septicaemia [10,11]. *Salmonella* has been reported to survive in acidic environments [12].

E. coli are a large and diverse group of bacteria. Although most strains are harmless, some can cause diarrhoea, urinary tract infections, respiratory illness, pneumonia, etc. A particular strain *of E. coli* known as *E. coli* O157:H7 causes a severe intestinal infection in humans. According to Massa et al. [13], Conner and Kotrola [14] and Ogwaro et al. [15], *E. coli* strains (including *E. coli* O157:H7) can survive milk fermentation and have been found to be present in yoghurt and traditionally fermented milk after 48–72 h of fermentation.

L. monocytogenes is a Gram-positive, pathogenic bacterium that is widely found in both agricultural and food processing environments. It is known to secrete a wide variety of virulence factors, which make the microorganism responsible for different infections, both localised and systematic [16,17]. It is a possible cause of subclinical mastitis and thus can be released into milk. *L. monocytogenes* has been isolated in raw milk from dairy farms. The main concern in the dairy industry is the ability of *L. monocytogenes* to withstand extreme conditions. It can grow in a wide range of pHs, between 4.3 and 10 [18].

S. aureus is a common contaminant in the dairy industry and one of the most common causative agents of food poisoning [19]. It is an opportunistic pathogen that can cause infections in warm-blooded animals and is a leading cause of nosocomial infections in humans and bovines with mastitis [20–22].

B. cereus group members are among the main organisms responsible for the spoilage of dairy products. At the farm, the presence of *B. cereus* is associated with the contamination of soil, fodder and bedding that are in contact with cow udders. At the time of milking, spores are conveyed in the milk [23].

Several researchers [24–27] have reported the presence of microorganisms in some Kenyan or Ugandan spontaneously fermented milk products. There is little modernisation of the production of traditional fermented products through the use of starter cultures under controlled conditions or exploitation of their probiotic and antimicrobial potential. Some data regarding milk quality and the incidence of pathogens in milk from large commercial

dairy farms is well documented, but there is limited or no data in the literature regarding microbiological quality or pathogen prevalence in Northern Uganda. It is necessary to understand which microorganisms are present in traditionally fermented, ready-to-drink milk since their presence is directly connected to the health of consumers. Assessment of the microbiological quality of traditionally fermented milk is required to protect consumers against exposure to any health hazard and to ensure that the product will not deteriorate microbiologically during its anticipated shelf life [27].

This assessment aimed to determine the microbiological quality of ready-to-drink fermented milk from Western Kenya and Northern Uganda. Few research studies have been focused on the microbiological quality of fermented milk from these areas, especially in Northern Uganda.

2. Materials and Methods

2.1. Collection of Samples

Six samples of full cream, 18-h-old, typical traditional African fermented milk were collected randomly in duplicate from two farms in each location: from the Kalenjin farm (Kenya), labelled as KE, and from the Karamojong farm, labelled UG 1, and the Acholi farm in Gulu (UG 2), both in Uganda. The samples were collected during the rainy season (July–September) in sterile plastic milk bottles. All the samples were produced in gourds. After collection, the samples were transported immediately at 4–5 °C in a cold box to the laboratory for analysis.

2.2. Sample Analysis

The fermented milk was 36 h old when it arrived in the laboratory. Within four hours of arrival, the pH, titratable acidity (TA) and microbiological content of the samples were analysed, and these tests were repeated after 24, 48, and 72 h at 25 °C to check the microbiological growth during storage. Broth-dilution and pour-plate methods were used for the microbial analyses [28]. The samples were prepared according to the *Official Methods of Analysis* published by the Association of Official Analytical Chemists (AOAC) [29] and were analysed in duplicate.

2.2.1. pH Measurement

The pH of the samples was measured with a Mettler Toledo Delta 320 pH meter, at room temperature (20 ± 2 °C). The pH electrode was calibrated before the measurements at pHs 4 and 7 with standard buffer solutions. The calibrated pH electrode was inserted into a 10 mL sample and the readings were recorded. All measurements were performed out in triplicate.

2.2.2. Titratable Acidity of Fermented Milk Sample

A 20 g sample of well-shaken yoghurt or unfermented milk was weighed accurately into a 250 mL Erlenmeyer flask, and 40 mL of boiled, cooled, distilled water was added to it. With a sterile pipette, two-to-three drops of phenolphthalein were added to the milk to act as an indicator of the endpoint. The flask contents were titrated against 0.1 N sodium hydroxide (NaOH) until the sample changed colour to persistent light pink. The initial and final readings on the meniscus burette were recorded prior to starting the titration and at the endpoint, respectively. The amount (ml) of 0.1 N NaOH that had been titrated was calculated by subtracting the final volume from the initial volume to give the amount of NaOH used to reach the endpoint. This was performed at least three times per sample. The percentage of lactic acid was then calculated through the use of Equation (1):

$$TA\ (\%) = V_t \times N \times 90 \times 100/Vs \times 1000 \quad (1)$$

in which:

V_t = volume of titrant (ml NaOH).

N = normality of titrant.
90 = equivalent weight for lactic acid.
Vs = volume of sample used (ml yoghurt/milk).

2.3. Isolation of Microorganisms

2.3.1. Sample Preparations

Ten millilitres of each sample were aseptically pipetted into 90 mL of sterile quarter-strength Ringer's solution (pH 7.2) and blended through the use of a Stomacher lab blender (Seward Medical, London, UK) for 2 min, then mixed thoroughly. Serial dilutions (10^{-1} to 10^{-8}) were prepared in sterile quarter-strength Ringer's solution.

2.3.2. Isolation of Microorganisms

Portions (1 mL) of the chosen dilutions were plated on the following media (Table 1).

Table 1. Summary of cultures and media used for the isolation of microorganisms in the samples.

No.	Medium for Growth	Microorganisms	Time (hour)	Growth Condition and Incubation Temperature (°C)
i	Plate count agar (Oxoid M325)	Total aerobic mesophilic bacteria	48 ± 2	aerobic 30 ± 1
ii	MRS agar (LAB098) (pH 6.5)	Mesophilic lactobacilli	48 ± 2	aerobic 35 ± 1
iii	Rogosa agar	Mesophilic lactobacilli	48 ± 2	anaerobic 35 ± 1
iv	MRS agar (LAB 098 + vancomycin)	Leuconostoc	48 ± 2	anaerobic 30 ± 1
V	MRS agar (pH 5.5) (LAB098)	Thermophilic lactobacilli	48 ± 2	anaerobic 42 ± 1
vii	MRS agar (pH 6.0) (LAB098)	Thermophilic lactococci	48 ± 2	anaerobic 42 ± 1
viii	M17 agar (LAB 092)	Streptococci	48 ± 2	anaerobic 30 ± 1
viii	Violet red bile lactose agar with MUG supplement BRO 71 E),	Non-sorbitol *E. coli*	24 ± 2	aerobic 37 ± 1
ix	Violet red bile agar	Coliform	24 ± 2	aerobic 30 ± 1
X	XLD	*Salmonella* and *Shigella* spp.	24 ± 2	aerobic 37 ± 1
xi	Baird-Parker's medium (Oxoid CM 0275 + SR054C)	*Staphylococcus aureus*	24 ± 2	aerobic 37 ± 1
xii	Listeria enrichment broths A and B	*Listeria monocytogenes*	24 ± 2	aerobic 30 ± 1
xiii	*Bacillus cereus* agar	*Bacillus cereus*	24 ± 2	aerobic 30 ± 1
xiv	1.5% malt extract and agar no. 2	Yeasts	120 ± 2	aerobic 25 ± 1
xv	PDA + chloramphenicol	Mould	120 ± 2	aerobic 30 ± 1

Key: MRS =Man Rogosa Sharpe; XLD = xylose lysine deoxycholate.

(i) Plate count agar (Oxoid M325, Basingstoke, Hampshire, UK), incubated in an inverted position at 30 ± 1 °C for 48–72 ± 1 h [28], for enumeration of total aerobic plate counts. This estimates the number of viable aerobic bacteria per gramme, or millilitre of the product measured, in colony-forming units per ml (cfu/mL) [29]. Plates that contained 30 to 300 colonies were counted.
(ii) de Man Rogosa and Sharpe agar [30] (MRS, LAB098 pH 5.5) incubated anaerobically for 48 ± 2 h at 42 ± 1 °C in anaerobic jars (Biolab and Oxoid) with gas generating kits (Oxoid BR 38B) for selective enumeration of thermophilic lactobacilli, particularly *Lactobacillus delbrueckii* subsp. *Bulgaricus*.
(iii) Rogosa agar [31] (E. Merck, D-61 Darmstadt, Germany), incubated anaerobically for 48 ± 2 h at 35 ± 1 °C, for enumeration of mesophilic lactobacilli. A further analysis was performed on MRS agar + vancomycin for the enumeration of *Leuconostocs* incubated anaerobically at 32 °C for 48 ± 2 h.

(iv) M17 agar [32] (Oxoid CM 0817, pH 6.5), incubated aerobically for 48 ± 2 h at 30 ± 1 °C for the enumeration of lactococci, especially *Streptococcus thermophilus*. Ten isolates were obtained randomly from the countable plates of MRS, M17, and Rogosa agars, incubated at 42 °C for thermophilic bacteria and at 35 °C for mesophilic bacteria. Isolates were cultivated in MRS broth (CM359) pH 5.5 and pH 6.5 at 37 °C for 48–72 h for selective enumeration of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *Bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium*.

(v) Xylose lysine deoxycholate (XLD) agar for the enumeration of *Salmonella* and *Shigella* spp. A 25 mL portion of the sample was pre-enriched with 225 mL of buffered peptone water and incubated for 24 h at 37 °C. A portion (0.1 mL) of the pre-enriched culture was transferred to 9.9 mL of Rappaport-Vassiliadis broth and incubated at 42 °C for 24 h. A loopful of the enrichment broth was then transferred to XLD agar and incubated at 37 °C for 24 h. Characteristic *Salmonella* colonies that each showed a slightly transparent zone of reddish colour and black centre were sub-cultured on nutrient agar and confirmed biochemically using triple sugar iron and Simon citrate agar according to the procedures of Gebeheyu et al. [11], with some modification.

(vi) Violet red bile agar (Oxoid CM 107 with added 4-methylumbelliferyl-β-D-glucuronide (MUG) supplement BRO 71 E), incubated aerobically for 24 ± 2 h at 37 ± 1 °C for enumeration of non-sorbitol *E. coli*. The use of the supplement that contained MUG enabled the separate enumeration of *E. coli* that show glucuronidase activity. Plates were examined under long-wave UV light (366 nm) for the presence of fluorescing colonies. The presence of *E. coli* was tested further using indole production in tryptone water (Oxoid, UK) with Kovac's reagent (Biolife, Sarasota, FL, USA), according to AOAC methods [29], and as previously reported by Moushumi and Prabir [33]. Further analysis of total coliform was performed using violet, red bile lactose agar incubated aerobically for 24 + 2 h at 30 ± 1 °C. Only plates containing 15 to 150 colonies for coliform were counted.

(vii) *Bacillus cereus* was enumerated through the use of the most probable number technique with the selective agar mannitol yolk polymyxin B and polymyxin pyruvate egg mannitol bromothymol blue [29].

(viii) Baird-Parker's medium (Oxoid CM 0275 + SR054C) was used to enumerate *S. aureus* according to the reference method of the International Dairy Federation [34] and ISO 6888, 1:2021 [35].

(ix) A listeria-selective medium was used for the enumeration of *L. monocytogenes*. The International Dairy Federation method [17] was used. A 25 mL sample was homogenised in 225 mL of listeria enrichment broths A and B, and then incubated for 24 h at 37 °C on listeria-selective medium (Oxford formulation CM856, Oxoid™, UK) adjunct with listeria-selective supplement (SR0140, Oxoid™, UK). The latter was then incubated for 48 h at 30 °C. A loopful of the enrichment culture broth was streaked in duplicate onto polymyxin acriflavine lithium chloride ceftazidime aesculin mannitol agar (Oxoid, CM877) and incubated for 48 h at 37 °C. Suspected *L. monocytogenes* colonies were characterised through the use of the Gram staining and catalase test. Five presumptive *L. monocytogenes* colonies were selected from each Petri dish of selective agar and cultivated on trypticase soy agar medium (CM0131, Oxoid, UK) that had been supplemented with 0.6% yeast extract. They were placed subsequently into an incubator for 24 h at 30 °C. *Listeria* spp. Colonies typically ranged from greyish green to brownish green with black zones of 1–3 mm diameter of aesculin hydrolysis.

(x) Malt extract agar (1.5% agar no 2) (Oxoid) and potato dextrose agar (+0.005 g/L chloramphenicol) were used to enumerate yeast and mould counts. The plates were incubated at 20 and 25 ± 1 °C for five days. Yeasts and mould colonies were counted separately. Plates that contained 10 to 200 yeast and mould colonies were counted [36].

Further analyses were performed, which included examination of non-spore Gram-positive coccobacilli strains for catalase positivity [37], umbrella growth in motility, nitrate reduction, performance in the methyl red/Voges Proskauer test, and β-haemolysis produc-

tion. The cultures were characterised based on the microscopic appearances of 24-h-old cultures through the use of Gram-stained preparations and analytical profile index (API) biochemical tests (acid formation from glucose, rhamnose, xylose, and mannitol fermentation). All media were prepared with deionised water. Glassware, such as Petri dishes, test tubes, pipettes, and flasks, was sterilised in a hot oven at 160 °C for one hour.

2.4. Analytical Profile Index Biochemical Test

API was used to identify the Gram-positive and Gram-negative bacteria and yeast to the species level. API strips are used usually to detect enzymatic activity, mostly related to the fermentation of carbohydrates or the catabolism of proteins or amino acids by the inoculated organisms. Identification of the lactic acid bacteria (LAB) was performed using API 50 CH and API 50 CHL media (bioMérieux sa). For the enteric and non-enteric bacteria, API 20E and API 20NE were used, respectively, according to the manufacturer's instructions.

Ten representative isolates of each microorganism were selected. From each plate, a single isolated colony (from pure culture) was suspended in sterile distilled water. The bacterial suspension was placed in each compartment by use of a Pasteur pipette, the strips were incubated, and the procedure was performed according to the manufacturer's instructions. The galleries were incubated at 30 or 37 °C and reactions were observed after 24 h and 48 h. All positive and negative test results were compiled to obtain a profile number, which was then compared with profile numbers in a commercial codebook (the APILAB PLUS database (bioMérieux sa) or online to identify the bacterial species.

2.5. Data Analysis

Microbial counts were transformed to logarithmic values (log cfu/mL) and the transformed values were analysed through the application of one-way analysis of variance in Prism Graph Pad (San Diego, CA, USA), version 9.0. All samples were collected in duplicate. The data were expressed as the mean ± standard deviation. Comparison tests were conducted to determine whether or not there were significant differences ($p \leq 0.05$) between and within the different fermented milk samples from the three regions.

3. Results

3.1. pH and TA

Table 2 represents means of the pH and TA values of the fermented milk samples. There were no differences in pH or TA readings in each location. The mean pH of the samples was 2.9 ± 0.1% in the UG 1 sample, 3.4 ± 0.1% in the UG 2 and 3.6 ± 0.1% in the KE. There was a significant difference ($p < 0.05$) among means. The mean TAs of the samples were 1.26 ± 0.1% in UG 1, 0.92 ± 0.1% in UG 2, and 0.7± 0.1% in KE. A highly significant difference ($p < 0.001$) among the means of the TA measurements was observed. The UG 1 fermented milk sample had a significantly lower pH (2.9 ± 0.01) and higher TA (1.26 ± 0.1%) than the others. The KE sample showed a higher pH (3.6) but a higher TA (0.92%) than did the UG 2, which had a pH of 3.4 but a TA of 0.71%. There was no significant difference ($p > 0.05$) among the acidities of samples from each region. During the three days of storage at 25 °C, the pHs of the samples did not change much from the pHs or TAs at the time of initial analysis (36 h) after collection (data not shown).

Table 2. Mean pH and TA of the fermented milk (±SD, $n = 6$).

Sample	pH	TA (%)
UG 1	2.9 ± 0.01	1.26 ± 0.1
UG 2	3.4 ± 0.01	0.71 ± 0.1
KE	3.6 ± 0.01	0.92 ± 0.1

3.2. Microbial Counts

Table 3 shows summaries of the microbial counts that were obtained from the traditional fermented milk samples that were 36 h old (considered ready for consumption). Values obtained in the counts were transformed into log cfu/mL 36 h old (considered ready for consumption). The total aerobic mesophilic bacteria count (AMBC) is an indicator of the sanitary conditions of the handling of raw milk and good-quality milk products [38]. The results show that the mean total AMBC in the samples was between 9.4 and 9.9 log cfu/mL. The mean counts of mesophilic anaerobic *lactobacilli* on MRS (35 ± 1 °C) were lower (6.33 log cfu/mL) in UG 2 and higher in UG 1 (7.8 log cfu/mL) in KE (Table 3). The counts of LAB in UG 2 were close to the total AMBCs, which suggested that most of the bacteria in UG 2 were LAB. The highest coliform counts were obtained from UG 1 samples (5.33 log cfu/mL). *E. coli* was detected in all the samples, and the highest count was obtained from UG 1 samples (4.08 log cfu/mL), while the same level of contamination was noted in UG 2 and KE (3.34 and 3.34 log cfu/mL), respectively. From the results, it can be assumed that *E. coli* was the major component of the coliform.

Table 3. Mean microbial counts of the probable microorganisms in the fermented milk samples (log cfu/mL).

No.	Microorganism	UG 1	UG 2	KE
i	Total aerobic mesophilic bacterial counts	9.35	9.68	9.69
ii	Mesophilic lactobacilli (aerobic)	7.24	7.28	7.28
iii	Mesophilic lactobacilli (anaerobic)	7.8	6.33	7.28
iv	*Leuconostoc*	5.77	5.68	5.70
v	Mesophilic lactococci	7.94	8.20	8.32
vi	Thermophilic lactobacilli.	8.23	8.29	8.32
vii	Thermophilic lactococci	7.85	7.76	7.76
viii	Streptococci	8.22	8.02	8.02
ix	Non-sorbitol *E. coli*	4.08	3.34	3.34
x	Coliform	5.33	4.33	4.24
xi	Salmonella spp.	2.97	2.87	2.93
xii	Shigella spp.	0.00	0.00	0.00
xiii	*Staphylococcus aureus*	4.41	4.35	4.11
xiv	*Listeria monocytogenes*	1.18	1.13	1.12
xv	*Bacillus cereus*	2.92	2.86	2.82
xvi	Yeasts	8.33	8.36	8.52
xvii	Mould	8.04	8.17	8.21

Key: UG1, UG2, Uganda samples 1 and 2. KE: Kenya yoghurt samples. $n = 6$ (samples analysed in duplicates). Values are means ± SD.

3.3. Microbial Analysis

A total of 22 different types of microorganisms were grouped according to their colony phenotypes and Gram stain reactions. The prevalence of each group of microorganisms is presented in a pie chart (Figure 1) expressed as percentage of the total number of groups of the isolates (n) obtained from the samples. Aerobic mesophilic bacteria were the largest group of microorganisms, comprising 34% of the total count (Figure 1). Mesophilic lactobacilli and the *Leuconostoc* group comprised 18%, while 8% of the isolates were thermophilic lactobacilli spp. And 9% were streptococci species. 11% of the total count was coliforms, whilst yeasts and moulds and other (unidentified) represented 9% and 11% respectively.

3.3.1. Identification of the Isolates with API Biochemical Analysis

The presence of multiple and closely related species in these products makes the differential or selective enumeration of bacteria difficult due to the similarity in growth requirements and overlapping biochemical characteristics of the species. However, after the Gram stain, the isolates were subjected to API biochemical analysis. Table 4 shows the presumed predominant microorganisms that were identified through the use of API biochemical analysis. Mesophilic aerobes grown on M17 and MRS agars at 35 °C dominated

the samples. Of the pathogens, *Bacillus cereus* had the highest number of microorganisms in the group (Table 4).

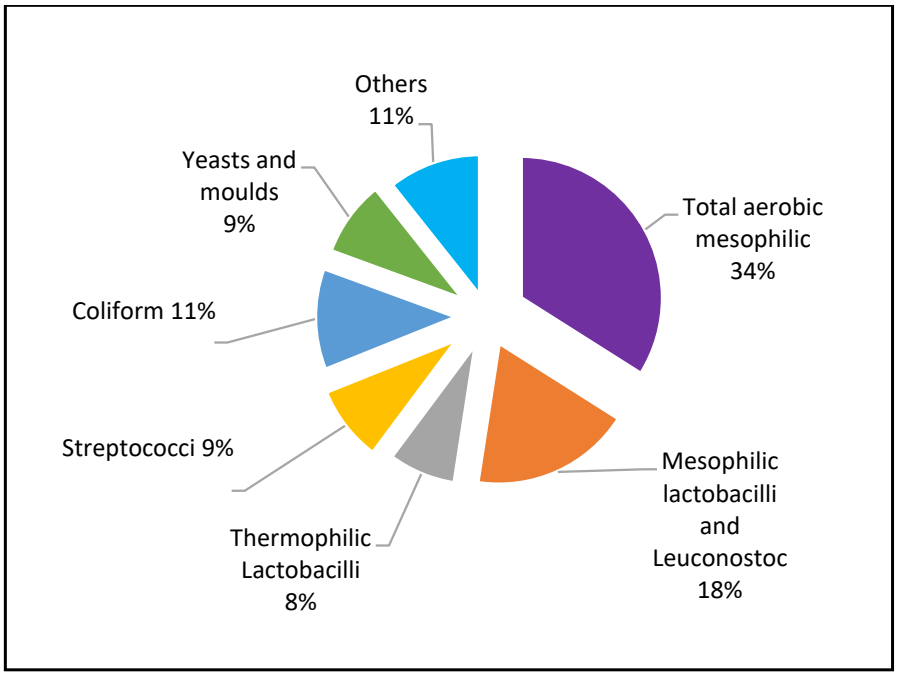

Figure 1. A pie chart of the diverse microorganisms from the traditional African yoghurt (n = 22). n = number of total groups identified.

LAB were the dominant groups of bacteria in all the samples, and these could be identified by their phenotypic and microscopic appearance. They were grouped under *Lactobacillus*, *Streptococcus*, and *Lactococcus* spp.

The phenotypical characteristics of the presumed *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were compared with those of the laboratory collections of *Lactobacillus bulgaricus* NCIMB 11778 and *Streptococcus thermophilus* NCIMB 10378, when Gram-stained and in API biochemical analysis. Ten isolates which were Gram positive, coccoid or spherical shaped were grouped under *Streptococci* spp., and another 10 under the lactobacilli, of which three isolates were identified preliminarily as beta-bacteria (heterofermentative *Lactobacillus*). Others included *Leuconostoc* and *Enterococcus* spp.

From the UG 1 samples, twelve different colonies were identified and grouped according to their Gram stain reactions. Three of the ten isolates were Gram positive, very tapered rod-shaped and according to the API 50 CHL biochemical test, they were identified as *Lactobacillus* spp. Ten of the twelve isolates were Gram-positive and coccoid or spherical in shape. They were grouped under the *Streptococcus* spp. Of the mesophilic lactobacilli, API tests indicated *L. cremoris*, *L. mesenteroides*, *Lactococcus* spp., and *L. lactis* spp.

Many of the Gram-positive mesophilic groups with large sporulated ends were identified with API 20E preliminarily as belonging to the *Bacillus*. The other group, which were also Gram-positive with round or coccoid in groups or chains, were identified as belonging to the *Staphylococcus* and *Enterococcus* spp., which are common in the cattle environment. *S. caprae*, which colonise healthy human skin, nails, and nasal mucosa, were identified in the UG 2 sample. The Gram-positive diplococci or pairs of short-chained isolates were grouped into the *Enterococcus* family. The coliforms were dominated by *E. coli*. With API 20E, presumed *E. faecalis*, *E. agglumeritus* and *E. durans* were preliminarily identified.

From the KE sample, 17 different isolates were preliminary identified. API analyses indicated that four of the isolates were of *lactobacilli* spp. Others were less distinct but identified as Staphylococci species. Table 4 shows the phenotypical and morphological characteristics of some of the yeasts that were isolated from the fermented milk.

Table 4. Phenotypic and morphological characteristics of yeasts and mould isolated from the samples.

Isolate	Macro-Colony Morphology (Margin, Colour, Elevation, Cell Appearance)	UG1	UG2	KE
1	Cream, smooth, oval shape entire and ellipsoidal cell	√	√	√
2	Undulating, white top with green base, slightly convex, spheroidal to short ellipsoidal. (Blue colony on Kluveymyces differential medium)	√	√	√
3	Yellow-green, powdery and pale yellowish on reverse *Aspergillus flavus*	√	ND	√
4	Dirty white with yellow spores at the centre, base orange, slightly radially furrowed (*Microsporum* spp.).	√	√	√
5	Cream-yellow, powdery, and pale yellowish on reverse, capsulate margin, slightly raised centre, filamentous cells	√	√	√
6	White to cream, yellowish, wrinkled, nearly flat elevation, oval cells and ellipsoidal	√	√	ND
7	White to cream coloured, flat with aerial mycelium (*Aspergillus* spp.)	√	√	√
8	Green with a red base	√	√	√
9	White at the base and black spores at the top	√	√	√
10	White pin head, clear zones around the colony	√	√	√
11	Black, yellow to pale cream in the centre (*Aspergillus*)	ND	ND	ND
12	White measuring 1–4 mm, opaque and flat. Ropy to the touch	√	√	√
13	Straw cream at the centre, base orange, slightly radially furrowed	√	√	ND
14	Well-formed white colonies (*Aspergillus* spp.)	√	√	√
15	Green and pale yellow on reverse (*Penicillium*)	ND	ND	ND
16	White base with black conidiophores	√	ND	√
17	Greenish black, white mycelia at the margin, white in the centre (*Rhizopus* sp.)	ND	ND	ND
18	Greenish surrounded by creamy-white ring at the margin (*Penicillium*)	ND	√	ND
19	White to cream, smooth, glaucous dark green on the obverse and pale yellow on the reverse	ND	ND	ND
20	Cotton white to cream on the obverse and yellow to orange on the reverse with dark brown exudate	√	√	√
21	White colony, opaque and flat	√	√	√
22	Bright red colonies	ND	ND	ND

Key: UG1, UG2, Uganda samples 1 and 2. KE: Kenya yoghurt samples. √ = Detected ND = Not Detected.

3.3.2. Yeast and Mould

Many fermented dairy products such as yoghurt and sour milk are contaminated with yeast species that become the major cause of spoilage. This is because the low pH of these products offers a favourable environment for their growth. Yeasts are known to be the major contaminants of products such as fermented or acidified milk. This can be seen in the results observed in this study, in which the isolated yeasts were identified but not to the species level. Identification was achieved mainly due to the phenotypical and morphological characteristics of the isolated yeasts and moulds. These characteristics showed that the most common yeasts and moulds present in the samples of the fermented milk from Northern Uganda were *Saccharomyces* and *Kluyvermnyces* spp., and in the samples from Western Kenya, *Kluyvermnyces; Penicillium* spp. and *Aspergillus* species. Although yeasts were isolated from UG 2 samples, *Aspergillus* flavus was not detected. *A. flavus* is associated with corn and groundnuts and produces toxins. In a traditional setting, each farmer produces corn and groundnuts for home consumption. *A. flavus* can be transferred from the farmer's hands or a cow's skin to contaminate the milk. This contaminant requires monitoring in dairy farming. However, the API system that was used did not identify most of the yeast and mould isolates.

3.4. Survival of Pathogens during Storage

The aim of this study was to assess the microbial quality of typical traditional African fermented milk that is ready for consumption. However, we also studied the survival of pathogens in milk that was stored for three days. Samples of UG 1, UG 2, and KE were stored at 25 °C for a further three days after the initial assessment of the fermented milk had been performed when it was 36-h-old and viable cell counts for general coliforms, *E. coli*, *L. monocytogenes*, *S.* Typhimurium, *S. aureus*, and *B. cereus* had been found (Table 3). Viable counts were performed very 24 h to assess the survival of these organisms. It is of note that traditional farmers in the areas of study consume the milk within 24–48 h of production. The milk storage aimed to observe the survival pattern of the pathogens for possible improvement.

Figure 2 shows that coliforms were still recovered after three days of storage (104 h after production). The viable counts declined by approximately two log units in the UG 1 and UG 2 samples and by approximately 1.5 log units in the sample (KE). *E. coli* counts declined after 36 h by three to four log units in all the samples during the three days of storage by 2.8 log units were still recovered in all the samples after 104 h of fermentation, which indicated some resistance even in the low pH environment of the products. *S. typhimurium* was not detected in KE samples after two days of storage (60 h old) but it survived after this time in UG 2 samples (Figure 2). *L. monocytogenes* was not detected after 60 h of production and storage. *B. cereus* survived for up to 60 h after production and storage and was not recovered after three days of storage. *S. aureus* survived in all the samples up to 84 h and were not detected after 104 h in the UG samples but were present in the KE samples.

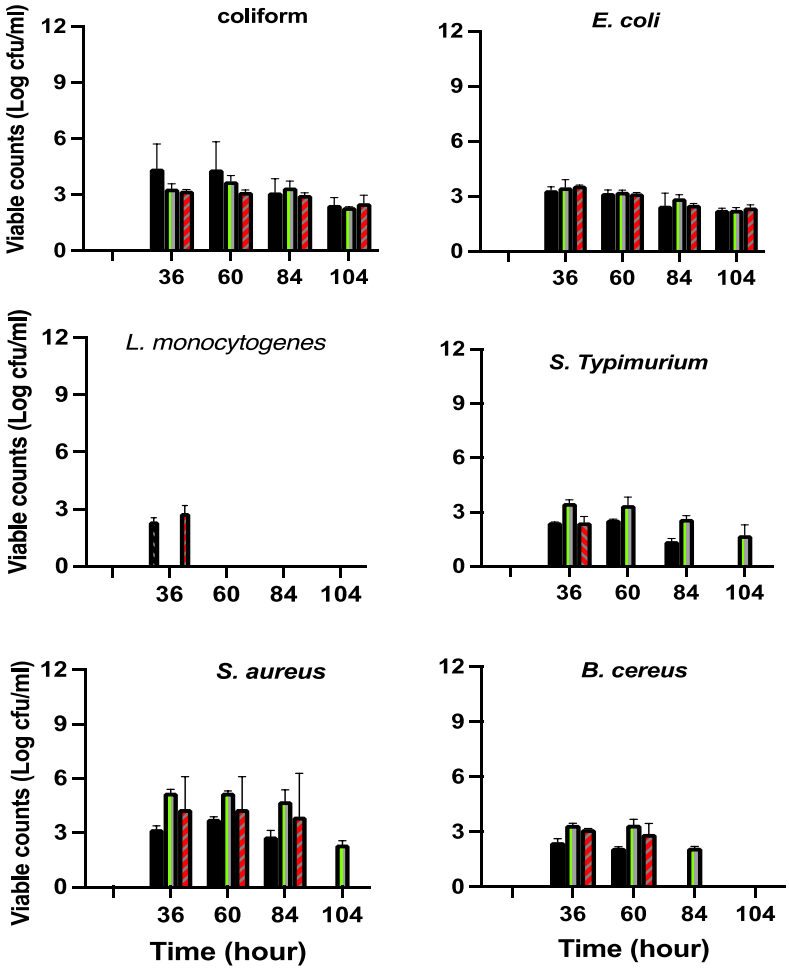

Figure 2. Survival of the coliform, *E. coli, L. monocytogenes, S. typhimurium, S. aureus and B. cereus* in traditionally fermented milk during storage for 104 h at 25 °C in UG 1 [■], UG 2 [■], and KE [■] samples.

Coliform bacteria are often used as indicator organisms for the hygiene status of milk on farms. Reports state increasingly that the total number of Gram-negative bacteria reflect accurately the hygiene status of processing environments on farms, yet this report shows that even Gram-positive bacteria such as *S. aureus*, which colonise healthy skin, nails and nasal mucosa of humans, and *B. cereus*, which is usually associated with soil, also reflect the hygiene status of farms and especially of the farmers.

4. Discussion

In this study, the physicochemical and microbiological attributes of traditional African yoghurt produced in Northern Uganda and Western Kenya were assessed to establish the status of the microbial risks that are associated with this product. Milk is processed at the household level to become sour; this is achieved by leaving fresh, raw milk to ferment naturally for one to three days at ambient temperature. Fermentation occurs spontaneously in gourds or earthenware pots. Sometimes sour milk from previous batches is added to speed up the fermentation process.

In the three days from production to analysis, the pH of the tested traditional fermented milk was low (2.9–3.6). Makut et al. [39], Nduko et al. [40], Ifeanyi et al. [41], and Digbabul et al. [42] reported that the pH of traditionally fermented yoghurt ranged from pH 3.5 to 5.11. The low pH in this study was reflected in the TA level, which was 1.26 $\pm$ 0.1, 0.71 $\pm$ 0.1, and 0.92 $\pm$ 0.1% for the UG 1, UG 2, and KE samples, respectively.

The AMBCs in fermented milk indicate the sanitary or quality conditions that prevailed during the production and handling of raw milk, or post-fermentation contamination. The average AMBC obtained in the current study was very high (10^9 cfu/mL), which indicated poor quality of the products. This number is over the microbial limit that is acceptable (10^6 cfu/mL) for such products according to the Health Protection Agency (HPA) guidelines [43]. Regarding the microbial quality of the tested samples, the AMBCs were not significantly different ($p > 0.05$) from each other. The mean counts for mesophilic lactobacilli were highest in the UG 1 sample (10^8 cfu/mL) followed by the KE (10^7 cfu/mL), and lowest in the UG 2 (10^6 cfu/mL). The thermophilic lactobacilli were 10^7 cfu/mL in UG 1, higher in UG 2 (10^9 cfu/mL), but lower (10^6 cfu/mL) in KE samples. The high AMBCs (10^6–10^9 cfu/mL) may have been due to the already high numbers of bacteria in the raw milk. This has been observed by other researchers who have studied raw milk taken from different areas of Africa [44]. Hot weather in the production areas also enhances the growth of microorganisms in milk if it is contaminated before or during processing [45]. Besides the high AMBC counts, the yoghurt samples had a rich diversity of microorganisms, predominantly LAB and yeasts.

In Africa, fermentation is spontaneous and back slopping is used, in which the previously fermented milk is used as a starter culture. Elsewhere in the world, specific milk starter cultures (*Lactobacillus bulgaricus* and *Streptococcus thermophilus)* are used to ferment milk into yoghurt under a controlled environment. Thus, it came as no surprise that these typical African fermented milk products harboured rich and diverse types of microbes, especially LAB. The amounts of the bacteria recovered from the samples were similar to those reported from Zambian milk by Yambayamba and Zulu [5]. Similarly, high bacteria count (5.6–7.5 log cfu/mL) were reported in the traditional yoghurt (10^8 cfu/mL) of Sudan and Egypt [46]; in the traditional fermented milk of Zimbabwe (10^8 cfu/mL) [47]; and in traditional fermented milk from Morocco [48]. In South Africa, a high number of microorganisms (10^8 cfu/mL) were also reported [49,50]. These high numbers of mesophilic bacteria could have been due to the warm ambient temperature (28–35 °C) at which the milk fermented at the time. The presence of microorganisms in traditional fermented milk depends on the nature of the milk and the temperatures of the regions from which they have been obtained [46]. It also depends on the level of contamination at the production site. Contamination can occur during milking, especially in cases in which hygiene practices such as washing the udders pre-milking are poor [51]. It is therefore important to remove visible dirt from the outer surface of the udder because this is likely to contribute to the

contamination of the raw milk. Most traditional herders in the region of study do not practise pre-milking udder washing.

Other researchers [52] have noted that mesophilic bacteria such as *Leuconostoc* spp. are found in traditional fermented milk products in regions with cold climates, whereas, in warm regions, thermophilic bacteria such as *Lactobacillus* and *Streptococcus* dominate [53]. This could explain the high numbers of mesophilic bacteria that were found in these samples, because they were fermented and collected during the rainy season and cooler months (25–35 °C) in Kenya and Uganda.

The amounts of LAB were in the range of 10^8 log cfu/mL. The counts of thermophilic lactobacilli and Lactococcus were 2.87×10^7 in the UG 1, 1.54×10^9 in the UG 2 and 1.74×10^8 in the KE samples. Obadai and Dodd [54] reported counts of LAB in the range 10^8 to $\times 10^{10}$ in *nyarmie*, in the traditional fermented milk of Ghana. These results agree with those reported by Owusu-Kwarteng et al. [55] in *nunu*, Ghana's traditional fermented milk product, and by Mathara et al. [56] in *kule naoto*, Kenyan traditional milk. In this report, the dominant Streptococci were *S. thermophilus*. The abundance of Lactobacillaceae and Streptococcaceae over other families suggested the dominance of LAB during the fermentation process, and this was equally reported in other studies [57,58]. In addition, this high number of LAB may have been due to natural selection and/or temperature of fermentation. The presence of fewer *Leuconostocs* suggests that this group is unable to compete with other LAB in mixed cultures [57]. This gives them a selective disadvantage over other LAB and a selective advantage over thermophilic bacteria. LAB are generally recognised as safe; they are part of the natural microbiota of various foods and are often used as starter cultures. Some lactic acid bacteria such as *Lactococcus*, *Leuconostoc*, *Pediococcus*, and *Lactobacillus* species are found to inhibit the growth of microorganisms and other pathogens in yoghurt which could be the same effect in traditionally fermented milk.

The numbers of coliforms were unsatisfactorily high in the UG 1 sample (10^5 cfu/mL) but at a borderline of acceptance in the UG 2 and KE samples (10^3 cfu/mL) in this study. Counts of coliform in UG 1 samples suggested poor handling and processing conditions of the milk. Levels of other pathogens such as *E. coli, Salmonella* species, *Bacillus cereus*, and *S. aureus* were also unsatisfactory according to the HPA guidelines, with counts of between 10^3 and 10^4 cfu/mL. However, *B. cereus* did not maintain its contamination after 72 h of storage, probably due to the acidity of the products. Hamama [59] reported similar results in 'lben' and 'jben', Moroccan traditional fermented dairy products.

Salmonella species are known pathogens that can cause food poisoning if contaminated milk or milk products are consumed. In the present study, *Salmonella* species were recovered in UG 1 samples irrespective of the low pH (pH 2.9). *Salmonella*, as enteric pathogens, encounter low pH values in the environment, especially during their transit in the host. According to Foster [60], *Salmonella* species such as *Salmonella* Typhimurium periodically confront acid environments during their lives. In an experiment, Liyuwork et al. [61] observed antimicrobial resistance in *Salmonella* species isolated from dairy products in Addis Ababa. Chatti et al. [12] reported the isolation of acid-resistant *Salmonella* from food and wastewater in Tunisia. Although it is generally thought that *Salmonella* is destroyed or inactivated during the fermentation of highly acidic products, such as yoghurt in which the pH value is less than 4.55, this was not the case in this study. This could be because *Salmonella* can survive in various environmental niches for long periods.

Many diseases are transmissible via milk products, and pathogenic and acid-tolerant bacteria in acidic foods have recently been the causes of public health concern. Unpasteurised milk has been found to be a major vehicle for the transmission of pathogens such as *E. coli, L. monocytogenes*, and *Salmonella*. It can be assumed that sources of contamination by microorganisms include dirty teats and milkers' hands, and the repeated use of milking and fermentation vessels [62]. The presence of coliforms has long been thought to indicate faecal contamination; however, recent reports regarding this diverse group of bacteria indicate that only a fraction are faecal in origin, while the majority are environmental contaminants [63]. Low counts of coliforms might be due to the high acidity of the products,

which restricted the growth of the microorganisms. However, coliforms were still recovered even in such high acidity conditions, suggesting adaptation to the acidic environment.

Yeasts and mould can build up on equipment surfaces and under the surface of package lids. These often contaminate fermenting milk [64]. The presence of yeasts and mould in milk, and their products, is undesirable as they can cause changes in the product that reduce the shelf life and render it unacceptable for consumption. In this study, many of the components of the microbial population were yeasts. The high number of yeasts in the products suggests a high presence of yeasts in the environment where the milk was fermented. In addition, it indicates that yeasts form a significant part of the microflora of these naturally fermented milk products in these areas. When milk is fermented according to good manufacturing practice, it should contain less than 10 yeast cells per gramme, and a shelf life of three to four weeks is expected if they are correctly refrigerated. However, milk that is contaminated with an initial load of 100 or more yeast cells per gramme will quickly spoil as the yeast cells multiply.

Yeasts may be a common part of the flora of the milking parlour and equipment and may impact the overall quality of the products. Yeasts and mould can produce toxic metabolites that are not destroyed during fermentation [65]. In this study, several yeast and mould species were recovered from the fermented milk, similar to the report of Savova and Nikolova [66]. Despite their undesirability due to spoilage effects, yeasts play an important role in foodstuffs. Getachew et al. [67] commented that the variety of microorganisms present in naturally fermented milk products created rich and full flavours that are hard to imitate. Yeasts can grow in a broad range of pH environments and usually adapt to coexistence with LAB in acidic environments [62]. *Saccharomyces cerevisiae*, a lactose fermenting yeast present in the yoghurt, may have contributed to lowering the acidity of the products [68].

The use of appropriate traditional equipment is crucial to pathogen control. A complex relationship exists between the microbiology of milk and milk products and their processing environment. The equipment must be easy to clean and sanitise, to prevent the formation of niches in which microorganisms can grow and settle, forming biofilms [64]. Only a few pathogens of concern associated with milk and milk products typically originate from the production environment: *Listeria monocytogenes*, *Salmonella* ssp., and most recently *Cronobacter sakazakii*. These pathogens have been widely reported to persist in processing plant niches for years and in some cases decades. Furthermore, lack of pasteurisation, inadequate storage and maturation conditions, the temperature of water used for cow udder washing, the practice of mixing milk lots, the type of milk container, use of refrigeration, and milk filtration are some of the major risk-enhancing factors in traditional milk fermentation [69].

To minimise contamination during milking, effective hygiene practices must be applied to the hands of the milkers, the udders of the animals and the milking equipment, and the general environment; for example, faecal contamination must be prevented [61]. Washing hands without detergent is insufficient to improve the cleanliness of milk or milk products. Poor drying practices after hand washing and the use of old, dirty clothes for other farm activities is a risk factor for milk contamination [67]. Traditional knowledge plays a role in the creation of awareness in the community to manage day-to-day activities in livestock management [69]. The main advantage of spontaneous fermentation processes is that they are appropriate to rural situations, where they were created.

Several reports on the microbiological quality of fermented milk in Africa provide knowledge of the various microorganisms in yoghurt and other traditional fermented milk. However, gaps remain regarding the knowledge of pathogen control in traditional milk fermentation environments as microorganisms continue to be identified in traditional dairy products. Although many countries have milk safety regulations and surveillance systems which monitor foodborne pathogens to ensure food safety, such surveillance of milk and milk products is not conducted routinely in most African countries. Consistency in the day-to-day implementation of milking procedures is an important part of good dairy farming

practices. Use of the guide developed by the US Food and Agriculture Organization [70] would help to improve the standard of milk quality and farming practices at traditional farms. The period of incubation and temperature of storage of the products must also be considered during assessment for improvement of fermented milk as well as the safety of traditional fermented milk. Some researchers [7–9,13–15] have reported that the numbers of pathogens decrease when the fermented milk is stored for more than seven days at 22 °C or they are eliminated when the milk is stored at 25 °C or atmospheric temperature for 15 days. This period must be considered together with pH or acidity of the product. The organoleptic characteristics of the product may become unsatisfactory at a pH below 5.0 because the increased acidity makes the product unacceptable to the consumer.

5. Conclusions

This study of traditional fermented milk of Northern Uganda and Western Kenya has shown that although the products are of low pH, they harbour high and variable loads of bacteria and therefore could pose health risks to consumers. In respect to food safety, the consumption of unpasteurised milk and dairy products poses significant health risks to consumers due to the persistence in the dairy chain in Africa of microbial pathogens of significant safety concern. The presence of microorganisms such as *E. coli*, *Salmonella* spp., *Bacillus* spp., and *Staphylococcus aureus* indicates the need to improve hygiene in traditional fermented milk production among farmers. The cross-contamination of milk products with microorganisms is an ongoing risk throughout traditional milk production.

To improve the quality of traditionally fermented milk, training must be conducted, and awareness raised regarding hygiene practices on the farm. Such practices include the cleaning and sanitisation of hands before and after milking, udder washing, drying the udder with clean dry cloths, and correct washing of milk equipment. Instruction in ways to avoid cross-product contamination from the environment and equipment should be stepped up. This should include everyone in the household who is involved in milking and processing, especially women. Rural people's preference for fermented milk with raw milk over boiled or pasteurised milk, and the fact that smallholder dairying and informal markets dominate dairying in Africa, must be recognised. Additionally, clear messages must be promulgated regarding the dangers of consuming dairy products made from raw milk.

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References

1. Materia, V.C.; Linnemann, A.R.; Smid, E.J.; Schoustra, S.E. Contribution of traditional fermented foods to food systems transformation: Value addition and inclusive entrepreneurship. *Food Secur.* **2021**, *13*, 1163–1177. [CrossRef]
2. Iwuoha, C.I.; Eke, O.S. Nigerian indigenous fermented foods: Their traditional process operation, inherent problems, improvements and current status. *Food Res. Int.* **1996**, *29*, 527–540. [CrossRef]
3. Moatsou, G.; Moschopoulou, E. Microbiology of Raw Milk. In *Dairy Microbiology and Biochemistry: Recent Developments*; Özer, B.H., Akdemir-Evrendilek, G., Eds.; Taylor & Francis Group, LLC: New York, NY, USA, 2015; pp. 1–38.

4. Aliyo, A.; Teklemariam, Z. Assessment of Milk Contamination, Associated Risk Factors, and Drug Sensitivity Patterns among Isolated Bacteria from Raw Milk of Borena Zone. *Ethiop. J. Trop. Med.* **2022**, *2022*, 3577715. [CrossRef]
5. Yambayamba, K.E.; Zulu, M.P. Influence of the milking environment on the microbial quality of raw milk produced by smallholder farmers in Magoye. *UNZA J. Sci. Technol.* **2011**, *15*, 37–43.
6. Leone, C.; Thippareddi, H.; Ndiaye, C.; Niang, I.; Diallo, Y.; Singh, M. Safety and Quality of Milk and Milk Products in Senegal—A Review. *Foods* **2022**, *11*, 3479. [CrossRef] [PubMed]
7. Moonga, H.B. Product Optimization of Zambian Traditionally Fermented Milk-Mabisi. Doctoral Dissertation, Wageningen University, Wageningen, The Netherlands, 2019.
8. Maleke, M.S.; Adefisoye, M.A.; Doorsamy, W.; Adebo, O.A. Processing, nutritional composition, and microbiology of amasi: A Southern African fermented milk product. *Sci. Afr.* **2021**, *12*, e00795. [CrossRef]
9. Landis, E.A.; Oliverio, A.M.; McKenney, E.A.; Nichols, L.M.; Kfoury, N.; Biango-Daniels, M.; Shell, L.K.; Madden, A.A.; Shapiro, L.; Sakunala, S.; et al. The diversity and function of sourdough starter microbiomes. *eLife* **2021**, *10*, e61644. [CrossRef]
10. Mazurek, J.; Salehi, E.; Propes, D.; Holt, J.O.; Bannerman, T.; Nicholson, L.M.; Bundesen, M.; Duffy, R.; Moolenaar, R.L. A multistate outbreak of *Salmonella enterica* serotype Typhimurium infection linked to raw milk consumption—Ohio, 2003. *J. Food Prot.* **2004**, *67*, 2165–2170. [CrossRef]
11. Gebeyehu, A.; Taye, M.; Abebe, R. Isolation, molecular detection and antimicrobial susceptibility profile of *Salmonella* from raw cow milk collected from dairy farms and households in southern Ethiopia. *BMC Microbiol.* **2022**, *22*, 84. [CrossRef]
12. Chatti, A.; Daghfous, D.; Landoulsi, A. Acid resistance of *Salmonella* isolated from animals, food and wastewater in Tunisia. *Ann. Saudi Med.* **2007**, *27*, 195–198. [CrossRef]
13. Massa, S.; Altieri, C.; Quaranta, V.; De Pace, R. Survival of *Escherichia coli* O157: H7 in yoghurt during preparation and storage at 4 °C. *Lett. Appl. Microbiol.* **1997**, *24*, 347–350. [CrossRef]
14. Conner, D.; Kotrola, J.S. Growth and survival of *Escherichia coli* O157:H7 under acidic conditions. *Appl. Environ. Microbiol.* **1995**, *61*, 382–385. [CrossRef] [PubMed]
15. Ogwaro, B.A.; Gibson, H.; Whitehead, M.; Hill, D.J. Survival of *Escherichia coli* O157:H7 in traditional African yoghurt fermentation. *Int. J. Food Microbiol.* **2002**, *79*, 105–112. [CrossRef]
16. Bashiry, M.; Javanmardi, F.; Taslikh, M.; Sheidaei, Z.; Sadeghi, E.; Abedi, A.S.; Mirza Alizadeh, A.; Hashempour-Baltork, F.; Beikzadeh, S.; Riahi, S.M.; et al. Listeria monocytogenes in Dairy Products of the Middle East Region: A Systematic Review, Meta-Analysis, and Meta-Regression Study. *Iran J. Public Health* **2022**, *51*, 292–305. [CrossRef]
17. Motarjemi, Y.; Moy, M.G.G.; Jooste, P.J.; Anelich, L.E. Milk and Dairy Products. Chapter 5. In *Food Safety Management*; Elsevier: Amsterdam, The Netherlands, 2014; pp. 83–117.
18. Sanaa, M.; Poutrel, B.; Menard, J.L.; Serieys, F. Risk factors associated with contamination of raw milk by *Listeria monocytogenes* in dairy farms. *J. Dairy Sci.* **1993**, *76*, 2891–2898. [CrossRef] [PubMed]
19. Le Loir, Y.; Baron, F.; Gautier, M. Staphylococcus aureus and food poisoning. *Genet. Mol. Res.* **2003**, *2*, 63–76.
20. Pazakova, J.; Turek, P.; Laciakova, A. The survival of *Staphylococcus aureus* during the fermentation and storage of yoghurt. *J. Appl. Microbiol.* **1997**, *82*, 659–662. [CrossRef] [PubMed]
21. Umoh, V.J.; Adesiyun, A.A.; Gomwalk, N.E. The occurrence of *Staphylococcus aureus* in fermented milk products (fura and manshanu) in Nigeria. *Int. J. Food Microbiol.* **1990**, *10*, 343–347. [CrossRef]
22. Peton, V.; Le Loir, Y. *Staphylococcus aureus* in veterinary medicine. *Infect. Genet. Evol.* **2014**, *21*, 602–615. [CrossRef] [PubMed]
23. Tirloni, E.; Stella, S.; Celandroni, F.; Mazzantini, D.; Bernardi, C.; Ghelardi, E. Bacillus cereus in Dairy Products and Production. *Plants Foods* **2022**, *11*, 2572. [CrossRef]
24. Washaya, S.; Jakata, C.; Tagwira, M.; Mupofu, T. Bacterial Milk Quality along the Value Chain in Smallholder Dairy Production. *Sci. World J.* **2022**, *21*, 7967569. [CrossRef]
25. Obafemi, Y.D.; Oranusi, S.U.; Ajanaku, K.O.; Akinduti, P.A.; Leech, J.; Cotter, P.D. African fermented foods: Overview, emerging benefits, and novel approaches to microbiome profiling. *npj Sci Food.* **2022**, *6*, 15. [CrossRef] [PubMed]
26. Nyambane, B.; Thari, W.M.; Wangoh, J.; Njage, P.M.K. Lactic acid bacteria and yeasts involved in the fermentation of amabere amaruranu, a Kenyan fermented milk. *Food Sci. Nutr.* **2014**, *2*, 692–699. [CrossRef] [PubMed]
27. Mukisa, I.M.; Kyoshabire, R. Microbiological, physicochemical, and sensorial quality of small-scale produced stirred yoghurt on the market in Kampala city, Uganda. *Nutr. Food Sci.* **2010**, *40*, 409–418. [CrossRef]
28. *EN ISO 8261:2001*; Milk and Milk Products-General Guidance for the Preparation of Test Samples, Initial Suspensions and Decimal Dilutions for Microbiological Examination: Lait et Produits Laitiers-Lignes Directrices Générales Pour la Préparaton des Échantillons Pour Essai, de la Suspension Mère et Des Dilutions Décimales en Vue de L'e-xamen Microbiologique. IOS (International Organization for Standardization): Geneva, Switzerland, 2001.
29. Association of Official Analytical Chemist. *Official Methods of Analysis*, 18th ed.; Association of Official Analytical Chemist: Washington, DC, USA, 2006.
30. De Man, J.C.; Rogosa, M.; Sharpe, E.M. A medium for the cultivation of Lactobacilli. *J. App. Bact.* **1960**, *23*, 130–135. [CrossRef]
31. Rogosa, M.; Mitchell, J.A.; Wiseman, R.F. A selective medium for the isolation of oral and faecal lactobacilli. *J. Bacteriol.* **1951**, *62*, 132–133. [CrossRef]
32. Terzaghi, B.E.; Sandine, W.E. Improved medium for lactic Streptococci and their bacteriophages. *Appl. Microb.* **1975**, *29*, 807–813. [CrossRef]

33. Moushumi, B.; Prabir, K.S. Microbiological quality of some retail spices in India. *Food Res. Int.* **2003**, *36*, 469–474.
34. International Dairy Federation. *Milk and Milk-Based Products—Enumeration of Staphylococcus Aureus (IDF Standard 1990)*; IDF145; IDF: Schaerbeek, Brussels, 1990.
35. *ISO 6888-1:2021*; Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Enumeration of Coagulase-Positive Staphylococci (*Staphylococcus aureus* and Other Species)—Part 1: Method Using Baird-Parker Agar Medium. ISO (International Organization for Standardization): Geneva, Switzerland, 2021.
36. Duncan, S.; Yaun, E.; Sumner, S.S.; Bruhn, J. Chapter 09 Microbiological Methods for Dairy Products. In *Standard Methods for the Examination of Dairy Products*; American Public Health Association: Washington, DC, USA, 2012. [CrossRef]
37. Reiner, K. *Catalase Test Protocol*; American Society Microbiology: Washington, DC, USA, 2016.
38. Sulaiman, I.M.; Hsieh, Y.H. Foodborne Pathogens in Milk and Dairy Products: Genetic Characterization and Rapid Diagnostic Approach for Food Safety of Public Health Importance. In *Dairy in Human Health and Disease across the Lifespan*; Springer: Berlin, Germany, 2017; pp. 127–143.
39. Makut, D.; Ogbonna, A.I.; Dalami, H. An Assessment of the Bacteriological Quality of Different Brands of Yoghurt Sold in Keffi, Nasarawa State, Nigeria. *J. Nat. Sci. Res.* **2014**, *4*, 19–22.
40. Nduko, J.M.; Matofari, J.W.; Nandi, Z.O.; Sichangi, M.B. Spontaneously fermented Kenyan milk products. A review of the current state and future perspectives. *Afr. J. Food Sci.* **2017**, *11*, 1–11.
41. Ifeanyi, V.O.; Ihesiaba, E.O.; Muomaife, O.M.; Ikenga, C. Assessment of microbiological quality of yoghurt sold by street vendors in Onitsha metropolis, Anambra State, Nigeria. *Br. Microbiol. Res. J.* **2013**, *3*, 198. [CrossRef]
42. Digbabul, B.; Shember, J.; Amove, J. Physicochemical, microbiological, and sensory evaluation of yoghurt sold in Makurdi me-tropolis. *Afr. J. Food Sci. Technol.* **2014**, *5*, 129–135.
43. Health Protection Agency (HPA). *Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods*; Health Protection Agency: London, UK, 2009.
44. Majoie, G.Ã.; Mousse, W.; Haziz, S.I.N.; Farid, B.A.D.; Ahouissou, O.R.; Adjanohoun, A.; Lamine, B.M. Microbial quality of artisanal yoghurt and Degue products collected in schools of Cotonou and Abomey-Calavi (Benin) *Afr. J. Food Sci.* **2020**, *14*, 112–118.
45. Jans, C.; Meile, L.; Kaindi, D.W.; Kogi-Makau, W.; Lamuka, P.; Renault, P.; Kreikemeyer, B.; Lacroix, C.; Hattendorf, J.; Zinsstag, J.; et al. African fermented dairy prod-ucts–overview of predominant technologically important microorganisms focusing on African *Streptococcus infantarius* var-iants and potential future applications for enhanced food safety and security. *Int. J. Food Microbiol.* **2017**, *250*, 27–36. [CrossRef]
46. Abdalla, M.O.M.; Abdel Nabi, S.Z. Evaluation of microbiological quality of Sudanese fermented dairy product 'mish' during storage. *Adv. J. Food Sci. Tech.* **2010**, *2*, 155–158.
47. Moonga, H.B.; Schoustra, S.E.; Linnemann, A.R.; Kuntashula, E.; Shindano, J.; Smid, E.J. The art of mabisi production: A traditional fermented milk. *PLoS ONE* **2019**, *14*, e0213541. [CrossRef]
48. Benkirane, G.; Ananou, S.; Dumas, E.; Ghnimi, S.; Gharsallaoui, A. Moroccan Traditional Fermented Dairy Products: Current processing Practices and Physiochemical and Microbiological Properties: A Review. *J. Microbiol. Biotechnol. Food Sci.* **2022**, *12*, e5636. [CrossRef]
49. Doslash, R.; Osvik, V.; Sperstad, S.; Breines, E.; Hareide, E.; Godfroid, J.; Zhou, Z.; Ren, P.; Geoghegan, C.; Holzapfel, W.; et al. Bacterial Diversity of aMasi, a South African Fermented Milk Product, Determined by Clone Library and Denaturing Gradient Gel Electrophoresis Analysis. *AJMR* **2013**, *7*, 4146–4158. [CrossRef]
50. Taye, Y.; Degu, T.; Fesseha, H.; Mathewos, M. Isolation and Identification of lactic acid bacteria from cow Milk and Milk products. *Sci. World J.* **2021**, *2021*, 4697445. [CrossRef]
51. Gleeson, D.; O'Brien, B.; Flynn, J.; O'Callaghan, E.; Galli, F. Effect of pre-milking teat preparation procedures on the microbial count on teats prior to cluster application. *Ir. Vet. J.* **2009**, *62*, 461. [CrossRef]
52. Haile, W.; Yilma, Z.; Teklegiorgis, Y. Incidence of pathogenic and indicator bacteria in raw and pasteurized milk in Hawassa city, rift valley of Southern Ethiopia. *Afr. J. Food Sci.* **2012**, *7*, 100–107.
53. Ismail Ahmed, A. Isolation and Identification of LAB from Sudanese Traditional Fermented Camel (*Camelus dromedarius*) Milk Gariss. *Open J. Nutr. Food Sci.* **2022**, *4*, 1022.
54. Obodai, M.; Dodd, C.E.R. Characterization of dominant microbiota of a Ghanaian fermented milk product, nyarmie, by cul-ture- and nonculture-based methods. *J. Appl. Microbiol.* **2006**, *100*, 1355–1363. [CrossRef] [PubMed]
55. Owusu-Kwarteng, J.; Tano-debrah, K.; Glover, R.L.K.; Akabanda, F. Process characteristics and microbiology of fura produced in Ghana. *Nat. Sci.* **2010**, *8*, 41–51.
56. Mathara, J.M.; Schillingera, U.; Kutima, P.M.; Mbugua, S.K.; Holzapfel, W.H. Isolation, Identification, and characterisation of the dominant microorganisms of kule naoto: The Maasai traditional fermented milk in Kenya. *Int. J. Food Microbiol.* **2004**, *94*, 269–278. [CrossRef]
57. Teuber, M. The Genus Lactococcus. In *The Genera of Lactic Acid Bacteria. The Lactic Acid Bacteria*; Wood, B.J.B., Holzapfel, W.H., Eds.; Springer: Boston, MA, USA, 1995; Volume 2.
58. Togo, C.A.; Feresu, S.B.; Mutukumira, A.N. Identification of Lactic Acid Bacteria isolated from Opaque beer (Chibuku) for potential use as a starter culture. *J. Food Technol. Afr.* **2002**, *7*, 93–97. [CrossRef]

59. Hamama, A. Moroccan Traditional Fermented Dairy Products. In *Applications of Biotechnology to Traditional Fermented Foods*; Ruskin, F.R., Ed.; National Academy Press: Washington, DC, USA, 1992; pp. 75–79.
60. Foster, J.W. Low pH Adaptation and the Acid—Tolerance Response of *Salmonella typhimurium*. *Crit. Rev. Microbiol.* **2008**, *21*, 215–237. [CrossRef]
61. Liyuwork, T.; Biruhalem, T.; Sefinew, A.; Hailleleul, N. Prevalence and antimicrobial resistance profile of *Salmonella* isolates from dairy products in Addis Ababa, Ethiopia. *Afr. J. Microbiol. Res.* **2013**, *7943*, 5045–5050.
62. Bereda, A.; Yesuf Kurtu, M.; Yilma, Z. Handling, processing and utilization of Milk and Milk products in Ethiopia: A review. *World J. Dairy Food Sci.* **2014**, *9*, 105–112.
63. Pyz-Lukasik, R.; Paszkiewicz, W.; Brodzki, P.; Belkot, Z. Microbiological quality of milk sold directly from producers to con-sumers. *J. Dairy Sci.* **2015**, *98*, 4294–4301. [CrossRef]
64. Fleet, G.H. Yeasts in dairy products. A Review. *J. Appl. Bacteriol.* **1990**, *68*, 199–211. [CrossRef] [PubMed]
65. Akabanda, F.; Owusu-Kwarteng, J.; Glover, R.L.K.; Tano-Debrah, K. Microbiological characteristics of Ghanaian traditional fermented milk product. Nunu. *Nat. Sci.* **2010**, *8*, 178–187.
66. Savova, L.; Nikolova, M. Isolation and Taxonomic study of yeast strain from Bulgarian Dairy products. *J. Cult. Collect.* **2000**, *3*, 59–65.
67. Getachew, A.; Tadie, A.; Chercos, D.H.; Guadu, T. Level of Faecal Coliform Contamination of Drinking Water Sources and Its Associated Risk Factors in Rural Settings of North Gondar Zone, Ethiopia: A Cross-Sectional Community Based Study. *Ethiop. J. Health Sci.* **2018**, *28*, 227–234. [CrossRef] [PubMed]
68. Bùchi, N.R.; Seller, H. Yeast and moulds: Yeasts in milk and dairy products. In *Encyclopedia of Dairy Sciences*, 2nd ed.; Academic Press: Cambridge, MA, USA, 2011; pp. 744–753. [CrossRef]
69. International Commission on Microbiological Specifications for Foods. *Microorganisms in Foods 5: Microbiological Specifications of Food Pathogens*; Blackie Academic and Professional: New York, NY, USA, 1996; p. 6.
70. Food and Agriculture Organisation of the United Nations (FAO). *Guide to Good Dairy Farming Practice: Milking Hygiene*; Food and Agriculture Organisation of the United Nations (FAO): Rome, Italy, 2023.