International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **8(12),** 16-26, December **(2019)** *Int. Res. J. Biological Sci.*

Microbial quality and antibiotic residues in pasteurised milk in and around Addis Ababa

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Available online at: www.isca.in, www.isca.me
Received 21st August 2019, revised 4th November 2019, accepted 10th December 2019

Abstract

Milk samples were collected in and around Addis Ababa city from nine milk processing plants and twenty three supermarkets. A total of 100 (27 from line, 27 from packed, 46 from supermarkets) pasteurized milk samples were collected. The overall average of total bacteria and coliform count of pasteurized milk from processing plants and supermarkets was 5.08 log_{10}cfu ml^{-1}, 4.059 log_{10} cfu $ml^{-}1$, 5.44 log_{10}cfu ml^{-1} and 3.89 log_{10}cfu ml^{-1} respectively. In all processing plants and supermarkets yeast and mould was not detected. Coliform and total bacterial count did not significantly vary (P>0.05) in pasteurized milk from the different categories of processing plants. The average total bacterial count in milk from different categories of supermarkets were significantly different (P<0.05). To ensure safety and quality of milk and health of the public, it is suggested to follow hygienic practice on milk production and handling.

Keywords: Antibiotic residues, coliform, shelflife, pasteurisation efficiency.

Introduction

The safety of milk is an important to protect human health. Milk safety issue that can pose health risk to human include microbial contamination and drug residues. Testing microbial quality of the milk can give information on the level of milk contamination. Normally milk has both gram negative and gram positive bacteria, but high population number of gram negative bacteria in milk or detection of antibiotic residues is an indication of hazard for health[1].

Milk secreted from healthy animal's alveoli is a sterile. However after secretion from alveoli and stored in udder for let downing, it become contaminated by microorganism those enter into the udder through the teat ducts. Milk cans and storage tanks are also another major source of biofilm for raw milk contamination at industry level. Microorganisms may easily spoil and reduce milk shelf life. Raw milk must be treated by heat to increase its shelf life and to avoid public health effect[2].

Milking cow which is under treatment is a main source of antibiotics in milk. Drug given for the cow either as treatment or vaccination has limited withdrawal and contaminates milk at various stages throughout its value chain. Some milk producers deliver milk from treated animal to collection centre and contaminate other bulky milk. Because of antibiotic negative impact on human health, fluid milk used for consumption must be free from antibiotic residues. The presence of antibiotics in milk also inhibits multiplication of starter culture during the production of fermented milk product such as yoghurt production[3].

Pasteurization, sterilization (in bottle) and UHT (ultra-high temperature) treatment integrated with aseptic packing are treatments used to eliminate gram negative bacteria. Sterilization is the term applied to a heat treatment process which has a bactericidal effect greater than pasteurization. It gives the processed milk a longer shelf life than raw milk. Because the milk is sterilized by high temperature, the product has a cooked flavour and a pronounced brown colour. Unlike sterilization, pasteurization is not intended to kill spore forming bacteria such as bacillus and Clostridium species in the food or liquid. Instead, pasteurization aims to reduce the number of viable pathogens so that they are unlikely to cause disease[4].

Milk normally has gram positive and negative bacteria. At suitable condition bacterial numbers increase within a short time and contaminate the milk by multiplying it and producing toxin in the milk. To eliminate those contaminant bacteria proper combined high temperature to short time pasteurization system is required. Due to under pasteurization some pathogenic psychotropic bacteria resist the heat and multiple its self within a short period of time during storage in cold room after pasteurization. Due to contamination milk shelf life may be reduce than the expected.

In addition utilizing of contaminated milk has its own impact on health of the consumer. So, thesis research were covered microbial and antibiotic contamination level of milk samples collected from processing plants and supermarkets. Due to contaminate its effect on shelf life of pasteurised milk were also observed.

The purpose of this quantitative study is to describe quality status of pasteurised milk interm of microbial load (total bacteria, coliform, yeast and mould), shelf life and antibiotic residue.

Materials and methods

Study area: The study was carried out from January to June, 2018 in and around Addis Ababa city, which has 4.6 million populations[5]. The city has 10 sub cities and located at a latitude of 8°58'N and longitude of 38°47'E. It is situated at an altitude/elevation of 2324 m above sea level. The regular rainy season extends from June to September. Mean Annual rain fall ranges between 800 -1200mm with mean annual temperature of 18°C.

Sampling method: This research covers milk processing companies under three main categories. List of milk processing company was obtained from the annual report of Ethiopia Meat and Dairy Industry Development Institute. Milk processing plants were categorized into three based on their number of employees, capital and actual daily processing capacity. Those categories are: small-scale (have less than 25 employees, capital < 50,000 birr and 500 - 3,000 litre/day), Medium-scale (has 26 – 50 employees, capital 50,000 - 26,000,000 birr and 3,001– 15,000 litre/day) and Large-scale processors have greater than 50 employees, capital >26,000,000 birr and >15,000 litre/day. According to the report there are 19 small scale processors (52.8%), 7 medium scale (19%) and 10 large scale (27.8%) milk processing companies in the country. Among large scale milk processing companies, Shola, Mama and Family milk processing, medium milk processing companies, Elemtu, Holland dairy and Nuredin Hassen and small milk processing companies, Berta milk, zagol milk and Ada milk had been purposively selected for sampling purpose. Each processing company were coded with a letter in order to avoid any bias. Each milk processing company were visited three times and two milk samples per visit were collected from each company on the course of study. Pasteurized milk from line before packing and pack pasteurised milk from outlet were collected.

In addition twenty three Supermarkets from fifty six supermarkets found in Addis Ababa city were randomly selected and categorised into four based on facility they use to extend the shelf life of milk. Basically in supermarket fridge and open cold display refrigerated systems are used to extend the shelf life of milk. Some supermarkets have standby generator to be used during power off. Based on those facilities supermarkets were categorised as those using standby generator with open cold display (Group 1), standby generator with fridge (Group 2), only open cold display (Group 3) and only fridge (Group 4). Each supermarket was visited twice and 46 milk samples were collected (Table-1). Samples were collected randomly. For microbial load and Sensory evaluation test 500ml of packed pasteurized samples were collected from processing plants and supermarkets. For pasteurization efficiency determination 250ml of milk samples were collected from line of processing plants before packing.

Total sample size required was determined by general formula[6]. It was calculated based on 95% confidence interval, which is only 5% standard error.

$N=0.25/SE^2=100$

Where: N=Total sample size and SE^2=Standard error.

Table-1: Sampling layout for Microbial and antibiotic residues test.

Type of sample	Processing plant			Retailer (Supermarket)	Overall
	S	M	L		
Line sample	9	9	9	-	27
Packed pasteurized milk	9	9	9	46	73
Total	18	18	18	46	100

S= Large Scale M= Medium Scale and L= Small Scale.

Sample collection and transportation: All milk samples were collected in the morning aseptically following standard procedure[7]. In order to control microbial multiplication during transporting icebox (at 4°C) was used. Samples were brought to Ethiopia Meat and Dairy Industry Development Institute dairy laboratory for microbial analysis and sensory evaluation within 6 hour interval. Antibiotic residue testing was carried out at Lame dairy private limited company dairy laboratory, Addis Ababa.

For total bacteria count, appropriate decimal dilutions were selected to gave the expected total number of colonies on a plate, i.e., between 30 and 300 colonies[7]. For analysis, Himedia brand (India) plate count agar and Himedia brand (India) peptone water were required. Both of them were prepared according to manufacturing instruction. Sterilized Agar was cool to 45°C after autoclaved and before pouring to petri-dish. One ml of milk sample was added into sterile test tube containing nine ml peptone water up to serial dilution 10^{-5} and mixed carefully by vortex. Then one ml of the sample from appropriate decimal dilution was placed on the petri-dish and by pouring method molten agars (10-15ml) was poured onto petri-dish, slowly shake and then incubated for 48h at 30°C[8].

Coliform count: For Coliform count, Oxoid brand (UK) Violet red bile agar and Himedia brand (India) peptone water were used. Both preparations were proceed according to manufacturing instruction. Sterilized Agar was cooled to 45°C before pouring to petri-dish. One ml of milk sample was added into sterile test tube containing nine ml peptone water up to

serial dilution of 10^{-5} and mixed carefully by vortex. Then one ml of the sample from appropriate decimal dilution was placed on the petri-dish and then molten agar (10-15ml) was add by pouring method onto petri-dish and slowly shock then incubated for 24 hours at 30°C. Finally, colony count was made using colony counter[9].

Yeast and mould testing: For yeast and mould test, I used Himedia brand (India) Malt extract agar and Himedia brand (India) peptone water were used. Both preparations proceed as according to manufacturer's instruction. Agar was cooled to 45°C after autoclaved and before pouring to petri-dish. One ml of milk sample was added into sterile test tube containing nine ml peptone water up to serial dilution of 10^{-4} and mixed carefully by vortex. Then one ml of the sample from appropriate decimal dilution was placed on the petri-dish and then molten agar (10-15ml) was poured onto petri-dish by pouring method and incubated at 30°C for five days by inverting the petri-dish. Finally, colony counter was used to count the colony[10].

The estimated number of colony per millilitre was calculated using the following formula:

$$\text{CFU/ml}= \frac{\Sigma C}{V}(n1 + 0.1n2)d$$

Where C= is the sum of colonies on all plates count, V= is the volume applied to each plate, n1 = is the number of plates counted at first dilution, n2 = is the number of plates counted at second dilution, d= is the dilution from which first count was obtained, CFU/ml= is the average plate count. The result was rounded to two significant figures and expressed as a number between1.0 and 9.9 multiplied by 10x where x is the appropriate power of 10.The best two consecutive dilutions, as n1 and n2 were used to calculate the results.

Phosphatase test: Reagent A and B need for the analysis were prepared as follows.

Reagent A was prepared by dissolving 2.65gram of sodium carbonate in 100ml of boiled water. In addition 100mg of di-sodium-phenyl phosphate was also dissolved in 80-90ml of boiled water. Three ml of sodium carbonate solution was added to the di-sodium phenyl phosphate solution and filled up to 100ml with boiled water.

Reagent B was prepared by dissolving 40-46 mg of di-bromide-chinon-chlorimide in 10ml of ethanol 96%. Duplicated sample was prepared by pippeting 0.5ml of milk in to clean test tubes. Control sample was boiled up to 100°C. To each test tube 5 ml of the reagent A was added and the test tubes were closed with well cleaned rubber stoppers to mix the content by swinging the test tubes around. Then test tubes were put into water bath of 35±2°C and left for 1 hour. To each test tube 6 drops (0.2 ml) of reagent B was added and mixed. After 5 minutes colour of the test tubes and control test tube (boiled milk) were compared. If test sample colour change to dark blue, it is positive otherwise negative[11].

Antibiotic residual analysis in milk: To test antibiotic residue in milk Copan testing method was pursued. All samples were well mixed before testing. Test strip held horizontally in Rosa incubator and peel at ''peel to here'' line. A 300µ pipette was used for sampling taking and micro pipette held vertically and pipette slowly into sample compartment at Rosa incubator. The samples were resealed and lid closed on Rosa incubator for 8 minute at 56°C. Finally based on colour of T and C line sample result was identified. Equal red colours of two lines indicate that the sample is positive for antibiotic otherwise T line is not red colour as C line and the result is negative. Positive sample is a sample which has antibiotic residue and negative sample not.

Sample testing (for total bacteria count, coliform testing, phosphatase testing and yeast and mould testing) was conducted at Ethiopian Meat and Dairy Industry Development Institute dairy Laboratory. In addition antibiotic residue testing also conducted at Lame dairy Private Limited Company Dairy Laboratory.

Shelf life for pasteurised milk: The sensory testing was carried out following Weibull Hazard method, where the initial number of panellists was n_0=3 and the constant with which the number of panellists was increased for each subsequent test was n=1. It was evaluated by ten experienced panellists. All participants are dairy exports and they well know characteristic of milk. The same batches of pasteurised milk were frequently evaluated at (1^{st}, 5^{th}, 7^{th}, and 9^{th}) day of its production. Generally, it was categorised into four (Excellent, Good, Fair and poor) groups based on pasteurised milk physical characteristic (colour, smell and appearance). Each physical characteristic were evaluated and scored at four evaluation days. Final acceptance of sensory evaluation was done based on ADSA scoring method and evaluation score < 36 not acceptable (Table 2 and 3). Consumer acceptability is usually determined by consumer tests' using a large number of untrained tasters in which samples with various ages are tasted together.

Table-2: Score Card for milk[12].

Score Card	
Contribution	Perfect Score
Odour	20
Flavour	40
Body(Consistency)	30
Colour and Appearance	10
Total	100

Table-3: General Score card guide[12].

Quality of pasteurised milk	Grade	Defect and Intensity	Approximate Score (%)
Excellent	A	No defect	More than 90%
Good	B	Flavour: Flat, slight cooked/ stale/ barny/ feed/ neutralized/ salty Texture: defects of only slight intensity	More than 80% but less than 90%
Fair	C	Flavour: Definite cooked/ barny/ neutralized/ feed/ flat. Slight rancid/ oxidized/ metallic/ fishy/ yeasty/ mouldy/ acidic Texture: Any texture defect of definite intensity	More than 60% but less than 80%
Poor	D	Texture: Pronounced defect	Less than 59%. The products are generally unacceptable at this score

Data management and analysis: Microsoft excel spread sheet were used to enter pasteurised milk sample data and to transform total bacterial count and coliform count to $\log_{10}$. To analysis data SPSS version 16.0 software were used for descriptive statistics, Chi-square (X^2) and Crosstabs between the variable to show their association.
For analysis, 95% CI and P-value < 0.05 was set for statistical significance of an estimate. The following model was used for statistical analysis;

$$Yijk= \mu + \beta j + Dj + \beta D(ij) + eijk$$

Where, Yij= individual observation for each test, μ= overall mean, Bj= the i^{th} milk source effect (Processing plants and Supermarkets)

Dj= the jth processing capacity effect
βD(ij)= interaction effect ,and eijk= the error term

Results and discussion

Microbial quality of pasteurised milk: No significant differences (*P>0.05*) were observed in total bacterial count and coliform between pasteurized milk from the different milk processing plants (Table-4). However, mean microbial count of each processing companies scale were different from one another. This difference may be due milk handling difference, material they used for pasteurization, transportation and etc. In within a scale large scale processing plants are more different within its scale in comparative to the other. No yeast and mould was detected in all pasteurized milk sample collected from the different processing plants. Total bacteria and coliform were detected in the range of 4.76 to 5.48 $\log_{10}$cfu ml^{-1} and 3.42 to 4.78 $\log_{10}$cfu ml^{-1} respectively.

The study on microorganisms from the supermarket shows that total bacteria count and coliform count ranged from 4.39 to 6.13 $\log_{10}$cfu ml^{-1} and 3.24 to 4.25 $\log_{10}$cfu ml^{-1} respectively (Table-4). Mean total bacteria count of supermarket at P< 0.05 is significantly different between groups of supermarket but mean coliform count at p>0.05 is not significantly different between groups of supermarket.

Group three supermarkets are highly significantly different from the other groups due to easily expose of milk for contamination during power off as comparing to other groups. This group use only open cold display which has less potential to hold refrigeration during power off.

The overall average total bacteria count in pasteurized milk from processing plants and supermarkets was 5.08$\log_{10}$cfu ml^{-1} and 5.44$\log_{10}$cfu ml^{-1} respectively. In Sudan found total bacteria count within a range of 5.81-14.81 $\log_{10}$cfu ml^{-1} which is higher than the current study result[13]. Very good quality pasteurised milk should contain less than 4.70 $\log_{10}$cfu ml^{-1} total bacteria counts and good quality milk should have population within a range of 4.70-5.0 $\log_{10}$cfu ml^{-1}.

According to Ethiopian standards, the current study result for total bacteria count (5.08 $\log_{10}$cfu ml^{-1}) is beyond the acceptable limit and it need controlling system. In milk samples collected from Addis Ababa and Bahir Dar 6.322 to 6.556 $\log_{10}$cfu ml^{-1} of aerobic plate count were found[5]. Their result is higher than the current study result and the limit set by the Ethiopian standard. The variation in the number of microorganisms reported between different authors could be due to factors such as raw milk quality, pasteurization efficiency and post pasteurization handling among others.

Post pasteurization contamination was the main source for pasteurized milk quality problem. It can be contaminated due to improper milk handling, transportation system and packaging material hygiene. Therefore, processing plants have to take proper actions in protecting the processed milk from post processing contamination.

Coli form result of the current study is 4.059 $\log_{10}$cfu ml^{-1}. In Sudan reported coliform count found with a range of 12.8 to 14.8 $\log_{10}$cfu ml^{-1} which states that storage temperature has significant effect on bacteria count[13]. According to US standards, coliform count in pasteurized milk should be less than 1 $\log_{10}$cfu/ml. The Ethiopian standard agency has stated that pasteurized milk must contain a faecal coliforms nil per ml or non faecal coliforms not more than 1 $\log_{10}$cfu ml^{-1}.

High coliform count in the pasteurized milk might be due to post-pasteurization contamination of the milk. Since coliform are associated with faecal contamination, sanitation and hygiene related issues need to be improved in the different milk processing plants in the country and the responsible bodies need to work towards ensuring this.

High temperature-short time combination pasteurized milk should contain less number of microorganisms within the range of 2-2.3$\log_{10}$cfu ml^{-1} and a coliform count below $\log_{10}$cfu ml^{-1}. A coliform count between 2 and 3 $\log_{10}$cfu/ml usually indicates poor milk handling and a coliform count >3 $\log_{10}$ suggest that bacterial growth is occurring on milk handling equipment[14]. The coliform count under the current study is high due to post process contamination that resulted in the growth of bacteria in pasteurized milk.

Normally, pasteurised milk from healthy udders contains < 3 log10cfu ml-1 of total bacteria count which will not have an adverse effect in the total numbers of microorganisms in the bulk milk during refrigerated store[15].

High contamination of pasteurised milk may be due to milk contamination after pasteurization by different factors. It may contaminate due to absence of cooling system, delay for cooling after pasteurization or absence of dust controlling system from packaging material during storage or packing time. Transporting pasteurized milk by normal car which has no refrigeration system could result in poor milk microbial quality. Such kind of milk contains high number of total bacteria count and coliform.

Interaction between milk processing plants category in term of their mean microbial load and antibiotics: In term of total bacterial and coliform count interaction between all milk processing plants category was not significantly different at $p > 0.05$. In addition antibiotic interaction between medium scales and small scale milk processing plants were also not significantly different. However, interaction between large scale to small scale and large scale to medium scale had significantly different at $p < 0.05$ (Table-5). This may be due to antibiotic contamination of milk samples collected from large scale milk processing plants. Large scale milk processing plants daily collect more than 20,000 litres and cannot test antibiotic residue as quality parameter.

In pasteurised 10.8% of antibiotic residues were occurred[16]. In Brazil indicated that 4.3% milk samples had detectable level of residue for antibiotic[17]. In Sweden indicated that 0.08-0.26% milk samples were positive for antibiotic residues which were lower than the findings of the current study[18].

In addition some literature indicate that antibiotics can induce cancer and other non-cancerous hazardous effects on the body after for a long period of antibiotic contaminated milk utilization and its toxin accumulations in the body[19]. Development of resistance to the class of antibiotics to which the consumers are exposed to is one of the adverse effects of consuming contaminated milk. In comparative to young people's children can use more milk. Pasteurised milk that contains antibiotic residue has more effect on children consuming milk frequently as compared to other consumers. Children and adult people using milk can be affected by the problem.

Antibiotics residue can also cause a wide variety of dairy manufacturing problems, including; inadequate milk curdling or suboptimal ripening during cheese production, inadequate acidity and flavour attributes during the manufacture of butter milk, suboptimal starter culture growth and false results during quality control testing due to the presence of interfering drug metabolites.

The current study result have shown that establishing effective monitoring system throughout the country is very critical issue to control the problem with antibiotics residues in milk and to produce a safe product with no health risks to consumers.

Table-4: Mean ± S.E. For microbial counts of pasteurised milk samples collected from different milk processing plants and Supermarkets expressed in cfu ml^{-1}.

Source	Category	TBC	CC
Processing plants	Large Scale	4.756^{a}±0.53	3.972^{a}±0.75
	Medium Scale	4.991^{a}±0.40	3.420^{a}±0.36
	Small Scale	5.483^{a}±0.40	4.783^{a}±0.47
	P-value	0.476	0.237
	Overall mean	5.077±0.24	4.059±0.33
Supermarkets	1	4.39^{a} ±1.22	3.57^{a}±0.69
	2	4.56^{a} ±0.09	3.24^{a}±0.33
	3	6.13^{b}±0.26	4.25^{a}±0.32
	4	5.68^{b}±0.25	4.19^{a}±0.54
	P-value	0.005	0.286
	Overall mean	5.44±0.20	3.89±0.24

*1= Standby Generator with open cold display, 2= Standby Generator with fridge, 3= only cold display, 4= only fridge, TBC=Total bacteria count and CC= Coliform count.

Table-5: Multiple comparison between processing plants category in term of their microbial load and antibiotics.

Dependent Variable		(I)	(J)	MD IJ	Std. Error	Sig.
Total bacterial count	LSD	Large Scale	Medium Scale	-0.24	0.60	0.70
			Small Scale	-0.73	0.60	0.24
		Medium Scale	Large Scale	0.24	0.60	0.73
			Small Scale	-0.49	0.60	0.42
		Small Scale	Large Scale	0.73	0.60	0.24
			Medium Scale	0.49	0.60	0.42
Coliform	LSD	Large Scale	Medium Scale	0.55	0.78	0.49
			Small Scale	-0.81	0.78	0.31
		Medium Scale	Large Scale	-0.55	0.78	0.49
			Small Scale	-1.36	0.78	0.09
		Small Scale	Large Scale	0.81	0.78	0.31
			Medium Scale	1.36	0.78	0.09
	LSD	Large Scale	Medium Scale	-0.3*	0.14	0.02
			Small Scale	-0.3*	0.14	0.02
		Medium Scale	Large Scale	0.3*	0.14	0.02
			Small Scale	0.00	0.14	1.00
		Small Scale	Large Scale	0.33*	0.14	0.02
			Medium Scale	0.00	0.14	1.00

Interaction between supermarkets category in term of their microbial load and antibiotics: Mean total bacterial count interaction between supermarkets category A and B, A and D, B and D & C and D is not significantly different at $p > 0.05$. However, interaction between supermarket A and C & B and C at variance Tukey HSD is significant different at $p < 0.05$. Similarly mean total bacterial count at variance LSD interaction between supermarket A and C, A and D, B and C & B and D is significantly different at $P < 0.05$.

In term of mean coliform count of supermarkets interaction between supermarket A and B, A and C, A and D, B and C, B and D, C and D at variance (Tukey HSD and LSD) is significantly not difference at $P > 0.05$ (Table- 6) and (Table-7). In addition at both variances antibiotic interaction between supermarkets is not significantly different at $p > 0.05$. In term of total bacterial count interaction between supermarkets C with other supermarkets category is significantly different except with D category. This may be due its less cold insulation capacity and loss of refrigeration and milk contamination at power off as compare to other supermarkets category.

Antibiotic residues in pasteurised milk: Among milk samples collected from nine milk processing plants only 11.1% of the samples were positive for antibiotic test. This result indicates that presence of antibiotics in pasteurized milk is direct correlated with volume of milk collected. Bulky raw milk collectors have probability to have antibiotic contaminated milk as compare to those collect less volume of milk. In addition out of 46 milk samples taken from supermarket only 4.3% samples were contaminated by antibiotics (Table-8). On average 6.8% antibiotic contaminated milk was detected under the current study.

Table-6: Multiple comparisons of four categories of supermarkets in term of bacterial load and antibiotics.

Dependent Variable		(I)	(J)	MD (IJ)	Std. Error	Sig.
Total bacterial Count	Tukey HSD	A	B	-0.170	0.655	0.994
			C	-1.741*	0.618	0.035
			D	-1.285	0.613	0.171
		B	A	0.170	0.655	0.994
			C	-1.571*	0.488	0.013
			D	-1.116	0.482	0.111
		C	A	1.741*	0.618	0.035
			B	1.571*	0.488	0.013
			D	0.456	0.430	0.715
		D	A	1.285	0.613	0.171
			B	1.116	0.482	0.111
			C	-0.456	0.430	0.715
	LSD	A	B	-0.170	0.655	0.797
			C	-1.741*	0.618	0.007
			D	-1.285*	0.613	0.042
		B	A	0.170	0.655	0.797
			C	-1.571*	0.488	0.002
			D	-1.116*	0.482	0.026
		C	A	1.741*	0.618	0.007
			B	1.571*	0.488	0.002
			D	0.456	0.430	0.295
		D	A	1.285*	0.613	0.042
			B	1.116*	0.482	0.026
			C	-0.456	0.430	0.295
Coliform	Tukey HSD	A	B	-0.063	0.877	1.000
			C	-1.068	0.827	0.573
			D	-1.006	0.820	0.614
		B	A	0.063	0.877	1.000
			C	-1.005	0.654	0.425
			D	-0.943	0.645	0.469
		C	A	1.068	0.827	0.573
			B	1.005	0.654	0.425
			D	0.062	0.575	1.000
		D	A	1.006	0.820	0.614
			B	0.943	0.645	0.469
			C	-0.062	0.575	1.000

The mean difference is significant at the 0.05 level. A=Standby Generator with Open cold Display, B= Standby Generator with fridge, C=Only Open Cold display, and D= Only Fridge, MD= mean difference, I and J= supermarkets.

Table-7: Multiple comparisons of four categories of supermarkets in term of bacterial load and antibiotics.

Dependent Variable		(I)	(J)	MD (IJ)	Std. Error	Sig.
	LSD	A	B	-0.063	0.877	0.943
			C	-1.068	0.827	0.204
			D	-1.006	0.820	0.227
		B	A	0.063	0.877	0.943
			C	-1.005	0.654	0.132
			D	-.943	0.645	0.151
		C	A	1.068	0.827	0.204
			B	1.005	0.654	0.132
			D	0.062	0.575	0.915
		D	A	1.006	0.820	0.227
			B	0.943	0.645	0.151
			C	-0.062	0.575	0.915
Antibiotics	Tukey HSD	A	B	0.000	0.116	1.000
			C	-0.067	0.109	0.928
			D	-0.062	0.108	0.938
		B	A	0.000	0.116	1.000
			C	-0.067	0.086	0.866
			D	-0.062	0.085	0.883
		C	A	0.067	0.109	0.928
			B	0.067	0.086	0.866
			D	0.004	0.076	1.000
		D	A	0.062	0.108	0.938
			B	0.062	0.085	0.883
			C	-0.004	0.076	1.000
	LSD	A	B	0.000	0.116	1.000
			C	-0.067	0.109	0.544
			D	-0.062	0.108	0.566
		B	A	0.000	0.116	1.000
			C	-0.067	0.086	0.443
			D	-0.062	0.085	0.467
		C	A	0.067	0.109	0.544
			B	0.067	0.086	0.443
			D	0.004	0.076	0.956
			A	0.062	0.108	0.566
			A	0.062	0.085	0.467
			C	-0.004	0.076	0.956

The mean difference is significant at the 0.05 level. A=Standby Generator with Open cold Display, B= Standby Generator with fridge, C=Only Open Cold display, and D= Only Fridge, MD= mean difference, I and J= supermarkets.

Table-8: Antibiotic residues in milk samples collected from supermarkets.

Source	Category	N (%)		Total
		Positive	Negative	
Processing plants	Large Scale	3(33.3)	6(66.7)	9
	Medium Scale	0	9(100)	9
	Small Scale	0	9(100)	9
	Total	3	24	27
	1	5 (10.9%)	0 (0.0%)	5 (10.9%)
	2	10 (21.7%)	0 (0.0%)	10 21.7%)
	3	14 (30.4%)	1(2.2%)	15 32.6%)
	4	15 (32.6%)	1 (2.2%)	16(34.8%)
	Total	44 (95.7%)	2 (4.3%)	46(100.0%)

*1 – Standby generator with cold display, 2 - Standby generator with fridge, 3 - Only cold displays, 4 - Only fridge.

Pasteurisation efficiency and shelf life: The phosphatase test result of all selected milk processing plants was negative indicating there was proper application of temperature and holding time.

Ten panellists were participated in evaluating the shelf life of pasteurized milk from the first day of production up to 9th day of production at 4 $\pm$ 1^{O}C according to Weibull Hazard method. At the first day of production all panellist gave excellent in term of smell, flavour and colour and appearance of milk. Depending on sensory evaluation pasteurized milk categorized into Excellent, Good, Fair and poor (Table-9). Based on smell at 9th day of its production score 21, at 7th day of its production score 84 and at 5th day of its production score 96.

Depending on Flavour at 9th day of its production score 33, at 7th day of is production score 86 and at 5th day of its production score 91(Table- 9). Colour and appearance of pasteurized milk is another point to evaluate its shelf life from first day of production up to 9th day of production. On the 9th day of production score 27, on 7th day of production score 85 and on the 5th day of production score 95. The overall results of the sensory test correlated well to the Weibull plot (r^2"0.78˝).

Fluid milk market is expected to remain fresh and appealing to customers for 12 - 14 days[20]. However, under the current study maximum shelf life of pasteurized milk was 7 days after production that was stored at 4^{o}C $\pm$ 1^{O}C. This indicated that the shelf life of the pasteurized milk is shorter than the expected one. This might have happened due to post pasteurization contamination problems. The result of this study showed that though the product had higher bacterial load than the acceptable limit, the sensory evaluation can judge as good and acceptable. From this it can be deduced that the microbial population does not always show the quality of milk. Therefore, using a microbial population as an indicator for shelf-life may not be always valid. The failure of total and psychrotroph microbial counts to predict the end of sensory shelf-life may be attributed to the fact that the species responsible to sensory retardation are only a part of the population. Since different microbes produce different off flavours, the nature of the dominant strain type (proteolytic, or lipolytic, etc.) is critical from the standpoint of sensory shelf life. Microbial load which is 10^{6}cfu/ml for the end shelf-life may not be correct and that perhaps a load of only 10^{4} cfu/mL of a particular species may result in an off flavour[21].

Pasteurized milk shelf life depends on several factors, such as, raw milk quality, storage condition, pasteurization method and efficiency, transportation system and etc. High level contamination of raw milk can affect pasteurization system and shelf life of pasteurized milk at room temperature for several hours in retail shops or household may lead to early spoilage of milk. Microbial spoilage of pasteurized milk can supports abundant growth of microorganisms, leading to development of off-flavours, coagulation and ropiness. Sensory change especially flavour changes of pasteurized milk mainly depend on the initial numbers and type of microorganisms present, the pasteurization conditions and the storage temperature.

Recontamination or post pasteurization contamination is also a factor for pasteurized milk quality problem.

Table-9: Shelf life evaluation results of pasteurised milk.

Criteria	Date after production N (%)		
	A	B	C
milk smell	Excellent	Good	Poor
Flavour	Excellent	Good	Poor
Colour and Appearance	Excellent	Good	Poor

A= at 5th day, B= at 7th day and C= at 9th day

Conclusion

Milk handling from production until consumption is a critical issue to keep quality of the milk. This study result indicate that total bacterial and coliform count result of pasteurised milk from both milk processing plant and supermarket were higher than the Ethiopia standard Agency recommendation. Among all milk samples collected from processing plants and supermarkets only 3(111.1%) and 2(4.3%) samples were positive for antibiotic test respectively. Pasteurization efficiency test result of all processing plants was negative. This indicates that they apply proper temperature and holding time.

Post pasteurization contamination is the main problem of processing companies selected for the study. Pasteurization efficiency of all processing plant under the current study was proper and in which short time to high temperature combination good to eliminate expected pathogenic bacteria in milk. However, due to several factors pasteurized milk was contaminated and reduces its expected shelf life to seven days at $4^{o}C \pm 1^{O}C$.

In conclusion pasteurised milk collected from both processing plant and Supermarket is microbiologically contaminated. However, it is free of Yeast and mould contamination. In case of antibiotic residue only 11.1% of the samples were positive. This implicate that concerning governmental and nongovernmental organization alone or together strongly can work on milk quality and safety to protect health of the consumers.

Abbreviation: CFU - Colony Forming Unit, UHT - Ultra-high temperature, CSA - Central Statistical Agency, EMDIDI - Ethiopia Meat and Dairy Industry Development Institute, Ml - millilitre, SE - Standard Error, UK - United Kingdom, ES - Ethiopia Standard, ADSA - America dairy science association, SPSS - Statistical Package for Social Sciences, US - United State, ISO - International Standard Organisation, TBC - Total Bacterial Count, CC - Coliform Count, Std. Error - Standard Error, Sig. - Signature, QSAE - Quality Standards Authority of Ethiopia.

Acknowledgement

I am greatly indebted to my instructor and research advisor Dr. Mestawet Taye for her Immeasurable helpful guidance, sincere criticism and constructive suggestions which she made during the course of this thesis task with no reservations. I also would like to thanks my co-advisor Mr. Haile Welearegay from Hawassa University who helped me in reviewing the thesis. I have special thanks to Ethiopian Meat and Dairy Industry Development Institute for sponsoring me research budget. In addition to this I like to thank Institute staff, especially w/o Azeb Tessema and w/o Mekalia Bezabeh for their laboratory assistance and creating good working environment during the laboratory work. I would like to thank Lame dairy private plc Industry management team especially w/o Mebrat for allowing me to use their laboratory with full facility to analysis antibiotic residue of my study samples. My heartfelt thanks also extend to those milk processing companies which sample were collected and Ethiopia Meat and Industry Development Institute Sanitation team for their overall assistance during the laboratory work. Last but not least, I am grateful to my family who send me to the school in my early child hood from the rural part of Ethiopia, and other relatives and friends who helped me in one way or another during the study.

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