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Research Paper

Contribution of Farm-level Hygiene and Handling Practices to Microbial Safety Profiles in the Informal Dairy Sector in Zimbabwe

S. Chimuti¹, D.T. Mugadza^{2,*}, V. Ntuli³, P.M.K. Njage⁴¹ Department of Food and Microbiology, Government Analyst Laboratory, Ministry of Health and Childcare, Harare, Zimbabwe² Department of Food Science and Nutrition, Midlands State University, Gweru, Zimbabwe³ Department of Food Science and Technology, School of Agriculture University of Venda, P. Bag X5050, Thohoyandou, Limpopo, South Africa⁴ Research Group for Genomic Epidemiology, National Food Institute, Technical University of Denmark, Denmark

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ABSTRACT

The current study assessed (i) the microbiological safety level profiles (MSLPs) of milkmen's hands and milking containers and (ii) the influence of hygiene and handling practices on MSLPs of raw and cultured milk, from six informal dairy farms in Zimbabwe. Interviews and direct observations were carried out during the assessment of hygiene and handling practices at six farms designated A to F. Microbiological criteria of the following six microbiological parameters: Total Bacterial Counts (TBCs), Coliform Counts (CCs), Total *Escherichia coli* Counts (TECs), *Salmonella* spp., *Listeria monocytogenes* and *Klebsiella pneumoniae*, were used to determine contamination level (CL) at four different critical sampling locations (CSLs). The CSLs were raw milk (CSL1), cultured milk (CSL2), milkmen's hands (CSL3), and milking containers (CSL4). The microbiological criteria of the six microbiological parameters were used to score CLs as: intolerable (0), poor to average (1), average (2), and good (3). MSLPs at each CSL for the six farms were computed based on the CL scores to a maximum score of 18. A total of 192 samples were collected and analyzed. *Salmonella* spp. and *L. monocytogenes* were not detected at all the CSLs. All the farms failed to achieve a maximum MSLP score of 18 at all the CSLs. The relationship between MSLPs and hygiene and handling practices was tested using point-biserial correlation coefficients. The correlation study revealed that handling and hygiene practices (such as the duration between milking and storage, the type of milking container utilized at farms, the frequency of cleaning the milking parlor, the water source for hand and equipment washing, and the use of hand sanitizers) generally influenced the MSLPs on the farms. Both training and improvement in infrastructure are needed to improve the quality of milk and its products produced and sold in the informal value chain in Zimbabwe.

The demand for milk and its products is increasing in Zimbabwe as the population grows and lifestyles change. Because of this need, both output and sales are rising, which is crucial for maintaining both the nation's economy as a whole and the livelihoods of dairy farmers. In Zimbabwe, milk is commercialized through formal and informal value chains, although the latter predominates (Hove, 2015). Approximately 4,528 registered small-scale dairy farmers in the country produce and sell milk, primarily through informal markets, accounting for <5% of the country's agricultural gross domestic product (Hove, 2015). The informal value chain in Zimbabwe sells raw milk and dairy products (particularly cultured milk from unpasteurized milk) directly to consumers through street vendors (Gweshe et al., 2020). Because the producers hardly ever implement food safety management systems

(FSMSs), safety and quality along this value chain remain daunting (Abunna et al., 2019). Informal dairy producers shun adopting a structured safety management system because of its complexities and other requirements, such as time and cost (Brown et al., 2019). The majority of developing nations, including Zimbabwe, have informal dairy farmers who produce and process milk using conventional methods. Several studies have demonstrated that the milk products produced by this sector are not sufficiently safe or of sufficient quality (Filipovic & Kokaj, 2009; Mossie, 2019; Welearegay et al., 2012). Furthermore, informal dairy producers are blamed for poor hygiene practices, resulting in poor milk quality due to a lack of knowledge and the implications of poor hygiene (Brown et al., 2019; Georgescu et al., 2014; Worku et al., 2014). Brown et al. (2019) reported that the informal

* Corresponding author.

E-mail address: mugadzadt@staff.msu.ac.zw (D.T. Mugadza).

dairy industry's low knowledge levels and negative attitudes affect their behavioral practices with regard to observing milk quality requirements and food safety laws.

Although there is a lack of appropriate data on the burden of food-borne and dairy-borne diseases in developing countries, Grace et al. (2020) compiled information from different sources to better reflect the estimates. Consuming pathogen-contaminated milk and dairy products in underdeveloped nations may have caused 20 disability-adjusted life years per 100,000 persons in 2010 (Grace et al., 2020). The likelihood that milk and dairy products sold by unregistered vendors played a significant role in this burden is strong.

Since milk is so nutrient-dense, it is an ideal growth medium for pathogenic microorganisms. Milk is subject to microbiological contamination risks at the farm level and throughout the dairy value chain due to several contamination sources and risk factors (Grace et al., 2020). At the farm, contamination of raw milk can emanate from within the udder (in the case of infected animals with subclinical mastitis), contaminated hands of farm personnel, milking environment, milk handling, and storage equipment (Ntuli et al., 2023). Therefore, the control of microbial contamination of milk depends on managing these risk factors. According to empirical research, increasing awareness of milk handling procedures at the farm level following intensive training has improved its quality (Lindahl et al., 2018). The study by Lindahl et al. (2018) provides evidence of the interrelatedness of end product quality and the performance of implemented food safety programs at the farm level. Therefore, analyzing the informal dairy farms concerning microbial contamination along the production and processing chain is essential for assessing their performance and providing evidence-based mitigation measures to reduce milk-borne diseases (Wambui et al., 2018). Once these measures are established, farmers can adopt milk quality and safety practices that are economically viable, technically feasible, and socio-culturally acceptable (Nyokabi et al., 2021).

Several strategies have been developed to assess the effectiveness of hygiene practices in controlling and managing microbiological contamination of milk; however, Jacxsens et al. (2009) developed a robust strategy using a microbiological assessment scheme (MAS). The MAS involves identifying critical sampling locations (CSLs), selecting appropriate microbiological parameters to be analyzed, assessing sampling frequency, selecting sampling and analytical methods, data processing, and interpreting results (Jacxsens et al., 2009). The MAS employs established microbiological criteria at each selected risk factor point (i.e., CSL) along the milk production and processing chain (Jacxsens et al., 2009). Microbiological data obtained at each established CSL are compared with microbiological limits to determine the microbial safety level profiles (MSLPs). Microbiological limits are a typical strategy that has been used to reduce consumer exposure to potentially pathogenic microorganisms in food preparation (Wambui et al., 2018). Based on MAS studies conducted in the dairy industry, CSL established throughout the production and processing chain included raw milk, milk products, food contact surfaces, as well as milkman's hands and milking containers (Opiyo et al., 2013). For microbiological parameters, the dairy industry uses pathogens (such as *Listeria monocytogenes* and *Salmonella* spp.) and indicator organisms (such as total bacterial counts, coliform counts, and *Escherichia coli* counts) to ascertain safety and quality. Although research has shown that milk and dairy products from Zimbabwe's informal dairy industry were severely contaminated with *E. coli* and other pathogens (Chimuti et al., 2016), no study has made an effort to gather data that could be used to develop a dairy quality management strategy based on a thorough analysis of the microbiological risk factors. Therefore, this study assessed (i) the microbiological safety level profiles (MSLPs) of milkmen's hands and milking containers and (ii) the influence of hygiene and handling practices on MSLPs of raw and cultured milk, from six informal dairy farms in Zimbabwe.

Materials and methods

Study area and characteristics of dairy farms

The current study was conducted from November 2020 to August 2021 across four provinces in Zimbabwe which are Manicaland, Mashonaland East, Mashonaland Central, and Harare (Fig. 1). Six dairy farms (denoted A to F) were purposively sampled based on their characteristics as informal dairy farms. Hove (2015) documented that various parameters, including the size of the herd, the number of employees, and the amount of milk produced daily, are used to classify dairy farms in Zimbabwe. The dairy farms included in the current study were categorized as small-scale since the dairy herd size was less than 30 cows, the number of farm workers was fewer than 30 people, and the daily output capacity was less than 100 L.

Survey instrument and data collection

The data collection instrument used in this study was designed as described by Berhe et al. (2020). The survey tool collected information on farm characteristics (number of cows and works per dairy farm, source of water used on the farm, availability of milking parlors), milking practices, milking hygiene, storage facilities, and milking equipment. A pretest survey was carried out on selected farms to assess the understanding of respondents to the questions provided. Based on the pretest results, the questions were adjusted accordingly.

Microbiological assessment scheme

In the current study, the MAS included identifying critical sampling locations, sampling methods, sampling frequency, methods for microbiological analysis, and selection of microbiological parameters.

Identification of critical sampling locations

Critical sampling locations (CSLs) were selected based on published information (DeVere & Purchase, 2007). The selected CSLs included milk containers, milkmen's hands, raw milk, and cultured milk. Raw milk and cultured milk were assigned as CSL1 and CSL2, respectively. Milkmen's hands and milking containers were considered as CSL3 and CSL4, respectively. Milkmen's hands (CSL3) were considered critical because they are a potential source of microbial contaminants such as *E. coli* (Opiyo et al., 2013). Because the milk and the containers come into direct contact, improperly cleaned milking containers have been recognized as critical sampling locations due to the potential for cross-contamination (Opiyo et al., 2013). Milk (CSL1 and CSL2) would become contaminated, resulting in poor milk quality if there were high microbial loads at CSL3 and CSL4. Microbial analysis of hand and container samples was done to give an indication of the actual performance of hygienic practices (Jacxsens et al., 2009). The actual microbiological quality of the milk (CSL1 and CSL2) was determined to establish the contribution of CSL3 and CSL4 and other practices along the chain.

Sampling method, frequency and methods of analysis

The horizontal method for collecting milk (raw and cultured) samples and swabs from milkmen's hands and milking containers was done in accordance with ISO 18593:2004 (2004) and Opiyo et al. (2013). To analyze the samples, analytical techniques based on standard methods were applied (Table 1). These methods were used to detect and enumerate viable microorganisms from milk and swab samples. The sampling frequency was the same as described by Jacxsens et al. (2009) with some modifications. According to Jacxsens et al.



Figure 1. Location of the six selected informal dairy farms (marked with black dots) in four provinces (Manicaland, Mashonaland East, Mashonaland Central, and Harare) in Zimbabwe.

(2009), sampling at different CSLs is done once at the food processing establishment. In the current study, sampling was done three times at each farm, i.e., once a month for three months (January, May, and August 2021), to provide a profile of microbiological contamination at each of the CSLs. Samples of raw milk were collected after milking while sampling for milkmen's hands, and milking containers were collected before milking (different individuals and containers). Samples of cultured milk were collected at the point of sale. A total of 192 samples were collected, which consisted of 54 raw milk, 54 cultured milk samples, 66 swabs from 22 milkmen's hands, and 18 swabs from milking containers. At each visit, samples (raw milk, cultured milk, swabs from milkmen's hands, and milking containers) were collected in triplicate, and each of the six farms was sampled once a month for three months. For swab samples, a 25 cm² surface area on milkmen's hands and milking containers was swabbed. After which, the swabbing stick was soaked in a test tube containing 9 mL of buffered peptone water. Milk samples were collected using 250 mL sterile bottles. The samples were transported for analysis in ice-packed cooler boxes to the Government Analyst Laboratory (ISO 17025:2017 Accredited), Ministry of Health and Childcare in Harare, Zimbabwe.

Selection of microbiological parameters

Microbiological parameters selected for the samples consisted of three categories of microorganisms: pathogens, hygiene and utility indicators. *Salmonella* spp., and *L. monocytogenes* were selected as pathogens (ICMSF, 2018; Opiyo et al., 2013), while total *E. coli* and coliform counts were used as hygiene indicators. *K. pneumoniae* is an opportunistic pathogen that has recently been recognized in food-borne outbreaks (Hartantyo et al., 2020; Zhang et al., 2018); hence, it was selected in the current study as a pathogen. Total bacteria counts were used as a utility indicator (Wambui et al., 2018). The selected pathogens were used in the study because they are frequently isolated from milk and dairy products (ICMSF, 2018; Opiyo et al., 2013).

Enumeration of indicator organisms

The pH of raw and cultured milk samples was determined before microbial counts. Enumeration of TBC, CC, and TEC was performed following ISO standard procedures (Table 1). Swabs were used to

Table 1

Legal requirements for the microbiological parameters that were analyzed at the selected sampling locations (raw and cultured milk and swabs from milkmen's hands and milking container)^a

Parameter	Analytical method	Milkmen's hand swabs	Milking container swabs	Raw milk	Cultured milk
Total bacterial counts	(ISO 4833-1:2013)	m ^b < 1.9 log CFU/cm ² ; M ^c > 3 log CFU/cm ²	m < 1.9 log CFU/cm ² ; M > 3 log CFU/cm ²	m = 0; M = 4.5 log CFU/mL	m = 0; M = 4.5 log CFU/mL
Coliforms	(ISO 4832:2006) (ISO 18593:2004)	Good, ≤1; average, ≤1.8; poor to average, ≤2.5; intolerable, >2.5 log CFU/cm ²	Good, ≤1; average, ≤1.8; poor to average, ≤2.5; intolerable, >2.5 log CFU/cm ²	m = 0; M = 1.3 log CFU/mL	m = 0; M = 1.3 log CFU/mL
<i>Escherichia coli</i>	(ISO 11866-2:2005)	Absent on tested surface	Absent on tested surface	Absent in 1 mL	Absent in 1 mL
<i>Salmonella</i> spp.	(ISO 6579-1:2017)	Absent on tested surface	Absent on tested surface	Absent in 1 mL	Absent in 1 mL
<i>Klebsiella pneumoniae</i>	(Badri et al., 2017; Yang et al., 2021)	Absent on tested surface	Absent on tested surface	Absent in 1 mL	Absent in 1 mL
<i>Listeria monocytogenes</i>	(Hitchins et al., 2022)	Absent on tested surface	Absent on tested surface	Absent in 1 mL	Absent in 1 mL

^a Microbiological criteria were based on the Zimbabwe Dairy Regulations RGN 886 and the values established by the Laboratory of Food Microbiology and Food Preservation at the University of Ghent (Jacxsens et al., 2009; Zimbabwe Dairy Regulations, 1977).

^b m – maximum level of bacteria per test volume considered acceptable.

^c M – maximum level bacteria per test volume considered marginally acceptable.

enumerate TBC, CC, and TEC on milkmen's hands and milking containers, according to prescribed standards (ISO 18593:2004). Plate count agar (Mast, UK), violet red bile agar (Mast, UK), and tryptone bile X-gluconoride (TBX) (Titanic Biotech, India) were used for enumeration of TBC, CC, and TEC, respectively. Swab samples collected were first transferred into 9 mL of 1% buffered peptone water and mixed using a vortex. All the samples were subjected to a ten-fold serial dilution using buffered peptone water (Mast, UK), after which 0.1 mL of the dilutions (10⁻¹–10⁻⁶) were spread on agar plates. TBC and CC plates were incubated at 37 °C for 24 h, while for *E. coli*, the plates were incubated at 44.5 °C for 24 h. After incubation, the counts for raw and cultured milk samples were expressed as colony-forming units (log CFU/mL), while for swabs, the counts were expressed as log CFU/cm².

Salmonella spp.

Isolation of *Salmonella* spp. was done according to a standard procedure (ISO 6579-1:2017). Milk (25 mL) and swab (1 mL) samples were enriched in 225 mL and 9 mL, respectively, of buffered peptone water and incubated at 37 °C for 18 h. For each preenriched sample, 0.1 mL and 1 mL were transferred to 10 mL of Rappaport Vassiliadis peptone broth (Oxoid, UK) and 10 mL of selenite cysteine broth (Oxoid, UK) and incubated for 22 h at 41.5 °C and 37 °C, respectively. Thereafter, a loopful of the culture was streaked on xylose lysine desoxycholate agar (XLD) and brilliant green agar (BGA) (Oxoid, UK) and incubated at 37 °C for 22 h. Typical *Salmonella* colonies show red with black centers on XLD and red colonies surrounded by a bright red medium on BGA.

Listeria monocytogenes

A method by Hitchins et al. (2022) was used for the isolation and identification of *L. monocytogenes* from both milk and swab samples. Milk (25 mL) and swab (1 mL) samples were homogenized in 225 mL and 9 mL buffered *Listeria* enrichment broth (oxid, Basingstoke, UK), respectively, and incubated at 30 °C for 4 h. Subsequently, selective supplement (*Listeria* selective enrichment supplement, SR0141) was added followed by incubation for 48 h at 30 °C. The preenrichment culture was streaked on *Listeria* selective agar base (oxid, Basingstoke, UK) supplemented with *Listeria* selective supplements (oxid, SR0140) and incubated at 30 °C for 48–72 h. Typical *L. monocytogenes* shows black zones around the colonies.

Klebsiella pneumoniae

K. pneumoniae was isolated and identified following a method by Yang et al. (2021) and Badri et al. (2017). Buffered peptone water was used to enrich (24 h at 37 °C) both the milk and swab samples before identification. After the enrichment process, the culture was streaked on MacConkey agar and incubated for 24 h at 37 °C. Lactose fermenting colonies exhibiting a pink mucoid appearance were selected for further biochemical identification and confirmation.

Microbiological safety level profile (MSLP)

Microbiological data were used to calculate MSLPs at each CSL from the different farms (Jacxsens et al., 2009). The MSLPs were calculated to give an overview of contamination at each CSL. This summary made it possible to compare the levels of microbial contamination at each CSL across the selected farms, providing some understanding of the general levels of microbial contamination that are associated with the hygiene and handling practices at farms (Opiyo et al., 2013). The results for each microbiological parameter at each CSL were expressed as log CFU/ml or log CFU/m² for indicator organisms and utility parameters, while pathogens were evaluated as present or absent. Although standard deviations were calculated for each microbiological parameter at each CSL at each farm, variations were not accounted for during the calculation of the MSLPs in the MAS (Jacxsens et al., 2009). The results were then interpreted based on the defined legal criteria established in Zimbabwe Dairy Regulations RGN 886 (Zimbabwe Dairy Regulations, 1977) and the values established by the Laboratory of Food Microbiology and Food Preservation at the University of Ghent (Jacxsens et al., 2009) (Table 1).

To obtain MSLPs at each CSL, data were further evaluated using a score attribution system (Table 2) developed by Jacxsens et al. (2009). Individual microbiological results (contamination level (CL)) after comparing with microbiological criteria presented in Table 1 were then evaluated across the CSLs by assignment of a score (from 0 to 3) to each type of microbiological parameter (Jacxsens et al., 2009; Opiyo et al., 2013; Wambui et al., 2018). For indicator and utility parameters, (i) a score of 0 (intolerable) was given when the microbiological criteria were exceeded for a microbiological parameter at a specific CSL according to Table 1; (ii) a score of 1 (a poor to average result) was given when the microbial results were equal to the maximum marginally acceptable level; (iii) a score of 2 (moderate/average result) was given whenever the results were less than the maximum level considered marginally acceptable, but more than maximum level considered acceptable; (iv) lastly, a score of 3 (good results) was given

Table 2

Attribution system for assignment of microbiological food safety level profile scores at each critical sampling location of the dairy farms

Score	Benchmark	Contamination level ^d
3	R ^a < m ^b	Good
2	m < R < M ^c	Average
1	R = M	Poor to average
0	R > M	Intolerable

^a R – results obtained from analysis.

^b m – maximum level of bacteria per test volume considered acceptable.

^c M – maximum level of bacteria per test volume considered marginally acceptable (values at or above M are unacceptable).

^d For pathogens, it is either zero (detected) or 3 (not detected) due to the severity of the concern.

when results were below the minimum acceptable value for a specific microorganism at a specific CSL. Considering the guidelines in Table 1, the scoring for pathogens was either 0 or 3. The MSLPs at each CSL for the six farms were calculated by summing up the scores attributed to each of the microbiological parameters. For example, if a farm got good results (3 scores) for all the six microbiological parameters at a CSL, then the maximum MSLP for that CSL would be 18.

Data processing, statistical analysis, and interpretation

The mean values of microbial counts and the standard deviations for the microbial parameters at each farm were determined. ANOVA, at $p < 0.05$, was used to assess for significant differences in mean microbial counts among the farms. The posthoc test, Tukey, was then performed for multiple comparisons. The magnitude of the association between MSLPs and hygiene and handling practices in all the farms was tested using point-biserial correlation coefficients. The MSLP scores at each CSL were computed as continuous variables, while the handling and sanitary practices were computed as binary variables. The binary variables ((1); (0)) were created as follows: time taken from milking to storage (5–30 min (1); > 30 min (0)), type of milking container (stainless steel (1); plastic (0)), consistent supply of potable water (yes (1); no (0)), frequency of milking parlor cleaning (cleaning each time after milking (1); once even if milking is conducted twice a day (0)), water source for cleaning equipment and hands (municipal tap water/borehole water (1); well (0)), cleaning detergents (use of sanitizers (1); no use of sanitizers (0)). The analysis was designated a significance threshold of $p < 0.05$. All analyses were conducted using R version 4.2.3 (RR Core Team, 2013).

Table 3

Characteristics of the selected six informal dairy farms in Zimbabwe, including their hygiene and handling practices

Variable	Farm					
	A	B	C	D	E	F
Number of workers	11	14	17	22	12	5
Number of dairy cows	16	9	15	11	13	18
Water source	Borehole	Borehole	Well	Borehole	Borehole	Tap ^b
Presence of a milking parlor	Yes	Yes	Absent	Absent	Absent	Yes
Type of milking containers	Plastic	Plastic	Plastic	Plastic	Plastic	Stainless steel cans
Milking practices	Hands	Hands	Hands	Hands	Hands	Hands
Frequency of milking per day	Once	Twice	Twice	Twice	Twice	Twice
Frequency of cleaning milking parlour per day	Once	Once	– ^a	–	–	Twice
Time from milking to storage	5–30 min	5–30 min	5–30 min	5–30 min	5–30 min	<5 min
Cooling facilities	Absent	Absent	Absent	Absent	Absent	Cold room
Cleaning detergents	Liquid soap and sanitizers	Liquid soap	Liquid soap	Solid soap	Liquid soap	Liquid soap and sanitizers

^a Milking parlor was absent from the farm. Milking was conducted in an open space.

^b Tap – Municipal tap water.

Results

Characteristics of the farms, hygiene, and handling practices

Most of the farms surveyed in this study were situated in peri-urban areas. The number of people working on the farms ranged from 5 to 17, and the dairy herds were between 9 and 18 cows. Only Farm F milk had documented standard operation procedures for hygiene and handling practices. However, the system's recordkeeping was not adequate according to the standards prescribed in most FSMSs. None of the farm workers received formal training on hygiene practices and used appropriate protective clothes. The survey revealed that hand milking was the standard procedure on all the farms, with five of them doing it twice a day. The majority of the farms used borehole water for cleaning purposes and one farm used municipal tap water (Table 3). All the farms except for Farm F reported a consistent supply of water. Most of the farms used plastic containers while one farm (Farm F) used stainless steel containers for milking and storing milk. According to the survey, Farm F also had a cold room with temperatures between 8 and 10 °C for milk storage. Generally, the time taken from milking to storage rooms was between 5 and 30 min. The storage rooms were very close to the milking area, hence the short time. Farms A, B, and F had milking parlors, and the cleaning schedule was mostly twice a day. Farm A used borehole water without disinfectant to clean the parlor. Farm F cleaned their parlor, milking equipment, and milkmen's hands using municipal tap water with disinfectant. Farms C, D, and E conducted milking in an open space. However, they used borehole water with disinfectants for cleaning purposes. Farm C did not have a milking parlor, and the farm used water from a well for cleaning. Detergents used at the farms differed, with some using liquid soap combined with sanitizers, while one farm used solid soap for hygiene and sanitary purposes.

Raw milk (CSL1)

The pH of raw milk ranged from 6.5 to 6.9. The mean TBC for raw milk ranged from log 5.1 CFU/mL – log 6.7 CFU/mL. Farm A had the lowest counts, while Farms B and C had the highest. In all the farms, the TBC means at CSL1 showed a significant difference at $p < 0.05$ (Table 4). The TBC at CSL1 for all the farms was above the prescribed microbiological criteria for this indicator, and a contamination level (CL) score of 0 was assigned. The range of means for CC was log 2.1 CFU/mL to log 5.7 CFU/mL, with Farm F exhibiting the lowest count and Farm B the highest. The CC at CSL1 for all the farms also exceeded the upper limit of indicator organisms deemed acceptable;

Table 4
Level of hygiene and utility indicators of raw milk obtained from the six selected informal dairy farms in Zimbabwe^a

Farm	Microbial counts (log CFU/mL) ^b		
	TBC ^c	CC ^d	TEC ^e
A	5.1 ± 0.3*	5.3 ± 0.1*	ND ^f
B	6.7 ± 0.9**	5.7 ± 0.1*	6.6 ± 0.1*
C	6.7 ± 0.1**	4.8 ± 0.5**	6.6 ± 1.1*
D	6.1 ± 0.3***	4.7 ± 0.2**	1.9 ± 1.0**
E	5.2 ± 0.3*	4.8 ± 0.8**	2.2 ± 0.4**
F	6.5 ± 0.1****	2.1 ± 0.6***	ND

^a Mean ± standard deviations are reported.

^b Means in a column with superscripts *, **, ***, and **** are significantly different at $p < 0.05$.

^c TBC – total bacterial count.

^d CC – coliform count.

^e TEC – total *E. coli* count.

^f ND – not detected.

as a result, a CL score of 0 was awarded, which reduced the MSLP of the farms for this respective CSL. There was a significant difference in the mean *E. coli* counts at CSL1 for the farms where the organism was identified (Table 4). *E. coli* was not detected in raw milk samples from Farm A and Farm F. The legal criteria provide that a CL score of 3 is awarded when *E. coli* is not present in a food sample, while a score of 0 is awarded when it is. *K. pneumoniae* was detected in raw milk samples from Farms B and D, which resulted in a CL score of 0 for both farms. There was no *Salmonella* spp. and *L. monocytogenes* detected in raw milk samples from all six farms, and because of this, the farms were awarded a CL score of 3. The MSLPs at CSL1 for all the six farms ranged from 6 to 12, with Farms B and D having the lowest score while Farms A and F recording the highest (Fig. 2).

Cultured milk (CSL2)

Cultured milk samples recorded pH values ranging from 4.6 to 4.9. There was a significant difference in mean TBC at CSL2 for all the farms. The mean TBC in cultured milk ranged from log 4.7 CFU/mL – log 7.0 CFU/mL (Table 5). Farm D had the lowest counts, while Farm B had the highest. The recorded TBC for all the farms exceeded the maximum level of counts per test volume and is considered marginally acceptable. Therefore, all the farms received a CL score of 0 at CSL2. The mean CC at CSL2 ranged between log 3.4 CFU/mL and log 5.4 CFU/mL (Table 5). The highest CCs were recorded in samples from Farm C. The mean CC for Farms A, B, D, E and F were not significantly different at $p < 0.05$. All the farms had CCs that were higher than the upper limit of counts per test volume, which is deemed somewhat acceptable; as a result, the assigned CL was 0. No *E. coli* was detected in cultured milk from Farm F. Nonetheless, the other farms recorded mean *E. coli* counts ranging from log 1.6 CFU/mL to log 6.4 CFU/mL. There was a significant difference ($p < 0.05$) in mean *E. coli* counts of cultured milk from farms where the indicator organism was detected. The farms that tested negative for *E. coli* had a CL score of 3, whereas the farms that tested positive for it had a score of 0. All farms received a CL score of 3 for pathogens since no pathogens were found in the cultured milk. MSLP scores at CSL2 for Farms A, B, C, D, and E were 9, while Farm F recorded the highest with 12 (Fig. 2).

Milkmen's hands (CSL3)

The means TBC on milkmen's hands ranged from log 2.0 CFU/cm² to log 6.1 CFU/cm² (Table 6). Farm B recorded the lowest counts, while Farm C had the highest. There was a significant difference ($p < 0.05$) in mean TBC at CLS3. Contamination level of TBC on milkmen's hands was above the tolerable value (>2.5 log CFU/cm²,

Table 1) prescribed by standards for Farms B, C, D, and E. These farms received a CL score of 0 at this CSL. However, for Farms A and F, the TBC (Table 6) was less than the maximum level considered marginally acceptable but more than the maximum level considered acceptable. Therefore, the CL score for both farms was 2. No coliforms, *E. coli*, or pathogens were detected on milkmen's hands. As a result, all farms were awarded the highest CL score (3) for these parameters. Considering CLS3, the MSLP ranged from 15 to 17, and the highest was observed at Farms A and F (Fig. 2).

Milking container (CSL4)

There were no hygiene indicator organisms (coliforms and *E. coli*) and pathogens that were detected at this sampling location. Nonetheless, TBC was found at concentrations between mean log 2.3 CFU/cm² and log 4.3 CFU/cm² (Table 6). Milking containers from Farm C recorded the highest TBC counts. There was a significant difference ($p < 0.05$) in the mean TBC of milking containers from the farms. All farms, with the exception of Farms F, had TBC contamination levels on milking containers that were higher than the acceptable limit (>2.5 log CFU/cm², Table 1) set by regulations. The TBC for Farm F (Table 6) was higher than the maximum level deemed acceptable but less than the maximum level deemed marginally acceptable. Therefore, at CSL4, Farm F was awarded a CL score 2 while the rest of the farms received a score of 0. Farm F received the highest MSLP score of 17 while the rest of the farms received an overall score of 15 (Fig. 2).

Relationship between the overall MSLPs at each CSL and hygiene and handling practices

Table 7 presents the point-biserial correlation coefficients between the overall MSLPs and hygiene and handling practices at the six different farms. The correlation coefficient values between the time taken from milking to storage and the overall MSLPs at all the CSLs ranged from 0.23 (CI: -0.63 to 0.63) to 0.89 (CI: 0.76–0.89). There was a strong positive relationship between the time taken from milking to storage and the MSLPs for raw milk (CLS1) and cultured milk (CSL2). Less time (5–30 min) was highly correlated with an increase in MSLPs. There was a statistically significant negative correlation found between the type of milking containers used on the farms and MSLPs (Table 7). Consistent water supply and overall MSLPs all CSLs had association values ranging from -0.40 (CI: -1.00 to (0.28)) to 0.47 (CI: 0.12–0.57). Generally, little correlation was found between the MSLPs at the CSL and the reliable availability of potable water. The frequency of cleaning the milking parlor and the overall MSLPs revealed positive correlations; however, the relationship between the variables was significantly positive at CSL1 (0.65 (CI: 0.17–0.98)) and CSL2 (0.68 (CI: 0.29–0.99)). The association between the overall MSLPs at all the CSLs and the sources of water for hygiene and sanitary purposes, and the use of sanitizers revealed significantly positive correlation coefficients (Table 7). Use of sanitizers on the farms and access to clean water sources like municipal and borehole water were strongly correlated with an increase in MSLPs at CSLs across the farms.

In general, there was a significantly positive correlation found between the overall MSLPs at the CSLs for all the farms and the duration between milking and storage, the type of milking container utilized at farms, the frequency of cleaning the milking parlor, the water source for hand and equipment washing and the use of sanitizers (Table 7).

Discussion

Understanding the risk factors at each stage of the milk production and processing chain is a prerequisite to evaluating performance and

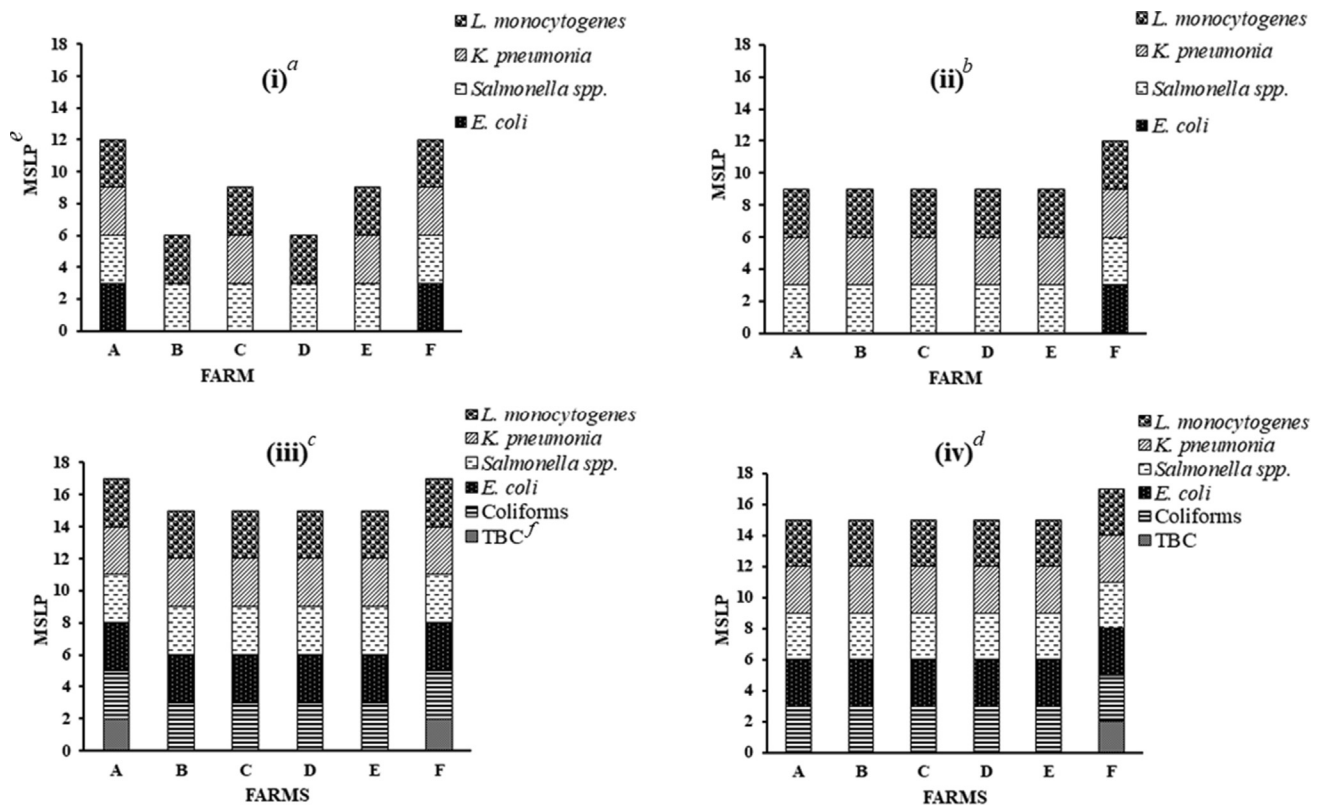


Figure 2. Microbial safety level profile scores at critical sampling locations for the six selected informal dairy farms in Zimbabwe. ^a(i) CSL1 – raw milk; ^b(ii) CSL2 – cultured milk; ^c(iii) CSL3 – milk men’s hands; ^d(iv) CSL4 – milking containers; ^eMSLP – microbial safety level profiles; ^fTBC – total bacterial count.

Table 5

Level of hygiene and utility indicators of cultured milk obtained from the six selected informal dairy farms in Zimbabwe^a

Farm	Microbial counts (log CFU/ml) ^b		
	TBC ^c	CC ^d	TEC ^e
A	4.9 ± 0.4*	4.1 ± 1.1*	5.9 ± 0.80*
B	7.0 ± 1.2**	4.2 ± 0.5*	6.2 ± 1.30*
C	6.8 ± 0.6**	5.4 ± 0.3**	6.4 ± 0.3*
D	4.7 ± 0.4***	3.4 ± 0.2*	1.6 ± 1.5**
E	5.1 ± 0.2*	4.7 ± 1.2*	1.9 ± 0.1**
F	5.4 ± 0.1****	4.1 ± 1.1*	ND ^f

^a Mean ± standard deviations are reported.
^b Means in a column with superscripts *, **, ***, and **** are significantly different at $p < 0.05$.
^c TBC – total bacterial count.
^d CC – coliform count.
^e TEC – total *E. coli* count.
^f ND – not detected.

Table 6

Level of utility indicators on milkmen’s hands and milking containers from the six selected informal dairy farms in Zimbabwe^a

Farm	Milkmen’s hands (log CFU/cm ²) ^b	Milking container (log CFU/cm ²)
A	2.3 ± 1.2*	4.1 ± 0.8*
B	3.0 ± 0.9**	3.1 ± 0.9**
C	6.1 ± 1.4***	4.3 ± 0.8*
D	4.8 ± 1.3****	3.9 ± 1.0*
E	4.4 ± 3.0****	3.1 ± 1.5**
F	2.0 ± 1.7*	2.3 ± 0.9**

^a Mean ± standard deviations are reported.
^b Means in a column with superscripts *, **, ***, and **** are significantly different at $p < 0.05$.

providing evidence-based mitigation solutions to enhance milk quality and reduce the occurrence of milk-borne diseases. The current study used an MAS to assess the impact that hygiene and handling practices have on the contamination of milk (both raw and cultured), milkmen’s hands, and milking containers from informal dairy farms in Zimbabwe.

Our study revealed that the farm characteristics, including their hygiene and handling practices, strongly influenced the contamination of milkmen’s hands, milking containers, and both raw and cultured milk produced at the farms. All the farms failed to reach the maximum MSLP scores of 18 at each CSL. This can be a result of farm features that were noted in our survey, which include lack of cold rooms, utilizing plastic containers, using unsafe water sources such as wells, milking in open areas with bare hands, lack of cold rooms, and generally not having FSMS (Knight-Jones et al., 2016; Millogo et al., 2010). Opiyo et al. (2013) employed a MAS for both large-scale (that use FSMS) and small-scale (that use some limited form of hygiene practices) dairy processing plants in Kenya to assess their performance. The authors reported results similar to those of our study, which revealed extremely low MSLPs for small-scale dairy farms. Small dairies, which are more likely to lack or seldom have a food safety program, have been reported to generate low-quality products (Njage et al., 2018), and this highlights the necessity of FSMS at dairy plants regardless of the production scale.

Farms B and D had the least MSLP scores, followed by Farms B and E at CSL1. According to the survey, these farms lacked suitable facilities or had inadequate facilities. Nonetheless, all the farms at this CSL received an attribution score of 0 for mean TBC and CC because these indicators in raw milk exceeded the upper limit of bacteria count deemed acceptable. The concentration of TBC and CC that are above legal limits and leading to microbial inadequacies of milk are mostly linked to poor sanitation and hygiene of the farm environment, milking, and storage equipment, as well as personnel hygiene (Chimuti et al., 2016; Ntuli, 2017; Ntuli et al., 2023). The characteristics of

Table 7

Point-biserial correlation coefficient establishing the relationship between overall microbial safety level profiles and the farm variables at the six selected informal dairy farms in Zimbabwe^e

Variable ^d	Raw milk	Cultured milk	Milkmen's hands	Milking containers
	CC^b(CI^c)	CC(CI)	CC(CI)	CC(CI)
T _m	0.57 (0.20–0.63)^e	0.89 (0.76–0.89)	0.23 (–0.63 to 0.63)	0.44 (0.20–0.63)
T _{yp}	–0.77 (–0.82 to –0.33)	–0.53 (–0.76 to –0.08)	–0.03 (–0.43 to 0.07)	–0.76 (–0.81 to –0.43)
P _b	0.02 (–0.10 to 0.10)	0.47 (0.12–0.57)	–0.44 (–1.00 to 0.32)	–0.40 (–1.00 to 0.28)
P _{lor}	0.65 (0.17–0.98)	0.68 (0.29–0.99)	0.32 (–0.72 – 0.99)	0.33 (0.14–0.50)
W _t	0.89 (0.76–0.89)	0.77 (0.40–0.93)	0.63 (0.63–0.83)	0.54 (0.20–0.63)
D _t	0.57 (0.30–0.63)	0.64 (0.27–0.88)	0.71 (0.66–1.00)	0.64 (0.40–0.91)

^a Significance threshold ($p < 0.05$).

^b CC – correlation coefficient.

^c CI – confidence interval.

^d Variables: T_m – Time from milking to storage, T_{yp} – Type of milking container, P_b – Consistent supply of potable water, P_{lor} – Frequency of milking parlor cleaning; W_t – Water source for cleaning equipment and hands, D_t – Cleaning detergents.

^e Figures highlighted in bold indicate correlation coefficients that showed a significantly strong relationship between microbial safety level profiles and the farm variables.

the farms that we observed throughout our survey may be additional contributing reasons to the milk's microbiological deficiencies.

Detecting *E. coli* in Farms B, C, D, and E at CSL1 was a concern because these organisms are an indication of fecal contamination and the presence of other pathogens, such as *Salmonella* spp., in food (Torres-Vitela et al., 2013). *E. coli* in milk violates food regulations in Zimbabwe which stipulates that the organism should not be detected in food (Zimbabwe Dairy Regulations, 1977). Farrokh et al. (2013) reported that two potential pathways lead to the presence of *E. coli* in raw milk, and these are (i) discharge of the organisms from the udder as a result of subclinical mastitis or (ii) fecal contamination either directly or indirectly. Regrettably, the farms under investigation in the study sold raw milk to the public. Therefore, to reduce safety risks, the milk should be pasteurized before being sold to consumers.

In the current study, all farms except Farm F recorded low MSLP scores of 9 for cultured milk (CSL2). TBC, CC, and the presence of *E. coli* invited an attribution score of 0 for these farms, reducing the MSLPs at CSL2. The recorded pH (4.6–4.9) of cultured milk allows the growth of most microbes such as *E. coli* (Wilks & Slonczewski, 2007). Cultured milk sold at informal small-scale dairy farms in Zimbabwe is produced by natural fermentation under rudimentary and uncontrolled conditions (Gran et al., 2003). A study of cultured milk products by Gran et al. (2003) in Zimbabwe reported *E. coli* counts $>5 \log$ CFU/mL (Gran et al., 2003). Cultured milk, because of its metabolites and low pH, is expected to inhibit the growth and survival of bacteria, which include pathogenic microorganisms (Gavrilova et al., 2019). However, the product may record microbial counts higher than the maximum level of counts per test volume that is considered to be marginally acceptable due to factors such as the use of low-quality raw materials, the use of natural fermentation under unsanitary conditions, and inadequate storage facilities (Gran et al., 2003).

In this study, MSLPs for each farm at all CSLs recorded better results because the attributed CL received the highest score of 3 for not detecting *Salmonella* spp. and *L. monocytogenes*. This indicates that the handling and hygiene procedures successfully kept these pathogens under control even with subpar farm facilities. The MAS study by Opiyo et al. (2013) also reported attribution scores of 3 for not detecting *Salmonella* spp. at all the CSLs of both the investigated small-scale and large-scale dairies in Kenya. The failure to detect pathogens such as *Salmonella* in the present study can be attributed to low levels of the organisms in milk and the contact surfaces and probably low sampling rates (D'Amico et al., 2008). *K. pneumoniae* was, however, found in raw milk from Farms B and D. The organism is shed in milk as a result of clinical mastitis. Studies have shown that wood products are the primary source of *K. pneumoniae* at farms

(Munoz et al., 2006). Applying good hygiene and sanitary practices at dairy farms and using alternative bedding materials for cows, such as sand, are recommended to lower the risk of *K. pneumoniae*, mastitis, and other pathogens (Munoz et al., 2006).

No indicator organisms or pathogens were detected at CSL3 and CSL4. However, TBC was recorded in ranges between 2.0 CFU/cm²–log 6.1 CFU/cm² and 2.3 CFU/cm² and log 4.3 CFU/cm², respectively. A study by Lues and Van Tonder (2007) detected TBC on the palms of the hands (98%) of food handlers at 35 outlets studies in South Africa. TBC on hands and surfaces that come into contact with food should be less than log 2 CFU/cm² (Lues & Van Tonder, 2007). In this study, using unsafe water sources such as wells and milking in open spaces with bear hands may cause a high TBC level on milkmen's hands and milking containers. Because of inadequate personal hygiene or cross-contamination, the hands of milkmen and milking containers can play a crucial role as vectors in the transmission of microbes that have the potential to cause food-borne illnesses (Lues & Van Tonder, 2007).

At all the CSLs, the highest MSLP score was achieved by Farm F. The features of the farm, along with its hygiene and handling procedures, helped to reduce the risk of raw and cultured milk contamination. These procedures included cleaning the milking parlor with detergents after each milking session, using municipal water, storing milk in stainless steel cans in a cold room, and minimizing the amount of time it took to store milk—less than 30 min. The farm also had documentation of a food safety program despite its implementation being inadequate according to prescribed FSMSs such as ISO 22000. The point-biserial correlation results revealed a strong relationship between MSLP scores and the farm variables. Farms with high MSLP scores were highly correlated with facilities, handling procedures, and hygiene standards that lower the risk of contaminating raw and cultured milk. The shorter the period between milking and storage, the lower the microbial growth, which results in better raw and cultured milk quality (Paludetti et al., 2018). This study revealed that the farms that used stainless-steel cans to store milk in a cold room recorded high MSLP scores. Research has indicated that milk producers using plastic containers generate milk with inadequate microbiological quality. This is because bacteria easily form biofilms on plastic material, encouraging the proliferation of microorganisms (Wafula et al., 2016). Research conducted in Zimbabwe on unconventional water sources showed that fecal coliforms were severely polluted in well water (Moyo, 2013). This could be why Farm C, which used water from wells, reported the lowest MSLP scores at all the CSLs. The frequency of cleaning the milking parlor and the usage of sanitizers were associated with higher MSLP scores, as indicated by the correlation coefficient values. The observation is in line with the findings

of Mogotu et al. (2022), who observed that good hygiene practices were associated with good-quality milk.

The current study revealed risk variables that can serve as a foundation for offering mitigation strategies based on emphatical evidence to improve milk quality. Based on the findings of this study, suboptimal hygienic practices contributed to reduced microbiological quality of raw milk in six informal dairy farms. Several critical sampling points were characterized by high microbial counts beyond regulatory limits, which may be a risk to public health. There was a protective effect of good handling practices such as the use of steel milk transport containers, the municipal water source for cleaning equipment, the use of sanitizers, and the frequency of milking parlor cleaning, which were associated with improved milk microbial quality. In order to improve the quality of milk and its products that are produced and marketed in Zimbabwe's informal value chain, both infrastructural development and training are required.

CRedit authorship contribution statement

S. Chimuti: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **D. T. Mugadza:** Writing – review & editing, Supervision, Formal analysis, Conceptualization. **V. Ntuli:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **P.K.M. Njage:** Writing – review & editing, Supervision, Software, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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