Research Article

Microbial Hazard Analysis in the Pasteurized Milk Production Value Chain at a Commercial Dairy Plant in Hawassa, Southern Ethiopia

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ABSTRACT

Previous works in Ethiopia largely focused on end product quality analysis of different brands of pasteurized milk from retail markets. The aim of this study was to assess microbial hazards in the value chain of pasteurized milk production at a dairy plant in Hawassa city, southern Ethiopia. Ten milk samples were collected and analyzed from each of four presumptive, critical control points in the pasteurized milk production chain. Critical control point (CCP1) was the raw milk at the receiver tank just before it goes to the processing line, CCP2 - pasteurized milk before being packed, CCP3 - pasteurized milk immediately after packed and the CCP4 - pasteurized milk in the market. The overall mean Aerobic mesophilic bacteria counts at the respective CCPs in $\log_{10}$ cfu ml^{-1} were 8.21 (CCP1), 5.04 (CCP2), 6.06 (CCP3) and 6.14 (CCP4). The aerobic mesophilic bacterial genera were dominated by isolates related to *Bacillus* followed by *Pseudomonas*, *Staphylococcus* and *Streptococcus*. Likewise the overall mean *Enterobacteriaceae* counts in $\log_{10}$ cfu ml^{-1} were 6.1 (CCP1), 0.00 (CCP2), 5 (CCP3) and 4.8 (CCP4). The overall mean total coliform counts in $\log_{10}$ cfu ml^{-1} were 4.09 (CCP1), 0.00 (CCP2), 3.22 (CCP3) and 3.48 (CCP4). More than 50% of the raw milk samples had mean fecal coliform count exceeding 5 $\log_{10}$ cfu ml^{-1}. *E. coli* was detected in 60% (6 of 10) of the raw milk samples. The observed counts of hygienic indicator microorganisms throughout the stages were above the maximum acceptable limit of both national and international standards, the raw milk being the primary culprit. Therefore relevant corrective measures are recommended to rectify the deviations and avoid potential disease outbreaks.

Keywords: Dairy plant; Ethiopia; Hawassa; Microbial hazard; Pasteurized milk; Value chain

INTRODUCTION

In Ethiopia, the estimated average per capita milk consumption is about 20 liters per year. This is quite very low compared to the African average of 40 liters per year and a world average of 105 liters per year [1]. Despite the high livestock population the productivity is very low and Ethiopia remains a net importer of dairy products and its milk market is essentially domestic. More than 97% of the total annual milk production in the country comes from the traditional smallholder milk production system that is dominated by indigenous breeds of cows that have low genetic potential [2]. Moreover, inadequate capital by dairy producers, scarce and poor quality animal feed, lack of cold chain transport and other infrastructures result in poor quality milk supply to processing centers [3].

On top of the production constraints a significant proportion of the output is lost due to microbial spoilage before it reaches processing plant. At farm level milk quality is affected by unhygienic milking by hand and poor cleanliness of milk handling equipments [4]. The handlers in the rural Ethiopian setting where most of the milk for processing is collected generally depend on traditional practices and lack formal training in food hygiene with little or no formal education [5,6]. As a result the milk collected from these sources for processing often is of poor hygienic quality and safety [7]. Contamination also occurs at collection centers and some study showed high level of adulteration and bacterial load in collection centers [8,9]. Several studies also have shown the occurrence of pathogenic microorganisms in raw milk in Ethiopia such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella* species [10-12]. Therefore, the demand for pasteurized milk with hygienic quality and safety in many urban centers has increased in parallel with the increase in the middle class population, who can afford the prices,

There are about two dozen dairy processing plants in Ethiopia with pasteurized milk production capacities ranging from 1200 to 60,000 liters of milk per day [13]. Although some of them keep their own dairy cow most of the milk processed in these plants are collected

from the small-scale traditional farmers in the rural areas where the basic amenities of hygienic handling and storage are lacking. The rapidly increasing urbanized affluent population leads to increasing demand for important food items like pasteurized milk. Since dairy processing enterprises are profit driven, the increase in demand for their products is likely to force the omission or laps in the internal quality and safety control program.

Microbial contamination and safety issues may arise at any one of the stages in the milk value chain. Good manufacturing practice and hazard analysis and critical control point system (HACCP) in the production and supply chain of raw and pasteurized milk is has been internationally acknowledged and accepted system for the effective food safety management [14]. Although it is being promoted in the growing agro-industries like dairy processing plants in Ethiopia, periodic monitoring systems and enforcement are lacking. This work aimed to assess the critical microbial hazard control points in the value chain of pasteurized milk production in a commercial dairy plant in Hawassa city, Southern Ethiopia.

MATERIALS AND METHODS

Description of the study area

The study was conducted in Hawassa city, the capital of the Sidama regional state of Ethiopia. The city is located about 275 km south of Addis Ababa, within the geographic coordinates 7° 3' N and 38° 28' E/7.050° N 38.467° E and has an elevation of 1697 m above sea level. According to the Ethiopian Central Statistical Agency [15], the estimated population of Hawassa for 2015 was 351,469. The city is one of the tourist destination hotspot for locals and foreigners as at is home to a rift a valley lake and diversity of cultures. It is home to the only dairy processing plant which is focus of this work. Currently it is processing about 2500 Liters of raw milk per day although it has the capacity to process up to 20,000L per day. It collects most of the raw milk for processing from neighboring rural areas.

Sampling points

The milk samples were collected from four presumptive, critical microbial hazard control points in the pasteurized milk production chain at the dairy plant (Figure 1). Accordingly, critical control point one (CCP1) was the raw milk samples at the receiver tank just before it goes to the processing line, CCP2 - pasteurized milk samples before packed.CCP3 was pasteurized milk sample immediately after packed and CCP4 was pasteurized milk from selling point (from two big supermarkets in the city).

Study design and sample size

A cross-sectional study design was employed based on laboratory analysis of milk samples from the dairy plant during Feb 2019 to August 2019. Convenient, non-probabilistic approach was used to collect a total of 40 samples consisting of 10 samples from each CCP and 100 ml per sample from the dairy plant. For samples from the retail market, ten packs of 500 ml pasteurized milk per sample of different production batches were considered. Sample collection was done once a week and during each collection trip one sample was collected from each of the four presumptive critical control points.

Sample collection

The Samples of both raw and pasteurized milk were collected
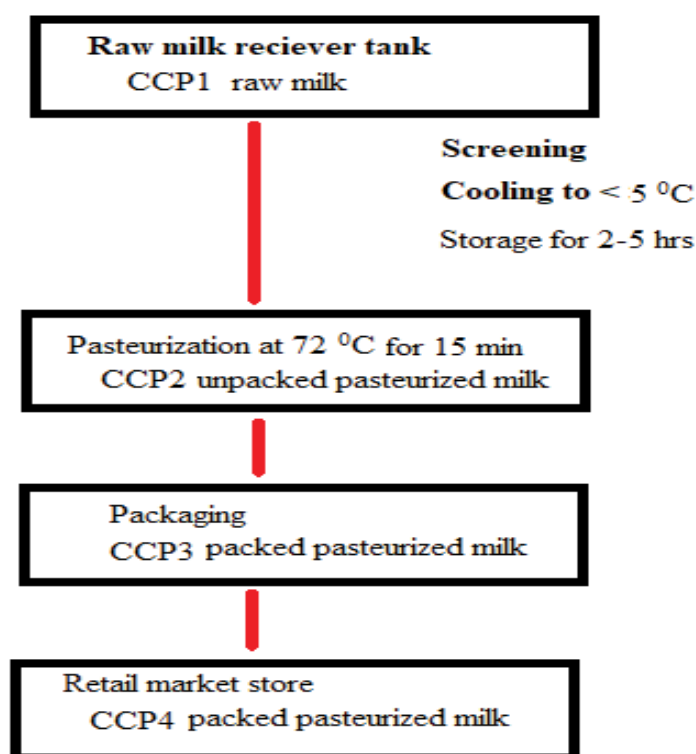
Figure 1: Flow chart indicating the presumptive Critical Control Points (CCPs) for microbial hazard analysis in the production of pasteurized milk in the dairy plant.

aseptically in a sterile bottle, transferred to icebox with ice packs and transported to the Microbiology laboratory of the Department of Veterinary medicine, Hawassa University. All samples were analyzed immediately on arrival to the laboratory and when there was delay it was stored in a refrigerator (4°C) and analyzed within 24 hours of collection.

Media preparation and storage

All the bacteriological growth media used in this study were prepared according to the instructions of the manufactures (HiMedia, India).

Preparation of decimal dilution of the milk samples

For all milk samples, the bottle was agitated manually by shaking repeatedly for about two minutes and a sterile pipette was used to aseptically transfer 10 ml of the well-mixed milk sample into a sterile bottle containing 90 ml of sterile buffered peptone water diluent. From this bottle, further tenfold serial dilution was prepared up to 10^{-6} by transfer of 1ml aliquots using micropipette into labeled tubes containing 9 ml sterile buffered peptone water [16]. Vortex mixing was done between each transfer into tubes to ensure uniform homogeneity.

Aerobic Mesophilic Bacterial count (AMBC)

From appropriate dilutions (10^{-5} and 10^{-6}) prepared as in 2.6 above, 0.1ml aliquots were aseptically transferred onto the surface of respectively labeled plate count agar (PCA, Hi Media labs, India) plates and spread plated with sterile bent glass rod (Sterilized by Dipping into absolute ethanol and burning off the alcohol). The inoculated plates were incubated at 37°C for 48 to 72 hrs. At the end of the incubation the colonies were counted using Quebec colony counter (Richert) and plates having between 30 and 250 colonies were considered to calculate the average AMBC.

The count of Enterobacteriaceae

Appropriate (10^{-3} and 10^{-4}) dilution was inoculated by spread plating as in 2.7 above on to violet red bile glucose with lactose agar (Hi Media labs, India) plates and incubated at 37°C. At the end of the incubation plates with countable colonies (all types) were considered for calculations

Total coliform count

From the plates in 2.9., countable pink colored colonies only were counted for calculations

Fecal coliform count

From the plates in 2.9., countable pink colonies surrounded by a zone of acid precipitated bile were considered for calculations.

Confirmation of *Escherichia coli*

From countable plates in 2.11, five to ten typical, distinct colonies were picked and purified by repeated subculturing. The purified isolates were streaked onto plates of Eosine methylen blue agar. Isolates that showed black colonies with green metallic sheen on EMB agar were putatively identified as *E. coli*. Further confirmation was done by standard Indole, Methyl red, Voges Proskauer and Citrate (IMVC) biochemical test [16].

Determination of Dominant Aerobic Mesophilic Bacterial

From countable plates in 2.7 above, five to ten distinct colonies were picked separately and purified by repeated sub culturing on nutrient agar plates. The purified isolates were maintained in 20% glycerol cryopreservation vials at -20^0 C freezers until further characterization by gram staining, microscopy and selected battery of biochemical tests. Cryopreservation was done by mixing 800 micro lt of the broth culture of each isolates with 200 micro lt of sterile glycerol. Standard methods were used for putative identification to generic level based on colony morphology, gram reaction and microscopy. The result of gram reaction was used to guide for further biochemical tests including Catalase test, oxidase test, reaction on Triple sugar iron agar (TSI), sulphide-Indole-motility (SIM) medium and indole test [16,17]. Briefly, for Catalase test a portion of the well isolated colony of the test bacterium was mixed with 3% Hydrogen peroxide on a clean glass slide. The formation of bubbles indicated a positive test while the absence showed a negative result [17].

For oxidase test, a portion of the well isolated colony was smeared on a filter paper strip impregnated with freshly prepared Kovac's oxidase reagent. Formation of a deep purple color within five to ten seconds constituted a positive oxidase test and the absence a negative test [16].

Urease test was done on urea agar slant by taking a portion of the colony of the test bacterium using inoculating needle and stabbing the butt and streaking the slant. After inoculation the tube was incubated at 37°C for 24 hours. At the end of the incubating the tube was examined for color change from yellow to pink that indicates positive test [16,17].

TSI test: Using inoculation needle a portion of the colony of the test bacterium was taken and stabbed into the center of the TSI agar butt and streaked on the slant [16,17]. The inoculated tube was then incubated at 37°C for 24 hours. At the end of the incubation

reactions are notes as acid/acid (yellow slant/yellow butt) indicates fermentation of dextrose, lactose and/or sucrose. An alkaline/acid (red slant/yellow butt) indicates the fermentation of dextrose only. An alkaline/alkaline (red slant/red butt) indicates absence of carbohydrate fermentation. Blackening of the medium occurs in the presence of hydrogen sulfide and bubbles or cracks in the agar indicated gas production. Likewise the SIM medium was inoculated by taking a portion of the colony of the test bacterium using inoculating needle and stabbing the center [16,17]. The inoculated tube was incubated at 37°C for 24 hours and examined. Blackening of the medium and growth away from the stab line indicated hydrogen sulfide production motility respectively. To determine Indole production two to three drops of Kovac's reagent was added and the appearance of red ring indicated a positive test.

Data analysis and presentation

All enumerations were done in duplicate plates and values were transformed into $\log_{10}$ unit for ease of manipulations. To calculate the average load from multiple plates the following formulae was used [18].

N = Summ of all colonies from all plates with countable plates

$(N_1 + 0.1N_2)$ D

Where: N_1 is the number of plates with countable colonies in the first dilutions

N_2 is the number of plates with countable colonies in the second dilution

D is the dilution factor corresponding to the first dilution

All generated data were entered and analyzed using SPSS, version 21. The average quantitative microbial parameters were compared for milk samples drown from the different CCPs and p vlue less than 0.05 were used to adjudge statistical significance for observed differences. .

RESULTS AND DISCUSSION

Aerobic Mesophilic Bacteria count (AMBC)

The aerobic mesophilic bacterial count (AMBC) of the milk samples drawn from raw milk receiver tank (CCP1 or RM) ranged from 6.3 $\log_{10}$ cfu ml^{-1} (log unit) to 9.34 log unit with the average value being 8.21 long unit (Table 1).The value for pasteurized milk samples drawn just before packaging (CCP2) declined to 5.05 long unit, with the range being 0 to 6.17 log unit. The mean AMBC of the pasteurized milk samples immediately after packaging (CCP3) and at selling point in the retail outlets (CCP4) were 6.06 log unit and 6.14 log unit, respectively (Table 1). The mean AMBC of milk samples drawn at CCP1 (RM) was significantly higher (P<0.05) than that of samples drawn at all other stages (Table 2). Although slight increase in the mean AMBC of the pasteurized milk samples were observed after packaging in the dairy plant and at retail points, the observed differences among the samples at CCP2, CCP3 and CCP4 stages were not statistically significant (P> 0.05).

The mean AMBC of the raw milk used for processing at the dairy plant (8.21 log10 cfu ml^{-1}) was much higher than the Ethiopian standard, which recommends that AMBC of raw milk intended for processing should be less than 5 log units [19]. All the ten raw milk samples (100%) showed mean AMBC that exceeded the recommended standard (Table 2). Not only the raw milk, but also

Table 1: The mean aerobic mesophilic bacteria counts in Log10 CFU ml^{-1} of milk samples from four Critical Control Points (CCP) in the pasteurized milk production in a dairy plant, Hawassa.

Sample number	CCP1	CCP2	CCP3	CCP4
1	9.34	5.78	5.59	6.07
2	7.98	5.49	6.29	6.25
3	6.30	5.47	5.98	6.45
4	9.15	5.47	6.01	6.31
5	9.27	6.17	6.45	6.20
6	7.82	0.00	5.75	6.31
7	7.59	5.54	6.46	6.25
8	9.06	5.49	5.71	6.23
9	7.22	5.57	5.89	5.57
10	8.40	5.50	6.47	5.78
Minimum	6.30	0.00	5.59	5.57
Maximum	9.34	6.17	6.47	6.45
Mean (± S.E.)	8.21 (±.320)	5.05 (±.565)	6.06 (±.105)	6.14(±.084)

Legends: CCP1=Critical Control Point 1 (Raw milk), CCP2=Critical Control Point 2 (Pasteurized Milk) before Packaging, CCP3=Critical Control Point 3 (Pasteurized milk immediately after packaging at the dairy plant, CCP4=Critical Control Point 4 (Pasteurized milk at Selling Point in the market), S.E=Standard Error

Table 2: Multiple comparisons of mean aerobic mesophilic bacterial counts of milk samples drown from four Critical Control Points of pasteurized milk production at a dairy plant Hawassa.

Dependent Variable: AMBC, Tukey HSD						
(I) Stages	(J) Stages	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CCP1	CCP2	3.16500*	.46941	.000	1.9008	4.4292
	CCP3	2.15300*	.46941	.000	.8888	3.4172
	CCP4	2.07100*	.46941	.000	.8068	3.3352
CCP2	CCP1	-3.16500*	.46941	.000	-4.4292	-1.9008
	CCP3	-1.01200	.46941	.155	-2.2762	.2522
	CCP4	-1.09400	.46941	.110	-2.3582	.1702
CCP3	CCP1	-2.15300*	.46941	.000	-3.4172	-.8888
	CCP2	1.01200	.46941	.155	-.2522	2.2762
	CCP4	-.08200	.46941	.998	-1.3462	1.1822
CCP4	CCP1	-2.07100*	.46941	.000	-3.3352	-.8068
	CCP2	1.09400	.46941	.110	-.1702	2.3582
	CCP3	.08200	.46941	.998	-1.1822	1.3462

*. The mean difference is significant at the 0.05 level.

Legends: CCP1=Critical Control Point 1 (Raw milk), CCP2=Critical Control Point 2 (Pasteurized Milk before Packaging, CCP3=Critical Control Point 3 (pasteurized milk immediately after packaging at the dairy plant, CCP4=Critical Control Point 4 (pasteurized milk at Selling Point in the market)

nine of the 10 pasteurized milk samples before packaging (90%) and all of (100%) the pasteurized milk samples after packaging and at retail points had mean AMBC that exceeded the recommended standard for raw milk (Table 2). This is a clear indication of gross problem in the raw milk used for processing at the dairy plant that led to microbiologically sub-standard quality and perhaps unsafe pasteurized milk product and calls for prompt intervention.

According to the East Africa standard for raw milk specification, a good quality raw milk should contain the AMBC of between $>2 \times 10^5$ and 1×10^6 cfu ml^{-1} [20]. The mean AMBC of the raw milk samples in the present study exceeded this recommended value by about 2 log units. The AMBC of the raw milk samples in this study was also not in compliance with the United States Food and Drug Administration guideline [21] that requires the AMBC of raw milk prior to pasteurization not to exceed 3×10^5 cfu ml^{-}

^{1}. High counts of bacteria in raw milk are common in countries that lack effective on farm cooling practice and efficient farm-to-processing plant refrigeration chain [22,23]. Similar observation regarding unhygienic handling, unclean milking equipments and faulty milking procedures were reported among small scale milk production system in many parts of Ethiopia before including Ezha district of the Gurage zone [24], Adigrat [25], Shashamene [26] and Bench-Maji zone [27].

The mean AMBC of the raw milk samples in this study was higher than the 7.7×10^6 cfu ml^{-1} (6.89 log units) and the 6.20 $\log_{10}$ cfu ml^{-1} values for raw milk samples collected before pasteurization in dairy processing plants in Addis Ababa [28,29]. The mean AMBC value in this work was also higher than the reported mean AMBC of 4.8 $\log_{10}$ cfu ml^{-1} for raw milk samples before pasteurization in Blue Nile Dairy plant, Sudan [30]. However, it was in agreement with the

reported AMBC of 1.9×10^8 cfu ml^{-1} (8.28 log units) for raw milk samples upon arrival at a dairy processing plant in Addis Ababa [7]. Considering the raw milk for processing by most dairy plants in Ethiopia is collected from private farmers scattered in rural areas where there is no standard hygienic procedure, the variations in mean AMBC reported by different studies may not be a surprise.

The mean AMBC of the pasteurized milk samples before packaging (CCP2) in this study was 5.04 log$_{10}$ cfu ml^{-1}. Nine of the ten pasteurized milk samples (90%) before packaging showed mean MABC greater than 5 log units. According to the United States Food and Drug Administration [31], pasteurization should kill more than 90% of total microbes (reduce the AMBC by one log unit) in the raw milk. In this context the pasteurization process *per se* at the dairy plant may be considered in compliance as it achieved a net reduction of the MABC by more than 3 log units. The reason for substandard microbial quality (high microbial load) of the pasteurized milk product in the dairy plant was therefore not due to faulty pasteurization process but due to gross contamination level of the raw milk used for processing. Similar observations were made by other previous workers elsewhere [32].

The mean AMBC of pasteurized milk samples before packed (5.04 log units) in the present study was not in compliance with both the Ethiopian (ES 3462:2009) [33] and East African standards (EAS 69:2006) [34] that require AMBC of pasteurized milk not to exceed 3×10^4 cfu ml^{-1}. It was also beyond the US FDA [21] acceptance limit that recommends the AMBC of pasteurized milk to be less than 2×10^4 cfu ml^{-1}. The mean AMBC of pasteurized milk sample before packed in the present study was less than the reported 7×10^5 cfu ml^{-1} [35] for pasteurized milk sample at a dairy plant in Addis Ababa. The mean AMBC of the pasteurized milk samples before packaging in this study was also higher than that reported (1.29 log$_{10}$ cfu ml^{-1}) at the Blue Nile Dairy plant in Sudan [30].

Concerning pasteurized milk samples immediately after being packed (CCP3), the mean AMBC was (6.06 log$_{10}$ cfu ml^{-1}) slightly higher than the mean AMBC of the unpacked pasteurized milk samples (CCP2). However, the observed differences in mean AMBC of the milk samples at CCP2 and CCP3 was not statistically significant (Table 2). The rise in count might be due to growth of pasteurization survivors as well as post-pasteurization contamination during the packaging process from the environmental source or unsterile packages. Similar finding has been reported by other studies elsewhere which was presumed to have occurred post pasteurization in the downstream production line, such as in regeneration or cooling section, in storage tanks and in the final packaging [36].

The AMBC of the packed pasteurized milk samples (CCP3) in this study was not in agreement with both the Ethiopian (ES 3462:2009) [33]and East African standards (EAS 69:2006) [34], which requires AMBC to be less 3×10^4 cfu ml^{-1} for pasteurized milk. Likewise, it also exceeded the maximum limit set by the United States Food and Drug Administration [21] that commands the AMBC of pasteurized milk to be less than 2×10^4 cfu ml^{-1}.The mean AMBC of the packed pasteurized milk in this study was also higher than the 4.65 log cfu ml^{-1} reported in Cairo, Egypt for packed pasteurized milk [37]. The observed data in the mean AMBC of the milk samples suggested the stages following pasteurization and the packaging process of pasteurized milk as well as sterility check in the packages are the second critical control points. However the impact of the grossly contaminated raw milk used for the pasteurization process still remained the most important critical control point.

The mean AMBC of the pasteurized milk samples drawn at retail points was (6.14 log$_{10}$ cfu ml^{-1}) slightly higher than that of the packed pasteurized milk samples at the dairy plant suggesting growth of pasteurization survivors as well as post pasteurization contaminants. The count at this stage exceeded the maximum acceptable regulatory limit of both the Ethiopia (ES 3462:2009) [33] and East Africa standards (EAS 69:2006) [34] for pasteurized milk that recommend the AMBC of pasteurized milk to be less than 3×10^4 cfu ml^{-1}. It was also higher than the 2×10^4 cfu ml^{-1}, a value which was recommended by FDA [21].

The observed increase in the mean MABC of the pasteurized samples at the retail market (CCP4) stage in the present study might be due to longer storage time of the batch of the samples used in the study as well as temperature abuse during storage of the packed pasteurized milk at the supermarkets. Similar findings were reported by other workers previously [38]. The mean AMBC of the packed pasteurized milk samples at the retail level in this study was higher than the 5.64 log$_{10}$ cfu ml^{-1} reported for packed pasteurized milk samples from market in Kenya [39]. However, it was lower than the 2.12×10^6 cfu ml^{-1} of packed pasteurized milk samples from a market reported in Addis Ababa [28].

Entrobacteriaceae counts (EC)

The mean *Entrobacteriaceae* counts (EC) of the milk samples drawn from raw milk receiver tank (CCP1) ranged from 5.46 log$_{10}$ cfu ml^{-1} (log unit) to 8.08 log units with the average value being 6.07 long units (Table 3). The value for pasteurized milk samples drawn just before packaging (CCP2) declined to undetectable level. The mean EC of the pasteurized milk samples immediately after packaging (CCP3) was 5.00 log units with the range being zero to 7.86 log units. Concerning pasteurized milk samples at selling point in the retail outlets (CCP4), the mean EC was 4.78 log units with the range being zero to 6.71 log units. Two of the 10 packed milk samples (20%) from the dairy plant had no detectable *Enterobacteriaceae*. Similar observation was also made for two different packed pasteurized milk samples from the retail market

Table 3: The mean *Entrobacteriaceae* counts of the milk samples in Log10 CFU ml^{-1} collected from four Critical Control Points in the pasteurized milk production at a dairy plant, Hawassa.

Sample number	CCP1	CCP2	CCP3	CCP4
1	5.96	.00	7.49	0.00
2	5.78	.00	5.96	5.60
3	5.76	.00	5.94	6.25
4	5.88	.00	5.69	6.31
5	5.83	.00	7.86	6.71
6	5.56	.00	5.86	5.91
7	5.74	.00	0.00	5.54
8	5.46	.00	5.64	5.71
9	6.67	.00	5.57	0.00
10	8.08	.00	0.00	5.85
Minimum	5.46	.00	.00	.00
Maximum	8.08	.00	7.86	6.71
Mean (± S.E.)	6.07(±.245)	.000(±.000)	5.00(±.870)	4.78(±.805)

Legends: CCP1=Critical Control Point 1 (Raw milk), CCP2=Critical Control Point 2 (Pasteurized Milk before Packaging, CCP3=Critical Control Point 3 (pasteurized milk immediately after packaging at the dairy plant, CCP4=Critical Control Point 4 (pasteurized milk at Selling Point in the market), S.E=standard error

– CCP4 but non corresponding samples (Table 3). The reason for the absence of EC in these different samples might be due to viable but not culturable state and /or differences in production batch of the samples considered from the retail market.

The mean *Entrobacteriaceae* counts (EC) of the raw milk samples (CCP1) was significantly higher ($P<0.05$) than that of pasteurized milk samples just before packaging - CCP2 (Table 4). This was another indication for the effectiveness of the pasteurization process at the dairy plant and that higher microbial load in the packed pasteurized milk samples very likely arose due to post processing contamination. However, no statistically significant differences ($P<0.05$) were observed in the mean EC among the raw milk samples and the packed pasteurized milk samples at the dairy plant (CCP2) and the retail market (CCP4). It is interesting to note here that the mean *Enterobacteriaceae* count of all of the raw milk samples (100%) exceeded the 5 $\log_{10}$ cfu/ml – the maximum standard acceptable limit for MABC for raw milk samples intended for processing in a dairy plant in the Ethiopian guideline [19]. (ES 3460:2009).

The mean *Enterobacteriaceae* counts (EC) of raw milk used for production of pasteurized packed milk at the dairy plant was 6.07 log10 cfu ml^{-1}. This value was comparable with the report that most of the raw milk samples collected for study in Ethiopia had EC of more than 5 $\log_{10}$ cfu ml^{-1} [40]. On the other hand the mean EC of the raw milk samples in the present study was less than the 6.86 log10 cfu ml^{-1} value reported for milk samples collected from urban area of Nirobi, Kenya [41].

The mean *Enterobacteriaceae* counts (EC) of pasteurized milk before packaged (CCP2), was zero. Therefore the observed count in this study was in compliance with maximum limit (less 1 cfu ml^{-1}) set for pasteurized milk by European commission (Council Directives 92/46/EEC], [42]. According to the guideline of Food safety of Australia and New Zealand [43] the *Enterobacteriaceae* count is usually employed to assess the status of hygienic handling and effectiveness of processing underlying heated treated foods. .

With regard to the pasteurized milk samples immediately after being packed (CCP3,), the mean *Enterobacteriaceae* counts (EC) was 5 $\log_{10}$ cfu ml^{-1}. This value was beyond the maximum regulatory limit set for pasteurized milk by the European commission Council [42]. Likewise the mean EC of the pasteurized milk samples at the retail outlets (4.78 $\log_{10}$ cfu ml^{-1}) also exceeded the above recommended limit by the European commission. Having such an elevated EC in the packed pasteurized milk indicated post pasteurization contaminants or the existence of viable but non culturable bacteria that survived pasteurization and which then multiplied following favorable storage temperature [44,45]. Likewise, the high EC of the milk samples from the retail outlets might be due to older production batch as well as temperature abuse during storage of milk products at the retail outlets. The mean EC at this stage (CCP4) in this study was higher than the 0.10 log10 cfu ml^{-1} reported for pasteurized milk samples collected from urban area of Nirobi region, Kenya [41].

Post pasteurization contamination of milk is known to occur during filling process [46]. Filling equipment has been identified by many studies as a main source of post pasteurization contamination of packed pasteurized milk [47-49]. This can occur due the formation of biofilms on the equipment, which are resistant to cleaning and sanitization, leading to persistent contamination over time [50]. In addition to the filling equipment, the packaging process and the packaging plastic bags could be sources of post pasteurization contamination [51]. Some packaging container, especially plastic, are convenient for bacteria to and adhere and form biofilm [44].

Total coliform count

The mean total coliform count (TCC) of the raw milk samples drawn from receiver tank (CCP1) ranged from zero to 6.70 log10 cfu ml^{-1} (log units) with the average value being 4.09 long unit (Table 5). Three of the 10 raw milk samples (30%) had no detectable coliform bacteria at all. The value for pasteurized milk samples drawn just before packaging (CCP2) declined to undetectable level (Table 5). The mean total coliform count (TCC) of the pasteurized milk samples immediately after packaging (CCP3) was 3.22 log10 cfu ml^{-1} with the range being zero to 7.86 log units. With regard

Table 4: Multiple Comparisons mean *Enterobacteriaceae* counts of milk samples drown from the four Critical Control Points in the pasteurized milk production at a dairy plant, Hawassa.

(I) Stage	(J) Stage	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
(Dependent Variable: EC , Tukey HSD)					Lower Bound	Upper Bound
CCP1	CCP2	6.07200*	.85642	.000	3.7655	8.3785
	CCP3	1.07100	.85642	.600	-1.2355	3.3775
	CCP4	1.28400	.85642	.448	-1.0225	3.5905
CCP2	CCP1	-6.07200*	.85642	.000	-8.3785	-3.7655
	CCP3	-5.00100*	.85642	.000	-7.3075	-2.6945
	CCP4	-4.78800*	.85642	.000	-7.0945	-2.4815
CCP3	CCP1	-1.07100	.85642	.600	-3.3775	1.2355
	CCP2	5.00100*	.85642	.000	2.6945	7.3075
	CCP4	.21300	.85642	.994	-2.0935	2.5195
CCP4	CCP1	-1.28400	.85642	.448	-3.5905	1.0225
	CCP2	4.78800*	.85642	.000	2.4815	7.0945
	CCP3	-.21300	.85642	.994	-2.5195	2.0935

*. The mean difference is significant at the 0.05 level.

CCP1=Critical Control Point 1 (Raw milk), CCP2=Critical Control Point 2 (Pasteurized Milk before Packaging, CCP3=Critical Control Point 3 (pasteurized milk immediately after packaging at the dairy plant, CCP4=Critical Control Point 4 (pasteurized milk at Selling Point in the market).

to pasteurized milk samples at selling point in the retail outlets (CCP4), the mean TCC was 3.48 log units with the range being zero to 6.53 log units. Five of the 10 packed pasteurized milk samples (50%) at the dairy plant and four (40%) of them at the retail market showed no detectable coliform bacteria (Table 5).

Pasteurization had significantly reduced the mean TCC of the raw milk samples. The mean TCC of the pasteurized milk samples just before packaging (CCP2) was significantly lower than that of milk samples at all other stages (Table 6). The mean total coliform count (TCC) of the raw milk samples was significantly higher (P<0.05) than that of the pasteurized milk samples drawn before packaging-CCP2 (Table 6). However, no statistically significant differences were observed in the mean TCC among the raw milk samples and packed pasteurized milk samples from the dairy plant as well as the retail market.

The presence coliform bacteria in foods at very elevated level indicate the unhygienic handling of the food [52]. The mean total coliform count (TCC) of raw milk before pasteurization at the dairy plant (4.09 $\log_{10}$ cfu ml^{-1}) was within the range of the maximum acceptable regulatory limits (10^3 to 5×10^4 cfu ml^{-1}) of both the Ethiopia and East Africa standards [33]. The mean TCC of the raw milk samples in the present study was comparable with the 4.06 log cfu ml^{-1} [29] and the 7×10^4 cfu ml^{-1} or 4.85 log units [7] reported for raw milk samples from dairy plants in Addis Ababa. On the other hand, it was lower than the 5.33×10^6 cfu ml^{-1} or 6.73 log units reported for raw milk samples taken from a dairy processing plant in Addis Ababa [28]. Elsewhere, mean TCC ranging from 10^3 cfu ml^{-1} to 9×10^3 cfu ml^{-1} values were reported for raw milk samples on arrival at a dairy plant in Khartoum, Sudan [53] which is much lower than that of the present study.

Table 5: The mean total coliform count of milk samples in Log10 CFU ml^{-1} collected from four Critical Control Points in the production pasteurized milk at dairy plant, Hawassa.

Sample number	CCP1	CCP2	CCP3	CCP4
1	6.6	0	0	0
2	5.53	0	0	5.6
3	5.5	0	7.66	0
4	0	0	0	0
5	5.49	0	7.86	6.53
6	5.61	0	5.5	5.64
7	5.47	0	0	5.54
8	0	0	5.64	5.7
9	6.7	0	5.57	0
10	0	0	0	5.85
Minimum	0	0	0	0
Maximum	6.7	0	7.86	6.53
Mean (± S.E.)	4.090 (±.90)	.000 (±.000)	3.223 (±1.10)	3.486 (±.95)

Legends: CCP1=Critical Control Point 1 (Raw milk), CCP2=Critical Control Point 2 (Pasteurized Milk) Before Packaging, CCP3=Critical Control Point 3 (pasteurized milk immediately after packaging at the dairy plant, CCP4=Critical Control Point 4 (pasteurized milk at Selling Point in the market), S.E=standard error

Table 6: Multiple Comparisons mean of total coliform counts (TCC) of milk samples drown at four Critical Control Points in pasteurized milk production at dairy plant, Hawassa.

(Dependent Variable: TCC , Tukey HSD)						
(I) Stage	(J) Stage	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CCP1	CCP2	4.09000*	1.21310	.009	.8229	7.3571
	CCP3	.86700	1.21310	.891	-2.4001	4.1341
	CCP4	.60400	1.21310	.959	-2.6631	3.8711
CCP2	CCP1	-4.09000*	1.21310	.009	-7.3571	-.82209
	CCP3	-3.22300	1.21310	.054	-6.4901	.0441
	CCP4	-3.48600*	1.21310	.033	-6.7531	-.2189
CCP3	CCP1	-.86700	1.21310	.891	-4.1341	2.4001
	CCP2	3.22300	1.21310	.054	-.0441	6.4901
	CCP4	-.26300	1.21310	.996	-3.5301	3.0041
CCP4	CCP1	-.60400	1.21310	.959	-3.8711	2.6631
	CCP2	3.48600*	1.21310	.033	.2189	6.7531
	CCP3	.26300	1.21310	.996	-3.0041	3.5301

*. The mean difference is significant at the 0.05 level.

Legends: CCP1=Critical Control Point 1 (Raw milk), CCP2=Critical Control Point 2 (Pasteurized Milk before Packaging, CCP3=Critical Control Point 3 (pasteurized milk immediately after packaging at the dairy plant, CCP4=Critical Control Point 4 (pasteurized milk at Selling Point in the market).

The mean TCC of the pasteurized milk before packaged (zero or undetectable) was in compliance with both the Ethiopian [33] ES3462, 2009 and East Africa standards [34] EAS 69, 2006 that require the TCC of pasteurized milk to be zero and less than 10 cfu ml^{-1}, respectively. It was also in agreement with maximum acceptance limit set by FDA [21] and the European Council Directives 92/46/EEC [42] that recommends the TCC of pasteurized milk to be less than 10 cfu ml^{-1}. The absence of coliform in pasteurized indicates the effectiveness of the process [52]. Similar findings were also reported for samples of pasteurized milk obtained before packaging at two dairy processing plants in Addis Ababa [29]. On the other hand, mean TCC ranging from5 cfu ml^{-1} to 1×10^2 cfu ml^{-1} were reported for samples of pasteurized milk drown before packaging at a dairy plant in Khartoum [53], Sudan which was not in compliance with the recommended standards.

The mean TCC of the pasteurized milk samples immediately after packaged – CCP3 was 3.22 log$_{10}$ cfu ml^{-1}. This value was a dramatic increase from non-detectable level in the unpacked pasteurized milk samples and was much higher than the maximum acceptable limit of 0.00 cfu ml^{-1} and less than 10 cfu ml^{-1} set respectively by the Ethiopian [33] and East African [34] standards for pasteurized milk. Similar to the trend observed for the count of *Enterobacteriaceae* above, this can be due to post pasteurization contamination or re growth of survivors which were in viable but not culturable state (VBNC) following favorable conditions. Coliform are known to enter VBNC state when exposed to extreme conditions [54]. Five of the 10 pasteurized milk samples immediately after packaged (50%) had no detectable coliform bacteria. The mean TCC of the pasteurized milk samples after being packed at the dairy plant in the present study was higher than the 2.60 log10 cfu ml^{-1}, reported for packed pasteurized milk samples obtained from two dairy processing plants in Addis Ababa [29]. It was also higher than that reported 'no detectable coliform bacteria' for packed pasteurized milk from a dairy company in Cairo, Egypt [37].

Likewise, the mean TCC of the pasteurized milk samples at the market point was 3.48 log10 cfu ml^{-1}. This value was higher than the 5.1×10^2 cfu ml^{-1} reported for pasteurized milk samples collected in Addis Ababa [55]. It was also higher than the 1.02 log$_{10}$ cfu ml^{-1} for pasteurized milk samples obtained from markets in Kenya [39]. The observed value in the present study exceeded both the Ethiopian [33] and East Africa [34] standard. Encountering of such unacceptable count in heat treated milk could be due to post pasteurization contamination, temperature abuse in cold chain during transportation and storage in the super markets. Similar reasons forwarded for the packed pasteurized milk samples at the dairy plant (post pasteurization contamination and VBNC) may explain this observations. Four of the ten pasteurized milk samples from the retail market (40%) had no detectable coliform bacteria in compliance with recommended standards.

Fecal coliform counts (FCC)

The fecal coliform counts (FCC) of the raw milk samples drawn from milk receiver tank (CCP1 or RM) ranged from zero to 5.76 log10 cfu ml^{-1} (log units) with the average value being 2.82 long unit (Table 7). Five of the 10 (50%) raw milk samples had no detectable fecal coliform bacteria. However, the FCC was declined to undetectable level all pasteurized samples (Table 7).

The pasteurization process at the dairy plant was effective in eliminating fecal coliform bacteria from raw milk samples and no post pasteurization contamination or growth of VBNC fecal

coliform bacteria were encountered. The mean fecal coliform count of milk samples drawn at CCP1 (RM) was significantly higher (P<0.05) than that of samples drawn at all other stages (Table 8).

Fecal coliform are the subgroup of total coliform bacteria both of which are member of the family *Entrobactriceae* which are used as indicator of fecal contamination. The fecal coliform group consists mostly of *Escherichia coli* that grow and ferment lactose at elevated temperature (45.5°C) and hence refers to thermotolerant

Table 7: The mean fecal coliform counts of milk samples in Log10 CFU ml^{-1} collected from four Critical Control Points in a dairy plant, Hawassa.

Sample number	CCP1	CCP2	CCP3	CCP4
1	5.55	.00	.00	.00
2	5.54	.00	.00	.00
3	.00	.00	.00	.00
4	5.74	.00	.00	.00
5	5.66	.00	.00	.00
6	.00	.00	.00	.00
7	.00	.00	.00	.00
8	5.76	.00	.00	.00
9	.00	.00	.00	.00
10	.00	.00	.00	.00
Minimum	.00	.00	.00	.00
Maximum	5.76	.00	.00	.00
Mean (± S.E.)	2.82 (±.941)	.000 (±.000)	.000 (±.000)	.000 (±.000)

Legends: CCP1=Critical Control Point 1 (Raw milk), CCP2=Critical Control Point 2 (Pasteurized Milk) Before Packaging, CCP3=Critical Control Point 3 (pasteurized milk immediately after packaging at the dairy plant, CCP4=Critical Control Point 4 (pasteurized milk at Selling Point in the market), S.E=standard error

Table 8: Multiple Comparisons mean of fecal coliform counts (FCC) of milk samples drown at four Critical Control Points in pasteurized milk production at a dairy plant, Hawassa.

Dependent Variable: FCC , Tukey HSD						
(I) Stage	(J) Stage	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CCP1	CCP2	2.82500*	.66604	.001	1.0312	4.6188
	CCP3	2.82500*	.66604	.001	1.0312	4.6188
	CCP4	2.82500*	.66604	.001	1.0312	4.6188
CCP2	CCP1	-2.82500*	.66604	.001	-4.6188	-1.0312
	CCP3	.00000	.66604	1.000	-1.7938	1.7938
	CCP4	.00000	.66604	1.000	-1.7938	1.7938
CCP3	CCP1	-2.82500*	.66604	.001	-4.6188	-1.0312
	CCP2	.00000	.66604	1.000	-1.7938	1.7938
	CCP4	.00000	.66604	1.000	-1.7938	1.7938
CCP4	CCP1	-2.82500*	.66604	.001	-4.6188	-1.0312
	CCP2	.00000	.66604	1.000	-1.7938	1.7938
	CCP3	.00000	.66604	1.000	-1.7938	1.7938

*. The mean difference is significant at the 0.05 level.

Legends: CCP1=Critical Control Point 1 (Raw milk), CCP2=Critical Control Point 2 (Pasteurized Milk) Before Packaging, CCP3=Critical Control Point 3 (pasteurized milk immediately after packaging at the dairy plant, CCP4=Critical Control Point 4 (pasteurized milk at Selling Point in the market)

coliforms [18]. *E. coli* is used to indicate recent fecal contamination or unsanitary conditions in processing. It is widely distributed in the intestines of humans and warm-blooded animals and is the predominant facultative anaerobe in the bowel and part of the essential intestinal flora. The origin of contamination milk could be from poor hygienic practice, such as cross contamination from handlers and from material that have been contaminated with feces [43]. The Fecal coliform bacteria like *E. coli* easily killed during pasteurization and their presences in heat treated food may indicate a post fecal contamination or inadequate pasteurization [21].

Identification of the dominant mesophilic aerobic bacterial flora

By selecting morphologically distinct colonies from countable PCA plates, a total of 173 mesophilic aerobic bacteria (MAB) were isolated. These consisted of 47 from raw milk samples –CCP1, 42 from unpacked pasteurized milk samples – CCP2, 37 from the pasteurized milk samples immediately after packaging at the dairy plant – CCP3 and 47 from pasteurized milk samples in the retail market – CCP4 (Table 9).

Gram staining, microscopy and biochemical tests allowed identification of 142 isolates (82.18%) into 10 putative genera. Overall, 89 (51.45%) of the isolates were gram negative bacteria whereas 84 (48.55%) were gram positive bacteria. Among the putatively identified genera the majority belonged to *Bacillus* (35/173 or 20.23%) followed by *Pseudomonas* (30/173 or 17.34%), *Staphylococcus* (27/173 or 15.61%) and *Streptococcus* (18/173 or10.40%). The other genera that occurred in significant number included isolates closely related *Shigella* (14/173 or 8.10%) and *Escherichia coli* (10/173 or 5.78%) whereas isolates related to, *Citrobacter, Enterobacter, Klebsiella* and *Salmonella* occurred in numbers less than five each (Table 9).

The dominant aerobic mesophilic bacteria in the raw milk samples (CCP1) were isolates related to the genera *Bacillus* (10/47 or 21.27%), *Staphylococcus* (9/47 or 19.15%), *Streptococcus* (7/47 or 14.89%), and *E. coli* (6/47 or12.77%). On the other hand isolates related to *Pseudomonas* (4/47 or 8.51%) and *Salmonella* (2/47 or

4.26%) occurred at less significant frequencies (Table 4). In addition to the putatively identified genera, the collective, the occurrence of isolates related to unidentified *Enterobacteriaceae* (9/45 or 20%) in the raw milk samples was equivalent to the frequency of the *Staphylococcus*. This is suggestive of the unhygienic handling and poor sanitary quality of the raw milk sample used for processing. Moreover, the occurrence of isolates related to *Salmonella* in two of the 10 raw milk samples (20%) should be of great concern. The incidence of salmonellae in the raw milk samples in this study was higher than that of the reported 11.9% incidence rate [56], but was lower than the 22.2% incidence in raw milk samples from India [57].

Members of the genus *Bacillus* are abundantly found in the environment owing to endospore formation, and can get into the milk *via* spreading from soil and animal feed to the udders of dairy cows and then into raw milk [58]. The other source of these bacteria may be unhygienic milking procedure, such as using unclean equipments and improper or inadequate cleaning the udder before milking [4]. *Bacillus* species, particularly *Bacillus cereus*, may play a dual role as spoilage agent (e.g. causing bitty cream) and as pathogen causing serious health problem in human [52].

Staphylococci are commonly found on the on the skin of humans and animals. Therefore, all foods including milk those handled by human may be contaminated by these bacteria [43]. The other potential source of these bacteria might be mastitic cows. *S aureus* is one of the members of *Staphylococci*, which is known to form a heat stable toxin that can cause inflammation in human [52]. However, large number of the bacteria is required to form sufficient amount of the toxin for intoxication to occur from consumption of contaminated food [59].

In the unpacked pasteurized milk samples (CCP2) *Bacillus* (10/42 or 23.81%) prevailed as the most frequently encountered genera as in the case of the raw milk samples. On the other hand the frequency of occurrences of *Staphylococcus* (6/42 or 14.3%) and *Streptococcus* (3 or 7.14%) declined while *E. coli* was not detectable in the unpacked pasteurized milk samples (Table 10). Surprisingly the frequency of occurrence of isolates related to the genus *Pseudomonas* (9/42 or 21.43%) more than doubled. Moreover, the collective frequency of occurrence of isolates related to the members of *Enterobacteriaceae*

Table 9: The dominant mesophilic aerobic bacterial flora of milk samples at four presumptive critical points in a dairy processing plant in Hawassa, Southern Ethiopia, 2019.

Putatively identified genera	Milk sampling point (Critical points - CCP)				
	CCP1	CCP2	CCP3	CCP4	Total
Bacillus	10 (21.27%)	10 (23.81%)	8(21.62%)	7 (14.89%)	35
Citrobacter	Nd	Nd	Nd	2 (4.26%)	2
E. coli	6 (12.77%)	Nd	1(2.70%)	3 (6.38%)	10
Enterobacter	Nd	Nd	Nd	1(2.13%)	1
[1]Nid Ent	9 (19.15%)	14 (33.33%)	1(2.70%)	3 (6. 38%)	27
Klebsiella	Nd	Nd	3 (8.11%)	Nd	3
Pseudomonas	4 (8.51%)	9 (21.43%)	11(29.73%)	6 (12.77%)	30
Salmonella	2 (4.26)	Nd	Nd	Nd	2
Shigella	Nd	Nd	6 (16.22%)	8 (17.02%)	14
Staphylococcus	9 (19.15%)	6 (14.29%)	4 (10.81%)	8 (17.02%)	27
Streptococcus	7 (14.89%)	3 (7.14%)	1 (2.70%)	7 (14.89%)	18
[2]Nid GP rod	Nd	Nd	2(5.41%)	2 (4.26%)	4
Total	47	42	37	47	173

Legends: Nd=Not Detected, CCP1=Raw milk, CCP2=Pasteurized milk before packaging, CCP3=Packed pasteurized milk at dairy plant, CCP4=Packed pasteurized milk in retail market [1]=unidentified *Enterobacteriaceae*, [2]=Unidentified, non-spore forming, Gram positive rod.

Table 10: The incidence of *Escherichia coli* in the samples of raw milk used for the production of pasteurized milk at a dairy plant in Hawassa, 2019.

Sample number	*Number of presumptive isolates screened	No of confirmed colonies that grew on EMB
1	5	1
2	5	0
3	0	0
4	0	0
5	5	3
6	5	1
7	0	0
8	0	0
9	5	2
10	5	2
Total	30	9 (30%)

Isolates randomly picked from fecal coliform count plates for confirmation by biochemical test

(14/42 or 33.3%) also increased and was higher than that of *Bacillus*.

Members of the genus *Bacillus* are among the theremoduric bacteria which can survive pasteurization [60]. Raw milk may be contaminated from an a mastitic mammary gland, udder, teat surfaces, milking utensils or storage tanks. The major concern to the dairy industry is *B. cereus* which forms heat resistant endospores. The spores are resistant to high temperature short time pasteurization and instead of being killed it germinate to vegetative form that will proliferate subsequently [59]. When ingested in numbers as high as 10^7 cells it may lead to diarrhea by production of enterotoxin in the intestine. On the other hand ingestion of food containing sufficient amount of the preformed enterotoxin causes emetic syndrome. *Pseudomonas* and members of the *Enterobacteriaceae* in general cannot survive pasteurization. Therefore, the presence of these bacteria in the pasteurized milk indicates the post pasteurization contamination. This finding reinforced that other than the raw milk, the process steps between pasteurization and packaging of the pasteurized milk are important critical points.

Pseudomonas (11/37 or 29.7%) and member of *Enterobacteriaceae* (32.4%) attained prominence in frequency of isolation in the pasteurized milk samples drown immediately after packaging at the dairy plant (CCP3). Moreover, isolates related to the *Shigella* (6/37 or 16.22%) occurred at significant frequency (Table 10). On the other hand *Bacillus* (8/37 or 21.6%) and the other gram positive bacteria, *Staphylococcus* and *Streptococcus* showed a decline in frequency. The source of *Pseudomonas* in packed pasteurized milk at dairy farm most likely was post pasteurization contamination that occurred during filling and packaging process [47]. They are important spoilage bacteria with proteolytic and lipolytic activity and causes defects, such as rancidity and bitter off flavor [61].

The predominant aerobic mesophilic bacterial genera in the pasteurized milk samples from the market (CCP4) were *Shigella* (8/47 or 17.02%)), *Staphylococcus* (8/47 or 17.02%), *Bacillus* (7/47 or 14.9%)), *Streptococcus* (7/47 or 14.90%)) and *Pseudomonas* (6/47 or 12.8%). The general pattern was a rise in the frequency of Gram positive bacteria back to predominance except that of isolates related to *Shigella* (Table 10). *Staphylococcus and Shigella* survives neither the effect of pasteurization nor refrigeration. Thus, their occurrence in milk sample at this stage might be due to temperature abuse or lack of cold chain during transportation and storage at retail market.

Any increase in temperature above 4°C will provide favorable condition for the growth of pasteurization survivors or post

pasteurization contaminants in milk [60]. Presence of both *Staphylococcus* and *Shigella* in packed pasteurized milk is hazardous and poses health risk to the public. Unlike the case of the raw milk samples, no salmonellae were detected in any of the pasteurized milk samples. A Previous work reported similar observations regarding the absence of salmonellae in pasteurized milk samples from Hawassa city [5]. Elsewhere, reported the detection of salmonellae in 20% of pasteurized milk samples from markets in India [57].

Detection of *Escherichia coli* in the milk samples

Escherichia coli accounted for about 30% (9 of 30) the fecal coliform count in the raw milk samples (2.82 ±.941 log units) and were detected in 60% (6/10) of the raw milk samples (Table 9). *E. coli* is part of the normal flora in the intestine of warm blooded animals including humans and its presence in food, including milk and milk products is commonly used as indicator of fecal contamination. The detection of *E. coli* in food samples is used to monitor the effectiveness of hygienic measures undertaken in food processing [43]. The incidence of *E. coli* in the raw milk samples in the present study (60%) was higher than the 24% reported for raw milk samples from a dairy plant in Khartoum, Sudan [53].

In the present study, *E. coli* was not detected in any of the pasteurized milk samples. According to the East Africa standard EAS 69/2006 [34] for pasteurized milk, the pasteurized milk must be devoid of *E. coli*. Therefore all the pasteurized milk samples from the dairy plant in this study were in compliance with the East Africa standard. Similar finding was reported for pasteurized milk samples collected from Khartoum, Sudan [53]. Previous works reported incidence rate of 25% and 60% for *E. coli* in pasteurized milk sample collected from markets in Hawassa and Addis Abeba, respectively [5,28].

CONCLUSION AND RECOMMENDATIONS

The aim of this work was to assess microbial hazard in the value chain of pasteurized milk production at a dairy plant by microbiological laboratory analysis of milk samples drawn from four presumptive critical control points. Based on the results and discussions in the foregoing sections the following conclusions may be drawn:

- The microbial quality of the raw milk samples (CCP1) used by the dairy plant was the most important critical control point as shown by nearly all the microbial load parameters that exceeded the local and international recommended standard limits

- The microbial load of the raw milk samples used by the dairy plant was so high that it undermined the effectiveness of the pasteurization process.

- Despite a 3 log unit reduction compared to the raw milk, 90% of the pasteurized milk samples immediately before packaging (CCP2) had mean MABC higher than 5 log units

- The mesophilic aerobic bacteria of the samples were dominated by Gram negative bacteria (*Pseudomonas*, lactose fermenter and non-lactose fermenter *Enterobacteriaceae*) that are normally known to be highly sensitive to pasteurization process; suggesting post pasteurization contamination.

- Since the pasteurization process achieved a 3 log reduction in the mean MABC, it can be considered in compliance with the standard efficacy that recommends a one log reduction

- The efficacy of the pasteurization process was affirmed by the reduction of the EC, TCC and FCC to undetectable levels in all of (100%) the pasteurized milk samples just before packaging (CCP2)

- *Escherichia coli* accounted for 30% of the TCC of the raw milk samples. Moreover isolates related to *Salmonella* species were detected in two of the 10 (20%) raw milk samples which is not acceptable according to recommended guidelines

- Paradoxically, Enterobacteriaceae and coliform bacteria were detectable in the majority of the packed pasteurized milk samples at the dairy plant (CPP3) and the retail market (CCP4) at levels higher than 5 log units suggesting gross post pasteurization contamination and temperature abuse

- Therefore the second most important critical control points are the process steps following pasteurization – between filling/packaging and storage in retail markets (CCP3 and CCP4)

- Likewise 80% of the packed pasteurized milk samples at the dairy plant (CCP3) and at the retail level (CCP4) had *Enterobacteriaceae* count higher than 5 log units

- Five of the 10 (50%) packed pasteurized milks samples at the dairy plant (CCP3) and 60% of them at the retail level had TCC higher than 5 log units

- All (100%) of the pasteurized milk samples had no detectable fecal coliform bacteria. However, a significant number of the packed pasteurized milk samples at the dairy plant as well as at the retail level had isolates related to pathogens like *Shigella* species and potential spoilage bacteria like *Pseudomonas* and *Bacillus* species.

- These findings calls for interventions for appraisal of internal quality control program in the dairy plant by reviewing the good manufacturing practice and critical microbial hazard control points monitoring with focus on the raw milk used for processing and stages following pasteurizations such sterility checking of plastic packages, filling system, cold chain transport and storage.

- Quality-based incentive payment to farmers should be instituted at raw milk collection centers so that farmers will recognize the value of keeping hygienic practices in their milk production system

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