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Bacterial Counts of Unprocessed Bovine Milk Produced by Small Scale Farmers in Ndivisi Ward, Bungoma County, Kenya

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was conducted to determine the bacterial counts in unprocessed bovine milk among different sub locations, milk sources and replicates in different months having different seasons.
Study Design: A cross-sectional study design was employed whereby milk samples from randomly selected production points and outlets were collected.
Place and Duration of Study: The study was carried out in Ndivisi ward, Bungoma County, Kenya, between October 2016 to December 2016.
Methodology: 100µl of each sample was placed onto plates with plate count agar (PCA) using pour plate method to determine bacterial counts. Bacterial communities were isolated from the samples cultured on MacConkey agar and Blood agar supplemented with 5% sheep blood which were later enriched and purified on nutrient agar. The bacteriological status of milk was assessed by total plate count, isolation and identification of pathogenic bacteria. Data on bacteriological quality of milk was summarized using statistical analysis; means, standard deviation and variance. The difference in

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bacterial counts (CFU/ml**)** between sub locations, sources of milk and replicates in the study was assessed using analysis of variance (ANOVA). Statistical significance was set at p=0.05 using a computer package, SPSS software version 20.0.

Results: A total 486 were collected but only 235 samples (48.4%) were contaminated. *Staphylococcus aureus* was (28.1%) in abundance, pathogenic *Escherichia coli* (21.7%), *pseudomonas aeruginosa (19.1%) Bacillus subtilis* (11.5%), *Citrobacter freundii* (10.2%), and *Klebsiella pnemoniae* (9.4%) and they cause mastitis, food poisoning and diarrhoea. The presence of bacteria in milk in Ndivisi ward is associated with poor milk handling practices and contamination.

Conclusion: Milk in Ndivisi ward is contaminated hence not suitable for human consumption. High bacterial counts at production (single animal) are the main cause of mastitis in dairy animals.

Keywords: Unprocessed bovine milk; contamination; small scale farmers.

1. INTRODUCTION

Milk production in Ndivisi Ward is heavily dependent upon small scale farmers. Milk is sold unpasteurized to the public either directly from producers, via informal markets or to Kenya co-operative creameries (KCC). Resources are extremely limited and small scale farmers produce milk with low levels of hygiene and productivity. Low levels of hygiene and productivity is a factor in milk contamination. The health and hygiene of the cow, the environment in which the cow is housed and milked, and the procedures used in cleaning and sanitizing the milking and storage equipment are all key in influencing the level of microbial contamination of unprocessed milk. The temperature and length of time of storage allow microbial contaminants to multiply and increase in numbers. Thus these factors will influence the total bacteria count or Total Plate Count (TPC) and the types of bacteria present in bulk unprocessed milk.

Moreover, milk and its products are known to contain rich nutrient contents, including carbohydrates, proteins, and minerals, which may promote the growth of microbes including some food-borne pathogens [1]. The consumption of pathogen containing products may cause illnesses ranging from stomach upset to more serious symptoms [2]. The occurrence of other types of bacteria may potentially affect the product nutritional and sensory quality properties and in turn result in significant economic losses [3]. Milk is synthesized in specialized cells of the mammary gland and until recently, it has been believed to be virtually sterile when secreted into the alveoli of the udder [4] and that microbial contamination can generally occur from within the udder, the exterior of the udder, and from the surface of milk handling and storage equipment. Thus, the health and hygiene of the cow, the environment in which the cow is housed and milked, and the procedures used in cleaning and sanitizing the milking and storage equipment are all key in influencing the level of microbial contamination of unprocessed milk. Equally important are the temperature and length of time of storage, which allow microbial contaminants to multiply and increase in numbers. All these factors will influence the total bacteria count (TBC) and the types of bacteria present in bulk unprocessed milk. Since milk is a medium permissive to the growth of many bacterial species, most prevalent mastitis-associated bacteria are able to multiply *in vivo* with a doubling time of 20–30 min during the first few hours following entry into the udder [5]. One implication of the above considerations is that once they are within the lumen of a lactating mammary gland, many bacterial species are able to proliferate and reach high concentrations, unless a prompt immune reaction hampers their growth. The consequence of such high concentration of bacteria is mastitis [6,7]. Beyond this stage of milk production, contamination is either intentional or unintentional. Intentional contamination includes additives such as antibiotics, water which is unsterilized and hydrogen peroxide. Unintentional contamination includes contamination from the environment, milk handlers, equipment and milking practices. The presence of food borne pathogens in milk is due to direct contact with contaminated sources in dairy farm environment and due excretion from the udder of an infected animal [8]. Similarly, detection of coliform bacteria and pathogens in milk also indicates possible contamination of bacteria either from the udder, milk utensils or water supply used [9]. The milk can carry dangerous bacteria such as *Salmonella spp*, *Corynebacterium diphtheria,* pathogenic *Escherichia coli, Campylobacter coli* and *Listeria monocytogenes* which are responsible for causing food borne diseases. However, the

bacteria in milk can be especially dangerous to people with weakened immune system, old adults, pregnant women and children. Pregnant women run a serious risk of becoming ill from bacteria *Listeria* that cause miscarriage, fetal death or illness or death of newborn [10]. [11] Assessed the bacteriological quality of milk in Harare and revealed that milk and milk products sold in various outlets contained a variety of bacteria which are of great concern to public health. Another research showed the importance of microbiology to dairy industry studying the outbreaks of food borne illnesses associated with consumption of milk and dairy products that had been contaminated with pathogenic organisms or toxins. Undesirable microorganisms constitute the primary hazard to safety, quality and wholesomeness of milk and dairy food. Consequently, increased emphasis has been placed on microbiological analysis of milk and dairy products designed to evaluate quality and ensure safety and regulatory compliance [12].

The study carried in and around Coimbatore District in India indicated that dominant microbial flora associated with raw milk samples were in the order of *Lactobacillus sp., Staphylococcus aureus, Escherichia coli; Bacillus sp., Pseudomonas fluorescens, Salmonella sp.,* among the isolated pathogens [13]. Milk safety is crucial for both public health and farmer income, with consumers paying more for safer food. Furthermore, improved hygiene reduces spoilage and wastage benefitting producers, traders and consumers.

This paper presents and discusses data and findings from my study of bacterial contamination of milk in Ndivisi Ward (Fig. 1). The objective of our study was to assess levels bacterial contamination along small scale farmers' milk value chains in Ndivisi Ward of Western Kenya, focusing on unprocessed bovine milk sold by small scale farmers in Bungoma County. We considered where failings occur and the scope for improving milk safety and quality.

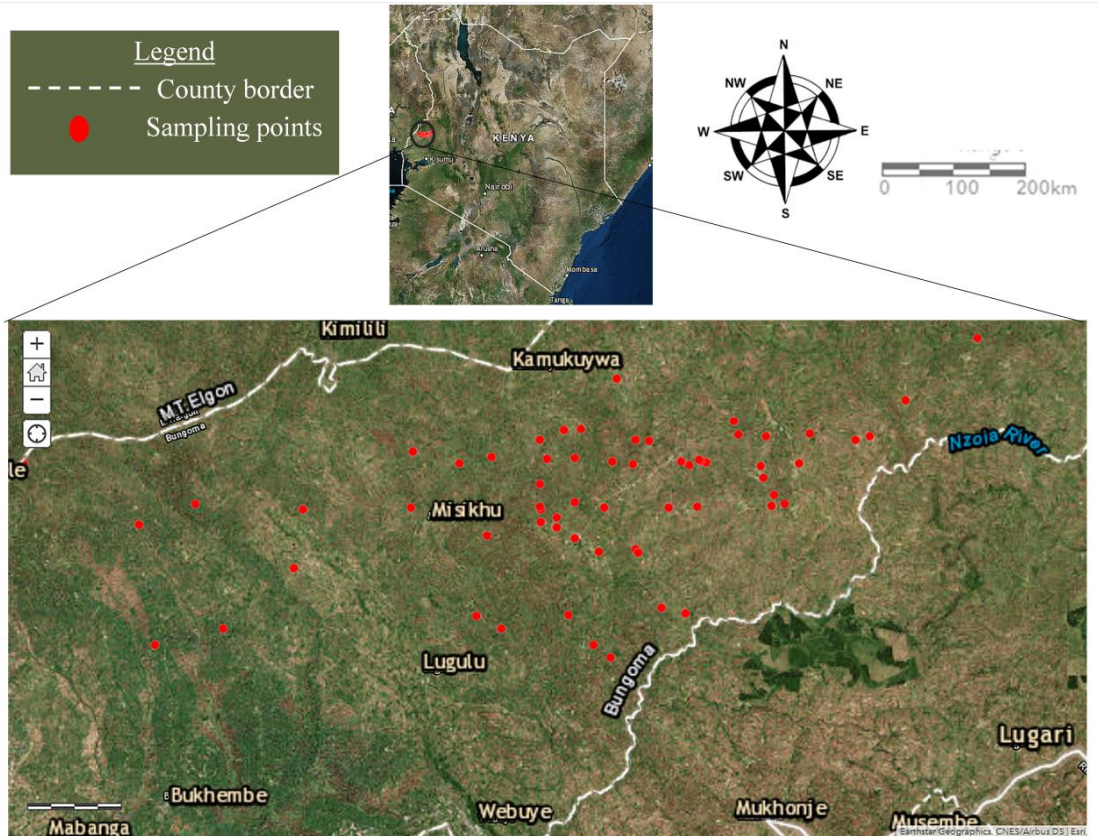


Fig. 1. Ndivisi Ward, Bungoma County in Western Province, Kenya.

2. MATERIALS AND METHODS

2.1 Selection

All producers supplying milk in Ndivisi Ward at the time of the study (September 2017) were identified and visited. They were enlightened on the importance of research and the expected benefits.

2.2 Field Visits

Steps in the milk value chain and points of sampling are summarized in Fig. 2.

On farm: Producers were visited once at milking time. Milk samples (25 mL) were collected aseptically from a single udder-quarter of all cows milked. After being milked, a cow's milk was poured into a container (Bulk milk) for that day. A milk sample was also collected from this bulk milk immediately after milking. Observations were made on milking practices including measures of hygienic practice and time. Milking and milk transportation to the outlets and KCC was done in the same manner.

KCC: After milking, the bulk milk was transported to the KCC and another sample was taken (25 mL).

Serial samples: To prevent further microbial growth during storage all samples taken were kept at 4·°C from point of sampling until culturing for microbiology.

Repeated sampling: Repeated sampling was carried out once every month from each of the sampling points. Out of 486 samples collected 235(48.4%) were contaminated but 251(51.6%) were not contaminated.

2.3 Microbiology and Quality Assessment

All samples were assessed for microbial density using the pour plate method. Microbiological analyses of unpasteurized bovine milk were performed at Masinde Muliro University of science and technology, Kenya.

Total plate Count (TPC) was determined using standard plate count agar using pour plate method hence incubated at 37ºC for 24 hours. Colony forming units (CFUs) were counted and expressed as; cells per 1ml [14]. MacConkey agar was used to differentiate between the lactose fermenters (coliforms) from the non-lactose fermenters. Fastidious bacteria were cultured on Blood agar supplemented with 5% sheep blood at 37ºC for 24 hours but also indicated the type of haemolysis. The bacteria were identified by colony characteristics, Gram's staining, biochemical tests namely triple sugar iron (TSI), Simmon's Citrate Agar, Motility lysine indole medium(MIL), oxidase test, catalase test and coagulase test. Kovacks reagent was used to confirm for production of indole by a microorganism. Standard reference strain of *Escherichia coli* and *Staphylococcus aureus* were used as controls. The confirmed isolates were then stored at -80°C in 10 % glycerol broth until used in other experiments.

2.4 Analysis

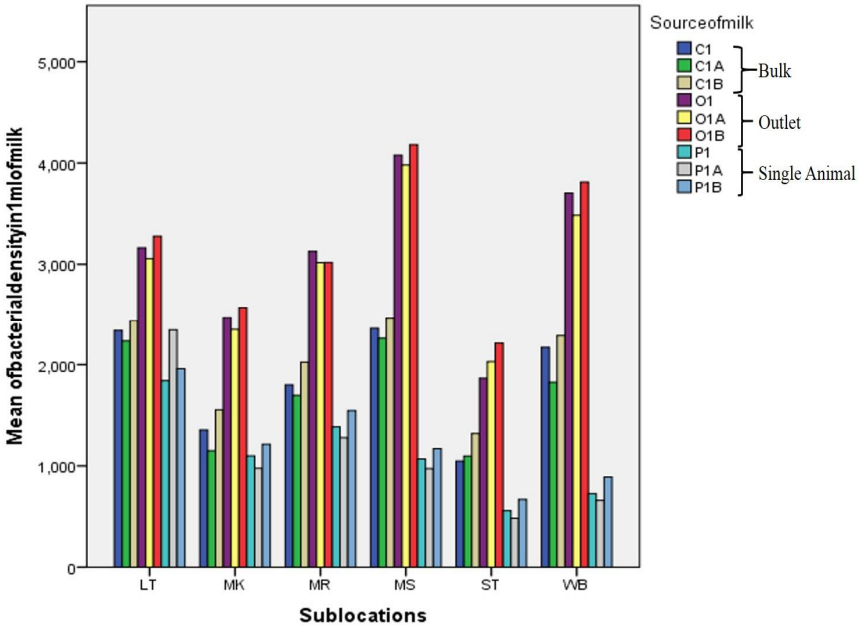
Data on bacteriological quality of milk was summarized using statistical analysis; means, standard deviation and variance. The difference in bacterial densities between sub locations and sources of milk in the study was assessed using analysis of variance (ANOVA). Statistical significance was set at $p<0.05$ using a computer package, SPSS software version 20.0.

Fig. 2. Diagram showing the flow of events during small scale milk production and transport to the outlets including KCC where unpasteurized milk was bought and sold to consumers.

3. RESULTS AND DISCUSSION

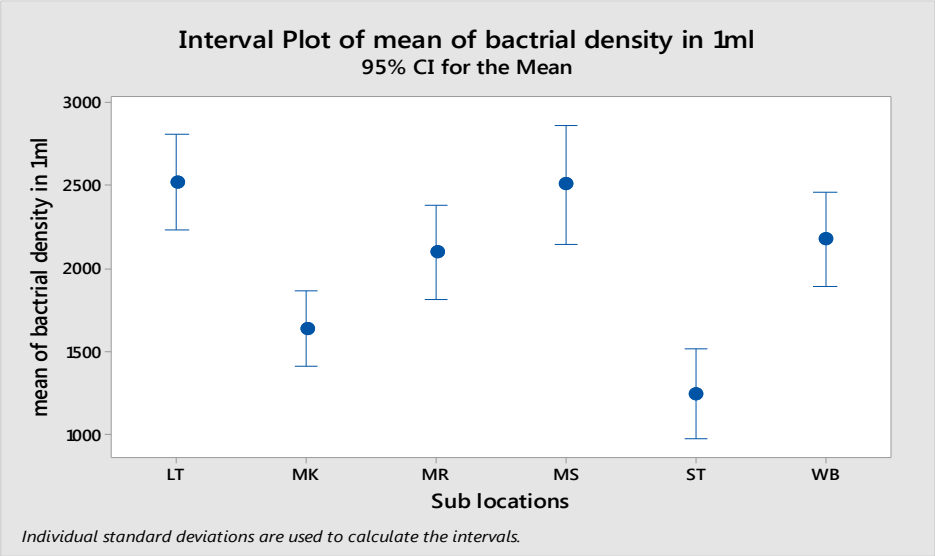
3.1 Mean Bacterial Counts in 1 ml of Milk

Mean of bacteria counts (CFU/ml**)** in 1 milliliter of milk in each of the sub-locations at production (P and C) and outlets (O) are presented in the following charts.



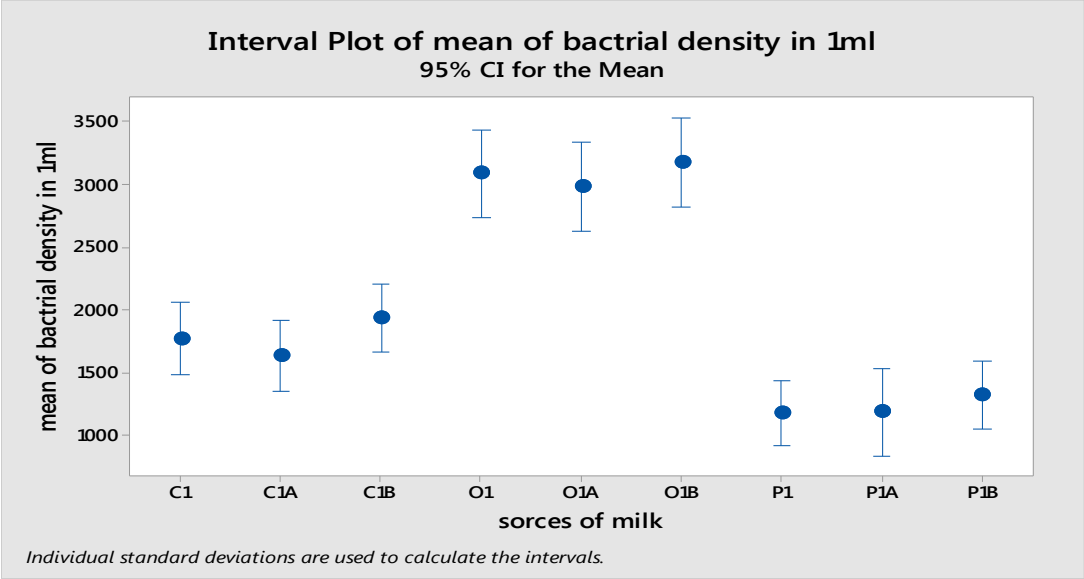
Lutacho (LT), Marinda (MR), Makuselwa (MK), Misemwa (MS), Wabukhonyi (WB) Sitabicha (ST) P-Single Animal, C-Bulk Milk,O-Outlets, 1-First Collection,1A-Second Collection,1B-Third Collection

Mean Bacterial Counts in 1 ml of Milk in Each Sub Location



Lutacho (LT), Marinda (MR), Makuselwa (MK), Misemwa (MS), Wabukhonyi (WB) Sitabicha (ST) CL - confidence interval

Mean of Bacterial Counts in 1 ml of Milk per Collection Point



P-Single Animal, C-Bulk Milk, O-Outlets, 1-First Collection,1A-Second Collection,1B-Third Collection, CL -confidence interval

Bacterial counts in milk from sub-location in Ndivisi ward between production (single cows P, bulk milk C) and outlet (O) over a time period of 3 months.

There was high bacterial counts in outlets (O) due to lack of cold chains (Refrigeration) the bacteria multiple rapidly, contamination arise majorly from the environment since milk is transported in open containers with the cups for measuring the quantity of milk sold to consumers left exposed to more contaminants, it is then followed by Bulk milk (C) in which bacterial counts are also higher because containers in which milk is poured from different animals within the farm are contaminated, also poor milking techniques like rubbing of the fur of the animal, using milked milk as lubricant on teats during milking play an essential role in milk contamination and lastly lowest at production (P) whereby milk from a single in most cases is sterile unless is from an infected animal. This is evidenced in all the sub-location of Ndivisi ward. There was also highest microbial density during the third collection (in December) being a dry season with relative limited pasture and water, milk produced was extremely in small quantities which do not meet the demand of the consumers thus was is prone to adulterations- water added to increase the quantity unfortunately some of this water created an opportunity for contamination, first collection (in October) followed. It was a rainy season, with abundance of water which was an important medium for bacteria to multiply hence higher bacterial counts and was lowest in the second collection (in November) the start of a dry season, water and pasture were now beginning to be a limiting factor and there no reported cases of adulteration. In this cases there was low bacterial load.

At production (P), there was high microbial density in Lutacho sub-location, followed by Marinda, Makuselwa, Misemwa, Wabukhonyi and Lowest in Sitabicha. At production (C), there was high microbial density in Lutacho sub-location, followed by Misemwa, Wabukhonyi, Marinda, Makuselwa, and Lowest in Sitabicha. At outlet (O), there was high microbial density in Misemwa, sub-location, followed by Wabukhonyi, Lutacho, Marinda, Makuselwa, and Lowest in Sitabicha.

In general, Lutacho sub-location had the highest microbial density, followed by Misemwa, Wabukhonyi, Marinda, Makuselwa, and Lowest in Sitabicha. Lutacho, Misemwa, Wabukhonyi are rural setups with only one cooperative society in each sub location, milk was solely sold to consumers since famers lack cold chains, milk is prone to bacterial multiplication hence high

bacterial counts. Similarly, there is little or no knowledge on bacterial contamination and poor milking practices like application of contaminated cow dung to teats to prevent calves from suckling were major causes of mastitis and contamination. Marinda, Makuselwa and Sitabicha are market places with many cooperative societies hence full aware of microbial contamination, here milk was sold solely to cooperative societies that require high standards to which farmers must comply to thus milk produced has low bacterial counts. On other hand, milk produced was mainly from hybrid dairy animals which require keen monitoring and treatment. Generally, milk in Ndivisi ward is not safe for human consumption since bacterial counts are higher than the recommended standards by English legislation which requires coliform counts of <100 CFU/mL for milk to be drunk raw [15]. This is shown in Table 1.

The standard deviations shows that there was a very huge difference between the highest and lowest values in each sub location.

3.2 Mean Bacterial Counts per ml of Milk in Collection Points in Three Replicates

Mean of bacteria counts per milliliter of milk in per collection point in three replicates is presented in Table 2. A total of 486 sampled were assessed.

The standard deviations shows that there was a very huge difference between the highest and lowest values per collection point. This is shown in Table 2.

There was no significant difference in the bacterial counts between and within the sub locations, replicates per month and milk sources of Ndivisi ward since the p- value is less than 0.05. This is shown in Table 3.

Table 1. Mean of bacterial counts per sub location

Bacterial counts per ml of milk				
Sub locations	**Mean(CFU/ml)**	**N**	**Std. deviation**	**Variance**
Lutacho(LT)	2516.91[a]	81	±1303.809	1699919.105
Makuselwa(MK)	1637.78[c]	81	±1022.662	1045837.500
Marinda(MR)	2099.01[b]	81	±1284.255	1649311.512
Misemwa(MS)	2504.07[a]	81	±1621.936	2630676.944
Sitabicha(ST)	1246.18[c]	81	±1223.391	1496686.273
Wabukhonyi(WB)	2173.21[b]	81	±1287.460	1657552.068
Total	2031.14	486	1374.178	1888363.870

N-Number of samples

Table 2. Bacterial counts ml of milk in collection point in three replicates

Bacterial counts per ml of milk				
Source of milk	**Mean (CFU/ml)**	**N**	**Std. deviation**	**Variance**
C1	1845.81[b]	54	±1062.424	1128744.154
C1A	1710.74[b]	54	±1054.820	1112644.724
C1B	2015.56[b]	54	±1066.638	1137715.723
O1	3090.57[a]	54	±1280.459	1639574.673
O1A	2985.74[a]	54	±1284.778	1650655.101
O1B	3178.15[a]	54	±1308.179	1711332.355
P1	1112.96[c]	54	±861.647	742436.338
P1A	1118.89[c]	54	±1244.315	1548319.497
P1B	1241.48[c]	54	±854.918	730884.556
Total	2031.14	486	1374.178	1888363.870

P-Single Animal, C-Bulk Milk, O-Outlets, 1-First Collection , 1A-Second Collection, 1B-Third Collection, N-Number of samples

Table 3. Significant test

		Sum of squares	df	Mean square	F	Sig.
Sub-Locations	Between Groups	937.100	309	3.033	1.111	.220
	Within Groups	480.400	176	2.730		
	Total	1417.500	485			
Milk Sources	Between Groups	255.450	309	.827	2.123	.000
	Within Groups	68.550	176	.389		
	Total	324.000	485			
Replicates per Month	Between Groups	207.433	309	.671	1.014	.465
	Within Groups	116.567	176	.662		
	Total	324.000	485			

df- degree of freedom, p-value-probability value, f- variance among means

3.3 Identification of Bacteria

Six bacterial species were identified using biochemical test up to the species level (see Table 4).

Staphylococcus aureus was found in 28.1% (66 samples) of the milk samples, *Pseudomonas aeruginosa* 19.1%(45 samples), *Bacillus subtilis* 11.5(66 samples) *Citrobacter freundii,* 10.2% (24 samples) *Escherichia coli* 21.7% (51 samples) and *Klebsiella pnemoniae* 9.4% (22 samples) were found at production (P and C) and at outlets in all the six sub-locations in all the three replicates. *Pseudomonas aeruginosa was* found only in Wabukhonyi and Lutacho at production (P and C) but lacks in outlets. *P. aeruginosa* is probably susceptible to the additives used since it never reached the outlets.

3.5 Accounting for Bacterial Presence in Milk

As was observed, there is high microbial density at outlets (O). This may be due to the following reasons.

Western Kenya has temperatures of about 27ºC on average. During the day when milk sellers are at the market (the outlet), milk is exposed direct sunlight so that temperature is much higher at about 37ºC which is optimum for bacterial growth. Temperature at the market and many nutrients in milk provides optimum conditions for *E. coli* and other human pathogens to multiply.

Cows were milked once a day. Time of milking varied with nine farmers milking between 6.00am and 8.00am. Milk was delivered to the outlets including KCC immediately after milking. Milking took 10–30 min, milking by hand into a bucket.

At production (C), contamination results from milk handlers, contaminated milk equipments, contaminated milking water, poor milking practices and contamination from the environment. High bacterial counts at production is the main cause of mastitis among the dairy animals in Ndivisi ward which may have resulted from poor milking techniques such as incomplete milking. Incomplete milking creates a favorable nutritious environment for bacterial growth and multiplication. Bedding used to house cattle is the primary source of environmental pathogens, but contaminated teat dips, intramammary infusions, water used for udder preparation before milking, water ponds or mud holes, skin lesions, teat trauma, and flies are all incriminated sources of infection. My findings are almost similar to other scholar's findings in that there is low bacteria counts at production (from single animal), followed by bulk milk and highest at outlets and also the bacterial load varies with environmental conditions having high bacterial counts during the rainy season and lowest during the dry season [15,16,17,18]. The uniqueness of my study proves that there was high bacterial counts during the dry season due additives majorly water which was added to increase the quantity of milk. I there recommend that farmers should the stop the use of additives like contaminated water and hydrogen peroxide, Consumers should buy milk at production (Single animal) since has low bacterial load and finally Farmers should employ cold chains in transportation and storage of milk.

Table 4. Identification of bacteria based on biochemical tests

Sourse	Gram stain	Haemolysis	Colony colour	Motility	Lysine	Indole	Citrate	Tsi	Catalase	Coagulase	Oxidase	Identity
P,C and O	+ve Cocci	Beta	Yellow	-ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	*S. aureus*
P and C	-ve Rods	Beta	Green	+ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	*P. aeruginosa*
P,C and O	+ve Rods Monopolar	Beta	White	+ve	-ve	-ve	+ve	+ve	+ve	-ve	+ve	*B. subtilis*
P,C and O	-ve rods	Beta	Pink Slow fermenter	+ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	*C. freundii*
P,C and O	-ve Rods	Beta	Pink Fast fermenter	-ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	*K. pnemoniae*
P,C and O	-ve Rods	Beta	Pink Fast fermenter	+ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	*E. coli*

P-Single Animal, C-Bulk Milk, O-Outlets, +ve-Positive, -ve-Negative

4. CONCLUSIONS

Most small scale farmers of Ndivisi Ward produce milk of good quality but in very small quantities (a litre or 3litres per cow/day and about 5L to 10L per bulk milk/day). Levels of hygiene are low with no refrigeration of milk even in outlets, where it is sold without pasteurization. Levels of bacterial counts in milk are high hence rapid spoilage milk. Low levels of hygiene, poor milking and milk handling practices are factors in the high bacterial counts in milk and mastitis which present in the dairy cattle population.

AWARENESS

To reduce the health risks associated with consumption of unprocessed bovine milk, it is necessary to raising community awareness on the importance of boiling milk before consumption, avoid the use of additives like contaminated water and hydrogen peroxide, Consumers should buy milk at production (Single animal) since has low bacterial load and finally, farmers to employ the use of cold chains (refrigeration) in transportation and storage of milk.

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ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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