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Integrated assessment of microbiological risks and compliance of raw milk from smallholder dairy systems in Manica Province, Mozambique

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ABSTRACT

Milk is widely recognized as a highly nutritious food. However, if produced under inadequate hygienic and sanitary conditions, it can pose a risk to public health. This study aimed to evaluate the microbiological safety and compliance of raw milk produced by small-scale producers in rural communities of Manica Province, one of the main dairy-producing regions of Mozambique. To the best of our knowledge, this is the first study to integrate microbial load quantification with compliance assessment against international standards in smallholder dairy systems in the districts of Gondola, Vanduzi, and Macate. A total of 34 raw milk samples were analyzed for mesophilic aerobic bacteria, total and fecal coliforms, coagulase-positive and coagulase-negative *Staphylococci*, molds and yeasts. The mean count of mesophilic aerobic bacteria was 7.27 ± 0.14 Log_{10} cfu/mL, exceeding the recommended limits of 10^4-10^5 cfu/mL. Total coliforms were detected in all samples (> $1.1x10^3$), exceeding acceptable levels (≤ 3 MPN/mL), while fecal coliforms were below 3 MPN/mL. The mean values for coagulase-positive *staphylococci* and coagulase-negative *Staphylococci* were 5.82 ± 0.14 and 5.97 ± 0.12 Log_{10} cfu/mL, respectively, which were above the recommended limits (10^2- 10^4 cfu/mL). Molds and yeasts were detected in all samples, with mean values of 5.09 ± 1.60 and 5.50 ± 1.05 Log_{10} cfu/mL, respectively, which are above the acceptable levels for dairy products.The findings demonstrate non-compliance with internationally accepted microbiological standards, highlighting deficiencies in hygiene and sanitation practices during the production, handling and storage of milk. This poses a potential risk to public health. These results provide novel scientific evidence regarding the microbiological safety of raw milk produced in small-scale systems in Manica Province, and emphasise the importance of implementing good milking practices, improving infrastructure, and strengthening sanitary monitoring to ensure product safety.

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1. Introduction

Raw milk is a high nutritious food and an important Source of proteins, fats, carbohydrates, vitamins, and minerals essential to human nutrition (1). However, its nutrient-rich composition, near-neutral pH, and high-

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water activity (aw) provide favorable conditions for microbial growth, making it highly susceptible to microbiological contamination and spoilage, particularly produced and handled under inadequate hygienic conditions (2,3).

Recent studies indicate that these physicochemical characteristics, combined with exposure to external contamination sources during milking and inadequate equipment sanitization, facilitate microbial proliferation and negatively affect milk quality and safety (3,4).

Microbiological contamination of raw milk may occur through endogenous and exogenous routes. Endogenous contamination is associated with the presence of intramammary infections, such as mastitis, caused by pathogenic microorganisms that can be directly excreted into milk. In contrast, exogenous contamination occurs after milking and results from contact with contaminated surfaces, inadequately sanitized equipment, microbiologically unsafe water, handlers' hands, or improper storage and transport conditions (2,5). These contamination sources contribute to increased microbial loads and compromise milk safety.

Among the main microorganisms associated with raw milk contamination, indicator bacteria of hygiene and sanitary quality, such as total and thermotolerant coliforms, are particularly relevant. Their presence and high counts are associated with poor hygienic practices during milking, handling, and storage. These microorganisms are widely used as indicators of fecal contamination and potential pathogen presence, reflecting deficiencies in hygienic and sanitary conditions throughout the dairy production chain (6-8).

The enumeration of mesophilic aerobic microorganisms is commonly used as a general indicator of microbiological quality, enabling assessment of hygienic conditions during milk production, storage, and transport (4).

Staphylococcus aureus is an important pathogen associated with failures in milking hygiene and is frequently linked to intramammary infections and contamination by handlers. This microorganism can produce enterotoxins responsible for food poisoning (9). The presence of molds and yeasts is also relevant, as these microorganisms contribute to milk spoilage and may pose health risks due to the potential production of mycotoxins (e.g., *Aspergillus*, *Penicillium*). These toxins may contaminate milk through contaminated food safety concern. Recent studies have demonstrated that mycotoxins can occur in milk and dairy products, posing risks to human health, especially in production systems with inadequate management practices (10,11).The consumption of contaminated raw milk has been associated with the transmission of several pathogens, including *Salmonella* spp., *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus*, representing a significant public health concern. Therefore, assessing the microbiological quality and safety of raw milk is essential to verify compliance with internationally established standards and to identify potential risks to consumers.Despite its growing importance in developing countries, small-scale dairy production is often hindered by poor hygiene practices, inadequate sanitation infrastructure and lack of systematic microbiological monitoring. Consequently, there is a scarcity of scientific data onthe microbiological quality

and safety of raw milk produced in these contexts, which limits effective risk assessment and the development of control strategies.

Against this backdrop, the present study sought to evaluate the microbiological safety and compliance of raw milk produced by small-scale producers in the districts of Gondola, Vanduzi and Macate in Manica Province, Mozambique. The evaluation was based on key microbiological contamination and hygiene indicators, providing evidence to inform risk assessment and the implementation of food safety control measures in small-scale production systems.

2. Materials and Methods

2.1.Study área

The study was conducted between June and July 2022 in rural communities in the districts of Gondola, Vanduzi and Macate in Manica Province. These districts were selected as they are priority areas for small-scale dairy production within the Land O'Lakes International Development support project.

2.2.Study design and sample collection

A cross-sectional study was conducted. Raw milk samples were collected from community-based milk collection and cooling points were small-scale producers delivered milk that had been stored in containers.

A total of 34 raw milk samples (100 mL each) were collected from 30 smallholder dairy production units located in the selected districts, with at least one sample collected from each unit. Samples were randomly selected at the time of milk delivery to the collection points.

Prior to sampling, the milk in the containers was carefully homogenized to ensure representative samples. Sampling was performed aseptically by transferring approximately 100 mL of milk into sterile, wide-mouth, polypropylene containers that been sterilized and properly sealed.

All samples were labelled with unique identification codes to ensure traceability and producer anonymity. The procedure was carried out under controlled hygienic conditions, with personnel wearing disposable gloves and surgical masks. Measures to minimize the risk of cross-contamination were implemented, including changing gloves between sampling points and disinfecting hands with a 70% alcohol solution.

2.3. Sample transport and preservation

After collection, samples were placed in insulated cool boxes with frozen ice packs to ensure a temperature of between 2 and 8^{o}C was maintained during transport.

Transport of the Microbiology Laboratory of the Catholic University of Mozambique (Chimoio) took no longer than 1 h. Upon arrival, the samples were stored immediately at 4± 1 ^{o}C and processed within 2 h of collection at the latest.

2.4. Materials and reagents

The following materials and culture media were used:

- Sterile bags
- Sterile plastic bottles
- Test tubes/dilution tubes
- Petri dishes (disposable)
- Pipettes (VITLAB and Eppendorf)
- Plate Count Agar (PCA)-Oxoid
- Inoculation loops
- MacConkey broth- Oxoid

- Brilhant Green Bile Broth (2%)- Oxoid
- *Escherichia coli* (EC) broth- Oxoid
- Sabouraud Dextrose Agar with chloramphenicol – Roth
- 3M Petrifilm™ Staph Express Count System – 3M
- Peptone Water – Oxoid

2.5.Laboratory equipment

The following items of laboratory equipment were used:

- Bacteriological incubator (BIOBASE)
- Stomacher 400 Circulator (SEWARD)
- Colony counter (Merck Chemicals)
- Tube shaker (LABASCO)
- Analytical balance (Merck)
- Laminar flow cabinet (Flow Fast H)
- Water bath (Memmert)
- Bacteriological incubators (37 °C and 44.5 °C) – standard microbiological laboratory equipment
- Laboratory refrigerator (4 ±1 °C)- microbiology laboratory

2.6. Microbiological analyses

2.6.1. Enumeration of mesophilic aerobic bacteria

Mesophilic aerobic bacteria were enumerated using the standard plate count method with the pour plate technique on plate count agar (PCA). Plates were incubated at 37°C for 48 h. Samples were analyzed in duplicate and at different dilutions levels. After incubation, colonies were counted and results were expressed within the range of 25 to 250 cfu per plate (12).

2.6.2. Total coliform determination using the multiple-tube (MPN) technique

The APHA method was followed using the multiple-tube technique, with three tubes per dilution, to determine the most probable number (MPN) of total coliforms per milliliter sample. MacConkey broth was used as the culture medium and incubated at 36 ± 1° C for 24-48 h. Tubes showing gas production and turbidity were considered positive. One milliliter from positive tubes was transferred to three sets of three tubes containing 2% brilliant green bile broth (BVB), which were incubated at 36 ± 1 °C for an additional 24-48 h. Positive results were indicated by gas production and color change to yellow. The MPN was calculated using probability combination tables and expressed as MPN/mL.

2.6.3. Fecal coliform determination using the multiple-tube MPN technique

Confirmation of fecal coliforms was performed using tubes that showed positive results in MacConkey broth. From these tubes, 1 mL aliquots were transferred to three series of tubes containing *Escherichia coli* (EC) broth culture medium. These tubes were incubated in a water bath at 44.5°C for 24-48 h, and tubes showing gas production and turbidity were considered positive (12). The most probable number (MPN) was then determined using probability combination tables and expressed as MPN/mL.

2.6.4. Enumeration of coagulase-positive and coagulase-negative staphylococci using the 3M Petrifilm method

Enumeration of coagulase-positive and coagulase-negative *staphylococci* was performed using duplicate Petrifilm™ plates. One milliliter of each prepared dilution was inoculated onto the plates, which were

incubated at 35–37 °C for 2 h and then transferred to an oven at 62 °C ± 2 °C for 1-4 h. After this period, thermonuclease-reactive discs were added, and the plates were incubated at 35-37°C for 1-3 h. Colonies showing red or blue coloration surrounded by a pink area were considered positive. Results were expressed as colony-forming units per milliliter (cfu/mL).

2.6.5. Quantification of molds and yeasts

Molds and yeasts were analyzed using serially prepared dilutions. For enumeration, 0.1 mL aliquots of each dilution were inoculated onto Sabouraud dextrose agar supplemented with chloramphenicol (400 mg/L) using the spread plate technique. Plates were incubated at 25 °C for at least 4 days for colony enumeration, and results were expressed as colony-forming units per milliliter (cfu/mL).

2.7. Statistical Analysis

Data were analyzed using descriptive statistics, including means, standard deviations, and frequencies. Analysis of variance (ANOVA) followed by Tukey's post hoc test was used to evaluate significant differences between means, while the chi-square (χ^2) test was applied for frequency comparisons. Statistical significance was considered at $p < 0.05$. All analyses were performed using SPSS software.

3. Results

Thirty-four raw milk samples collected from small producers in the districts of Gondola (n = 10), Macate (n = 10), and Vanduzi (n = 14), in Manica Province, were analyzed. Microbiological parameters evaluated included mesophilic aerobic bacteria, total and fecal coliforms, coagulase-positive staphylococci, coagulase-negative staphylococci, molds, and yeasts. Microbial counts varied among the studied districts, as shown in Table 1.

3.1.Aerobic-mesophilic bacteria

Mesophilic aerobic bacteria counts were similar among districts, with values ranging from 7.27 to 7.28 Log_{10} cfu/mL, with no statistically significant differences ($p>0.05$).

3.2. Total coliforms and Fecal coliforms

All samples showed total coliform levels greater than 1.1×10^3 MPN/mL, while fecal coliforms showed values lower than 3 MPN/mL in all districts.

3.3.Coagulase-positive and coagulase-negative *staphylococci*

The average count of coagulase-positive staphylococci ranged from 5.81 to 5.83 Log_{10} cfu/mL, with no significant differences observed between districts ($p>0.05$). Similarly, coagulase-negative staphylococci count ranged from 5.92 to 6.0 Log_{10} cfu/mL, also showing no statistically significant differences ($p>0.05$).

3.4.Molds and yeasts

Statistically significant differences were found in mold and yeast counts ($p<0.05$). The Gondola district showed significantly lower mold (3.83 Log_{10} cfu/mL) and yeast (4.47 Log_{10} cfu/mL) counts compared to Macate and Vanduzi districts, where mean values were higher than 5.5 Log_{10} cfu/mL.

Table 1. Microbiological counts (mean ± SD) of raw milk samples produced by small producers in the districts of Gondola, Macate, and Vanduzi, Manica Province, Mozambique.

Districts	No. of samples	Aerobic-mesophilic bacteria (cfu/g)	Total coliforms (MPN/mL)	Faecal coliforms (MPN/mL)	Coagulase-negative positive (Log_{10} cfu/mL)	Coagulase-negative *staphylococci* (Log_{10} cfu/mL)	Molds (Log_{10} cfu/mL)	Yeasts (Log_{10} cfu/mL)
		Mean load±SD	Mean load±SD	Mean load±SD	Mean load±SD	Mean load±SD	Mean load±SD	Mean load±SD
Gondola	10	$7,28^{a}$±0,10	>$1,1x10^{3}$	< 3	$5,82^{a}$±0,16	$6,00^{a}$±0,16	$3,83^{b}$ ±2,67	$4,47^{b}$ ±1,80
Macate	10	$7,27^{a}$±0,12	>$1,1x10^{3}$	< 3	$5,83^{a}$±0,10	$5,92^{a}$±0,07	$5,66^{a}$±0,13	$5,88^{a}$±0,11
Vanduzi	14	$7,27^{a}$±0,13	>$1,1x10^{3}$	< 3	$5,81^{a}$±0,16	$5,98^{a}$±0,13	$5,58^{a}$±0,09	$5,78^{a}$±0,07
Overall	34	$7,27^{a}$±0,14	>$1,1x10^{3}$	< 3	$5,82^{a}$±0,14	$5,97^{a}$±0,12	$5,09^{a}$ ±1,60	$5,50^{a}$±1,05

[a,b] Means in the same column with different superscript are statistical different at 5% significance level ($p<0.05$).

4. Discussion

4.1. Microbiological analysis of raw milk

The microbiological analysis of raw milk is divided into several sections as follows:

4.2. Mesophilic aerobic bacteria

The high counts of mesophilic aerobic bacteria (BAM) observed in raw milk samples from Gondola, Macate, and Vanduzi districts, with mean values ranging from 7.27 to 7.28 Log_{10} cfu/mL (≈ 1.9×10^{7} cfu/mL), suggest severe microbiological contamination. These values far exceed the internationally recommended limits for raw milk intended for processing, which is generally between 10^{4} and 10^{5} cfu/mL (13,14). According to established classification criteria, values above 10^{6} cfu/mL are considered unacceptable (15), confirming poor hygienic quality of the milk.

High MAB levels are widely recognized as indicators of inadequate hygiene during milk production. Previous studies have reported similar patterns in small-scale dairy systems, with microbial loads ranging from 3.16 $\times10^{5}$ to above 8×10^{8} cfu/mL (16, 17), especially in the absence of cooling systems and limited hygiene practices.

In the present study, the absence of immediate refrigeration is likely to be a key factor in bacterial proliferation. Milk is transported in metallic containers for 20-30 min at tropical temperatures of 30-37 ^{o}C, which are optimal for mesophilic bacterial growth. Such conditions allow rapid microbial multiplication, as previously demonstrated in studies where poor

temperature control led to exponential increases in bacterial load (18,19).

In addition, the structural condition of milking sites plays a critical role in microbial contamination. Open environments, dusty surroundings, unpaved floors and limited access to potable water increase milk's exposure to environmental microorganisms. Evidence from smallholder dairy system in South Africa and other African countries shows that inadequate hygiene, a lack of refrigeration, and poor handling practices are consistently associated with elevated mesophilic bacterial counts in raw milk (2, 3, 5, 20).

Further evidence from international and regional studies indicates that systemic limitations in dairy production systems contribute to sustained high mesophilic loads, beyond general hygiene conditions. Evidence from Ethiopia shows that inadequate sanitary infrastructure and limited access to quality control measures often result in bacterial counts that exceed regulatory thresholds (21). Furthermore, animal heath factors, particularly subclinical mastitis, may influence contamination levels by increasing the presence of somatic cell infections and pathogenic microorganisms in raw milk (22).

Due to the high levels observed, it is essential to implement corrective measures. Studies indicate that reducing the mesophilic aerobic bacterial load can be achieved by adopting good hygiene practices during milking, such as cleaning and disinfecting utensils, maintaining proper udder hygiene and using potable water. The introduction of simple, immediate post-milking refrigeration systems has also been identified as an effective strategy for limiting \bacterial growth, particularly in small-scale production systems (21-24). The continuous training of producers in hygienic management practices is also a critical intervention for improving the microbiological quality of milk.

4.3. Total and fecal coliforms

No specific microbiological criteria for raw milk have been officially established in Mozambique. However, international standards, including those established by the Codex Alimentarius Commission and Council Directive 92/46/EEC, indicate that acceptable levels of total coliforms in raw milk intended for processing should generally be below 10^2 cfu/mL or 3 NMP/mL depending of the classification system adopted. In the present study, all 34 raw milk samples from small productions units were contaminated with total coliforms, with values exceeding 1.1×10^3 MPN/mL, indicating levels above internationally accepted benchmarks. In contrast, no sample showed detectable fecal coliforms at 44.5 ^{o}C.

The absence of fecal coliforms in the presence of high levels of total coliforms suggest that the observed contamination was predominantly of environmental origin (secondary contamination), associated with deficiencies in hygiene and sanitation during milking, handling and transport, rather than recent direct fecal contamination or clinical herd problems. This pattern aligns with the widely accepted understanding that total coliforms function as general hygiene indicators, where fecal coliforms indicate recent fecal contamination.

Similar findings have been reported in various contexts, with total coliform values ranging from 8.5×10^4 to 3.5×10^7 cfu/mL in raw and pasteurized milk from small-scale production units in Senegal. These

values exceeded the national regulatory limit of 10^3 cfu/mL. It was also observed that 62.2% of the 210 raw milk samples analyzed exceeded this limit, regardless of the milking method used (23, 24).

In South Africa, average counts of 1.9 $\times 10^3$ cfu/mL were reported in milk from small, peri- urban producers, which exceeds national and international standards. Similarly, elevated levels of coliforms were documented in milk sold in Chad, which was associated with poor hygiene practices during milking and marketing (18, 20).

A systematic review showed that the high prevalence of coliforms in raw milk in various African contexts is strongly linked to structural deficiencies in primary production. These include inadequate udder hygiene, the use of non-potable water, contaminated utensils and the absence of immediate post-milking refrigeration. These factors were also observed in the districts evaluated in this study (21).

From technological and economic perspectives, high levels of total coliforms compromise the microbiological stability of milk, causing premature spoilage and potentially affecting its sensory characteristics. This can lead to financial losses for small-scale producers. High levels of microbiological contamination in artisanal systems have been associated with direct economic losses due to product rejection and accelerated deterioration (25).

The conditions observed in the studied districts, including unpaved corrals, the presence of dust and fecal matter, the use of untreated water, poor hygiene of utensils, and the absence of a cold chain at the milking sites, represent typical sources of secondary contamination factors. These findings are consistent with the literature, which demonstrates that the microbiological quality of raw milk is influenced more by hygiene and management practices than by intrinsic animal factors alone.

Strengthening hygienic practices through the production chain is essential to reduce coliform contamination. Studies have shown that the interventions such as washing the udder prior to milking, using treated water, properly sanitizing storage containers and improving environmental conditions in milking areas can significantly reduce these microbiological indicators (6, 24). Implementing targeted training programmers for smallholder producers has also been shown to positively impact the reduction of microbiological contamination in milk in similar production systems.

4.4. Coagulase-positive and coagulase -negative staphylococci (CPS and CNS)

Analysis of raw milk samples revealed a high presence of *staphylococcal* species, including coagulase-positive (CPS) and coagulase-negative (CNS) staphylococci, in all three evaluated districts. The mean counts overall were 5.82 ± 0.14 Log_{10} cfu/mL for CPS and 5.97 ± 0.12 Log_{10} cfu/mL for CNS, with no statistically significant differences between districts ($p > 0.05$). These values correspond to approximately 6.6 $\times 10^5$ cfu/mL, indicating a consistently high staphylococcal load across the study areas.

When compared with international standards, the observed levels were substantially above the recommended microbiological limits. Acceptable values have been established between 5×10^2 cfu/mL (2.7 Log_{10}) and 10^4 cfu/mL (4 Log_{10}), depending on milk

classification. Conversely, an approximate limit of 10^3 cfu/mL (3 Log_{10}) is recommended for raw milk intended for processing (14).

Thus, the counts observed in this study exceeded these benchmarks by approximately two to three logarithmic units, corresponding to bacterial concentrations 100 to 1000 times higher than the recommended limits. This indicates significant sanitary non-compliance.

The presence of CPS in raw milk may be due to primary sources, such as clinical or subclinical mastitis, or secondary contamination during milking. Studies on bovine mastitis caused by *Staphylococcus aureus* indicate that intramammary infections can contribute to bacterial shedding into milk, even in the absence of clinical symptoms (26).

Beyond primary contamination sources, post-milking factors may favor microbial proliferation. These include prolonged transportation at ambient temperature, a lack of an efficient cold chain and inadequate storage conditions, which can create an environment conducive to the maintenance and multiplication of *Staphylococcus* species in raw milk. Previous studies of dairy production systems have associated poor management and hygiene practices with the prevalence of *Staphylococcus* contamination in bovine milk (27).

In comparative terms, an average of 3.4 Log_{10} cfu/mL of *Staphylococcus aureus* has been reported in raw milk, which is substantially lower than the value observed in this study (20). Mik obtained from farms with good milking hygiene practices generally shows counts ranging from 2.0 to 2.3 Log_{10} cfu/mL, which can increase to 4 Log_{10} cfu/mL in cases of intramammary infection (28). The mean value of 5.82 Log_{10} cfu/mL observed in this study suggest that, in addition to possible intramammary colonization, factors relating to hygiene and post-milking handling may significantly contribute to the high microbial load.

Contamination along the production chain was also evident, showing that *Staphylococcus aureus* can be introduced and spread from milking to subsequent processing stages, especially if good hygiene practices are not strictly followed (29).

The results revealed *Staphylococcus* counts exceeding 5 Log_{10} cfu/mL, a threshold frequently associated with the potential production of heat-stable staphylococcal enterotoxins (30). Given that the milk was stored in conditions that promote bacterial growth, it is biologically plausible that toxigenic risk occurred.

Regarding coagulase-negative *staphylococci* (CNS), the mean count of 5.97 Log_{10} cfu/mL observed in this study suggests high environmental contamination and potential hygiene practice failures during milking and milk handling. Although *CNS* are traditionally considered less virulent than coagulase-positive *staphylococci* (CPS), they are currently recognized as important agents of subclinical mastitis and as indicators of poor hygiene conditions.

CNS isolated from bovine milk have demonstrated significant prevalence, as well as the presence of genes associated with virulence and antimicrobial resistance (31). These findings suggest that CNS should not be considered mere environmental contaminants, but microorganisms with the potential to impact animal health and the microbiological quality of milk.

These absence of statically significant differences between districts ($p > 0.05$) suggest that contamination is structural and systemic, reflecting similar

production, handling, and preservation conditions across the three areas studied.

Due to the high levels of *Staphylococcus aureus* identified in this study, controlling mastitis within herds is paramount, which can be achieved by implementing sanitary monitoring programs and early diagnostic strategies. Practices such as pre-and post-milking teat disinfection (pre-and post-dipping), segregation of infected animals and improved handler hygiene can significantly reduce contamination levels (26). Furthermore, maintaining the cold chain during storage and transportation is essential to limit bacterial proliferation and reduce the risk of enterotoxin production (31).

4.5. Mold and Yeasts

Analysis of the results revealed high levels of molds and yeast in the raw milk collected in the evaluated districts, with overall averages of 5.09 ± 1.60 Log_{10} cfu/mL and 5.50 ± 1.05 Log_{10} cfu/mL, respectively. These levels suggest that the milk has been exposed to environmental conditions conducive to fungal growth for an extended period, particularly in small-scale production systems where hygiene and sanitary controls, as well as post-milking thermal preservation, are often limited.

Statistically significant heterogeneity between districts ($p < 0.05$) suggests that microbiological contamination is associated with local determinants of milk production, including ecological, infrastructural and behavioral factors. The Macate district had the highest levels of fungal contamination, possibly due to greater exposure of the milk to environmental aerosols, soil characteristics, regional topography and reduced efficiency of community refrigeration points used for the product's temporary storage.

The result obtained in the present study exceeds those previously reported in literature, where mean mold and yeast count of between 3.4 and 3.5 Log_{10} cfu/mL were found in raw milk produced under conventional agricultural conditions (32). While the presence of fungi in raw milk is well documented, the substantially higher levels observed in Manica district suggest increased environmental contamination and deficiencies in hygienic and sanitary practices through the production process.

Similarly, variations in yeast counts ranging from 1.52 Log_{10} cfu/mL to values exceeding 4 Log_{10} cfu/mL have been reported in raw milk (33). Furthermore, evidence from studies conducted on thermally processed milk demonstrates that such treatments significantly reduce the fungal load. In this context, counts of up to 10^4 cfu/mL have been reported in dairies with different management, environmental and hygiene conditions (34-37).

Despite the absence of specific regulatory standards for mold and yeasts in raw milk, the concentrations observed exceed the established microbiological criteria for dairy products, including the Brazilian limit of 10^3 cfu/g for cheeses. These results suggest that the microbiological quality of milk produced by smallholder farms in Manica is compromised, which is probably due to inadequate milking conditions, such as poor infrastructure and inadequate cleaning of utensils used for manual milking.

Overall, the elevated microbial loads observed across the analyzed parameters suggest significant shortcomings in hygiene and sanitation control

through the raw milk production chain. These conditions are associated with poor adherence to good milking practices, poor environmental conditions at production sites, inadequate sanitation of equipment and long distances between production and collection points. All of these factors increase the risk of contamination. Additionally, post-harvest handling practices, such as the use of non-refrigerated metal containers and inappropriate transport methods like bicycles in high ambient temperatures, promote microbial proliferation and accelerate product deterioration.

The absence of an effective cold chain from milking to initial storage is a critical factor in the deterioration of the microbiological quality of raw milk (24). Refrigeration is widely recognized as an essential intervention for maintaining milk hygiene and safety, so the implementation of cooling systems, particularly bulk milk cooling tanks at production sites, is strongly recommended.

Overall, these findings should be interpreted as an indicator of the compromised microbiological safety of raw milk in the studied regions. The highlight the need for targeted interventions, including strengthening producer training in good hygiene practices, improving milking and collection infrastructure, establishing adequate cooling systems and reinforcing sanitary surveillance of artisanal milk production. This will help to safeguard public health and reduce economic losses associated with microbial spoilage.

Reducing fungal contamination requires improvements in environmental conditions and milk storage practices. Using properly sanitized containers that are protected from environmental exposure and implementing immediate post-miking refrigeration are effective measures for limiting the growth of molds and yeasts. Additionally, improvements to milking infrastructure, such as paved floors and dust control measures, can significantly reduce environmental contamination (10). It is also essential to raise producer awareness and provide training on appropriate hygiene and milk preservation practices to minimize these risks.

5. Conclusion

The results of this study demonstrate that raw milk produced in the analyzed districts presents high levels of microbiological contamination, characterized by mesophilic aerobic bacteria counts of approximately 7.0 $\log_{10}$ cfu/mL, total coliforms levels above $1.1x10^3$ MPN/mL, and significant loads of coagulase-positive staphylococci (CPS), coagulase-negative staphylococci (CNS), molds, and yeasts. The universal detection of coliforms and the high staphylococcal counts indicate substantial failures in hygienic and sanitary conditions during milking, handling, storage, and transportation.

The CPS levels observed exceed threshold frequently associated with the potential production of heat-stable staphylococcal enterotoxins, reinforcing the public health relevance of these findings, particularly when milk is consumed raw or subjected to inadequate heat treatment.

Overall, the findings demonstrate non-compliance with internationally recommended microbiological criteria and highlight the need for effective implementation of good milking practices, improved infrastructure, enhanced technical training for producers, and the establishment of continuous sanitary control systems. This study provides relevant scientific evidence

regarding the microbiological quality of raw milk in the region and can support strategic interventions aimed at protecting consumers and strengthening the milk production chain.

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Authorship contribution

All authors contributed equally

Declaration of competing interest

The authors declare that they have no competing interests

Data availability

Data will be available on demand.

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