

## Assessment of Raw Milk Microbial Quality at Different Critical Points of Oromia to Milk Retail Centers in Addis Ababa

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### Abstract

Milk microbial quality was assessed at different critical points of Oromia special zone surrounding finfine, Sebeta, Sululta and Holeta districts with the objective of assessing microbial load of raw milk at different critical points of milk marketing chain and to assess milk handling mechanism and associated factors at different points in the study points. Multi-stage purposive sampling method was used to conduct the bacteriological quality of raw milk from different critical points from peri-Addis Ababa districts of Oromia region to retail centers in Addis Ababa. A total of 60 raw milk samples were collected hygienically from each presumed critical points and examined for their microbial quality. The mean total bacterial counts were:  $6.48 \pm 1.06$ ,  $7.2 \pm 1.15$ ,  $7.02 \pm 0.17$  and  $6.7 \pm 0.64$ ,  $7.88 \pm 0.41$ ,  $7.20 \pm 0.05$  log *cfu/ml* at farmer and retail shop of Sebeta, Holeta and Sululta, respectively. The overall mean coli form counts ranged from  $5.42 \pm 1.73$  to  $5.78 \pm 0.95$ ;  $5.53 \pm 1.03$  to  $5.63 \pm 0.62$  and  $4.18 \pm 1.22$  to  $6.35 \pm 0.43$  log *cfu/ml* from farmer and retail shops of Sebeta, Holeta and Sululta respectively. E.coli was detected 26 (43.3%) of the samples at different critical points. Staphylococcus species was isolated from 17(28.3%) of samples collected from different critical points in the study sites. However, no Salmonella was found in all the samples. Mean value of yeast and mold counts were varied from  $3.77 \pm 0.47$   $2.46 \pm 1.15$ ,  $2.16 \pm 1.26$  and  $3.45 \pm 0.26$ , and  $2.30 \pm 0.19$ ,  $2.99 \pm 0.8$  log *cfu/ml* at farmer level of Sebeta, Holeta and Sululta respectively. Generally, the present was revealed that milk samples contained higher microbial load than different standards and considered as substandard which will result in public health hazard to the consumer. Therefore, intensive study on microbial status of milk in the study sites should be conducted to assure safety and quality policies to be set to assure the supply of quality milk in the area.

**Keywords:** Food safety, bacterial count, Critical points, Mold, coliform count.

### Introduction

Milk is a compensatory part of daily diet especially for the expectant mothers as well as growing children [Adil and Iman, 2011, Ahmed, 2011 and Ahmed, 2009]. Milk is virtually a sterile fluid when secreted into alveoli of udder. However, beyond this stage of production, microbial contamination might generally occur from three main sources; within the udder, exterior to the udder and from the surface of milk handling and storage equipments, but the surrounding air, feed, soil, feces and grass are also possible sources of contamination [Ali and Abdelgadir, 2011, Almaz et al, 2001 and APHA,1985].

Food spoilage is also a worldwide economic problem. Through microbial activity alone, approximately 25% of world's food supply is lost. These risks must be assessed and managed to meet growing and increasingly complex sets of national objectives. The agreement on the application of Sanitary and Phytosanitary Measures Agreement (SPMA) permits countries to take legitimate measures to protect the life and health of consumers provided such measures can be justified scientifically and do not unnecessarily impede trade [APHA,1992].

Milk is a complex biological fluid and by its nature, a good growth medium for many microorganisms. Because of its physico-chemical properties, it needs strict hygienic condition to avoid contamination of milk with microorganisms. Therefore, the microbial content of milk is a major feature in determining its quality [Argudin, 2010].

Food quality and safety standards in Ethiopia are one of the most concern areas because producers need to minimize loss while the general public would like to have a fair idea of what standard of food to buy for consumption. Also the safety of the food supplied for consumption especially for foods like milk is of paramount concern. Microbial load is a major factor in determining milk quality. It indicates the hygienic level exercised during milking, cleanliness of the milk utensils, condition of storage, manner of transport as well as the cleanliness of the udder of the individual animals [Asperger and Zangerl, 2003 and Aycicek, 2005].

The initial microbiological quality of milk can vary substantially based on factors such as the health of the animal, the sanitary condition of the milking environment and milker [Aydin et al, 2011]. Microbial contamination of milk can therefore originate from within the udder; the exterior of the teats and udder; and

from the milk handling and storage equipment [Aydin et al, 2011 and Benkerroum, 2004]. Hygienic practices during production, processing and handling of milk and milk products in the central Ethiopia are substandard [Beyene, 1994].

However, there is scanty information on the microbial properties and composition of raw milk in Ethiopia [Bintsis et al, 2008 and Biruk et al, 2009]. Such reports coupled with notion of problems related to milk supply chain and detection of milk microbial in dairy products after transportation and storage in the of Peri-Addis Ababa to milk retail in the city calls for systematic study and remedy for the malady. Therefore, the objective of this study was to assess microbial load of raw milk at different critical points of milk marketing chain and to assess milk handling mechanism and associated factors at different points in the study points.

## **Materials and Methods**

### **Study Area Description**

The study was conducted in three Peri-Addis Ababa districts (*Sululta, Holeta and Sebeta*) of Oromia Regional States of Ethiopia. The study sites were selected based on their milk production potential as well as their lion's share to milk retail market at Addis Ababa. Sululta is located between 9°4'30"N to 9°30'59"N and 38°31'26"E to 38°58'49"E. Animal production system is mainly mixed crop-livestock type of farming system (CSA, 2004). Holeta is situated at a distance of 31 km West of Addis Ababa and located at 9°02' N latitude and 38°29' E longitude in Oromia National Regional State (ONRS) of Ethiopia. It is found at an average altitude of 2449 m a.s.l. The area is one of the major dairy potential sites in Oromia Regional State Sebeta is located 24km from South west of Addis Ababa at a latitude and longitude of 8°55'N38°37'E and an elevation of 2356 masl. These areas take the lion's share in terms of their milk production potential and contribution to Addis Ababa milk market.

The main agricultural practices of the study areas are mixed, crop-livestock production system, in which *Teff*, wheat, lentil and chickpea are widely grown. Agriculture is strictly rain fed. The areas' rainfall and temperature ranges between 800-1500mm year<sup>-1</sup> and 10-25°C, respectively. Animal products, especially dairy products, play a headstone role in household food security both by direct consumption and purchasing of other food items in the area (WARDO, 2012).

### **Study population**

A total of 60 milk samples were collected from different critical points (farmers, collection centers, informal merchants, and dairy cooperative unions) and following the route milk retail centers in Addis Ababa were also engaged. Totally 12 samples were collected from each critical points following milk marketing chain.

### **Sample Collection and Transportation**

The study was conducted from December 2013 to April 2014 to assess the bacteriological quality of raw cow milk. Raw milk samples were collected from different critical points (farmers, collection centers, informal merchants, milk cooperative unions and retail centers). The samples were collected aseptically in sterilized universal bottles in cold icebox with ice bag and transported to Ethiopian Public Health Institute (EPHI) Food microbiology laboratory and then stored in refrigerator at 4°C before 24hrs of sampling as described by APHA (1992) and analyzed within 6hrs of sampling.

### **Bacteriological Laboratory Analysis**

The bacteriological tests considered for determination of the bacterial load in raw milk samples were Total Aerobic Bacterial Count (TABC), Coliform Count (CC), *E. coli* counts, salmonella and *S. aureus*. The total plate count agar used for determination of total aerobic bacteria in milk was sterilized by autoclave sterilization while the Violet Red Bile Agar (VRBA) used for determination of CC and *E. coli* count was sterilized by boiling. Each plate was marked by water proof marker with respect to sample number and dilution. XLD was used for isolation of salmonella species and MSA was used for isolation of *Staphylococcus aureus*. Besides yeast and mold counts were by using PDA with chloromphenicol. For enumeration of TABC and TCC, peptone water was used for dilution of each raw milk sample. Dilutions were selected so that the total numbers of colonies on a plate were not difficult to count. For both tests the media were prepared according to the guidelines given by the manufacturers as indicated by American Public Health Association (APHA) (1992).

The bacterial counts were made after plating and incubation of appropriate dilutions of milk samples in the standard Plate Count Agar (PCA) medium and in VRBA medium at 37°C for 48 hrs and 24 hrs respectively, following the standard procedures recommended by APHA (1992). After incubation, all colonies including those of pin point size in SPCA medium and purplish red colonies in VRBA medium were counted under colony counter and results from each standard PCA plates which contained 25 to 250 colonies per plate whereas, less than 100 coliform colonies VRBA were recorded. For colonies beyond this count the next

dilutions were plated and similar procedure was followed. When computing TAPC and CC, only the first two significant digits were recorded and the bacterial count was reported as colony forming unit per milliliter of milk (CFU/ml).

### Data Management and Analysis

Microsoft excel spread sheet was employed for raw data entry. Then  $\text{Log}_{10}$  transformation of bacterial count was done, before the analysis, and SPSS version 16.0 software was used for descriptive statistics. For all analysis, 95 % CI and  $P$ -value $<0.05$  was set for statistical significance of an estimate.

### RESULTS AND DISCUSSIONS

The microbial quality of milk indicates the hygienic levels during milking that include cleanliness of the milking utensils, proper storage and transport as well as the hygienic status of the udder of the individual cow (Spreer, 1998). Standard plate count (SPC) is one of the most commonly used microbial quality tests for milk and milk products.

The total aerobic bacterial counts (TABC) obtained from farmer level raw milk sample ranged from  $4.78 \times 10^4$  to  $8.29 \times 10^6 \log \text{ cfu/ml}$  with an average value of  $6.88 \pm 0.46 \log \text{ cfu/ml}$  and the total aerobic bacterial count of milk samples obtained from dairy cooperative union and retail shop ranged from  $3.85 \times 10^2$  to  $7.79 \times 10^6 \log_{10} \text{ cfu/ml}$  at Sululta to  $6.86 \times 10^5$  to  $7.88 \times 10^6$  at Holeta, respectively and  $7.55 \times 10^5$  to  $8.49 \times 10^7$  and  $7.14 \times 10^5$  to  $7.26 \times 10^5 \log \text{ cfu/ml}$  at Holeta and Sululta retail shops respectively. However, lower total aerobic bacterial count was obtained from Sebeta retail shop with the mean  $\pm$  SD of  $6.7 \pm 0.694 \log \text{ cfu/ml}$ . On other hand, the mean value of total aerobic bacterial count obtained from informal merchant at Sululta and Holeta was  $8.07 \pm 0.834 \log \text{ cfu/ml}$  and  $7.45 \pm 0.264 \log \text{ cfu/ml}$ .

The value of total aerobic bacterial count for present study revealed lower than that reported by Tola (2002) in Eastern Wollega that had average count  $7.4 \times 10$ ; Beyene (1994) in Southern Ethiopia that had average count of  $7.7 \log \text{ cfu/ml}$ ; Tassew & Seifu (2011) at Bahir Dar Zuria with the overall mean of  $7.58 \log_{10} \text{ cfu/ml}$ ; Worku *et al.* (2012) who reported bacterial count from  $7.36 - 7.88 \log_{10} \text{ cfu/ml}$  of raw cows' milk in Borana, Ethiopia and Mosu *et al.* (2013) at selected dairy farms in Debre Zeit town that had the average total bacterial count of  $7.07 \log \text{ cfu/ml}$ .

However, the mean total bacterial count of milk samples obtained from present study was higher than Tesfay *et al.* (2013) at Dire Dawa town with mean total bacterial count of  $5.84 \pm 0.629 \text{ cfu/ml}$ . On the other hands, mean values of total bacterial counts obtained from informal merchants and retail shops were higher than that reported by Tesfay *et al.* (2013) with mean value of  $9.137 \pm 0.885 \text{ cfu/ml}$ . The total aerobic bacterial count obtained from retail shop were significantly higher ( $p < 0.05$ ) than milk samples collected from households/farmers.

The higher total aerobic bacterial count observed in the present study may be attributed to the initial contamination of milk samples either from of the cow, milkers hand, milking areas and container itself. On the other hand, high bacteria count observed in milk samples collected from informal merchant and retail shop could probably be due to further contamination of the milk during transportation, extremely high transportation temperature, the use of poorly cleaned milk containers, lack of and improper cooling systems at milk vending areas and poor personnel hygiene.

The higher count indicates substandard hygienic conditions practiced during milking and subsequent handling. This implies that the sanitary conditions in which milk has been produced and handled are substandard subjecting the product to microbial contamination and multiplication. It is indicated that total bacterial count is a good indicator for monitoring the sanitary conditions practiced during production, collection, and handling of raw milk (Fatine *et al.*, 2012).

Hence training of milk handlers about hygiene can significantly reduce the bacterial load in milk. A good example for this could be reduced total bacterial count observed in milk sampled from farmers who received training on hygienic milk production and handling (Nebiyu, 2008; Sintayehu *et al.*, 2008). Milk produced under hygienic conditions from healthy cows should not contain more than  $4.7 \log_{10} \text{ cfu/ml}$  (O' Connor, 1994). Table 1 below indicates aerobic bacterial counts in different critical points and study district

**Table 1: Mean ( $\pm$  SD) Aerobic mesophilic bacteria counts of raw milk samples ( $\log_{10}$  cfu/ml) collected from different Value chain/critical points of the study sites.**

Sample sources	Study districts			
	Sebeta	Holeta	Sululta	Standard
Farmer	6.48 $\pm$ 0.06 <sup>a</sup>	7.2 $\pm$ 1.15 <sup>a</sup>	7.02 $\pm$ 0.17 <sup>a</sup>	3x10 <sup>4</sup>
Collection center	6.80 $\pm$ 0.03 <sup>b</sup>	7.64 $\pm$ 0.03 <sup>b</sup>	7.87 $\pm$ 0.49 <sup>b</sup>	
Informal merchant	6.89 $\pm$ 0.18 <sup>b</sup>	7.45 $\pm$ 0.26 <sup>a</sup>	8.07 $\pm$ 0.834 <sup>b</sup>	
Milk cooperative union	-	6.10 $\pm$ 1.08 <sup>b</sup>	5.96 $\pm$ 1.160 <sup>c</sup>	
Retail shops	6.7 $\pm$ 0.69 <sup>b</sup>	7.88 $\pm$ 0.42 <sup>c</sup>	7.20 $\pm$ 0.06 <sup>a</sup>	

The results were from duplicate values. Mean $\pm$ SD values indicated by different superscript with in a column have significant difference at ( $p < 0.05$ ).

The present result revealed that there is increment of bacterial count at each critical points. The mean ( $\pm$  SD) bacterial count was 6.48 $\pm$ 0.065, 7.2 $\pm$ 1.152 and 7.02 $\pm$ 0.869  $\log_{10}$  cfu/ml in dairy farmers, 6.7 $\pm$ 0.694, 7.88 $\pm$ 0.416 and 7.20 $\pm$ 0.056  $\log_{10}$  cfu/ml in milk vending/retail shops of Sebeta, Holeta and Sululta, respectively. This could be due to improper handling, storage and transport time after the milk leaves the dairy farms. There is a significant difference in the total aerobic bacterial counts in different critical points in the study areas and between districts at ( $p < 0.05$ ).

Coliform counts can indicate fecal contamination or contamination from equipment that has not been properly cleaned and sanitized (Schmidt, 2008; Bintsis *et al.*, 2008; Biruk *et al.*, 2009). As indicated in Table 8, the overall mean ( $\pm$  SD) of fecal coli form counts of present study at farmer level ranged from 5.42 $\pm$ 1.7352, 5.53 $\pm$ 1.0345, 4.18 $\pm$ 1.2286  $\log_{10}$ cfu/ml at Sebeta, Holeta and Sululta, respectively.

The coli form count obtained in the current study is higher than Tassew and Seifu (2011) at Bahir Dar Zuria with the mean value of 4.49  $\log$  cfu/ml; Fekadu (1994) who found coli form counts of 3.8, 4.0 and 3.8  $\log_{10}$ cfu/ml for cow milk produced in Aneno, Gulgula and Dongora districts of Southern region respectively; Worku *et al.* (2012) found overall coli form counts of 6.88 $\pm$ 0.040 and 7.786.88 $\pm$ 0.040 at cow udder and storage containers respectively in Borana pastoral community of Oromia region; Tesfay *et al.* (2013) with the mean value of 4.13  $\pm$  0.757  $\log_{10}$  cfu/ml for milk samples collected from dairy farms at Dire Dawa town; Tola (2002) raw cow's milk sampled from smallholder producers contained coli form counts of about 4.46  $\log$  cfu/ml; but it was lower than Zelalem and Faye(2006) who reported higher coli form count of 6.57cfu/ml for cow milk collected from different producers in central highlands of Ethiopia. On the other hand, the mean coli form counts obtained from retail shops of present study was higher than the above research works.

**Table 2: Mean ( $\pm$  S.D) value of Coli form counts of raw milk samples ( $\log_{10}$  cfu/ml) collected from different sampling points of the study areas.**

Source of Sample	Study districts				Standard Authority
	Sebeta	Holeta	Sululta	Standard	
Farmer	5.42 $\pm$ 1.70 <sup>a</sup>	5.53 $\pm$ 1.03 <sup>a</sup>	4.18 $\pm$ 1.23 <sup>a</sup>	10 <sup>3</sup>	ICMSF <sup>2000</sup>
Collection center	5.44 $\pm$ 0.98 <sup>a</sup>	5.92 $\pm$ 0.60 <sup>a</sup>	7.13 $\pm$ 0.30 <sup>b</sup>		
Informal merchants	5.47 $\pm$ 1.46 <sup>ab</sup>	6.10 $\pm$ 0.92 <sup>b</sup>	6.38 $\pm$ 0.60 <sup>c</sup>		
Milk cooperative union union	-	7.68 $\pm$ 0.51 <sup>c</sup>	6.15 $\pm$ 0.90 <sup>c</sup>		
Retail shops	5.78 $\pm$ 0.99 <sup>b</sup>	5.63 $\pm$ 0.63 <sup>a</sup>	6.35 $\pm$ 0.44 <sup>c</sup>		

The results were from duplicate values. Mean $\pm$ SD values indicated by different Superscript within the same column were significantly different at ( $p < 0.05$ ).

The mean coli form counts was ranged from 5.42 $\pm$ 1.735 to 5.78 $\pm$ 0.985 for sample collected from farmer and retail shop of Sebeta site. Sample from critical point retail shop had showed significantly higher ( $P < 0.05$ ) coli form count than farmer sample. Besides, sample collected from Sululta retail shop had showed significantly higher counts than farmer level sample. Coli form counts had showed significant difference among critical points and between districts. This might be due to cross contamination of milk along different critical points in chain and initial fecal contamination of the sample together with poor handling during transportation and storage.

The presence of *E. coli* organisms in milk and milk products is an indication of unsanitary production and/or improper handling of either milk or milk utensils (El-zubeir and Ahmed, 2007 & Olfa *et al.*, 2013). Milking udder with sub-clinical mastitis and wet environment lead to contamination of bulk tank milk and hence raw milk reaches the consumers with elevated Coliform count (FAO,2008; Zadoks *et al.*, 2007).

Unclean hands of workers, contaminated milk, unhygienic conditions of the manufacturing unit and

water supplied for washing the utensils could be the source for accelerating the bacterial contamination of milk products beside the post manufacturing contamination (Elmahmood *et al.*, 2007). Recovery and counting of *E. coli* is used as reliable indicator of fecal contamination and a possible presence of enteropathogenic and/or toxigenic microorganisms which constitute a public health hazard (Kaper *et al.*, 2004). The values of *E. coli* counts of present study from critical points was presented in table 3

**Table 3: Mean ( $\pm$  S.D) *E. coli* counts of raw milk samples (Log<sub>10</sub> cfu/ml) collected from different critical points of the study sites.**

Sample sources	Study districts				
	Sebeta	Holeta	Sululta	Standard	Authority
Farmer	3.19 $\pm$ 1.70 <sup>a</sup>	1.53 $\pm$ 0.00	1.19 $\pm$ 0.26 <sup>a</sup>	1x10 <sup>5</sup>	FDA,2001
Collection center	3.30 $\pm$ 0.42 <sup>a</sup>	1.61 $\pm$ 0.42 <sup>a</sup>	2.29 $\pm$ 1.28 <sup>b</sup>		
Informal merchants	-	2.56 $\pm$ 1.96 <sup>b</sup>	2.16 $\pm$ 0.06 <sup>b</sup>		
Milk Coop. Union	-	1.90 $\pm$ 1.54 <sup>c</sup>	2.86 $\pm$ 1.80 <sup>c</sup>		
Retail shops	4.17 $\pm$ <sup>b</sup>	1.53 $\pm$ 0.14 <sup>a</sup>	1.76 $\pm$ 0.03 <sup>d</sup>		

The results were from duplicate values. Mean $\pm$ SD values indicated by different Superscript within the same column were significantly different at ( $p < 0.05$ ).

The finding of the present study indicated that milk samples collected from different critical points in the study sites were highly contaminated with *E. coli*. From the total of 60 samples collected from different critical points in the study site; *E. coli* was isolated from 26(43.33%) of the samples with varying levels; 26.92, 7.96, 3.84, 3.84, 3.84, 3.84, 7.69, 7.69, 11.53, 11.53, and 11.53% were isolated from Sululta dairy cooperative, Sululta informal merchant, Sebeta retail hop, Sululta retail shop, Holeta farmer, Sululta farmer, Holeta dairy union, Sebeta farmer, Holeta informal merchant, Holeta retail shop and Sululta Collection center, respectively.

The result of present study is in line with Endale *et al.* (2013) at different critical points in Mekelle (44.4%); 11.1% at farm level, 11.1% at milk vending shops, 22.2% at cafeteria and Vahedi *et al.* (2013) in 42 (42%) from raw cow milk; but it is higher than that reported by Olfa *et al.* (2013) 13 out of 50 milk samples (32.5%) were contaminated with *E. coli* in Sfax, Tunisia from raw cow's milk from different localities.

The mean value of *E. coli* from present study from Sebeta retail shop is higher than Tesfay *et al.* (2013) who reported *E. coli* count of raw milk samples collected from dairy farms were 3.64  $\pm$  0.776 cfu/ml at Dire Dawa; but it is lower than his report at other critical point.

In contrast to this, the value of *E. coli* from present study is lower than the reported value for *E. coli* (3.93  $\pm$  0.01 cfu/ml) by Ali and Abdelgadir (2011) from raw milk samples. However, samples collected from dairy cooperative union in the present study had implicated higher *E. coli* counts than other critical points. It indicates that there is increment in microbial load along different critical points of milk marketing from farmer to the consumer level.

This may be due to cross contamination of milk during transportation, lack of sanitation of storage container and lack of temperature control through the chain that create conducive environment for multiplication of particular microorganism. *E. coli* count in milk samples obtained from retail shop was significantly higher ( $p < 0.05$ ) than milk samples obtained from dairy farmer for Sebeta site.

Milk and dairy products are frequently contaminated with enterotoxigenic *Staphylococcus* species, which are often involved in SFP, especially in areas characterized by a high level of consumption of these products, since staphylococci are often involved in cases of subclinical mastitis of ruminants resulting in contamination of milk (Salandra *et al.*, 2008). Raw milk is a potential source of staphylococci, especially in the case of mastitic milk and defective pasteurization (Kaloreu *et al.*, 2007).

The findings of present study revealed that *Staphylococcus aureus* was isolated from 17(28.33%) of samples collected from different critical points in the study sites. The findings of the present study was in line with that of Endale *et al.* (2013) at Mekelle *Staphylococcus aureus* was isolated from 48 samples (26.7%), milk samples collected from dairy farms and vendors from Mekelle.

However, it is higher than that reported by Mekuria *et al.* (2013) reported prevalence of 51 (15.5%) *S. aureus* out of the total samples examined from selected dairy farms around Addis Ababa; Aydin *et al.* (2011), reported 10.2% of *S. aureus* in raw milk samples collected from Turkey and Vadehi *et al.* (2013) 22(22%) of *S. aureus* in the raw milk samples from farms. But present finding was lower than that of Mekonnen (2009) who reported the prevalence of *Staphylococcus* 33% and 46% from buckets milk and tanks milk from Debre Zeit, respectively; Hempen *et al.* (2004) from Gambia, reported 33.3% of the raw milk samples showed counts of coagulase-positive *Staphylococci spp.* above 2x10<sup>3</sup> cfu/ml.

The findings of the present study may be due to lack of hygienic bedding condition as reported from majority of the study participants which is predisposing factor for mastitis that is complex of soiling of udder that favors further contamination and growth of bacteria, lack of washing udder and teat before and after milking, occurrence of sub-clinical mastitis and lack of overall hygienic condition during milking, storage and transportation.

*Salmonella* is an enteric bacteria and is the most common food-borne pathogen (Weigel *et al.*, 2004 and Mizumoto *et al.*, 2005). *Salmonella* are mostly facultative anaerobes, oxidase-negative, catalase-positive and gram negative rods. Most strains are motile and ferment glucose with production of both acid and gas. *Salmonella* have several sub species. *Salmonella enterica* is the most responsible for 99.9% infection in humans and most of infection are zoonotic in origin (Yan *et al.*, 2003).

According to Jayaroo and Henning (2001) *Salmonella* was isolated from 6.1% of bulk tank milk sample from dairy herds in South Eastern Dakata and Western Minnesota. In other study conducted in Addis Ababa salmonella was isolated from 2.1% of milk samples collected from different supper market in Addis Ababa (Tesfaw *et al.*, 2013). Tesfay *et al.* (2013) reported 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 from Dire Dawa; Van Kassel *et al.* (2004) reported a 2.6% occurrence of *Salmonella* spp. in raw milk samples collected from US dairies. However, *Salmonella* was not detected in the present study.

In the findings of yeast and mold of present study, highly varied from farmer to retail levels. However, the values of present findings were lower than that reported by Ahmed (2011), who reported 6.1 and 7.4log *cfu/ml* for yeast and mold for raw camel milk from Mieso districts of Oromia region. It was higher than that Karmen and Slavica (2008) and Fadda *et al.* (2004) who reported 2.3 log *cfu/ml* and 2.64 log *cfu/ml*, respectively.

The findings of yeast and mold was highly varied from farmer to retail levels and showed in the table below (Table 4).

**Table 4: Mean ( $\pm$  S.D) Mold and Yeast counts of raw milk samples (Log10 *cfu/ml*) collected from different critical points of the study sites.**

Sample Source	Districts					
	Sebeta		Holeta		Sululta	
	yeast	mold	yeast	mold	yeast	mold
Farmer	3.77 $\pm$ 0.47 <sup>a</sup>	3.45 $\pm$ 0.26 <sup>a</sup>	2.46 $\pm$ 1.15 <sup>a</sup>	2.30 $\pm$ 0.19 <sup>a</sup>	2.16 $\pm$ 1.25 <sup>a</sup>	2.99 $\pm$ 0.82 <sup>b</sup>
CC	3.76 $\pm$ 0.44 <sup>a</sup>	3.46 $\pm$ 0.08 <sup>a</sup>	3.24 $\pm$ 0.46 <sup>a</sup>	3.26 $\pm$ 0.04 <sup>b</sup>	3.16 $\pm$ 0.91 <sup>b</sup>	3.79 $\pm$ 0.70 <sup>c</sup>
IM	3.76 $\pm$ 0.41 <sup>a</sup>	3.51 $\pm$ 0.10 <sup>a</sup>	3.73 $\pm$ 0.42 <sup>b</sup>	2.43 $\pm$ 0.17 <sup>a</sup>	3.78 $\pm$ 0.10 <sup>c</sup>	4.72 $\pm$ 1.16 <sup>d</sup>
DCU	-	-	3.55 $\pm$ 0.52 <sup>c</sup>	3.45 $\pm$ 0.26 <sup>c</sup>	4.16 $\pm$ 0.34 <sup>c</sup>	3.73 $\pm$ 1.10 <sup>c</sup>
RS	3.85 $\pm$ 0.42 <sup>b</sup>	3.82 $\pm$ 0.76 <sup>b</sup>	3.59 $\pm$ 1.44 <sup>c</sup>	3.38 $\pm$ 0.48 <sup>c</sup>	2.26 $\pm$ 1.07 <sup>a</sup>	2.41 $\pm$ 0.15 <sup>a</sup>

The results were from duplicate values. Mean $\pm$ SD values indicated by different Superscript within the same column were significantly different at ( $p < 0.05$ ). CC=collection center, IM=informal merchant, DCU=Dairy Cooperative Union and RS=Retail shop

The mean $\pm$ SD of yeast counts varied between 3.77 $\pm$ 0.47 and 3.85 $\pm$ 0.42 for samples collected from Sebeta district (Table 4). Samples due to critical point retail shop was significantly higher than other points ( $p < 0.05$ ), mean $\pm$ SD of mold counts were varied between 3.45 $\pm$ 0.26 and 3.82 $\pm$ 0.76 for the sample Collected from Sebeta district. The mean $\pm$ SD of yeast counts were varied between 2.46 $\pm$ 1.15 and 3.59 $\pm$ 1.44 for the sample collected from Holeta district. Samples due to critical point informal merchant was significantly higher than other critical points ( $P < 0.05$ ).

Mean $\pm$ SD counts of mold counts were varied between 2.30 $\pm$ 0.19 and 3.38 $\pm$ 0.48 for samples collected from Holeta district. Samples collected from retail shop was significantly higher than all other critical points ( $p < 0.05$ ).

However, their values increased significantly at retail shops following the chain in the respective districts except for retail level of Sululta. The value for mold count was varied from 3.45 to 3.82, 2.30 to 3.38 and 2.99 to 2.41 at farmer and retail level of Sebeta, Holeta and Sululta, respectively. The value for yeast count was varied from 3.77 to 3.85, 2.46 to 3.57 and 2.16 to 2.26 at farmer and retail level of Sebeta, Holeta and Sululta, respectively. Due to sample collected from retail level of Sebeta and Holeta had showed significantly higher ( $p < 0.05$ ) yeast and mold counts than farmer level. However, the value for yeast in the case of Sululta was not significantly different between farmer and retail level.

This might be due to location of the areas; high altitude together with high relative humidity that favors the growth of molds, feeding stored feed that developed molds, lack of hygienic practices especially washing milking and milk storing utensils, mixing of cold and newly drawn milk and storing together.

## CONCLUSION AND RECOMMENDATION

The result obtained in this study concluded that milk available to the consumer in Addis Ababa via different supply chain critical points have a high bacterial load beyond acceptable critical limits according to American and European community member states. Also the milk considered from the study areas were contaminated with most hazardous agents such as *Staphylococcus species.*, *E. coli*, yeast and mold. It indicates that hygienic procedures were not strictly followed during milk production to supply route. The magnitude of the problem of

bacterial contamination deserves more elaborative studies from the point of production of milk to the point of milk retail for consumption.

The results of the present study indicate that strict preventive measures should be adopted to ensure contamination free milk and its products for the good health of all consumers. Therefore, stakeholder authorities should regularly monitor the overall hygienic conditions of the milk production and conduct frequent inspections of milk marketed in Addis Ababa to check whether or not the minimum legal standards are met. Remedial actions can be taken by:

Milk marketing actors especially from collection center to retail shop and/vendors should use refrigerated vehicle and cold chain in place of open container and vehicle to maintain bulk tank temperature there by minimize microbial growth during transportation and storage. Actors in each critical point should perform basic laboratory test for at least indicator microorganisms that are frequently detected in raw milk available for direct human consumption.

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