

Research Article

Laboratory Tests Revealed High Contamination of Raw Milk Sampled from Selected Nodes of the Dairy Value Chain in Mbulu District, Tanzania

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Abstract

This study was carried to investigate the levels and types of microbial contamination in raw milk from selected nodes of the dairy value chain in Mbulu District, northeastern, Tanzania. A total of 185 raw milk samples were collected from dairy farmers, vendors, and restaurants across four wards namely Endagikot, Imboru, Hydrom, and Dongobesh. The target bacteria were *Escherichia (E.) coli*, *Staphylococcus spp.*, *Klebsiella (K.) pneumoniae*, and *Enterococcus (E.) faecalis*. A total of 185 milk samples were collected from dairy farms, vendors and restaurants across four wards. Total viable bacteria count and bacterial isolates were analysed using culture, biochemical test and Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS). Contamination increased up the chain with the lowest mean bacterial load (7.78×10^3 cfu/ml) in milk samples collected directly from udder and the highest (2.09×10^4 cfu/ml) in farmers bulking containers. *E. coli* and *Staphylococcus spp.* were the most prevalent bacteria detected at rates of 32.1 and 17 percent respectively of total samples from the vendors as well as 18.9 and 17 percent of total samples collected directly from udder. The *E. faecalis* and *K. pneumoniae* were detected at much lower rates (3.77 and 1.89 percent respectively). The bacteria levels determined in the present study are sufficiently high to contribute to milk-borne illnesses among consumers. The findings highlight public health risks associated with poor hygiene practices, inadequate milk handling, and environmental contamination. Enhanced training for milk handlers, improved hygiene protocols and regular surveillance are recommended to ensure milk quality and safety.

Keywords

Contamination, Microbial Quality, Public Health, Dairy Value Chain

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1. Introduction

Globally, milk-borne diseases remain a significant concern, particularly in developing countries where milk handling systems are largely informal [12]. Milk is a traditional natural food of a large proportion of households in many tropical countries including Tanzania. It is more common, precious, and highly nutritious dependable food particularly among the rural communities [1]. However unfortunately, in such communities over 90 percent of milk and milk products are obtained in informal distribution channels which are less or not regulated thus compromising both quality and safety [2-4]. Because of this situation it has been published extensively that dairy products including raw milk which is the most consumed product and are highly susceptible to microbial contamination at different nodes of the distribution or supply chain [5]. Studies that has been conducted in Tanzania have shown high levels of bacterial contamination in milk, including pathogens with antimicrobial resistance traits [13]. Factors that lead to contamination had been mentioned to be; poor milking hygiene, use of unclean containers, poor storage and unhygienic handling practices by vendors and restaurant operators [6]. Milk is frequently transported in reused plastic containers under conditions that promote bacterial growth, particularly in the warm tropical climate [7]. In addition to poor hygiene during milking and handling, milk vendors commonly sell milk door-to-door or in open markets, increasing the chances of exposure to contaminants [8]. Restaurants and informal eateries also purchase raw milk from

vendors and farmers, where it is either sold raw, boiled, or used in preparation of tea and other beverages [9]. Each stage of handling, transport and processing introduces further opportunities for microbial contamination. Microbial contamination of milk poses a serious public health risks. A number of pathogenic bacteria such as *Escherichia* (*E.*) *coli*, *Staphylococcus* spp., *Klebsiella* (*K.*) *pneumoniae*, and *Enterococcus* (*E.*) *faecalis* has been associated with severe gastrointestinal infections and other foodborne illnesses [10]. The presence of fecal indicator organisms such as *E. coli* further highlights the risk of zoonotic pathogen transmission from animals to humans through milk consumption [11]. The mentioned previous findings underscore the need for context-specific assessments to understand contamination risks unique to different districts and value chain structures. Mbulu District, located in north-eastern Tanzania, is home to a significant population engaged in smallholder dairy farming. Despite the importance of milk safety, limited studies have examined the levels of bacterial contamination along the dairy value chain in the District. Understanding the bacterial load, prevalence, and co-occurrence of pathogenic species across different handling stages is essential for developing effective interventions to safeguard milk quality and public health. This study was therefore conducted (i) to assess the microbial contamination of cow milk in Mbulu District, (ii) to identify key bacterial contaminants, and (iii) to evaluate contamination patterns at critical points along the value chain.

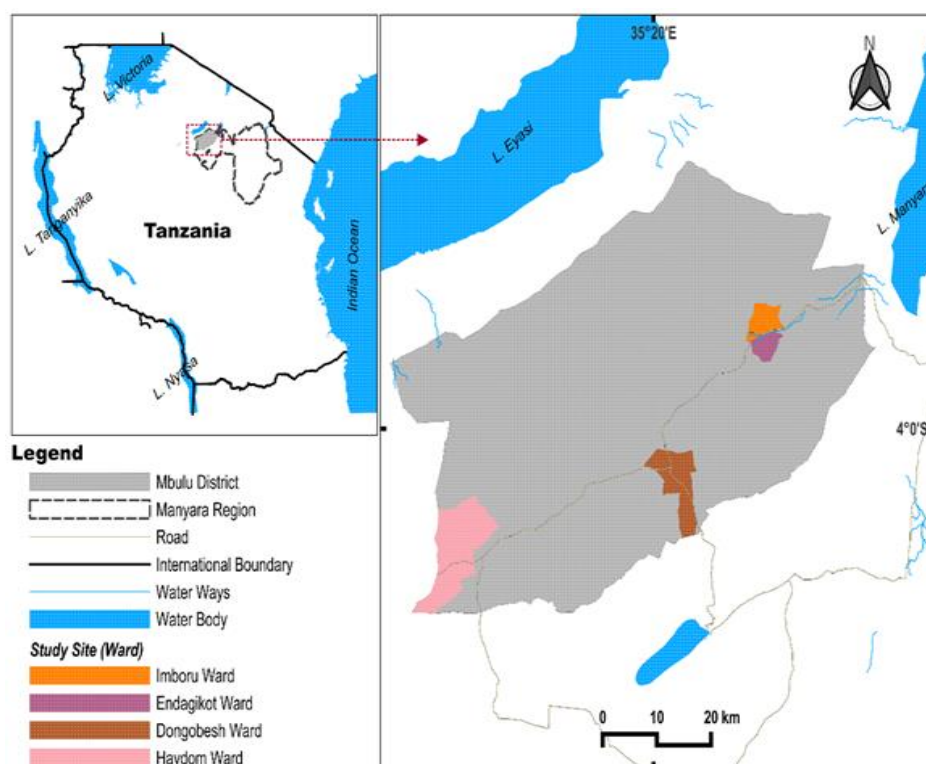


Figure 1. Map of Tanzania showing the study District and enlarged map of Manyara with the specific sites where sampling was carried out.

2. Materials and Methods

2.1. Description of the Study Area

This study was carried out in Mbulu District, an administrative district of Manyara Region located between latitudes 3.80 °S and 4.50 °S and longitudes 35.00 °E and 36.00 °E. The altitude of Mbulu is between 100 and 2400 metres above sea level (masl). The District has two administrative units which are Mbulu Town Council (TC) and Mbulu District Council (DC). The TC has 17 wards, 34 villages, and 58 streets whereas Mbulu DC has 18 wards and 76 villages. To a large extent, the District is characteristically rural area with few growing urban centres. During the 2022 National census, the population reached 376,865 people comprising of 193,494 males and 183,371 females. The population of cattle comprised of 336,005 heads of indigenous ones and 4,105 heads of dairy cattle (Mbulu District Veterinary Officer, unpublished data, 2023). The District location within the Map of Tanzania and wards involved in the study are shown in Figure 1.

2.2. Sample Size and Sampling Procedure

Multistage sampling was used to enroll milk samples from three nodes in the dairy value chain in Mbulu District. The nodes under consideration were the dairy farms (farmers), vendors, and restaurants (Figure 2). A total of 185 milk samples were collected from the nodes including 51 samples which were collected directly from the cows, 43 from milk bulking containers at the farms, 53 from containers of street vendors and 38 restaurants storage facilities across four wards namely Endagikot, Imboru, Hydrom, and Dongobesh. After securing a permit from ministry responsible for local governments, samples were collected from individuals of the study population upon their consent.

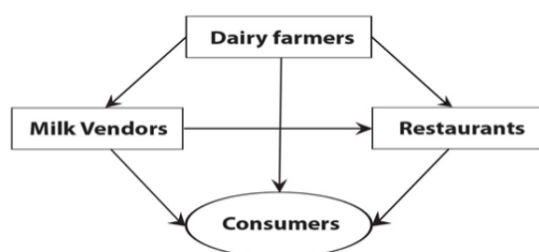


Figure 2. Raw milk value chain in Mbulu district.

2.3. Milk Sample Collection and Shipping

At the dairy farmer's level, raw milk was sampled direct from cow teats and the milked bulked in farmers' containers. From vendors' milk was sampled at their selling points which

were public roads, markets or in the streets. In the restaurant milk samples were collected from previously boiled and cooled milk ready to be served. At each stage 15mLs of milk was aseptically collected using a sterile labelled falcon tubes which were immediately placed in cool box packed with ice blocks. The temperature of the cool box during sampling was maintained at 4 °C. After sampling (approximately 2 hours later), the samples were stored in a deep freezer (20 °C) at Mbulu District Veterinary Laboratory for a few days before transferring them to Sokoine University of Agriculture (SUA), Veterinary Microbiology, Parasitology, and Biotechnology Laboratory for storage before analyses which were conducted within three days after arrival of samples.

2.4. Isolation of Bacteria from a Milk Sample and Biochemical Analyses

Raw Milk samples were prepared for isolation and identification of selected bacteria of public health importance in the dairy value chain. Bacterial culture was done by taking a loopful of milk sample, and inoculated on Mannitol salt agar (Oxoid® Ltd., Basingstoke, Hampshire, England) to support growth of *Staphylococcus* species, MacConkey agar (Hi media laboratories pvt ltd., Mumbai, India) to support growth of *E. coli*, Slanetz Bartley medium (Oxoid® Ltd., Basingstoke, Hampshire, England) to support growth of *Enterococcus* species, and Xylose lysine deoxycholate XLD (Oxoid® Ltd., Basingstoke, Hampshire, England) to support growth of *Klebsiella*. Thereafter, culture plates were incubated for 24 to 48 hours at 37 °C followed by reading the macro morphology of bacteria colonies. Then subculture was done until pure culture of bacteria colonies was obtained. Identification was done by reading the micro morphology of bacteria cells by using gram staining technique followed by microscopy (Olympus BH-2, Olympus Optical Co., Ltd., Tokyo, Japan) under oil immersion (100x objective) to confirm the shape. Subsequently the Gram-negative species were confirmed using biochemical essays, including the Indole, Methyl Red, Voges-Proskauer, and Citrate (IMViC) series tests and the Triple Sugar Iron (TSI) test, to differentiate them from other Enterobacteriaceae members. The IMViC and TSI tests above was extended to Confirm Gram-positive bacteria using coagulase, catalase, and motility tests, a method that was adopted and modified from [14].

2.5. Bacterial Load by Total Viable Count (cfu/mL)

Bacterial load, were assessed by taking 1mL of milk sample and mixed with 9mLs of sterile normal saline. Then, 1mL was serially diluted in 10-fold using 10 universal bottles containing sterile normal saline. In each dilution, 1mL was poured on the plate count medium Petri dishes in duplicate. The plates were incubated at 37 °C for 24 hrs. Then, colonies were

counted and the average colony counts were used to establish the colony forming unit per milliliter (cfu/mL) using the formula (1) adopted from Mpatswenumugabo et al. (2019).

$$\text{Total bacterial count } \left(\frac{\text{CFU}}{\text{mL}} \right) = \frac{(\text{Number of colonies counted})}{(\text{Dilution factor} \times \text{Volume plated})} \quad (1)$$

2.6. MALDI-TOF MS Test for Identification and Confirmation of Isolates

The Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) was utilized for the detection and verification of bacterial species. Thirty-one colonies of presumptive *Klebsiella* sp, *Staphylococcus* sp, *Enterococcus* sp, and *E. coli* of the isolates were transferred onto MALDI-TOF Ms sample plates, and analyzed using the VITEK®-MS system (bioMérieux, Vienna, Austria) at the Central Veterinary Laboratory (CVL) of Tanzania Veterinary Laboratory Agency (TVLA) in Temeke, Dar es Salaam. A reliability threshold of 95 to 100 percent was applied for species confirmation.

2.7. Data Management and Analysis

All data were organized in Microsoft excel and analysed using R software version 4.5.0 [15]. After assessing the normality of data, bacterial counts at different levels of the value chain were determined by comparing the respective means using one-way analysis of variance (ANOVA) and Tukey post hoc analysis. To test the significance difference in prevalence between levels and the bacteria isolates in the value chain Chi-square was used. The results were deemed to be significant at $P \leq 0.05$.

3. Results

3.1. Bacterial Load Across the Milk Value Chain

Results on the bacterial load from each when compared to the samples from cow teats revealed a non-significant association ($P > 0.05$) despite of having the highest mean cfu/ml from farmers bulking container samples. While the lowest load observation was observed from the samples collected from cow teats directly (Table 1).

Table 1. Summary of bacterial count (cfu/mL) and mean correlation between milk obtained from teats and other levels of the raw milk in the milk chain.

Source of samples	n	Mean (cfu/mL)	D.F	SEM	P-value
Cow Teats	51	7.78×10^3	50	4.808×10^3	-
Farm milk containers	43	2.09×10^4	42	8.63×10^3	0.509
Milk Vendors	53	1.43×10^4	52	6.03×10^3	0.884
Restaurants	38	1.67×10^4	37	8.07×10^3	0.803

3.2. Biochemical Analyses and Characteristics of Bacteria

Bacterial colonies cultured on different media exhibited varying biochemical characteristics presented in Figure 3. The *E. coli* colonies were grown on MacConkey agar and appeared as lactose fermenters, forming smooth, dry colonies of medium size, indicative of typical *E. coli* morphology (Figure 3A). *Staphylococcus* spp. cultured on Mannitol Salt Agar, exhibited golden-yellow colonies of medium size, consistent with mannitol fermentation and typical of *Staphylococcus aureus* (Figure 3B). The *K. pneumoniae* colonies appeared as large, mucoid colonies, characteristic of the organism's prominent capsule production on Xylose lysine deoxycholate (XLD) (Figure 3C). In contrast, *Enterococcus* spp. formed

small, translucent colonies with a slightly raised appearance, reflecting typical phenotypic traits on selective media (Figure 3D). This was followed by biochemical and phenotypic characterization of bacterial isolates. The results are presented in Table 1. Biochemical Test (IMViC) and TSI Test Results, revealed distinct patterns of reactivity across species. The *E. coli* exhibited positive results for indole and methyl red tests but was negative for Voges-Proskauer and citrate tests. It produced acid in both the slant and butt of triple sugar iron (TSI) agar and generated carbon dioxide (CO_2) but no hydrogen sulfide (H_2S). Conversely, *K. pneumoniae* demonstrated positive results for the Voges-Proskauer and citrate tests but was negative for indole and methyl red. It similarly produced acid in both the slant and butt of TSI agar with CO_2 but without H_2S production.

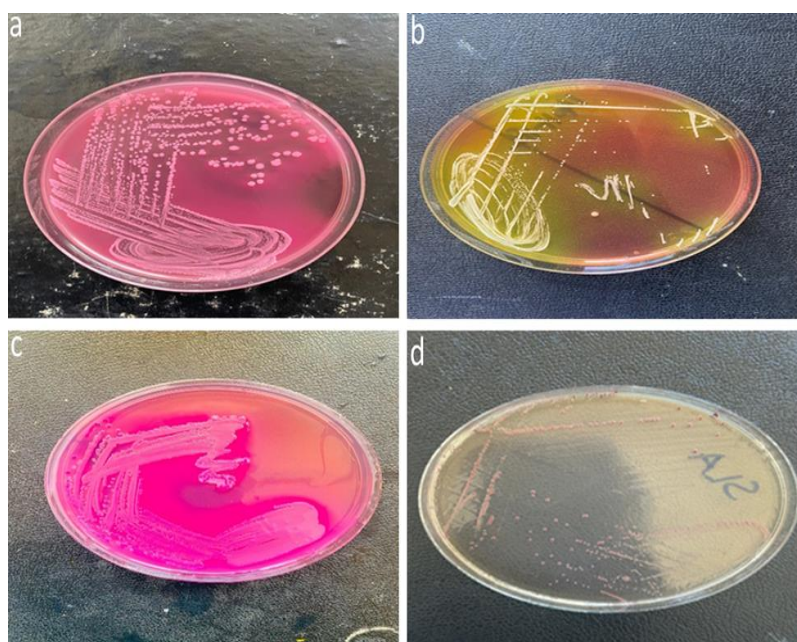


Figure 3. Characteristic appearance of bacterial colonies on selective media. A. *Escherichia coli* Colonies on MacConkey agar. B. *Staphylococcus* Colonies on Mannitol salt agar. C. *Klebsiella pneumoniae* colonies on Xylose lysine deoxycholate (XLD) medium. D. *Enterococcus* colonies on Slanetz Bartley medium.

Table 2. Biochemical test (IMViC) and TSI test results.

Bacteria	Indole	Methyl Red	Voges- Proskauer	Citrate Test	Slant	Butt	CO ₂	H ₂ S
<i>E. coli</i>	+	+	-	-	Acid	Acid	+	-
<i>K. pneumonia</i>	-	-	+	+	Acid	Acid	+	-

(+) indicate positive results, (-) indicate negative results.

3.3. Tests for Gram-positive Bacteria

Gram-positive isolates were evaluated using catalase, coagulase, and motility tests. While *S. saprophyticus* and *E. faecalis* were both catalase-negative and non-motile, *S. aureus* revealed catalase-positive results. Excluding *S. aureus* coagulase tests were negative across all gram-positive isolates which included *S. saprophyticus*, and *E. faecalis* bacteria. (Table 2).

Table 3. Summary Test for gram positive bacteria (*Staphylococcus saprophyticus*, *Staphylococcus aureus* and *Enterococcus faecalis*).

Test	<i>S. aureus</i>	<i>S. saprophyticus</i>	<i>E. faecalis</i>
Catalase test	+	+	-
Coagulase test	+	-	-
Motility	-	-	-

(+) indicate positive results, (-) indicate negative results.

3.4. Intensity of Contamination in a Single Sample

Four nodes of the value chain tested positive for more than one bacteria species. Samples collected from a farmers' container, tested positive for *Staphylococcus spp.* and *E. coli*, and *E. faecalis* while *K. pneumoniae* were not detected. Samples collected from restaurants, exhibited contamination by *Staphylococcus spp.* and *E. coli*, with no detection of *E. faecalis* or *K. pneumoniae*. On the other hand, in Hydrom ward, sample collected from cow teats tested positive for *Staphylococcus spp.* and *E. coli*, but no *E. faecalis* or *K. pneumoniae* contamination was detected. Similarly, in Endagikot ward samples sourced from cow teats, revealed the presence of *Staphylococcus spp.* and *E. coli*, but the absence of *E. faecalis* and *K. pneumoniae*. In the Imboru ward, co-contamination by more than one bacteria was observed from samples collected from milk-vendors. The analysis revealed contamination by *Staphylococcus spp.* and *E. coli*, while *E. faecalis* and *K. pneumoniae* were absent (Table 4).

Table 4. Results for intensity of bacterial contamination per single sample.

ward	Sample ID	Source	<i>Staphylococcus spp</i>	<i>E. coli</i>	<i>E. faecalis</i>	<i>K. pneumoniae</i>
Dongobesh	A106	FC	1	1	-	-
	A118	FC	1	1	1	-
	A71	R	1	1	-	-
	A85	R	1	1	-	-
Hydom	A148	CT	1	1	-	-
Endagikot	A179	CT	1	1	-	-
Imboru	A33	V	1	1	-	-

FC Farmers container, R restaurant, CT cow teats, V vendors, (1) present, (-) absent.

3.5. Prevalence of Bacterial Contamination in Milk Samples

Milk from cow teats showed the presence of *Staphylococcus spp.* (18.90%), *E. coli* (17.00%), and *Klebsiella pneumoniae* (1.89%), but no *E. faecalis* was detected. Contami-

nation increased further down the value chain, particularly among samples from vendors where *E. coli* was the most prevalent (32.10%). Contamination levels were generally lower in milk from restaurants, with *Staphylococcus spp.* and *E. coli* detected at rates of 13.20% and 21.10%, respectively. Across all nodes, *E. faecalis* and *K. pneumoniae* contamination remained minimal (Table 5).

Table 5. Prevalence of bacterial contamination in milk samples from cow teats, farmers' containers, vendors, and restaurants.

Source of sample	Bacterial Isolates	Prevalence n (%)	95% C.I	P-value
Cow Teats (N=53)	<i>Staphylococcus spp</i>	10 (18.90)	9.44- 32.00	<0.01
	<i>E. coli</i>	9 (17.0)	8.07- 29.80	
	<i>E. faecalis</i>	0 (0.00)	0.00-6.72	
	<i>K. pneumoniae</i>	1 (1.89)	0.05- 10.10	
Farmers container (N=43)	<i>Staphylococcus spp</i>	9 (20.90)	10.00- 36.00	<0.01
	<i>E. coli</i>	6 (14.00)	5.30- 27.90	
	<i>E. faecalis</i>	2 (4.65)	0.56- 15.80	
	<i>K. pneumoniae</i>	0 (0.00)	0.00-8.22	
Milk Vendors (N=53)	<i>Staphylococcus spp</i>	9 (17.00)	8.07- 29.80	<0.001
	<i>E. coli</i>	17 (32.10)	19.90- 46.30	
	<i>E. faecalis</i>	2 (3.77)	0.46- 13.00	
	<i>K. pneumoniae</i>	0 (0.00)	0.00-6.72	
Restaurant (N=38)	<i>Staphylococcus spp</i>	5 (13.20)	4.41- 28.10	<0.01
	<i>E. coli</i>	8 (21.10)	9.55- 37.30	
	<i>E. faecalis</i>	0 (0.00)	0.00-9.25	
	<i>K. pneumoniae</i>	0 (0.00)	0.00-9.25	
Total (N=185)		78 (10.43)	8.33-12.84	P>0.05

3.6. Bacterial Identification Confirmation

MALDI-TOF MS, confirmed the presence of *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Staphylococcus saprophyticus*, all with confidence values of 99.9%.

Table 6. Confirmation from MALD-TOF MS test.

Genus	Frequency	Specie	Confidence Value (%)
Enterococcus	5	Faecalis	99.9
Klebsiella	1	pneumoniae	99.9
Escherichia	17	Coli	99.9
Staphylococcus	3	saprophyticus	99.9
Staphylococcus	5	Aureus	99.9

4. Discussion

Phenotypic assessment in selective and differential media provided initial evidence of species identity in the milk samples. The study has revealed *E. coli*, *Staphylococcus spp.*, *E. faecalis* and *K. pneumoniae*. These are among of the common bacteria that contribute to milk borne illness [16, 17]. This visual differentiation further supported by biochemical testing the IMViC tests. The inverse biochemical profile reliably distinguished between *E. coli* and *Klebsiella pneumoniae* of the family enterobacteriaceae which are closely related species, highlighting the robustness of IMViC testing in milk contamination surveillance [18, 19]. For Gram-positive bacteria, *S. aureus* distinguished from *S. saprophyticus*, and *E. faecalis* through its catalase-positive and coagulase-positive results, a hallmark feature of *S. aureus* [20]. The selected bacteria in this study have been isolated as well from milk sample in the similar study conducted in Kilosa and Mvomero, Tanzania [21]. Quantitative microbial analysis across the milk value chain revealed a progressive increase in bacterial counts as milk moved from collection at the cow teat to downstream points of sale and consumption. Milk contamination by bacteria was for the first time reported in Tanga, Morogoro and Pwani, the three regions found in Tanzania [4]. In this study it was reported that five bacteria isolate namely, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Listeria innocua*, *Listeria ivanmovii* and *Klebsiella spp.* was observed in the milk value chain. Highest contamination in our study has reported the higher prevalence with of *E. coli* compared to the rest of the species. and Similar findings was observed in the conducted study to assess *E. coli* across the bovine milk value chain in Eastern Poland [22]. Despite the fact that the mean bacterial count at each level of the value chain was within the

allowable range of the East African standards for bacterial count where by threshold amount has to be less than 2.0×10^5 cfu/ml [23]. The mean bacterial load was lowest in the milk direct from cow teats (7.78×10^3 cfu/ml), consistent with initial microbial exposure being limited under hygienic milking conditions. However, contamination intensified insignificantly at subsequent stages, with mean bacterial counts reaching 2.09×10^4 cfu/ml in farm bulking containers, 1.43×10^4 cfu/ml from vendor samples, and 1.67×10^4 cfu/ml in restaurant samples. Despite these numerical differences, the absence of statistically significant variation ($p > 0.05$) in bacterial load across the different levels of the value chain suggests that contamination events may occur at multiple points, and that once contamination is introduced, bacterial populations stabilize and persist throughout handling and distribution [24]. This finding underscores the importance of maintaining strict hygiene at all stages rather than focusing exclusively on a single point in the chain. However, these findings have revealed a lower total bacterial compared to the study which was conducted in 2017, where by it was noted that the total bacteria count was surmounted to 1.0×10^7 cfu/ml [4]. The analysis of individual milk samples revealed instances of co-contamination, with multiple bacterial species present within single samples. This was particularly evident in samples collected from Dongobesh ward, where a single sample from either farmers' containers or restaurants harbored *Staphylococcus spp.* and *E. coli*, often alongside *Enterococcus faecalis* at the same time. Similar patterns were observed in samples from Hydom and Endagikot wards, where *Staphylococcus spp.* and *E. coli* were co-detected directly from cow teats milk samples. These results altogether imply that initial contamination might have arose during the milking process [12]. The observation of *Staphylococcus spp.* and *E. coli* co-occurrence across multiple wards and points in the value chain is of particular concern, as it reflects potential fecal contamination combined with poor hygiene practices during milk handling [25, 26]. The absence of *K. pneumoniae* in these co-contaminated samples may indicate that *Klebsiella* contamination is episodic or originates from more specific environmental reservoirs, such as water sources or contaminated equipment, rather than from the cow or the milking process itself [27]. The prevalence data further contextualize the findings on bacterial load and co-contamination. *Staphylococcus spp.* and *E. coli* were the dominant contaminants across all points in the value chain. Which put more emphasis on the unsanitary milking handling procedures resulting from human skin and fecal matter largely [11]. Their prevalence was highest in vendor samples (32.1% for *E. coli*), with slightly lower rates in milk from restaurants and farmers' containers. Interestingly, milk collected directly from cow teats still showed measurable contamination, with 18.9% testing positive for *Staphylococcus spp.* and 17% for *E. coli*. This suggest that initial contamination might have occurred at the source, likely from environmental exposure, infections or

suboptimal milking hygiene [28]. The *E. faecalis* and *K. pneumoniae* were detected at much lower rates, with *E. faecalis* present in only 3.77% of vendor samples and completely absent from restaurants and direct milk from cow teats in the chain. The *K. pneumoniae* was similarly rare, found only in 1.89% of cow teat samples and absent in all downstream samples. The findings imply that milk were likely to be contaminated with the bacteria during washing of the udder and the hygiene of the milker [6]. These low rates may reflect the limited niche that these species occupy within the milk environment, particularly in comparison to *E. coli* and *Staphylococcus* spp., which are more resilient and better adapted to survive the conditions present during milk handling and storage [20]. The results collectively highlight several important public health and food safety concerns in the milk value chain. The presence of fecal indicators such as *E. coli*, alongside *Staphylococcus* spp., demonstrates that both environmental and human-associated contamination routes contribute to microbial burdens in milk. The increasing bacterial load along the value chain further underscores the cumulative risk introduced by each additional handling step, particularly among vendors and in restaurants, where milk may be exposed to varying degrees of hygiene controls. The low prevalence of *Klebsiella pneumoniae* and *Enterococcus faecalis*, while reassuring in some respects, does not negate their potential risk, as these opportunistic pathogens can still cause severe illness, especially in vulnerable populations [29, 30]. These findings collectively emphasize the urgent need for enhanced hygiene interventions, regular microbial surveillance and targeted training for all actors in the milk value chain to minimize contamination risks and ensure consumer safety.

5. Conclusion

The findings of this study demonstrate that microbial contamination is prevalent along the raw milk value chain, with contamination levels progressively increasing from the initial milking stage at the cow teats to handling points including farm containers, vendors and restaurants. The presence of *Escherichia coli* and *Staphylococcus* spp. as the most prevalent contaminants highlights the combined risks of fecal contamination and poor hygiene practices during milk collection, storage, transport and sale. However, this study has failed to illuminate on pathogenicity of the bacterial isolates. Therefore, we recommend that other studies should be conducted in an area for an intensive surveillance and assessing the relative pathogenicity of the bacterial isolates in an area.

Abbreviations

ANOVA	Analysis of Variance
DC	District Council
XLD	Xylose Lysine Deoxycholate

SUA	Sokoine University of Agriculture
TC	Town Council
TSI	Triple Sugar Iron

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Author Contributions

Magdalena Gwandu: Conceptualization, Resources acquisition, Field work, Formal analysis and writing original draft

Charles Lymo: Supervision, Reviewing and editing

Elisa Mwega: Supervision, Methodology, Laboratory work

George Msalya: Supervision, Reviewing and editing, Methodology, Validation and conceptualization

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Data Availability Statement

The data is available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

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