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Available online at www.ijit.net.**Research Article****QUALITY AND SAFETY OF COW MILK PRODUCED AND MARKETED IN DIRE DAWA TOWN, EASTERN ETHIOPIA****TEKLEMICHAEL TESHAY*¹AMEHA KEBEDE², EYASSU SEIFU³**¹ Department of Biology, College of Natural and Computational Sciences, Aksum University, Ethiopia² Department of Biology, College of Natural and Computational Sciences, Haramaya University, Ethiopia³ Department of Animal Science, College of Animal and Range Sciences, Haramaya University, Ethiopia.**ABSTRACT**

The present study is primarily aimed at assessing the microbial quality of cow milk samples sold by dairy farms and milk vendors in Dire Dawa Town. A total of 30 cow milk samples were collected and examined. The average total aerobic bacterial count (TABC), coliform count (CC), *Escherichia coli* count (EC) and spore forming bacterial count (SFBC) of milk samples obtained from dairy farms were 5.84 ± 0.629 cfu/ml, 4.13 ± 0.757 cfu/ml, 3.64 ± 0.776 cfu/ml and 4.798 ± 0.745 cfu/ml, respectively. Whereas, the corresponding values of milk samples obtained from milk vendors were 9.137 ± 0.885 cfu/ml, 6.198 ± 0.418 cfu/ml, 5.001 ± 0.44 cfu/ml and 6.392 ± 0.154 cfu/ml for TABC, CC, EC and SFBC, respectively. On the other hand, the respective values for pasteurized milk samples obtained from the distribution center of Hamdael farm were 5.425 ± 0.103 cfu/ml, 4.079 ± 0.076 cfu/ml, 2.516 ± 0.055 cfu/ml and 4.257 ± 0.091 cfu/ml. TABC, CC, EC, and SFBC of milk samples obtained from milk vendors were significantly higher ($P < 0.05$) than milk samples obtained from dairy farms. The pathogenic bacteria detected from milk samples of the dairy farms were *Salmonella* spp. 3(18.75%) and *Staphylococcus aureus* 4(25%) and the corresponding values for milk samples obtained from milk vendors were 5(41.7%) and 6(50%) respectively. However, *Streptococcus pyogenes* were not isolated from any of the milk samples. The present study showed that dairy farm milk producers and milk vendors follow poor milk handling practices.

KEY WORDS: Cow milk, *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus***INTRODUCTION**

Ethiopia possesses 45 million heads of cattle, 1.1 million camels, 20 million goats and 26 million sheep and the total annual milk production in Ethiopia from about 10 million milking cows is estimated at about 3.2 billion liters¹. However, the dairy development of the country is very low compared to other African countries.

Under normal condition, milk is sterile until it reaches the milk ducts of the udder of the healthy milking animals. It is a highly nutritious food and ideal for microbial growth. As a result, it often deteriorates and becomes inappropriate for human consumption².

In Ethiopia due to the highly perishable nature of milk and poor handling techniques, the amount of milk produced is subjected to high post-harvest losses and it

is reported that losses of up to 20–35% have been confirmed from production through consumption including activities during collection, transportation, chilling, processing and distribution³. The overall milk consumption in Ethiopia is very low, compared with other least developed African countries and it was reported that the annual per capita milk consumption was 19–20 litres in 1993/1994⁴.

Compared to raw milk pasteurized fluid milk presents little health hazard. However, several food-borne disease outbreaks have been linked to pasteurized milk and this is traced to inefficient pasteurization temperature, poor packaging material and storage temperature abuse⁵. Though most bacteria are destroyed by pasteurization, there are certain types and certain bacterial stages that are not destroyed. Bacteria in milk, originating from different sources such as the cow or

the environment, can significantly influence the quality of dairy products and therefore consumer acceptance.

The objective of the study is to evaluate the quality and safety of cow milk produced and marketed in Dire Dawa town.

MATERIAL AND METHODS

Sample collection

A total of 30 milk samples each containing 500 ml of raw and pasteurized milk were collected using sterile bottles and placed in an ice box. Raw milk samples were collected directly from bulk milk containers used by the dairy farms (16 samples) and the milk vendors (12 samples). Two pasteurized milk samples were, however, collected from Hamdael Dairy Farm. After aseptically collecting the milk samples with sterile bottles, they were transported to Haramaya University Dairy Technology Laboratory.

Enumeration of microorganisms

Total aerobic bacterial count

The total aerobic bacterial count was made by adding 1 ml of milk sample into a test tube containing 9 ml of peptone water. After thoroughly mixing, the sample was serially diluted up to 10^{-7} (dairy farms and pasteurized milk) and 10^{-10} (milk vendors). Duplicate samples (1 ml) were pour plated using 15-20 ml molten standard plate count agar and mixed thoroughly. The pour plates were allowed to solidify and then incubated at 32°C for 48 hours. Colony counts were made using a colony counter ⁶.

Coliform count

One ml from each of the above 10^{-7} (dairy farms and pasteurized milk) and 10^{-10} (milk vendors) serial dilutions was transferred into duplicates of sterile Violet Red Bile Agar (VRBA) plates by the pour plate technique. The resulting inoculated plates were then incubated at 32°C for 24 hours. Pink to dark red colonies with bile precipitation around them were counted as coliforms ⁶.

Escherichia coli count

For the enumeration of *Escherichia coli*, 25 ml of milk sample was blended in 225 ml of lactose broth and serial dilutions were made by adding successively 1 ml of the sample suspension into 9 ml of sterile saline solution. 0.1 ml of each of the appropriately diluted milk sample from all sources was then spread plated on sterile Eosin Methyl Blue Agar (EMB agar). The resulting EMB agar plates were incubated at 37°C for 24 hours. Finally, blue black colonies with metallic green sheens colonies were counted as *E. coli* ⁶.

Spore -forming bacteria count

The enumeration of spore-formers was done using plate count agar following the methods recommended by ⁷. Milk samples were heated at 80°C for 10 minutes in water bath and volumes of 1 ml of appropriate dilutions were pour plated as for the standard plate count using plate count agar. All plates were incubated in an inverted position for 3 days at 30°C and colonies were counted.

Detection of Some Pathogenic Bacteria

The detection of pathogenic bacteria was done following methods described by ⁶. In this method, cell morphology (cell grouping), KOH test, catalase test, motility test, glucose broth confirmatory tests were conducted after cultivating of bacterial colonies in appropriate selective and differential media.

Salmonella spp.

A portion of 25 ml of milk was pre-enriched in 225 ml of buffered peptone water at 35°C for 24 hours. Then, 10 ml of pre-enriched sample was incubated in 100 ml Selenite Cystein broth at 35°C for 24 hours. Samples from enrichments were streaked on to Xylose Lysine Desoxycholate (XLD) agar media. These selective media were incubated at 37°C for 24 h and typical colonies with large, glossy black centers or colonies that appeared almost completely black or dark pink to red were considered as *Salmonella* spp. ⁶.

Staphylococcus aureus

Milk samples (25 ml) were diluted in 225 ml of buffered peptone water and mixed in a shaker for 2 minutes⁸. Following this, appropriate dilutions were surface plated on manitol salt. *Staphylococcus aureus* was detected on mannitol salt agar incubated at $35\pm 2^{\circ}\text{C}$ for 24-48 hours. A yellow zone around the colony indicates mannitol has been fermented. Further, colonies surrounded by yellow zones were transferred to blood agar base to see β hemolysis *i.e.* observing clear zones around the colony.

Streptococcus pyogenes

For the detection of *Streptococcus pyogenes*, milk samples were serially diluted in sterile saline solution ⁹. Using sterile pipette, 0.1 ml aliquots of samples were taken from appropriate dilutions and spread-plated on to 5% sheep Blood Agar. The resulting plates were then incubated at 37°C for 24 h. *Streptococcus pyogenes* was generally detected by observing colony morphology, size of the colony and β - hemolytic characteristics (observing large distinct zone of β -haemolysis) ¹⁰.

Statistical Analysis

The arithmetic means of the microbiological data for raw and pasteurized milk were compared by the Tukey test, at a probability level of 5% ¹¹.

RESULTS AND DISCUSSION

The total aerobic bacterial count (TABC) of milk obtained from dairy farms ranged from 1.88×10^4 to 3.15×10^6 cfu/ml with an average value of 5.84 ± 0.629 cfu/ml and the total aerobic bacterial count of milk samples obtained from milk vendors ranged from 3.5×10^7 to 2.05×10^{10} cfu/ml with an average value of 9.137 ± 0.885 cfu/ml (Table 1). On the other hand, the average total aerobic bacterial count of pasteurized milk was 5.425 ± 0.103 cfu/ml. This indicates that the total aerobic bacterial count obtained from milk vendors were significantly higher ($P < 0.05$) than milk samples collected from dairy farms and the pasteurized milk collected from Hamdael farm (Table 1).

Table 1 shows that the total aerobic bacterial count (TABC) of raw milk from dairy farms in the present study is lower than that reported by ¹² who found high total aerobic bacterial count of (9.089 ± 0.281 cfu/ml) in milk samples collected from dairy farms of Khartoum State. Furthermore, higher total aerobic bacterial count (10^6 cfu/ml) was reported by ¹³ for bulk tank milk of dairy farms in Burkinafaso. In the present study, the TABC obtained from pasteurized milk was lower than that reported by ¹⁴ who found from pasteurized milk a TABC of 6.5×10^5 to 6.5×10^{14} cfu/ml. The TABC of pasteurized milk samples was not significantly different from that of dairy farm milk samples.

The higher total aerobic bacterial count observed in the present study might be attributed to the initial contamination of the milk samples either from the udder of the cow, the milkers hand, the milking area and the milking containers. On the other hand, the very high bacterial count observed in milk samples collected from vendors' could probably be due to further contamination of the milk during transportation, the use of poorly cleaned milk containers, lack of and improper cooling systems at milk vending areas and poor personnel hygiene. On The other hand, the presence of high number of total aerobic bacteria in the pasteurized milk could be due to improper pasteurization temperature, failure of pasteurizing machine, post-processing contamination and poor packaging material of the product.

The milking process, especially the equipment used for milking or storage of milk introduces the greatest proportion of microorganism in cow milk ¹⁵. The health of the dairy herd, milking and pre-storage conditions are also basic determinants of milk quality ¹⁶.

The mean value coliform count of raw milk samples collected from dairy farms was 4.13 ± 0.757 log₁₀ cfu/ml (Table 1). This result is similar with values

reported by ¹⁷ who found total coliform count of 4.18 ± 0.01 log₁₀ cfu/ml from raw milk samples. Lower average values of coliform counts (2.23 ± 0.136 log₁₀ cfu/ml) from milk samples collected from Khartoum State dairy farms were reported by ¹⁸. In the current study, the highest mean value of coliform count was obtained in milk samples obtained from vendors (6.198 cfu/ml) (Table 1). The mean value for coliform count of the pasteurized milk samples were (4.079 ± 0.076 log cfu/ml) (Table 1). However, higher coliform counts ranging from 6.5×10^{12} to 6.5×10^{14} cfu/ml for pasteurized milk samples produced in Khartoum State were reported by ¹⁴.

Coliform count in milk samples obtained from vendors was significantly higher ($P < 0.05$) than milk samples obtained from dairy farms and distribution center for pasteurized milk in Dire Dawa *i.e.* Hamdael's farm (Table 1). However, the coliform counts (CC) obtained from pasteurized milk was not significantly different ($p > 0.05$) from that of the raw milk samples obtained from dairy farms.

Table 1: Mean (\pm SD) microbial counts (log₁₀ cfu/ml) of raw and pasteurized cow milk samples collected from different sources in Dire Dawa town.

Variables	Pasteurized milk	Raw milk		
	Hamdael farm n=2	Dairy farms (n=16)	Vendors (n=12)	Overall (n=28)
TABC	5.425 ± 0.103^{bc}	5.840 ± 0.629^b	9.137 ± 0.885^a	7.240 ± 1.835
CC	4.079 ± 0.076^{bc}	4.130 ± 0.757^b	6.198 ± 0.418^a	5.021 ± 1.211
EC	2.516 ± 0.055^{bc}	3.640 ± 0.776^b	5.001 ± 0.44^a	4.223 ± 0.941
SFBC	4.257 ± 0.091^{bc}	4.798 ± 0.745^b	6.392 ± 0.154^a	5.468 ± 1.015

Means followed by different superscript letters within a row are significantly different ($p < 0.05$). TABC= Total aerobic bacterial count, n= number of samples, CC= Coliform count, EC= *Escherichia coli* Count, SFBC= spore forming bacteria count

The high coliform count in the pasteurized milk might be due to post-pasteurization contamination of the milk. According to US standards, coliform count in raw and pasteurized milk should be less than 100 and 10 cfu/ml, respectively ¹⁹. However, Coliform count in the present study exceeds the US standards. The presence of coliforms in dairy farm milk samples could be associated with contamination of the milk with animal faeces. The higher coliform count in milk collected from vendors could be due to the poor personnel hygiene, poor sanitation of the milk collecting containers and increased contamination level during transportation.

The average mean value of *Escherichia coli* count of raw milk samples collected from dairy farms were 3.64 ± 0.776 cfu/ml (Table 1). This is lower than the reported value for *E.coli* (3.93 ± 0.01 cfu/ml) by ¹⁷ for raw milk samples. *Escherichia coli* count in milk samples obtained from vendors was significantly higher ($p < 0.05$) than milk samples obtained from dairy farms and the pasteurized milk. According to US standards,

E.coli must be absent from pasteurized milk samples. However, in the present study the pasteurized milk had very high *E.coli* count ($2.516 \pm 0.05 \log_{10}$ cfu/ml). The high *Escherichia coli* count in the pasteurized milk could be due to post-pasteurization contamination and poor handling practices of the pasteurized milk. The poor hygienic condition of milking environment, milkers hand, poor udder preparation, and lack of boiling milk, poor hygienic condition of the milking container and lack or inefficient use of cooling system in the study area might have contributed to the contamination of milk by *E.coli*. The presence of *Escherichia coli* and other pathogens in pasteurized milk is either due to insufficient pasteurization or indication of post-pasteurization contamination of milk²⁰.

The average values of spore-forming bacteria counts (SFBC)/ml of milk samples collected from vendors were significantly higher ($p < 0.05$) than raw milk samples obtained from dairy farms and the pasteurized milk collected from Hamdael farm (Table 1). The observed high SFBC from milk samples of all sources of the study area might be due to either contamination of raw milk before pasteurization or due to post-pasteurization contamination with spore-forming bacteria. The relatively higher spore-forming bacterial count in milk samples obtained from vendors may indicate that there was poor environmental sanitation and poor handling practice at the vending sites.

In the current study, pathogenic bacteria were detected from milk collected from vendors and dairy farms with higher frequency in milk from vendors (Table 2)

Table 2: Frequency and percentage of occurrence of pathogens in raw and pasteurized milk collected from different sources in Dire Dawa town.

Sample type	Sample source	N	<i>Salmonella</i> spp.		<i>S. aureus</i>		<i>S. pyogenes</i>	
			+ve	-ve	+ve	-ve	+ve	-ve
A	Dairy farms	16	3(18.8%)	13(81.5%)	4(25%)	12(75%)	-	16(100%)
	Vendors	12	5(41.7%)	7(58.3%)	6(50%)	6(50%)	-	12(100%)
B	Hamdael farm	2	-	2(100%)	-	2(100%)	-	2(100%)

A = Raw milk, B = Pasteurized milk, N = Number of samples, +ve = Positive, -ve = Negative,

Raw milk samples were positive for *Salmonella* spp. with a percentage of detection of 18.8% and 41.7% for milk samples obtained from dairy farms and vendors, respectively. Thus, the prevalence of *Salmonella* spp. in dairy farm bulk milk samples is higher than the findings of²¹ who reported a 2.6% occurrence of *salmonella* spp. in raw milk samples collected from US dairies. The

higher frequency of detection of *Salmonella* spp. in milk samples obtained from dairy farms could be associated to poor udder preparation, milkers' hygiene, poor milk handling practices, poor environmental sanitation and sanitation of milking equipment.

Table 2 also shows milk samples collected from dairy farms and vendors were positive for *Staphylococcus aureus*. The percentage of detection of this pathogen in milk samples obtained from dairy farms and vendors was 25% and 50%, respectively. The occurrence of *Staphylococcus aureus* in raw milk may not be unusual as it is one of the pathogens that is commonly isolated from mastitic cows. Lower rate (10.4%) of *Staphylococcus aureus* in bulk tank milk of dairy farms in Brazil²². In contrast to this higher rate (61.7%) of isolation of the organism from raw milk samples collected from Southern India dairy farms²³. The rate of isolation of *Staphylococcus aureus* from milk samples obtained from vendors is higher than that of²⁴ who reported 19.4% rate of isolation for *Staphylococcus aureus* in Khartoum State. The higher rate of isolation of *Staphylococcus aureus* milk from vendors could be due to poor milk handling practices during selling and increased contamination of milk during transportation to vending sites. The occurrence of *Staphylococcus aureus* in dairy farm milk samples could be associated with poor udder preparation and poor milking hygiene.

Attempts have also been made to detect *Streptococcus pyogenes* in milk samples collected from dairy farms, milk vending sites and the pasteurized milk of Hamdael's farm. However, none of the samples were found to be positive for this pathogen.

CONCLUSION

Generally, this study showed that the quality of milk obtained from the different sources (dairy farms and vendors) was poor. Therefore, concerned bodies should regularly monitor the overall hygienic conditions of the milk production and conduct frequent inspections of milk marketed in Dire Dawa town to check whether or not they are meeting the minimum legal standards.

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