# Quality Evaluation of Some Libyan Raw Milk

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## Abstract

Milk is highly nutritive and one of the most complete single foods available in the nature for health and promotion of growth. On other hand, milk provides essential nutrients for excellent growth of many bacterial species. This study **aimed** at assessing microbial quality of raw cow's milk and determined antimicrobial susceptibility of the selected common milk-borne bacteria isolated in Alharsha, Sabriha, Abi Issa, Rain, Surman, Sabratha cities.

**Method** we collected 27 samples of raw milk from farms with additional 12 samples from shops in west coast Libya for laboratory analysis including microbial quality assessment and antimicrobial susceptibility tests.

**Result** The milk was typically transported by traditional car without refrigeration until reaching the point of sale, although microbiological contamination was initially low, with almost all samples culture positive for NLF (Non-lactose fermentation) and *Escherichia coli*, all isolates were sensitivity to Levofloxacin but Resistant to Augmentin, with highly resistance reach against 8 of 10 antibiotics.

**Conclusion**: main source of microbial contamination of raw milk form the farms and increasing prevalence of multidrug resistant strains with reduce susceptibility to antibiotics. Also, after milking, milk was kept for long hours at temperatures favoring microbial growth and sold without a microbial kill step with low boiling temperature of milk help kept some types of bacteria.

**Key words:** Raw milk, Non-lactose fermentation, Antibiotics sensitivity test.

## 1. Introduction

Milk is one of the oldest foods known to human being, it is essential not only for the health development of infants but also, for all ages, as it contains all digestible nutrients required by the body in a proper and well-balanced properties **(Javaid *et al*., 2009).**

Milk production in west coast of Libya, is heavily dependent upon smallholder production. Fresh milk is often sold unpasteurized to the public either directly from producers or via markets. Resources are extremely limited and smallholder production is under-developed with low levels of hygiene and productivity.

At present, a major problem facing the dairy products is to ensure of raw milk good production, partly because of public requirement of its safety and quality. However, the extent of risk posed by consumption of raw milk in the country is not well documented.

Regulations concerning proper hygienic handling of milk and its pasteurization are not generally implemented in developing countries and consequently making milk-borne diseases a higher health risk to public. Milk quality is determined by its composition and hygienic level exercised during milking, such as, cleanliness of the milking utensils, condition of storage, manner of transport as well as the cleanliness of the udder of the individual animal. Production of milk and various milk products under unsanitary conditions and poor production practices can exert both a public health and economic constraints (**Swai ES and Schoonman L. 2000**).

Milk safety is crucial for both public health and farmer income, with consumers paying more for safer food (**Roesel, K. et al., 2014; Jabbar, M.A. et al., 2010**). Furthermore, improved hygiene reduces spoilage and wastage benefitting producers, traders and consumers. When untreated fresh milk is kept at ambient temperature it rapidly turns into sour milk through proliferation of lactic acid producing bacteria (**O'Connor, C.B. et al., 1992**). This is consumed as curd. Sour milk remains highly nutritious and the acidity inhibits many bacteria responsible for disease and spoilage. However, fresh milk is a particularly high-risk perishable food, especially when consumed unpasteurized (**Lund, B.M. et al., 2000**). Hence was the focus of this study.

Milk is nearly sterile during secretion from healthy animals, but foreign components may enter during or after milking as well as any changes occurring in the milk that are often detrimental to its quality. Therefore, raw milk has been a known vehicle for pathogens for more than 100 years **(Gillespie *et al*., 2003)**

Bacterial contamination of raw milk can originate from different sources: air,

milking equipment, feed, soil, faces and grass (**Coorevits et al., 2008**). The number and types of micro-organisms in milk immediately after milking are affected by factors such as animal and equipment cleanliness, season, feed and animal health (**Rogelj, 2003**). It is hypothesized that differences in feeding and housing strategies of cows may influence the microbial quality of milk (**Coorevits et al., 2008**). Rinsing water for milking machine and milking equipment washing also involve some of the reasons for the presence of a higher number of micro-organisms including pathogens in raw milk (**Bramley, 1990**). After milking, milk is cooled, which additionally influence the dynamic of microbial process (**Rogelj, 2003**).

Other bacterial sources are from milkers, handlers, drugs or chemicals used during treatment of animal and from water used for adulteration by unscrupulous and unfaithful workers/sellers which may be contaminated and may cause additional health problems (**Karimuribo et al., 2005**).

Common bacteria reported to be isolated from milk include *Staphylococcus* spp., *Listeria* spp., *Salmonella* spp., *E. coli* spp., Campylobacter spp., *Mycobacterium* spp., *Brucella* spp., *Coxiella* burnetii, *Yersinia* spp., *Pseudomonas aeroginosa* and *Corynebacterium ulcerans*. Others are *Proteus* spp., *Leptospira* spp., *Clostridium* spp., *Streptococcus* spp, *Klebsiella* spp., *Enterobacter* spp. and *Bacillus* spp. (**Shirima et al., 2003; Sivapalasingams et al., 2004; Al-Tahiri, 2005; Donkor et al., 2007; Parekh and Subhash, 2008**). All these are pathogenic bacteria that pose serious threat to human health and contribute up to 90% of all dairy related diseases (**De Buyser et al., 2001; Sivapalasingams et al., 2004; Donkor et al., 2007**).

**Objectives:**

- Assess safety of smallholder fresh cow's milk around west coast Libya. This involved observation and sampling of milk from milking to point-of-sale.
- To establish the possible risk factors for microbial contaminations of raw cow milk at farm level.
- assessing the microbial quality of raw cow milk collected from farmers and dairy producers from six districts in west coast Libya.
- To determine antimicrobial susceptibility of the common milk-borne bacteria isolated from raw cow milk.

## 2. Methodology

### 1- Planning and Sampling Collection

All subjects gave informed consent for inclusion before they participated in the study at the time of the study (November

2018).

The samples collected from **Alharsha, Sabriha, Abi Issa, Rain, Surman, Sabratha** cities. Approximately 30 ml of milk for each sample was aseptically taken and introduced aseptically in sterilized bottles then the whole kept in an icebox and carried to laboratory as soon as possible for microbial analysis.

**On Farm:**

Producers were visited once at milking time. We collected (**27**) samples from the fresh milk of the farms from **6** regions of the west coast of Libya.

**On Milk Markets:**

Market were visited at the morning. We collected (**12)** samples of raw cow's milk from **6** regions of the west coast of Libya.

2- **Data Collection**

Basic data on the smallholding were collected (owner, herd-size, location). Observations were made on milking practices including measures of hygienic practice, storage temperature and time.

**3- Microbiology and Quality Assessment**

All samples were assessed for recognize bacterial colony. Microbiological analyses of raw milk samples. 6 to 7 hours after collection samples were plated onto petri dish plates using standard methods. one loopful of milk was plated on Nutrient agar, Blood agar, Macconkey agar and incubated at 37 °C for 24 h. The visible colonies were then identified. The bacteria were identified by colony characteristics.

**4- Antibiotics sensitivity**

Used 10 antibiotics (Amikacin, Bactrim, Tetracycline, Imipenen, Levofloxacin, Ceftriaxone, Ciprofloxacillin, Gentamycin, Cefotaxime, Augmentin) on different culture media on Nutrient agar and Blood agar at 37 °C for 24hrs.

**5- Analysis**

- **Characteristics** of cows (age, breed, milk production and number of calves) and **farms** (gender of owner, herd size, location, husbandry and milking practices) were **described**.
- **Hygiene**, including temperature, was described for milk production.
- **Microbial** presence or absence, and colony counts were described for **different points** of sampling.
- The **relationship** between **cattle**, **farm** and **hygiene** characteristics, and the **microbial** quality of the milk were investigated graphically and using simple univariate statistics.

## 3. Results

**Descriptive Analysis**

- **Herd and Cattle Characteristics**

A total of **(27**) cows were sampled from 14 herds, with 1 to 2 cows milking per herd. Most farmers (13/14, 93%) were husband

and wife with one (7%) male run farms.

Characteristics of milking cows sampled (n = 27), including cow age (3-12 yr.) and volume of milk collected from a cow on daily (3-15 liters).

- **Milking**

Cows were milked twice a day. Time of milking with twelve farmers (86%) milking at 7am and 4pm, the coldest time of day. Milk was delivered to the market after milking.

Milking took 35–90 min, milking by hand into a bucket plastic or metal. Milk was then poured into a plastic (seven farmers, 50%) or metal (seven farmers, 50%) container that could be sealed. Although contamination of the pooled herd milk with cattle hair was not seen, some visible dirt contamination was observed for 2/14 (14%) farms.

Milking was done by hand without wear the gloves on 10/14 farms (71%), in addition without cleaning cow udder before milking on 1/14 farms (7%).

- **Transport the milk to markets**

Total herd milk volume at the daily milking varied from 3 to 15 L. About two-thirds (9/14) of farmers transported the milk to the market. Milk is kept without refrigerator from the start of milking to arrival at the **market** on 5/14 farms (36%).

- **Milk markets**

Sampled collected from (12) milk markets, most milk was fresh on (9/12, 75%) were store the milk from few hours to 48 hrs., in addition wear the gloves on 7/12 milk markets (58%).

- **Milk Microbiology and Quality**

A total of 39 milk samples were cultured for isolating bacteria. It was found bacteria growth as the following

**Table** 1: Microbial profile in raw milk from farms in six districts in Libya. **NLF** (Non-lactose fermentation, *Escherichia coli*. *Staphylococcus epidermidis* **nd**: not detect

| Districts | Alharsha | Rain | Abi Issa | Sabriha | Surman | Sabratha |
|---|---|---|---|---|---|---|
| **NLF** | **1** | **nd** | **nd** | **1** | **2** | **2** |
| ***E.coli*** | **nd** | **nd** | **1** | **nd** | **2** | **nd** |
| ***Staph. epidermidis*** | **1** | **1** | **1** | **nd** | **nd** | **nd** |

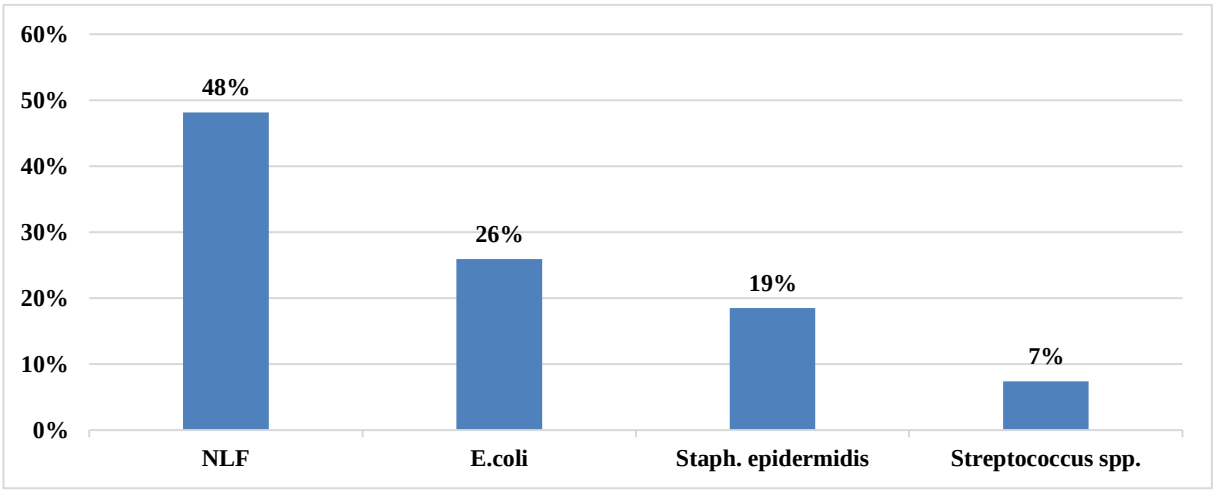


**Figure** 1. Frequency of occurrence of the isolates in the samples of milk from farm.

**Table** 2: Microbial profile in raw milk from markets in six districts in Libya. **NLF**: Non-lactose fermentation and *Escherichia coli*. **nd**: not detect.

| Districts | Alharsha | Rain | Abi Issa | Sabriha | Surman | Sabratha |
|---|---|---|---|---|---|---|
| **NLF** | **1** | **1** | **1** | **1** | **2** | **7** |
| ***E.coli*** | **3** | **nd** | **2** | **nd** | **1** | **1** |
| ***Staph. epidermidis*** | **1** | **nd** | **nd** | **nd** | **nd** | **4** |
| ***Streptococcus spp.*** | **nd** | **nd** | **nd** | **nd** | **nd** | **2** |

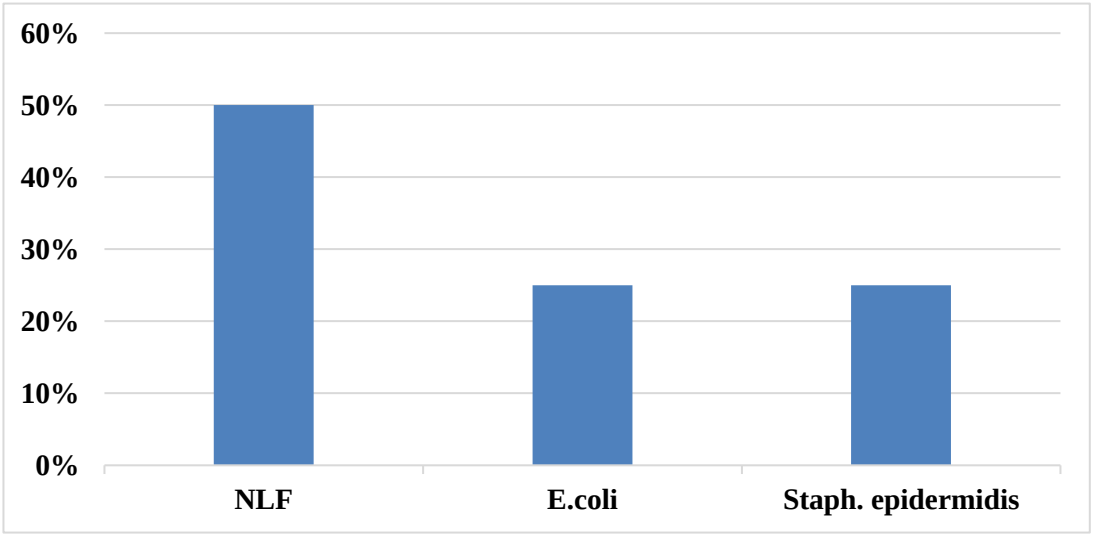


**Figure** 2. Frequency of occurrence of the isolates in the samples of milk from market.

**Table** 3: Percentage of distribution and frequency of isolation of different bacterial species from all milk samples.

| No. | Bacterial species | No. of isolates | % |
|---|---|---|---|
| 1 | NLF | 19 | 49 |
| 2 | *E.coli* | 10 | 26 |
| 3 | *Staph. epidermidis* | 8 | 21 |
| 4 | *Streptococcus spp.* | 2 | 5 |

- **Antimicrobial susceptibility profile**

Four (4) bacterial strains (NLF, *Escherichia coli*, *Staph. Epidermidis* & Streptococcus *spp.)* were isolated and tested against ten (10) antibiotics.

NLF isolates were the most predominant in dairy farms. The results declared that (NLF, *Escherichia coli & Staph. Epidermidis*) isolates were more sensitive to Levofloxacin (100%). On the other hand, all strain isolates from markets observed resistant manner to Tetracycline (100%) and Augmentin (92%).

**Table (4):** Antibacterial drug susceptibility of bacterial strains from raw milk samples. 100-80 Highly sensitive; 79-50 Quite; 49 – 40 moderate; Less than 40 weak; 0 = Resistant.

| Antibiotics used | Sensitivity (Degree %) | | | |
|---|---|---|---|---|
| | NLF | E.coli | Staph. epidermidis | Streptococcus spp. |
| AK (Amikacin) | 13.93% | 29.17% | 39% | 22.50% |
| SXT (Bactrim) | 9.29% | 15% | 16% | 22.50% |
| TE (Tetracycline) | 18.57% | 15% | 49% | 10% |
| IMP (Imipenen) | 65.71% | 74.17% | 90% | 55% |
| LEV (Levofloxacin) | 90% | 90% | 90% | 90% |
| CRO (Ceftriaxone) | 31.43% | 40.00% | 62% | 67.50% |
| CIP (Ciprofloxacillin) | 63.93% | 78.33% | 20% | 45% |
| CN (Gentamycin) | 18.57% | 21.67% | 4% | 32.50% |
| CTX (Cefotaxime) | 23.21% | 18.33% | 36% | 45% |
| AMC (Augmentin) | 6.07% | 0% | 12% | 0% |

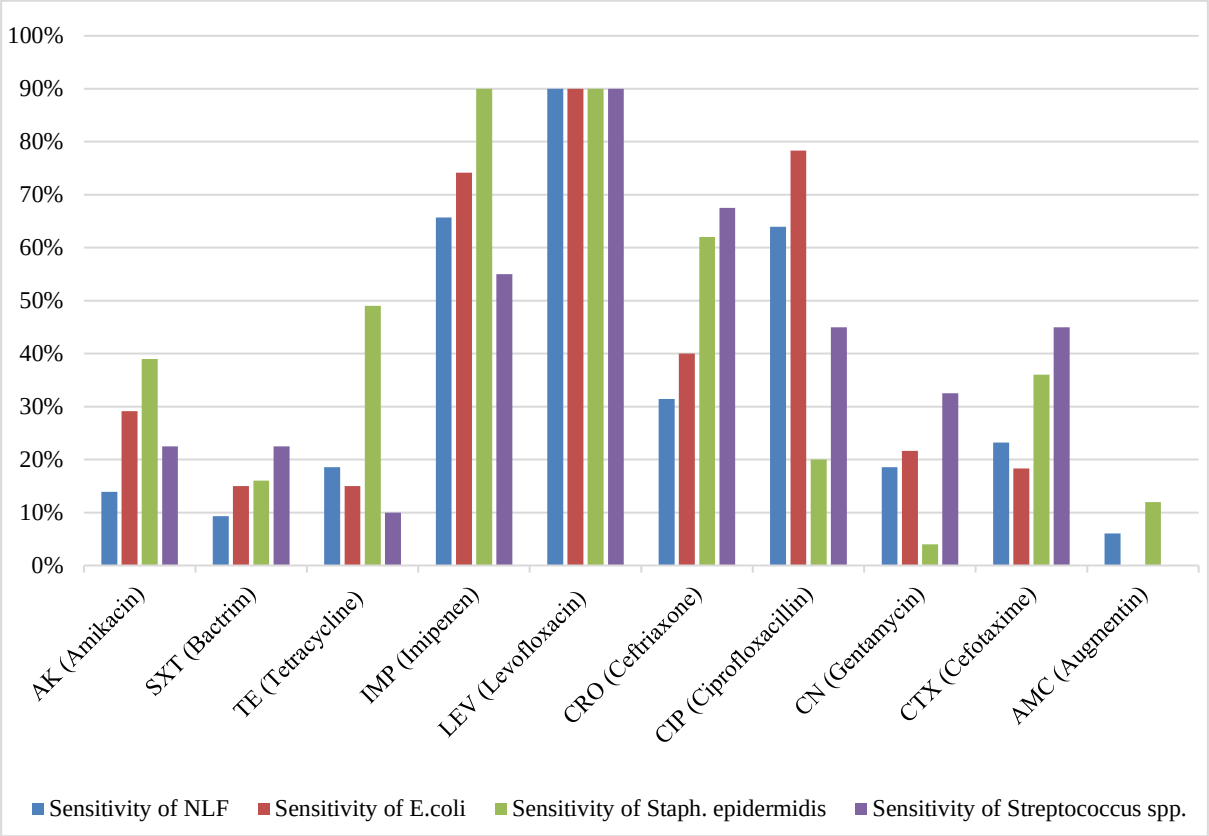


**Figure** 3. Sensitivity (Degree %) of bacterial strains from raw milk samples.

**Table (5):** Antibacterial drug susceptibility of bacterial strains from raw milk markets samples. 100-80 Highly sensitive; 79-50 Quite; 49 – 40 moderate; Less than 40 weak; 0 = Resistant.

| Antibiotics used | Sensitivity (Degree %) | | |
|---|---|---|---|
| | NLF | E.coli | Staph. epidermidis |
| AK (Amikacin) | 40% | 45% | 75% |
| SXT (Bactrim) | 10.83% | 0% | 13.33% |
| TE (Tetracycline) | 3% | 0% | 0% |
| IMP (Imipenen) | 59.17% | 43.33% | 36.67% |
| LEV (Levofloxacin) | 90% | 90% | 90% |
| CRO (Ceftriaxone) | 55.83% | 30% | 60% |
| CIP (Ciprofloxacillin) | 75% | 75% | 90% |
| CN (Gentamycin) | 50% | 28.33% | 36.67% |
| CTX (Cefotaxime) | 51.67% | 45% | 66.67% |
| AMC (Augmentin) | 3% | 0% | 0% |

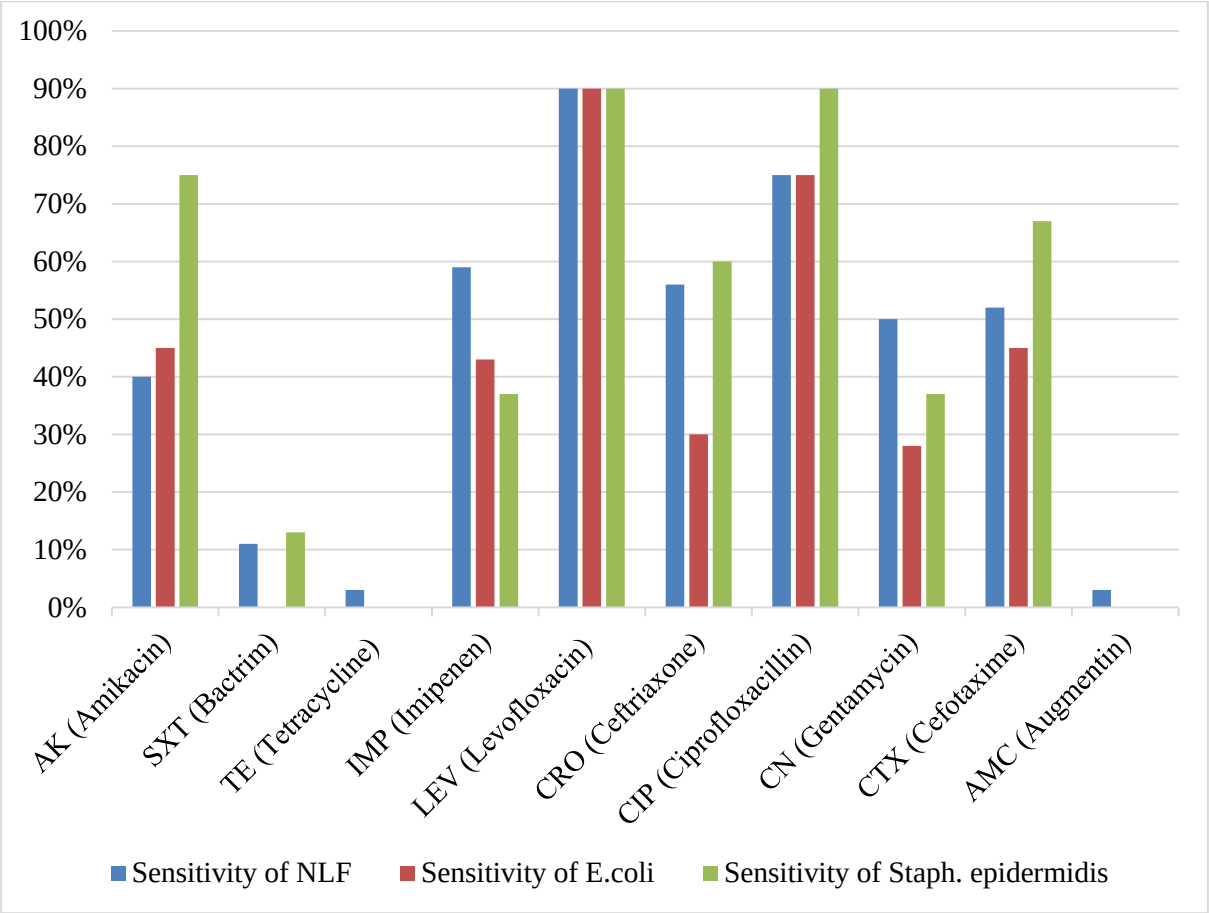


**Figure** 4. Sensitivity (Degree %) of bacterial strains from raw milk markets samples.

## 4. Discussion

Based on the objective of our study in verifying the raw health of raw cow milk to avoid its risks to public health, we have studied the methods of work in the care of cows and milking and how to save and transfer milk from the farms to the markets and cleanliness of the shop and equipment and how save the quantities of milk you deal with daily the identified of the dominant bacteria in raw milk such as (NLF, *E.coli*, *Staph. epidermidis* and *Streptococcus spp*.)..

Although smallholder fresh milk initially had bacteria of low levels to resistance antibiotics, absence of (1) refrigeration between milking and arrival at point of sale and (2) nonpasteurization after hours to two days from milking allowed a bacteria to high resistant to antibiotics. the practise of hygiene have a negligible effect on microbial quality and safety if hygiene is still limited and milk is kept at temperatures that favour bacterial to become more resistant to many types of antibiotic.

**4.1. Milk Microbiology Assessment of hygienic quality of raw milk**

Regarding to the identification of different isolated coliform strains which represented in table (3) the percent of **NLF**, ***E.coli***, ***Staph. epidermidis*** and ***Streptococcus spp***. were 49%, 26%, 21% and 5%, respectively. This implied that, raw cow milk from all districts had poor microbiological quality.

Based on data made throughout the collection of samples, we concluded that the improper hygiene practice and poor management before and during milking may have contributed to the contamination of milk with NLF. The NLF incidence at a considerable high percentage indicates the alarming situation both for dairy farming and for public health.

The incidence of *E.coli* was 26%. Lower percent were obtained in other researches as **Adesiyun** (**1994**). Which considered generally Presence of E. coli can be an indication of inadequate processing and/or post process recontamination by raw materials, dirty equipment or poor hygienic handling (**NSW,2009**). However, *E.coli* is one of the main in habitants of the intestinal tract of most mammalian species, including humans and animals, most *E.coli* are harmless but some are known to be pathogenic bacteria , causing sever intestinal and extra intestinal diseases in man. Several strains of *E.coli* are known to produce toxins that can cause diarrhea.

**4.2. Antimicrobial susceptibility profile**

Despite the important role that antibiotics play in the control of infection, there have only been the rapid resistance of the antimicrobial agent. In addition, the results indicated that the increasing prevalence of multidrug resistant strains in raw milk at markets more than fresh raw milk at farms. Consequently, the reducing susceptibility to antibiotics adds urgency to the search for new bacteria fighting strategies. Hence, there is a need to investigate the antibacterial properties of drugs that have not been done.

Results of *Staph. epidermidis* are shown in Table 5. They revealed that the gentamycin was weak sensitive to this species (37%), in contrast to reported by **Suzan 2016** who revealed that the resistant of Staphylococcus epidermidis against gentamycin was a quite sensitive to this species (69.2%).

In general; the three antibiotics; Levofloxacin, Ciprofloxacillin and Imipenen, have the highest antibacterial effects with mean values equal to 90%, 66% and 59%, respectively, while a moderate activity was shown against Ceftriaxone and Cefotaxime with mean values equal to 49% and 43%, respectively and weak activity was recorded to Amikacin, Gentamycin, Tetracycline, Bactrim and Augmentin with mean values equal to 40%, 29%, 12%, 12% and 3%, respectively.

**4.3. Distribution of raw milk samples collected in six districts and Observed Hygienic Practice**

Most critical to milk safety was the lack of pasteurization or boiling, which would kill off almost all microbial pathogens present (**Holsinger, V.H. et al., 1997**). The long time that milk was kept without refrigeration, on farm and during transport was also important (approximately ≥45 min). Farmers attempted to minimize contamination during milking, however, they lacked the resources to do this effectively.

Overall the findings are not surprising. Milk typically has little contamination when sampled directly from healthy cows. Poor hygiene results in bacterial contamination, with initial bacterial growth from the inherent antibacterial, and high microbial growth from antibacterial of milk from markets.

The longitudinal sampling approach adopted in this study allowed observation of changes in bacterial contamination along the milk value chain. Sampling at a single point may have led to different conclusions about the levels of milk contamination, i.e., low contamination on farm and at arrival at the markets, variable contamination a one day consistently high levels of bacterial contamination.

**4.4. Risk Factors**

No significant risk factors for final milk microbial quality were detected. Even when better hygiene is practiced at one point of the value chain, limited hygiene at other steps allowed microbial contamination with subsequent bacterial multiplication, resulting in similar microbial quality of the end product regardless of variation in upstream hygienic practices. Ensuring end-product microbial safety requires (1) all aspects of production to be hygienic or (2) a final kill-step to remove upstream contamination, with subsequent hygienic handling and refrigeration.

Limitations of the study also contributed to the absence of detected risk factors. Production methods were similar for all farms resulting in limited variation in risk factors; the small number of farms sampled led to limited power for detecting herd-level effects; and the microbial culture assays used have inherent variability, leading to reduced power to detect differences between groups.

**4.5. Options for Improved Milk Safety**

Much is already known about the requirements for safe milk production and future work should look at the effect and feasibility of interventions to improve milk quality**.**

**4.5.1. Funding and Pricing**

Funding is required to improve safety. This reduce disease arising from safer milk.

Currently all farmers receive a single milk price, regardless of milk quality. Paying

producers more for safer and higher quality milk would create an incentive for producer investment in milk quality.

**4.5.2. Refrigeration**

High costs and inadequate infrastructure (roads, electricity) make refrigeration at point of production or refrigerated transport seemingly unfeasible. Transporting milk at deliberately raised temperatures to prevent microbial growth would be a novel approach that could be studied.

**4.5.3. Pasteurisation**

Pasteurization at processing center is likely to be effective and would be easier to implement compared to interventions applied to all farmers. However, some of the milk is sold directly from the farm or consumed by the farmer's family.

A problem with on-farm pasteurization is that subsequent microbial proliferation will occur during unrefrigerated transport to the markets. Furthermore, it is harder to ensure pasteurization is done correctly by many producers compared to one central processor. However, options for pasteurization at the markets need to consider the limited infrastructure (reliable electricity, technical support, etc.), and funding.

It must be noted that poor hygiene cannot be entirely mitigated by pasteurization particularly if milk is heavily contaminated or if handling after pasteurization is unhygienic. Also, pasteurization does not inactivate toxins produced by some strains of ***S. aureus*** (**Dinges, M.M. et al., 2000**). commonly found in poorly handled smallholder milk (**Dessisa, F. et al., 20**14). As milk does not display a visible change when pasteurized, there is a risk that pasteurization will not be done adequately. Boiling requires more energy but is easily observed and may therefore, be more reliable (**Makita, K. et al., 2010**). when temperature cannot be easily monitored (**Safapour, N. et al., 1999**).

**Conclusions**

The main source of microbial contamination of raw milk form the farms and increasing prevalence of multidrug resistant strains with reduce susceptibility to antibiotics.

Smallholders in Western coast produce milk of good initial quality but in very small quantities (11 litre per cow/day and about 30 L per herd/day). However, levels of hygiene are low with no refrigeration of milk until it arrives at the point of sale, where it is sold without pasteurization. The result is a high-risk product with rapid spoilage.

In this under-developed setting, options for improving milk safety are limited. However, sustainable methods of milk pasteurization should be investigated as a microbial kill-step is needed to mitigate upstream contamination.

**Recommendation**

In order to enhance milk safety, regular such as on-site pasteurization should be introduced to facilitate the production of milk of high quality and safety. and consistent monitoring of microbiological quality should be established.

Strongly suggests the need to improve hygienic conditions and adequate sanitary measures that should be taken from stage of production to consumption.

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