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# Assessment of microbiological quality of raw milk produced and commercialized around INES Ruhengeri, Musanze district, Rwanda

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## Abstract

Milk contains high nutrients for humans and animal, but due to these high nutrients content and water it allows the growth of many types of microorganisms. In Rwanda, the dairy products are still commercialized in formal and informal markets. The milk sold in informal markets cause significant human health risks. The aim of the present study was the assessment the microbiological quality of raw milk produced and commercialized around INES Ruhengeri, Rwanda. Thirty milk samples were collected from 3 sectors of Musanze district, Rwanda and analyzed in food microbiology lab of INES Ruhengeri. The Total Viable Counts (TVC), *E. coli* and *Staphylococcus aureus (S. aureus)* had range from $2.3 \times 10^5$ cfu/ml to $9.1 \times 10^5$ cfu/ml, $1.8 \times 10^2$ to $2.9 \times 10^2$ cfu/ml and $1.8 \times 10^2$ to $2.2 \times 10^2$ cfu/ml respectively. The level of significant was not significant between TVC, *S. aureus* and *E. coli* count at *p* > 0.05. 3.3% of all samples were contaminated by *Salmonella* spp. It was concluded that high counts of bacteria were found in raw milk. The standards for the raw milks should be established and the raw milk producers should be given the appropriate training in hygienic handling of milk.

***Keywords:*** *Milk, Microorganisms, TVC, S. aureus, E. coli, Salmonella spp.*

## 1. Introduction

Milk is a fluid secreted from mammary gland of female. It is obtained from a variety of animal sources includes human, cattle, goats, sheep and buffalo; it is rich in nutrition content necessary to sustain animal or human life. Technically, the term milk is restrict to mean cow's milk and the milk from other animals must be spelled out by adding the name of animal from which it is drawn, for example sheep milk, goat milk, camel milk (Belitz *et al.,* 2009). However, the high nutrient content of those milks, which has proteins, fats, carbohydrates, vitamins, minerals and essential amino acids, all at a close to neutral pH and at a high water activity, provides a perfect growth environment condition for many microorganisms. Some of these nutrients can be directly used by all microorganisms, whereas others are transformed by some microorganisms and release them for being used by other microorganisms (Claeys *et al.,* 2013; and Quigley *et al.,* 2013).

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Raw milk can carry pathogenic bacteria such as *Salmonella* spp., *E. coli*, *Listeria monocytogenes, Campylobacter jejuri, Bacillus cereus, Yersinia enterocolitica, Staphylococcus aureus* (*S. aureus*) that cause foodborne illness, often called food poisoning. Microbial contamination of milk and dairy products causes a big health and economic challenges in both developed and developing country where 20 million of foodborne infection cases are reported annually (Pal *et al.,* 2016; and Ugochukwu and Niyibizi, 2019). The low quality milk and milk spoilage are of concern in the developing country like Rwanda. These countries rely on local small holder farms to supply milk to local consumers. Often, these Products are transported with unreliable refrigeration. Interruptions in the cold chain allow bacteria to quickly multiply, eventually reaching of levels unsafe for consumption. The low quality milk has negative impact on the milk consumers and processors. The milk marketing in Rwanda currently is in both informal and formal with 85% and 15% respectively (Doyle *et al.,* 2015).

Normally, the pure milk is produced by the healthy cow. The level of contamination can be influenced by different ways like the health and the hygiene of the cow, the environment or cowshed in which the cow is housed and milked and then the storage area and condition of the raw milk (Ugochukwu and Niyibizi, 2019). The temperature and the length of milk also influence the microbial growth and then the increasing of microbial load. Newly drawn milk from the udder of healthy cow contains less load of microorganisms which generally is lesser than 1,0000 microorganisms in one milliliter of milk (Bekuma and Galmessa, 2018).

It is normally accepted that the Lactic Acid Bacteria (LAB), a group of bacteria that ferment lactose to lactate, are a dominant population in bovine, goat, sheep and buffalo milk, prior to pasteurization. And the most common types of LAB in milk include *Lactococcus, Lactobacillus, Leuconostoc, Streptococcus and Enterococcus* (Quigley *et al.,* 2013). However Psychotropic populations, which particularly establish themselves during cold storage, are also a major component and frequently include *Pseudomonas* and *Acinetobacter* spp. To minimize the level of milk contamination, some improvements must be made, among those improvements include using improved methods of handling and processing of milk such as closed milking systems, use of bulk tanks to store and transport raw milk and changes in refrigeration systems (Nero and Carvalho, 2019; and Ledenbach and Marshall, 2009).

In Rwanda, row milk is commonly boiled before consumption for eliminating most pathogenic microorganisms; however the possible re-contamination is still the risk of consumer exposure to pathogenic bacteria. For example, *S. aureus* contamination is usually occurred after the milk is already boiled because *S. aureus* is heat-labile and does not compete well with other microorganisms (Kamana *et al.,* 2014). The dairy sector contributes 6% to the Rwanda's national GDP (Nishimwe *et al.,* 2015). However, the dairy products are still commercialized in formal and informal markets. The milk sold in informal markets cause significant human health risks especially transmission of diseases such as tuberculosis and brucellosis. Many small holder dairy farmers lack basic knowledge of sanitation, hygiene, and do not have access to clean water, adequate feed, and trainings. The resulting low quality milk potentially exposes consumers, especially infants, children, pregnant, and nursing women to health risk (Clay and King, 2019).

The fresh raw milk produced and commercialized around INES Ruhengeri (Musanze, Muhoza and Kimonyi sectors) follow the informal milk marketing channel where the risk of microbial contamination is very high; currently there is no available research showing the state of contamination or showing the quality of milk produce and commercialized around INES Ruhengeri. Therefore, microbiological assessment of milk is essential to establish the degree of contamination and recommend some corrective measures as a way to overcome these problems. It is in this framework that this study was conducted with aim of assessing the microbiological quality of raw milk produced and commercialized around INES Ruhengeri, Musanze district, Rwanda.

## 2. Materials and methods

### 2.1. Study site

The study was carried out in Musanze district Northern Province part of Rwanda, specifically in three sectors around INES Ruhengeri. The purposively selected sites were Musanze, Muhoza and Kimonyi sectors because the major agricultural activity is mixed with farming and has many small scale dairy farmers and venders than other sites.

## 2.2. Sample collection and handling

Three sectors were involved in the study namely Kimonyi, Musanze, and Muhoza. 30 milk samples from three sectors were collected from the storage containers used by farmers and venders in the visited areas, markets and kiosks. Labelled pre-sterile 30 mls bottles were used to collect the milk sample. The samples were immediately cooled and transported in cool boxes filled with ice packs to the INES Microbiology Laboratory and were stored at $4^{\circ}$C until analyses.

## 2.3. Microbiology analysis of milk samples

Analyses were carried out in INES Ruhengeri Food Microbiology Laboratory. Laboratory analyses of milk samples were performed where analysis for microbial quality of raw milk which involved establishing the Total Viable Counts (TVC) and isolation of some common milk-borne bacteria namely Enterobacteriaceae (specifically *E. coli* and *Salmonella* spp.) and *S. aureus* were performed.

### 2.3.1. Media preparation and storage

All the media used in this study were prepared according to manufacturer's instructions.

### 2.3.2. Total Viable Counts (TVC)

TVC was determined by following the ISO 4833-2:2013 method (ISO, 2013). Using a sterile pipette 25 ml of the milk sample were transferred into a conical flask containing 225 ml of Buffered Peptone water (BPW) and mixed well. Five-fold serial dilution of the inoculums from $10^{-1}$ to $10^{-5}$ was done into sterile BPW solution using disposable sterile pipettes tips. One millitre of the prepared inoculum was transferred into test tube containing 9 ml of BPW ($10^{-1}$ dilution). Then using another sterile pipette, 1 ml of the resulting dilution was transferred into a second test tube containing 9 ml of BPW ($10^{-2}$ dilution). The procedure was repeated for further dilutions up to $10^{-5}$ dilution and in the last dilution 1 ml of inoculum was discarded. The dilutions were mixed using a vortex mixer for 5-10 sec.

The already prepared Plate Count Agar (PCA) plates were removed from the refrigerator, kept at room temperature and labeled prior to inoculation. From each dilution (starting with the last dilution), two sterile PCA plates were each inoculated with 0.1 ml of the test sample. With the aid of sterile swab the sample was spread on the media surface and the plates were allowed to dry with their lids for about 15 min. The plates were inverted and incubated at 30 °C under aerobic condition for 72 h to allow bacterial growth. By using a new sterile pipette for each dilution, the procedure was repeated as above with further dilutions up to the first dilution and for the remaining test samples. The dilutions were also used for detection and enumeration of Enterobacteriaceae and *S aureus*, and the remained initial sample suspensions in the conical flasks were used in the initial preparation for isolation and presumptive identification of *Salmonella* spp.

### 2.3.3. Counting of bacterial colonies

Counting of bacterial colonies was performed by following the ISO 4833-2:2013 method (ISO, 2013). After the incubation period, bacterial colonies on the culture plates were countered manually. Only plates with colonies between 15 and 300 colonies were counted after incubation. After counting, the number of colonies was multiplied by the reciprocal of the dilution factor to give the count per ml of original milk sample, e.g., a count of 37 colonies on a petri dish of $10^{-3}$ means a count of $37 \times 10^3$ or 37 000cfu/ml. Lastly, the average of the dilution series was calculated to get CFU/ml number in the milk sample before dilution.

### 2.3.4. Expression of results

The countable bacterial colonies from two consecutive plates of each sample were converted into colony forming units per milliliter (cfu/ml) using a formula given by ISO 7218:2007(E) (ISO, 2007).

$$N = \frac{\sum C}{V \times 1.1 \times d}$$

*N*: Number of microorganism per ml or per gram of product.

*d*: Is the dilution corresponding to the 1st dilution retained.

*V*: Volume of inoculum placed in each dish in milliliters.

$\Sigma C$: Is the sum of colonies on the two dishes retained from two successive dilutions at least one of which contains a minimum of 10 colonies.

*2.3.5. Detection of* Salmonella *spp.*

Identification of *Salmonella* spp. in milk samples was done by using ISO 6579:2002 (ISO, 2002).

**Stage 1: Pre-enrichment in a non-selective medium:**

As described the remained initial sample suspensions in the conical flasks after serial dilution were used in the initial preparation for isolation and presumptive identification of *Salmonella* spp. BPW was used as a pre-enrichment liquid medium and the initial suspensions was incubated at 37 ℃ for 18 h.

**Stage 2: Enrichment in a selective liquid medium:**

Briefly 0.1 ml of the culture obtained in stage 1 was inoculated in a test tube containing 10 ml of RVS broth. Also, about 1 ml of the same culture was inoculated in a test tube containing 10 ml of Muller-Kauffmann Tetrathionate Novobiocin (MKTTn) broth. The inoculated MKTTn and Rappaport-Vassiliadis-Soya (RVS) broths were incubated at 37 ℃ and 42 ℃ respectively for 24 h.

**Stage 3: Plating out and identification:**

Using a sterile loop RVS broth culture obtained in stage 2 was inoculated onto Xylose-Lysine *Deoxycholate* (XLD) and MacConkey agar plates (already prepared plated media). The same was done using the culture obtained in the MKTTn broth (stage 2). The plates were inverted and incubated at 37 ℃ for 24 h. After incubation period, the plates were examined for typical colonies of *Salmonella* and atypical colonies that may be *Salmonella*. Typical colonies of Salmonella grown on XLD agar are red colonies with black centers due to xylose fermentation, lysine decarboxylation and production of $H_2S$ gas. The procedure was repeated as above with further dilutions up to the first dilution and for the remaining test samples. Each dilution a new sterile pipette was used. Only consecutive critical dilution steps were chosen for the inoculation on plates.

**Stage 4: Confirmation of *Salmonella*:**

Suspected Salmonella colonies from each XLD agar plate was confirmed using biochemical tests.

*2.3.6. Detection and enumeration of Coagulase Positive Staphylococci (CPS)*

Identification of *S. aureus* in milk samples was done by using ISO 6888-1:1999 (ISO, 1999) protocols through the following stages:

**Stage 1: Preparation of initial suspension and serial dilutions:**

The dilutions were prepared for initial suspensions from each sample; a serial dilution was carried out using peptone water as diluents. A one in five dilution of the sample solution was carried out by adding 1 ml of the sample solution to 9 ml of peptone water with a sterile pipette. The dilution was continued from $10^{-1}$ up to $10^{-5}$.

**Stage 2: Inoculation, incubation and isolation:**

Two sterile Baird-Parker (BP) agar plates (already prepared plated media) were removed from the refrigerator kept at room temperature and labeled prior to inoculation. Using a sterile pipette, 0.1 ml of the test sample was transferred from the last dilution to each of the two media plates. Then, a fresh sterile swab was used to spread the sample on the surface of the media. The plates were allowed to dry with their lids on for about 15 min. Thereafter, the plates were inverted and incubated at 37 ℃ for 24 h. After 24 h, all typical and atypical colonies present were counted and the plates were incubated at 37 ℃ for a further 24 h (48 h). After that, again, all typical and atypical colonies present were counted. Typical colonies grown on BP agar are black or grey, shining due to reduced action of tellurite, convex shaped and surrounded by a clear zone. After incubation for at least 24 h. An opalescent ring (due to proteolysis) in contact with colonies may appear in the clear zone. Atypical colonies are shining black colonies with or without a narrow white edge, the clear zone and opalescent ring are absent or hardly visible. By using a new sterile pipette for each dilution, the procedure was repeated as above with further dilutions up to the first dilution and for the remaining test samples, where only consecutive critical dilution steps were chosen for the inoculation on plates.

## Stage 3: Confirmation of CPS (*S. aureus*):

Confirmation of CPS was done by sub-culturing selected typical and/or atypical colonies obtained in stage 2 on Brain Heart Infusion (BHI) broth and by Coagulase test as biochemical test for identification of *S. aureus.*

### Sub-culturing onto Brain-Heart Infusion (BHI) Broth:

Using a sterile loop, an inoculum was picked from the surface of each selected colony and transferred into a sterile test tube containing 5 ml of BHI broth and incubated at 37 °C for 24 h.

### Coagulase Test:

Coagulase is a protein enzyme produced by microorganisms, among them is *S. aureus*. The enzyme protease converts fibrinogen to fibrin resulting to blood clotting. The BBL Coagulase Plasma, Rabbit with EDTA was used for the test. From incubated test tube containing BHI broth, 0.1 ml of each test culture was aseptically added to 0.2 ml of the rabbit plasma in eppendorf tube and incubated at 37 °C for 4-6 h. After the incubation period, eppendorf tubes were examined for clotting and if the test was negative, tubes were re-examined again at 24 h of incubation. The test was considered to be positive if the clot occupied more than half of the original volume of the liquid. Parallel with test samples, controls were performed simultaneously. As a positive control, 0.1 ml of sterile BHI broth inoculated with a known *S. aureus* was added to 0.2 ml of rabbit plasma in eppendorf tube and incubated. Also, for a negative control 0.1 ml of sterile BHI broth was added to 0.2 ml of rabbit plasma and incubated without inoculation.

### Stage 4: Counting and calculation of CPS:

After the incubation period, plates containing less than 300 colonies at two successive dilutions were selected for counting. Manual counting was done and the condition was at least one of the plates must contain at least 15 colonies. For each plate, the number of identified CPS was calculated According to ISO 6888-1:1999 (ISO, 1999) protocol by following equation:

$$a = \frac{bc}{Ac} \times Cc + \frac{bnc}{Anc} \times Cn$$

where

*a* = The number of identified CPS in plate,

*Ac* = The number of typical colonies submitted to the coagulase test,

*Anc* = The number of atypical colonies submitted to the coagulase test,

*bc* = The number of typical colonies, which have been shown to be coagulase positive,

*bnc* = The number of atypical colonies, which have been shown to be coagulase positive,

*Cc* = The total number of typical colonies seen on the plate, and

*Cnc* = The total number of atypical colonies seen on the plate.

### *2.3.7. Determination of* E. coli

This was carried out according to the procedures described by ISO 7251:2005(E) (ISO, 2005). For *E.coli* MacConkey Agar HIMEDIA REF M081-500G was used. Agar was prepared by dissolving 51.53 g of the Agar in 1 L of distilled water. The solution was pre-heated to allow dissolving later was autoclaved at 121 °C for 15 min. It was cooled to 45 °C in water bath ready for inoculation. Milk sample in a collecting bottle was carefully shaken. The sample bottle was aseptically opened then sample dilution series was carried out from $10^{-1}$ to $10^{-5}$ while holding the test tubes and sample bottle near a Bunsen burner to avoid contamination. The sterilized pipettes were used in sample taking to avoid contamination. The single sterile pipette was used per each dilution transfer. 1 ml of each dilution was pipetted into separate, labeled empty petri dishes, opening dish only enough to let the pipette in. This was done near the Bunsen burner. The plating was done in triplicate. The inoculated dishes were added with 15-20 mls of the MacConkey Agar. The petri dishes were gently moved at figure eight movements to mix the inoculum with the Agar then they were allowed to solidify. The petri dishes were inverted and incubated at 37 for 24 h. After incubation, all dishes with colonies 15 and 150 were counted. Only colonies that had reddish ring were counted.

### 2.3.8. Confirmation of E.coli

The biochemical test for confirmation was done to confirm the presence of *E.coli* in the milk samples which the organism grows.

### 2.3.8.1. Gram staining test

This test was done on the sterile glass slides and a drop of normal saline was firstly added on a slide then well-isolated colonies were spread on the prepared slides to make smears. These smears were dried on air and then followed by gentle fixing and flaming. Fixed smears were soaked in a crystal violet stain for two minutes then washed with a running tap water. Slides were then soaked in Lugol's iodine for two minutes and then washed with a running tap water. Thereafter these slides were decolorized by using acetone alcohol and washed on running tap water followed by covering of these fixed smears with neutral red for two minutes and then washed on running water. These slides were then dried on a draining rack after drying a drop of oil immersion was added to these slides then examined under a light microscope with 100X objective to examine the morphology of the bacteria.

### 2.3.8.2. Biochemical tests

### 2.3.8.3. Indole test

Peptone water solution was prepared by diluting 15.23 g of peptone water powder into 1000 ml of the distilled water and then 3 mls of this solution was added in a test tubes by using a sterile plastic disposable pipette. Fresh sterile plastic loop was used to inoculate the colonies to test tubes then these tubes were incubated at 37 °C for 48 h. After incubation 0.5 mls of Kovac's reagent (Loba Chemie Pvt. Ltd, Lot LM 01131303) were added to the incubated tubes. Tubes were then gently shaken and examined for red colored ring formation on the surface of the tube. Formation of this red ring is an indication of positive Indole reaction.

### 2.3.8.4. Citrate biochemical test

Simmon citrate agar (TM media, India) was prepared using 24.28 g/l in conical flask. The solution was heated on a heater (Model 690/5 India) to dissolve the medium and the mixture was sterilized in autoclave (Huatai YX280D) at 121 °C for 15 min. The medium was poured in labeled test tubes in inverted position. After media solidification, an inoculation loop was sterilized using a flame. The loop was inserted into the Petri dish for the selection of an isolated colony to be cultured in each test tube referring to the labels. The test tubes were incubated at 37 °C  for 24-48 h. The observations were then noted down.

### 2.3.8.5. Glucose and lactose fermentation tests

57.52 g of Kligler iron agar (TM media, India) and 50 ml of distilled water was mixed in a conical flask of 500 ml.  The media were then dissolved on a heater (Model 690/5) and sterilized in autoclave (Huatai YX280D) at 121 °C in 15 min. The medium was transferred in labeled test tubes and solidified as slant. Via a flame, an inoculation loop was sterilized and used to select an isolated colony to be cultured in test tubes. At 37 °C, the test tubes were incubated for 24-48 h and the results were noted down.

### 2.3.8.6. Sulfide production and motility tests

30 g of sulphide production, indole and motility (SIM), (BBL, India) medium was transferred in 1 L using conical flask of 250 ml. The mixture was warmed up on a heater (model 690/5) to dissolve the medium. At 121 °C, the solution was sterilized in autoclave for 15 min. The medium was conveyed in labeled test tubes in no inverted position then waited for solidification. An inoculation was done using isolated bacteria. After incubation at 37 °C for 24-48 h, the observations were recorded.

## 2.4. Statistical analysis

Data collected were analyzed using a descriptive statistical analysis approach with Statistical Packages for Social Sciences (SPSS), version 23 and one way ANOVA at $p > 0.05$.

## 3. Results and discussion

### 3.1. Total Viable Counts (TVC)

The results showed the minimum TVC was $1.7 \times 10^3$, $7.3 \times 10^2$ and $1.9 \times 10^4$ for Kimonyi, Musanze and Muhoza sectors respectively and maximum TVC was $1.06 \times 10^6$, $1.2 \times 10^6$ and $3.3 \times 10^6$ for Kimonyi, Musanze and Muhoza sectors respectively. From the results of this study, it was found that the majority of the milk samples had lower TVC than the maximum recommended level of $2.0 \times 10^6$ cfu/ml as given by East Africa

Community standards (EAS 67:2015) (AEAC SECRETARIAT, 2015). The low bacterial counts observed could have been due to clean milk handling by the farmers and vendors through proper cleaning of milk utensils, udder and hygiene observation by the milkers. As reported by (Khademi, 2019; and Khademi et al., 2019) flesh milk contains some microorganisms which are its normal biological constituents, its load changes easily depending on different conditions. The good quality milk is the one with lower bacterial load(Keefe, 2012).

## Staphylococcus aureus

The results showed isolation rate of *S. aureus* in the 30 raw milk samples in (cfu/ml) were 180, 220 and 200 for Kimonyi, Musanze and Muhoza respectively. In Gram's staining, smears from MCA, Gram's negative, pink color, mostly rood shape organisms were revealed. Many studies conducted in different areas implicated *S. aureus* as the common mastitis causing organism in lactating cows. The consumption of milk contaminated with *S. aureus* can be a health hazard because the main threat is based on the fact that about 10% of mastitis staphylococci are known to be producers of enterotoxins which are heat stable toxins (Rainard et al., 2018).

## E.coli

The result were $1.8 \times 10^2$, $2.1 \times 10^2$ and $1.9 \times 10^2$ in cfu/ml for Kimonyi, Musanze and Muhoza respectively. Gram's positive, violet color, short cocco-bacilli or rod were found within bundles and singly arranged while gram staining. So detected *E.coli* in milk is of great health concern as it indicates contamination with the faecal materials (Ruegg, 2017). But the use of hot water and detergents in washing hands of the milker, cleaning milk utensils with hot water and detergents is the advisable method of avoiding milk contamination as *E.coli* can easily be killed with high temperature and using hot water. Also, farmers with milk contamination of *E.coli* need to be informed on the possible sources of contamination of their milk like udder with faeces due to uncleaned animal houses so as they will be able to control the milk contamination.

The level of significant was recorded for raw milk is not significant between TVC, Total *S. aureus* and Total *E. coli* count at $p > 0.05$.

| Table 1: Average number of colonies grown on PCA, PB and MCA for each sample | | |
|---|---|---|
| **Sample** | **TVC in cfu/ml** | ***S. aureus* in cfu/ml** | ***E.coli* in cfu/ml** |
| K1 | $1.06 \times 10^6$ | $1.01 \times 10^2$ | $1 \times 10^2$ |
| K2 | $1.9 \times 10^5$ | $1.6 \times 10^2$ | $5.8 \times 10^2$ |
| K3 | $4.4 \times 10^4$ | $2.2 \times 10^2$ | $3.6 \times 10^1$ |
| K4 | $3.5 \times 10^3$ | $2.5 \times 10^2$ | $3.7 \times 10^2$ |
| K5 | $4.7 \times 10^3$ | $1.2 \times 10^1$ | $2.1 \times 10^1$ |
| K6 | $3.4 \times 10^3$ | $1.8 \times 10^1$ | $3.9 \times 10^2$ |
| K7 | $1.7 \times 10^3$ | $2.9 \times 10^1$ | $1.7 \times 10^2$ |
| K8 | $1.5 \times 10^5$ | $5.8 \times 10^2$ | $2.1 \times 10^1$ |
| K9 | $5.8 \times 10^4$ | $1.9 \times 10^1$ | $2.5 \times 10^1$ |
| K10 | $8.1 \times 105$ | $3.8 \times 10^2$ | $1.8 \times 10^2$ |
| Z1 | $1.01 \times 10^6$ | $6.1 \times 10^2$ | $4.5 \times 10^1$ |
| Z2 | $1.1 \times 10^6$ | $4.1 \times 10^2$ | $9.5 \times 10^2$ |
| Z3 | $1.07 \times 10^6$ | $2.6 \times 10^1$ | $4.7 \times 10^1$ |
| Z4 | $7.3 \times 10^2$ | $3.7 \times 10^2$ | $2.9 \times 10^2$ |
| Z5 | $1.1 \times 10^6$ | $4.5 \times 10^1$ | $3.6 \times 10^1$ |
| Z6 | $1.28 \times 10^5$ | $4.6 \times 10^2$ | $4.9 \times 10^2$ |
| Z7 | $6.9 \times 10^4$ | $6.7 \times 10^1$ | $3.7 \times 10^1$ |
| Z8 | $6.5 \times 10^5$ | $7.1 \times 10^2$ | $3.9 \times 10^1$ |

| Table 1 (Cont.) | | | |
|---|---|---|---|
| **Sample** | **TVC in cfu/ml** | ***S. aureus* in cfu/ml** | ***E.coli* in cfu/ml** |
| Z9 | $8.6 \times 10^3$ | $4.1 \times 10^2$ | $2.8 \times 10^2$ |
| Z10 | $1.2 \times 10^6$ | $1.06 \times 10^2$ | $2.9 \times 10^2$ |
| M1 | $7.4 \times 10^4$ | $1.6 \times 10^1$ | $4.9 \times 10^1$ |
| M2 | $1.02 \times 10^6$ | $1.8 \times 10^2$ | $5.4 \times 10^2$ |
| M3 | $4.7 \times 10^5$ | $1.5 \times 10^2$ | $2.7 \times 10^1$ |
| M4 | $7.4 \times 10^4$ | $6 \times 10^2$ | $2.1 \times 10^2$ |
| M5 | $4.6 \times 10^5$ | $7.1 \times 10^2$ | $3.7 \times 10^1$ |
| M6 | $1.05 \times 10^6$ | $2 \times 10^2$ | $3.6 \times 10^1$ |
| M7 | $1.9 \times 10^4$ | $6.1 \times 10^2$ | $5.9 \times 10^2$ |
| M8 | $5.5 \times 10^5$ | $6.2 \times 10^1$ | $5.7 \times 10^1$ |
| M9 | $3.3 \times 10^6$ | $3.9 \times 10^2$ | $2.4 \times 10^1$ |
| M10 | $2.2 \times 10^6$ | $2.8 \times 10^1$ | $3.9 \times 10^2$ |

| **Table 2: One way-ANOVA of TVC, *S. aureus* and *E. Coli* count** | | | | | | |
|---|---|---|---|---|---|---|
| *Source of variation* | **SS** | **df** | **MS** | ***F*** | ***p*-value** | ***F* crit** |
| Between groups | 3.53839E+6 | 2 | 8.22762E+6 | 0.9122 | 0.07132 | 1.3871 |
| Within groups | 2.20102E+12 | 10 | 1.30183E+12 | | | |
| Total | 2.44485E+14 | 12 | | | | |

### *Salmonella* spp.

This study found that 3.3% of milk samples corrected in all sectors contained *salmonella* spp. According to the presented biochemical results, raw milk contained different species such as *Salmonella* species, *E.coli* spp and *S.aureus.* Salmonella species are important bacteria known to cause food poisoning through consumption of contaminated milk and milk products (Alegbeleye et al., 2018). As described earlier that presence of these contagious pathogens in developing countries may be related to poor animal houses and poor milking hygiene practices by most small-scale livestock keepers (Lowenstein *et al.,* 2016).

## 4. Conclusion

Even if the average of TVC for both farmers and vendors in Musanze, Muhoza and Kimonyi sectors were within the acceptable ranges as per East African Community Standards. The milk was found to contain *E. coli, Staphylococcus* spp. and *Salmonella* spp. which can be originated either from animals themselves or from farmers and vendors. The presence of *E. coli, Salmonella* spp. in the sample is an indicator that microorganisms are present. Due to the milk contamination observed, the milk was found to have a public health concern and that milk must not be consumed as raw.

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