

Evaluation of Microbiological Quality of Raw Milk from Farmers and Dairy Producers in Six Districts of Djibouti

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Abstract

Objectives: The present study was carried out to evaluate the microbial quality of raw milk taken at different sampling points from farmers in Ali Sabieh, Arta, Dikhil, Djibouti, Obock and Tadjourah districts of Djibouti.

Methods: Two hundred samples of milk extracted from goats, cows and camels were collected and analyzed for the presence and enumeration of microorganisms using standard methods. Total bacterial counts exceeded the national standard in 56% of collected samples and Tadjourah district had higher milk samples (82%) with unacceptable total bacterial count.

Results: Key data demonstrated the presence of food-borne microorganisms. The mean value of aerobic mesophilic bacteria ($6.78 \log \text{cfu mL}^{-1}$), coliform counts ($3.91 \log \text{cfu mL}^{-1}$) and *E. coli* count ($2.58 \log \text{cfu mL}^{-1}$) was higher than maximum recommended value. The count of yeast and moulds ($5.54 \log \text{cfu mL}^{-1}$) was also very high. *Salmonella* spp. ($0.10 \log \text{cfu mL}^{-1}$) was only detected in the samples from Ali Sabieh district whereas *Staphylococcus aureus* and spore of *Clostridium* spp. were below the detection level in all samples. Count of *Brucella* spp. and Mycobacteria were $0.56 \log \text{cfu mL}^{-1}$ and $0.86 \log \text{cfu mL}^{-1}$ respectively suggesting the existence of mastitic animals. Pearson correlation matrix of microorganism's distribution in raw milk showed highest positive correlation between Yeast and moulds and *Streptococci* at $r=0.929$. Analysis in principal components exhibited the variability of microbial groups with 80.797% cumulative values of the variance and Eigen values ranging between 1.553 and 3.622.

Conclusion: The data suggested that raw milk samples collected from the six districts were non suitable quality and most of bacteria identified in the milk sampled could be potential food-borne pathogens.

Keywords: Raw milk; Djibouti; Hygienic; Microbiological quality

Introduction

Republic of Djibouti count about millions of small ruminants, 40000 cattles and 50000 camels; with a level of consumption of the breeding products increasing annually [1]. Among the products, milk with annual consumption per capita of 10 L equivalent liquid milk/kg, has lighting increased in last years. The strong request of milk causes an intensification of its production. The amount of milk produced in Djibouti was 16075 tons during 2013 [2].

Microorganisms enter milk from a variety of sources can be beneficial or harmful, for example *Lactococcus*, *Lactobacillus*, *Streptococcus*, *Propionibacterium* and fungal populations facilitate dairy fermentations, lactobacilli and bifidobacteria promote health while *Pseudomonas*, *Clostridium*, *Bacillus* and other spore-forming or thermophilic microorganisms cause spoilage and *Listeria monocytogenes*, *Salmonella*, Shiga toxin producing *Escherichia coli* (STEC), *Campylobacter* and mycotoxin-producing fungi cause disease [3]. Despite the outstanding nutritional quality and health benefits of milk, it serves also as an excellent vehicle for transmission of milk-

borne pathogens which may cause serious health risk to consumers. In fact, milk is a perishable product and an ideal medium for the growth of a wide variety of bacteria [4].

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 [5].

Studies done on selected farms show that raw milk may be contaminated by a wide range of bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Bacillus* spp., *Brucella* spp., *Listeria monocytogenes*, *Salmonella* spp. and *Corynebacterium* spp. as well as various yeasts and moulds [3]. In some cases, infections of milk with viable pathogenic bacteria can cause contamination and milk spoilage that rendering it unsafe. The main life-threatening illnesses associated with milk includes gastroenteritis, diarrhea, typhoid, or bovine tuberculosis [6].

Many developing countries including Djibouti use physical and chemical tests such as milk density and chemical composition to evaluate milk quality while studies including hygienic and/or microbiological criteria at the farm level are rather scanty [7]. Lack of updated data on milk hygienic quality in Djibouti may be harmful to the whole sector in comparison with other countries where this element represents a key factor towards sustainable dairy production [8].

At present, a major problem facing the dairy products in Djibouti 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 like Djibouti and consequently making milk-borne diseases a higher health risk to public.

In order to enhance milk safety, regular and consistent monitoring of microbiological quality should be established. Therefore, this study aimed at assessing the microbial quality of raw cow, goat and camel milk collected from farmers and dairy producers from six districts in Djibouti.

Material and Methods

Collection of samples

This study was conducted in Ali Sabieh, Arta, Dikhil, Djibouti, Obock and Tadjourah districts showed in Figure 1. The milk samples of raw cow, goat and camel were collected from six districts of Djibouti in October to November 2015. Milk samples collected from Cow, Goat and Camel respectively were 92, 66 and 42. A formula of Kothari [9] for unknown population (i.e. $n = Z^2SD^2/e^2$) was used to calculate the sample size for this study. Where Z, is the estimated standard variation at 95% confidence interval (CI) which was considered as the point of the normal distribution corresponding to the level of significance ($Z=1.96$). Standard deviation (SD) was estimated at 15% and e, is the estimated error and was considered at 5%. A total of 200 milk samples were obtained from the different sites of production. The numbers of the collected samples were respectively 70, 36, 32, 33, 15, 14 from Djibouti, Arta, Tadjourah, Ali Sabieh, Dikhil, Obock districts. Sampling has been done according the ISO/DIS 707 [10] method. Approximately 1L of milk for each sample was aseptically taken and introduced aseptically in sterilized bottles then the whole kept in an icebox and transported at 4°C to the laboratory. The time elapsed between obtaining sample and its analysis was eight (08) hours. Analysis was after sampling in duplicates.

Microbial analysis

Analysis for microbial quality of raw milk involved count of aerobic mesophilic bacteria, total coliform, yeast and moulds and common milk-borne pathogens namely Shiga toxin producing *Escherichia coli* (STEC), *Salmonella* spp., *Staphylococcus aureus*, *Streptococci*, *Brucella* spp. and Mycobacteria.

Research and enumeration of micro-organisms

The samples were serially decimal diluted with Quarter Strength Ringer's solution (Merck, Germany) and appropriate dilutions plated on media using the pour plate method ISO/FDIS 8261 (E) [11]. The

presence and number of total bacterial count were evaluated on Plate Count Agar (PCA) (Merck, Germany) with the addition of 0.1% w/v of milk. The plates were incubated at 30°C for 72 h for aerobic mesophilic. Determination of Aerobic mesophilic bacteria was done by using ISO 4833-1 [12] protocols. Enumeration of total coliform was carried out at 37°C for 48 h on Violet Red Bile Lactose (VRBL) agar (Merck, Germany) according to the standard ISO 4832 protocols [13]. Yeast and moulds were enumerated on Yeast Glucose Chloramphenicol (YGC) agar (Merck, Germany) at 30°C for 120 h according to the standard ISO 6611E method [14].

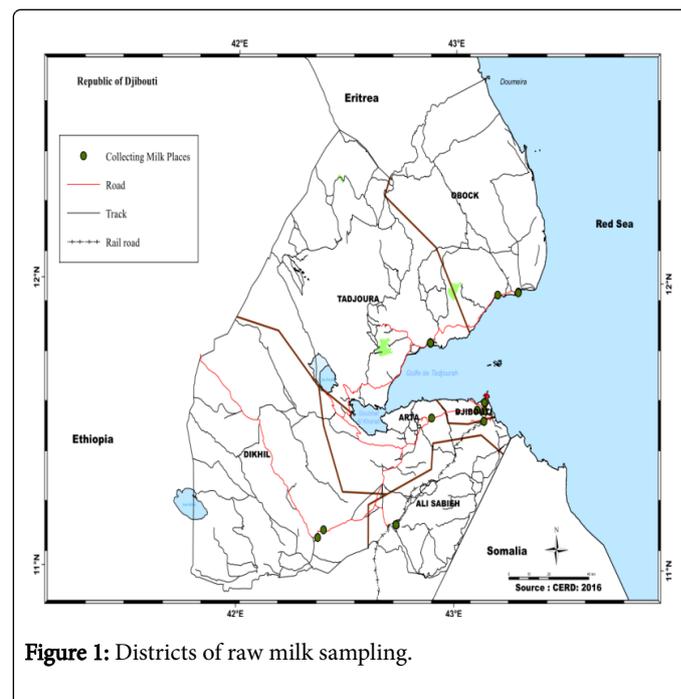


Figure 1: Districts of raw milk sampling.

Raw milk samples were examined and those suspected as *E. coli* colonies during detection were removed, thawed at room temperature and used for detection of *E. coli* (STEC). MacConkey (Biokar Diagnostics, France) agar medium was used for identification of *E. coli* (STEC) at $44 \pm 0.5^\circ\text{C}$ for 48 h according method ISO 4832 [13]. Detection of *Salmonella* spp. in milk samples was done at 37°C for 48 h according to ISO 6579 protocols [15]. Suspected *Salmonella* colonies from each Xylose Lysine Deoxycholate (XLD) agar plate (Merck, Germany) was confirmed using *Salmonella* Test kit. For the enumeration of coagulase-positive *Staphylococci* (CPS) (*Staphylococcus aureus*) the Baird Parker with Rabbit Plasma Fibrinogen (RPF) supplement agar (Biokar Diagnostics, France) was used (SIST EN ISO 6888-2) [16].

Identification of *Staphylococcus aureus* in milk samples was done according to ISO 6888-1 protocols at 37°C for 48 h [17]. For enumeration of *Streptococci*, Man, Rogosa and Sharpe (MRS) Agar (Fluka Biochemika 69966) incubated anaerobically at 42°C for 48 h was used according to ISO 15214 standard [18]. Enumeration of spore of *Clostridium* spp. was carried out on Tryptone-Sulfite-Cycloserine (TSC) agar (Biokar Diagnostics, France) (ISO 7937-V08-019) [19]. Method of Ko [20] was used to detect *Brucella* spp. and was enumerated on Phosphate-Buffered Saline (PBS) and *Brucella* agar (Biokar Diagnostics, France). Incubation was done at 37°C for 24 to 48 h aerobically or in CO₂-enriched atmosphere. Enumeration of Mycobacteria was realized on Lowenstein-Jensen medium (Biokar

Diagnostics, France) at $35 \pm 2^\circ\text{C}$ under 7-10% CO_2 and examined for growth up to 21 days incubation, according to method of Levidiotou [21].

Statistical Analysis

Descriptive statistics were established to report the variability of the different parameters involved in the evaluation of the milk hygienic quality. Bacterial counts were transformed into logarithmic decimals.

Log transformed counts of microbiological indicators data were analyzed using factorial analysis of variance between means of microorganism's number with respect to different sources. Correlation between different microbial indicators counted was determined. A p-value of <0.05 or 0.0001 was considered statistically significant. Associations between microbial groups were performed through Pearson correlation at 5%. The Pearson's correlation coefficients between variables log number of different groups of tested microorganisms in milk samples were calculated. Principal component analysis was performed in order to identify the microbial groups or districts, which are best represented.

Principal component analysis and principal coordinate analysis plots were generated, showing distinction among the contamination level of microbial groups and regrouping of districts.

Results and Discussion

Distribution of raw milk samples collected in six districts and according to animals

The percentage of raw milk samples collected in different districts was ranged between 7% and 35% and was relative to the level of animal livestock and ability of milk production in each district. The ranking order of districts according to the site of breeding and milk production was significantly different ($p < 0.0001$) and classified in the decreasing way as 35%, 18%, 16%, 8%, 7% respectively for Djibouti, Arta, Ali Sabieh, Tadjourah, Dikhil, Obock districts. The different percentages were mentioned in the Figure 2.

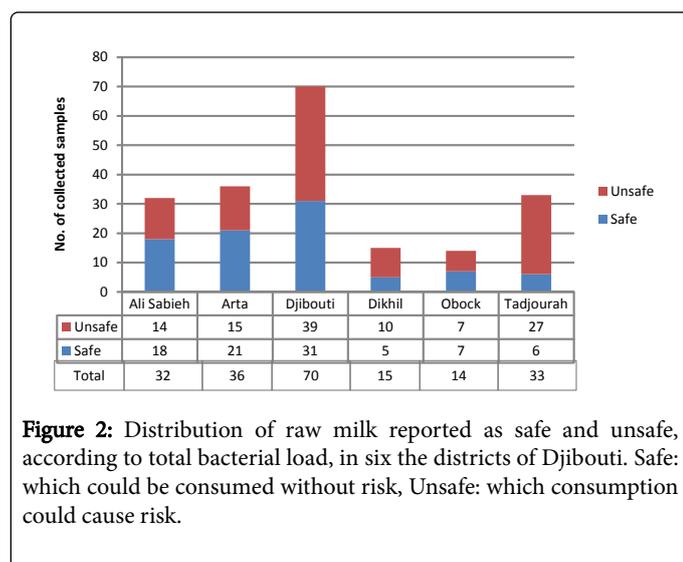


Figure 2: Distribution of raw milk reported as safe and unsafe, according to total bacterial load, in six the districts of Djibouti. Safe: which could be consumed without risk, Unsafe: which consumption could cause risk.

The assessment of samples collected according to animals showed that in the 200 raw milk samples the percentage of milk production

was 46%, 33% and 21% respectively from cow, goat and camel. These animals are an important part of stock farming in republic of Djibouti. It was clear that, most production of milk was coming from cow followed by goat which might be due to the easiness of their breeding.

Assessment of hygienic quality of raw milk

The differences in bacterial load between the raw milk samples in different districts were demonstrated to assess their hygienic quality. The overall distribution rates of microorganisms, reported as safe and unsafe, in different districts are shown in Figure 1.

From the 200 raw milk samples, 56% were found unsafe for consumption as they exceeded the national standard. The total bacterial counts varied considerably according to the region. The percentage of unsafe quality of milk was significantly different ($p < 0.0001$) and respectively ranged as 82% (Tadjourah), 67% (Dikhil), 56% (Djibouti), 50% (Obock), 44% (Ali Sabieh) and 42% for Arta district. It was clear that milk samples from all districts had strikingly high proportions (42-82%) of milk with unacceptable bacteria count. Considering the total milk samples which consumption could cause risk, Djibouti district, as urban region, had the highest (35%) samples with food-borne microorganisms count. The main microbial groups were counted and converted to log cfu mL^{-1} in milk analysis and results are summarized in Table 1.

When comparing the average bacteria enumerated over acceptability thresholds, a significant difference ($p < 0.0001$) was observed with aerobic mesophilic bacteria count which ranged from $6.40 \log \text{cfu mL}^{-1}$ (Arta district) to $7.48 \log \text{cfu mL}^{-1}$ (Tadjourah district). The mean value was $6.78 \log \text{cfu mL}^{-1}$ which was significantly ($p < 0.0001$) higher than maximum recommended value ($5.48 \log \text{cfu mL}^{-1}$) by the FAO. However, this value was comparable to value reported by Aaku [22] as $6.74 \log \text{cfu mL}^{-1}$, higher than that obtained by Torkar and Teger [23] as $4.51 \log \text{cfu mL}^{-1}$ and lower than that determined by Hadrya et al. [24], Tassew and Seifu [25] and Tankoano et al. [26] as respectively 6.90 , 7.58 and $8.95 \log \text{cfu mL}^{-1}$. Aerobic mesophilic bacteria count is a good indicator of general hygiene, permitting the appreciation of microbial pollution and the general quality of the product. The high contamination in raw milk samples indicated insufficient hygiene at milking or infection occurred from the skin of animals, milkers hands, animals shed and milking utensils [27].

Milk samples were also cultured for *Enterobacteriaceae* count and for isolation of *E. coli*. Results showed that the total coliform and *E. coli* count was ranged in significant difference ($p < 0.0001$) respectively from $3.74 \log \text{cfu mL}^{-1}$ (Arta district) to $4.07 \log \text{cfu mL}^{-1}$ (Tadjourah district) and from $2.06 \log \text{cfu mL}^{-1}$ (Dikhil district) to $2.98 \log \text{cfu mL}^{-1}$ (Djibouti district) respectively. The mean value of coliform counts ($3.91 \log \text{cfu mL}^{-1}$) was significantly higher ($p < 0.0001$) than the maximum coliform counts recommended level ($3 \log \text{cfu mL}^{-1}$) of Directive 92/46/EEC [28]. The mean value of *E. coli* count ($2.58 \log \text{cfu mL}^{-1}$) was also significantly higher ($p < 0.0001$) than standard value ($2 \log \text{cfu mL}^{-1}$). Furthermore, these results were in line with those done in raw cows' milk by Fekadu [29] who found that, coliform counts produced in Aneno, Gulgula and Dongora districts of southern region of Djibouti was $3.8 \log \text{cfu mL}^{-1}$. Moreover, the mean level of total coliform counts in our study was lower than the findings reported by Tankoano et al. [26] at Ouagadougou (Burkina Faso) ($8.95 \log \text{cfu mL}^{-1}$) and higher than that reported by Kas et al. [30] from the central part of Ivory Coast ($2.7 \log \text{cfu mL}^{-1}$). The presence of high numbers of coliforms in milk, which is mainly associated with unclean udder and

teats arise from a variety of sources such as manure, soil, food, personnel and even water and thus associated with unclean udder and teats, provides an index of hygienic standard used in the production of milk [31]. When the amount of total coliforms is increased, it may lead to food poisonings [27]. Sporadic high coliform counts may also be a

consequence of unrecognized coliform mastitis, mostly caused by *E. coli* [23]. Detection of *E. coli* in milk often reflects fecal contamination and is the known causative agent of diarrhoea and other foodborne-related illnesses through the ingestion of contaminated foodstuffs [32,33].

Districts	Ali Sabieh	Arta	Djibouti	Dikhil	Obock	Tadjourah	Mean	p-value
Range of count of microorganisms per ml for all sample, in log ₁₀ (cfu mL ⁻¹)								
AMB	6.46	6.4	6.42	6.45	7.45	7.48	6.78	<0.0001
TC	3.88	3.74	3.94	3.82	4.02	4.07	3.91	<0.0001
<i>E. coli</i>	2.76	2.41	2.98	2.06	2.67	2.63	2.58	<0.0001
<i>Salmonella</i> spp.	0.1	nd	nd	nd	nd	nd	0.1	
<i>Streptococci</i>	3.7	3.6	3.92	3.94	3.69	3.93	3.79	<0.0001
<i>S. aureus</i>	nd	nd	nd	nd	nd	nd	nd	<0.0001
YM	5.57	5.28	5.37	5.34	5.96	5.7	5.54	<0.0001
Spore of <i>Clostridium</i> spp.	nd	nd	nd	nd	nd	nd	nd	<0.0001
<i>Brucella</i> spp.	0.28	0.15	1	1	0.76	0.18	0.56	<0.0001
<i>Mycobacteria</i>	0.97	0.78	0.78	0.97	1	0.66	0.86	<0.0001

Table 1: Microbial profile in raw milk from six districts in Djibouti. AMB: Aerobic mesophilic bacteria, TC: Total coliforms, YM: yeast and moulds. nd: not detect, p<0.0001 indicated statistical significant difference, Results represent the mean of three independent assay, CFU: Colony Forming Unit.

Enumeration of *Streptococci* ranged significantly (p<0.0001) from 3.60 log cfu mL⁻¹ (Arta district) to 3.94 (Dikhil district) and with mean value 3.79 log cfu mL⁻¹. It was further observed that all milk samples with the exception of that from Ali Sabieh district were devoid of *Salmonella* spp. Although the level of *Salmonella* in the milk samples from Ali Sabieh district seemed to be very low (0.10 log cfu mL⁻¹), the potential for this organism to grow in improperly stored raw milk and in products made from raw milk presents a public health risk [33].

Also is it remarkable to underline that no germs of *S. aureus* and spore of *Clostridium* spp. were detected in all samples. This is a favorable finding because, *Clostridium* spp. is responsible for the food poisoning and food spoilage in certain products [34] and *S. aureus* is considered as the third most important cause of disease in the world among the reported food borne illnesses due to its capability to produce a wide range of heat stable enterotoxins [35]. *S. aureus* can gain access to milk either by direct excretion from udders with clinical or subclinical staphylococcal mastitis or by contamination from the environment during handling and processing of raw milk [35].

Enumeration of yeast and moulds ranged significantly (p<0.0001) from 5.28 log cfu mL⁻¹ (Arta district) to 5.96 (Obock district) and with mean value 5.54 log cfu mL⁻¹ which was comparatively higher than the Codex Alimentarius [36] required count (4 log cfu mL⁻¹) and those reported by Tokar and Teger [23] (4.1 log cfu mL⁻¹) and Tankoano et al. [26] (4.86 log cfu mL⁻¹), hence indicating poor milking hygiene. Lues et al. [32] reported that high counts of yeasts and moulds in milk is rather uncommon, as the pH of milk is neutral, causing bacteria to predominate and their presence in excessive amounts in milk is considered undesirable due to its ability to sensorially degrade milk.

Total count of *Brucella* spp. and *Mycobacteria* was ranged with significant difference (p<0.0001) respectively from 0.18 log cfu mL⁻¹ (Tadjourah district) to 1 log cfu mL⁻¹ (Dikhil and Djibouti districts) and from 0.66 log cfu mL⁻¹ (Tadjourah district) to 1 log cfu mL⁻¹ (Obock district) and with the mean values 0.56 and 0.86 log cfu mL⁻¹. These results were correlated with Donkor et al. investigation [37], who have detected at Ghana, the presence of *Mycobacterium* spp. in the marketed raw milk. Quigley et al. [3] reported that milkborne pathogens like *Brucella* spp. are a particular cause for concern as they are able to survive and multiply at refrigeration temperatures and may cause severe diseases.

In fact, the presence of *Brucella* spp. and *Mycobacteria* suggested the presence of mastitic animals. Mastitis-associated pathogens typically infect the teat canal and pass into the milk during milking [3].

So the global microbiological characteristics have shown that raw milk samples collected in the six districts of Republic of Djibouti could be an important source of infection with a wide range of microorganisms, particularly enteric pathogens. The highest source of microbial contamination of the milk was the aerobic mesophilic bacteria followed by coliform and yeast and moulds, contamination arising probably from dung or poor healthy animals and unsatisfactory hygiene/sanitation practices.

The distribution of microorganisms in raw milk is presented in Table 2 and showed positive and negative correlations (p=5%) among microbial parameters studied. The highest positive correlation was observed between the presence of Yeast and moulds and Streptococci (r=0.929).

	AMB	Total Coliform	<i>E. coli</i>	<i>Salmonella</i> spp.	<i>Streptococci</i>	<i>S. aureus</i>	Yeast and moulds	Spore of <i>Clostridium</i> spp.	<i>Brucella</i> spp.	<i>Mycobacteria</i>
AMB	1									
Total Coliform	0.594	1								
<i>E. coli</i>	0.126	0.4	1							
<i>Salmonella</i> spp.	0.434	-0.154	0.453	1						
<i>Streptococci</i>	0.302	0.409	-0.18	-0.517	1					
<i>S. aureus</i>	-0.572	-0.672*	-0.872*	-0.538	0.037	1				
Yeast and moulds	0.318	0.646*	-0.148	-0.647*	0.929*	-0.042	1			
Spore of <i>Clostridium</i> sp.	-0.291	0.439	0.356	-0.435	0.191	-0.25	0.357	1		
<i>Brucella</i> sp.	-0.167	0.104	-0.199	-0.509	0.59	0.196	0.551	0.677*	1	
<i>Mycobacteria</i>	0.228	-0.277	-0.025	0.647*	-0.427	-0.126	-0.515	-0.066	0.119	1

Table 2: Pearson correlation matrix of microorganism's distribution for six district of Djibouti. * Correlation is significant at the 0.05 level.

They also had positive correlation with total coliform ($r=0.646$) and negative correlation with *Salmonella* spp. ($r=-0.647$). The latter had contrary positive correlation with *Mycobacteria* ($r=0.647$). Also spore of *Clostridium* and *Brucella* spp. were positively correlated with $r=0.677$. *S. aureus* had a negative correlation with total coliform ($r=-0.672$) and *E. coli* ($r=-0.872$) suggesting that there does not necessarily exist a relationship between the *S. aureus* and *E. coli* or coliform in heavily contaminated milk.

Parameters	Principal components		
	F1	F2	F3
AMB	-0.004	-0.423	-0.488
Total Coliform	0.257	-0.433	-0.052
<i>E. coli</i>	-0.094	-0.425	0.34
<i>Salmonella</i> spp.	-0.433	-0.265	-0.046
<i>Streptococci</i>	0.425	-0.067	-0.242
<i>S. aureus</i>	0.067	0.58	-0.134
Yeast and moulds	0.498	-0.127	-0.221
Spore of <i>Clostridium</i> spp.	0.292	-0.128	0.624
<i>Brucella</i> spp.	0.364	0.081	0.311
<i>Mycobacteria</i>	-0.295	-0.052	0.171
Eigen values	3.622	2.904	1.553
% Variance	36.224	29.042	15.531
% Cumulative	36.224	65.267	80.797

Table 3: Coordinate of 10 microbial parameters and their contribution to identification of hygienic quality of the raw milk in six districts of

Djibouti. AMB: Aerobic mesophilic bacteria, *E. coli*: *Escherichia coli*, *S. aureus*: *Staphylococcus aureus*.

Principal components analysis

Analysis of principal components exhibited the variability of microbial groups within raw milk samples from Djibouti. Table 3 contained the relevant results. The cumulative values of the variance of the first three principal components (F1, F2 and F3) for the 10 microbial parameters were 80.797%, with Eigen values ranging between 1.553 and 3.622 (Table 3). Principal component F1 had an Eigen value 3.622 and contributed for 36.224% of the variation of the parameters. This principal component (F1) is associated positively to total coliform, *Streptococci*, *S. aureus*, yeast and moulds, spore of *Clostridium* spp. and *Brucella* spp. Principal components F2 and F3 had respective Eigen values 2.904 and 1.553, accounted for 29.042% and 15.531% to the total variation and were associated positively with rate of *S. aureus* and *Brucella* spp. for F2 and *E. coli*, spore of *Clostridium* spp., *Brucella* spp. and *Mycobacteria* for F3.

Projection of variables on factorial plan

According symmetrical scaling of component analysis score in the Figure 3, the axis 1 x 2 and 1 x 3 explained respectively 97.55 % and 95.59% the total inertia. The samples of each district distinctively responded to the majority of the variation detected in the samples across two axes.

The projection of variables corresponding to unsafe milk of six districts revealed the effects of food borne pathogens on the milk quality. The projection of variables on axis 1 x 2 showed the regrouping of districts according to quality of milk. The projection plan 1 x 2 showed a classification of 3 principal groups according to aerobic mesophilic bacteria and total coliform count in the samples of six districts.

The first group (Arta and Ali Sabieh districts) correlated negatively with axe 1 and positively on axe 2, was same level unsafe milk. This was opposite to second group (Dikhil, Djibouti, Tadjourah districts) which was correlated negatively with the two axis. The third group represented by Obock district was proximate to axis 2 and revealed the high level of total coliform count.

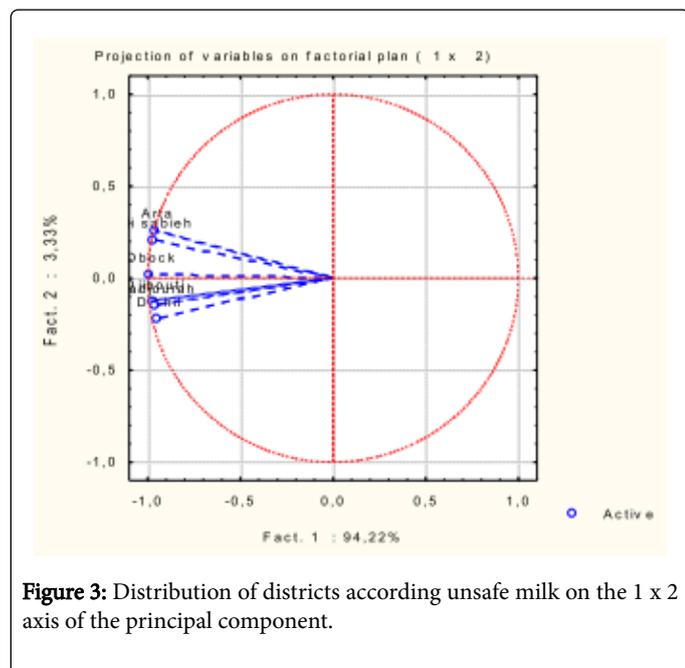


Figure 3: Distribution of districts according unsafe milk on the 1 x 2 axis of the principal component.

The projection plan 1 x 3 showed single group according Aerobic mesophilic bacteria and *E. coli* count in the samples of six districts. The first group (Arta and Ali Sabieh, Djibouti districts) correlated negatively with axe 3, was same level unsafe milk. Also two separated groups were represented by districts Dikhil and Tadjourah. These were respectively correlated negatively and positively with axis 3 that demonstrate the influence of *E. coli* count in unsafe quality of milk.

Conclusion

In conclusion, the present study highlighted the poor microbiological quality of raw milk collected from six districts of Djibouti. This was evident from the high values of aerobic mesophilic bacteria, total coliform and yeast and moulds in the samples which are not in conformity with official standards and therefore indicated serious pathogenic germ that endanger keeping quality and safety of raw milk. Furthermore, this result strongly suggests the need to improve hygienic conditions and adequate sanitary measures that should be taken from stage of production to consumption. The poor microbiological quality observed in the present study requires further investigation of the status of the animals' health, especially mastitis and the significance of the effect of containers to ascertain their contribution on microbial quality. Furthermore and from a microbial safety point of consideration, it is recommended not to consume raw milk and pasteurization of the milk is strongly advised. More food safety education should be given to producers, handlers and consumers. The limits of this study reside to the size of samples and also the classic technics of detection of microorganisms.

Conflict of Interests

All the authors contributed to the study have not declared any conflict of interests.

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The views expressed herein can in no way be taken to reflect the official opinions of FAO or the European Union.

References

1. Directorate of Livestock and Veterinary Services (2016) Report on Production and Animal Health.
2. FAO-ONU (2013) Food production: Milk production statistics in Djibouti.
3. Quigley L, O'Sullivan O, Stanton C, Beresford TP, Ross RP, et al. (2013) The complex microbiota of raw milk. FEMS Microbiol Rev 37: 664-698.
4. Parekh TS, Subhash R (2008) Molecular and bacteriological examination of milk from different milchanimals with special reference to Coliforms. Curr Res Bacteriol 1: 56-63.
5. Swai ES, Schoonman L (2011) Microbial quality and associated health risks of raw milk marketed in the Tanga region of Tanzania. Asian Pac J Trop Biomed 1: 217-222.
6. Al-Khatib IA, Al-Mitwalli SM (2009) Microbiological quality and sample collection policy for dairy products in Ramallah and Al-Bireh districts, Palestine. East Mediterr Health J 15: 709-716.
7. Srairi MT, Moudnib J, Rahho L, Hamama A (2006) How do milking conditions affect the hygienic quality of raw milk? Case study from Moroccan dairy farms. Livest Res Rural Dev 18.
8. Djemali M, Kayouli C (2003) Dairy farming in Tunisia. In Djemali M, Guellouz M (edn) The milk chains in the Mediterranean: challenges for a sustainable future. Wageningen.
9. Kothari CR (2004) Research Methodology, Method and Techniques. New age international publishers, India.
10. ISO/DIS 707 (1995) Milk and milk products-Guidance on sampling. International Organization for Standardization, Geneva, Switzerland.
11. ISO/FDIS 8261 (E) (2001) Milk and milk products-General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination. International Organization for Standardization, Geneva, Switzerland.
12. ISO 4833-1 (2013) Microbiology of the food chain-Horizontal method for the enumeration of microorganisms. International Organization for Standardization.
13. ISO 4832 (2006) Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of coliforms-Colony-count technique. International Organization for Standardization, Geneva, Switzerland.
14. ISO 6611E (2004) Milk and milk products-Enumeration of colony-forming units of yeasts and/or moulds-Colony-count technique at 25°C, ISO, FIL/IDF, International Organization for Standardization, Geneva, Switzerland, International Dairy Federation, Brussels, Belgium.
15. ISO 6579 (2002) Microbiology of food and animal feeding stuffs-Horizontal method for the detection of Salmonella spp. International Organization for Standardization.
16. SIST EN ISO 6888-2 (1999) Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) Part 2: Technique using rabbit plasma fibrinogen agar medium. International Organization for Standardization, Geneva, Switzerland.

17. ISO 6888-1 (1999) Microbiology of food and animal feeding stuffs–Horizontal method for enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species). International Organization for Standardization.
18. ISO 15214 (1998) Microbiology of food horizontal Method for the enumeration of the mesophilic lactic acid bacteria Technical by counting of the colony at 30°C.
19. ISO 7937-V08-019 (2005) Microbiology of food-Horizontal method for the enumeration of Clostridium perfringens-Colony-count technique.
20. Ko J, Gendron-Fitzpatrick A, Ficht TA, Splitter GA (2002) Virulence Criteria for Brucella abortus Strains as determined by interferon regulatory factor 1-deficient mice. *Infect Immun* 70: 7004-7012.
21. Levidiotou S, Papamichael D, Gessouli E, Golegou S, Anagnostou S, et al. (1999) Detection of mycobacteria in clinical specimen using the mycobacteria growth indicator tube (MGIT) and the Lowenstein Jensen medium. *Microbiol Res* 154: 151-155.
22. Aaku EN, Collison EK, Gashe BA, Mpuchane S (2004) Microbiological quality of milk from two processing plants in Gaborone Botswana. *Food contr* 15: 181-186.
23. Torkar KG, Teger SG (2008) The microbiological quality of raw milk after introducing the two day's milk collecting system. *Acta Agric Slovenica* 92: 61-74.
24. Hadrya F, Elouardi A, Benali D, Hami H, Soulaymani A, et al. (2012) Bacterial Quality of Informally Marketed Raw Milk in Kenitra City, Morocco. *Pak J Nutr* 11: 662-669.
25. Tassew A, Seifu E (2011) Microbial quality of raw cow's milk collected from farmers and dairy cooperatives in Bahir Dar Zuria and Mecha district, Ethiopia. *Agric Biol J N Am* 2: 29-33.
26. Tankoano A, Kabore D, Savadogo A, Soma A, Fanou-Fogny N, et al. (2016) Evaluation of microbiological quality of raw milk, sour milk and artisanal yoghurt from Ouagadougou, Burkina Faso. *Afr J Microbiol Res* 10: 535-541.
27. Aggad H, Bridja M, Aek B, Benaouali M, Djebli A (2010) Some quality aspects of pasteurized milk in Algeria. *World J Dairy Food Sci* 5: 21-24.
28. Directive 92/46/EEC (1992) Council Directive 92/46/EEC of 16 June 1992 laying down the health rules for the production and placing on the market of raw milk, heat-treated milk and milk-based products. *Official JL* 268: 1-31.
29. Fekadu Beyene (1994) Present situation and future aspects of milk production, milk handling and processing of dairy products in Southern Ethiopia. Food production strategies and limitations: The case of Aneno, Bulbula and Dongora in Southern Ethiopia. Department of Food Science. Agricultural University of Norway, Norway.
30. Kas K, Mégnanou RM, Akpa EE, Assidjo NE, Niamké LS (2013) Evaluation of physico-chemical, nutritional and microbiological quality of raw cow's milk usually consumed in the central part of Ivory Coast. *Afr J Food Agric Nutr Dev* 13: 7888-7904.
31. Bille PG, Haradoeb BR, Shigwedha N (2009) Evaluation of chemical and bacteriological quality of raw milk from neudamm dairy farm in Namibia. *Afr J Food Agric Nutr Dev* 9: 1511-1523.
32. Lues JFR, Venter P, van der Westhuizen H (2003) Enumeration of potential microbiological hazards in milk from a marginal urban settlement in central South Africa. *Food Microbiol* 20: 321-326.
33. Reta MA, Bereda TW, Alemu AN (2016) Bacterial contaminations of raw cow's milk consumed at Jigjiga City of Somali Regional State, Eastern Ethiopia. *Int J Food Contam* 3: 4-10.
34. Garde S, Arias R, Gaya P, Nunez M (2011) Occurrence of Clostridium spp. in ovine milk and Manchego cheese with late blowing defect: Identification and characterization of isolates. *Int Dairy J* 21: 272-278.
35. Normanno G, Firinu A, Virgilio S, Mula G, Dambrosio A, et al. (2005) Coagulase-positive of raw staphylococci and Staphylococcus aureus in food products marketed in Italy. *Int J Food Microbiol* 98: 73-79.
36. Codex Alimentarius (2004) Code of hygienic practice for milk and milk products. *CAC/RCP* 57-2004.
37. Donkor ES, Aning KG, Quaye J (2007) Bacterial contaminations of informally marketed raw milk in Ghana. *Ghana Med J* 41: 58-61.