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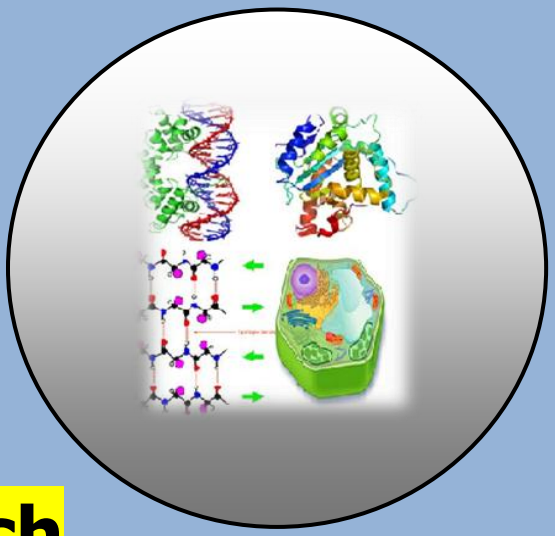
ISSN 0970-4973 Print
ISSN 2319-3077 Online/Electronic

Global Impact factor of Journal: 0.756
Scientific Journals Impact Factor: 3.285
Index Copernicus International Value
IC Value of Journal 6.01 Poland, Europe

J. Biol. Chem. Research
Volume 32 (1) 2015 Pages No. 313-322



Journal of Biological and Chemical Research

An International Journal of Life Sciences and Chemistry

Indexed Abstracted and Cited in about 25 different Scientific Databases around the World

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 32, No. 1: 313-322, 2015
(An International Journal of Life Sciences and Chemistry)

ISSN 0970-4973 (Print)
ISSN 2319-3077 (Online/Electronic)

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RESEARCH PAPER
Received: 09/01/2015 Revised: 16/02/2015 Accepted: 28/02/2015

Microbial Quality of Raw Cow Milk Collected from Farmers and Dairy Cooperatives of Haramaya District, Ethiopia

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ABSTRACT

This study was carried out to investigate the microbial quality of raw cow milk collected from farmers and dairy cooperatives in Haramaya district. For this purpose a total of 20 raw cow milk samples were collected and analyzed for microbiological qualities including milk grading by Methylence blue reduction (MBR) test, Total Bacterial Count (TBC), Coliform Count (CC), detection and enumeration of pathogenic bacteria. According to the result of MBR test, among the raw milk samples, 60% were poor, 35% were fair and 5% were of good quality. The mean of TBC, CC, Escherichia coli, Staphylococcus aureus, and Salmonella of the raw milk collected from farmers were found to be (6.52, 5.59, 6.21, 5.61and 5.18log$_{10}$ cfu/ml) respectively. Means of TBC, CC, E. coli, S. aureus and Salmonella Spp of the raw milk collected from dairy cooperatives in the study area were (6.14, 5.19, 5.85, 5.34 and 5.17log$_{10}$ cfu/ml) respectively. Milk samples collected from the dairy cooperatives were likely to be fair in quality as compared to the samples collected from farmers. Mean values of all the above parameters were not significantly different (P>0.05) among the farm groups. The microbial profiles indicated that the microbiological quality of milk produced by farmers and dairy cooperatives in the study area was poor and had non-conformance to the standards. Accordingly, it could be concluded that this milk type could possibly poses a serious health risk in the study area. Thus, adequate hygienic measures should be taken during production and handling processes of the milk.

Key words: Bacterial Count, Coliform Count, Raw Milk and Pathogenic Bacteria.

INTRODUCTION

Milk is a complex biological fluid and by its nature, a good growth medium for many microorganisms. Cow's milk has long been considered a highly nutritious and valuable human food and is consumed by millions daily in a variety of different products. Its nutrient composition makes it an ideal medium for bacterial growth and therefore it can be considered one of the most perishable agricultural products because it can so very easily be contaminated (Bryan, 1983, Bramley and McKinnon, 1990; Heeschen, 1993). Raw Milk (RM) often contains microorganisms which may cause food borne diseases (Adesiyun *et al*., 1995; Steele *et al*., 1997; Headrick *et al*., 1998).

Because of the specific production it is impossible to avoid contamination of milk with micro-organisms; therefore the microbial content of milk is a major feature in determining its quality (Rogelj, 2003). He also stated that the number and types of microorganisms in milk immediately after milking are affected by factors such as animal and equipment cleanliness, season, feed and animal health. Bacterial contamination of raw milk can originate from different sources: air, milking equipment, feed, soil, faeces and grass (Coorevits *et al*., 2008). It is hypothesized that differences in feeding and housing strategies of cows may influence the microbial quality of milk (Coorevits *et al*., 2008). Rinsing water for milking machine and milking equipment washing also involve some of the reasons for the presence of a higher number of microorganisms including pathogens in raw milk (Bramley, 1990).

The safety of dairy products with respect to food-borne diseases is of great concern around the world. This is especially true in developing countries where production of milk and various milk products takes place under unsanitary conditions and poor production practices (Mogessie 1990; Zelalem and Faye, 2006; Alganesh et al., 2007; Asaminew and Eyassu, 2011). There is high level of cow milk production in the farmers and pastoralist areas of eastern Hararghe. However; there was no adequate study conducted on microbial quality of raw cow milk in Haramaya district. Therefore, the purpose of this study was to investigate the microbial quality of raw cow milk sampled from farmers and dairy cooperatives of Haramaya district.

MATERIAL AND METHODS

Description of the Sampling site

This study was conducted in Haramaya district of eastern Hararghe, Ethiopia. Haramaya district is one of the woredas in the Oromia Region of eastern Hararghe, Ethiopia. The altitude of this district ranges from 1400 to 2340 meters above sea level. Haramaya District located about 500 Km distanced from Addis Ababa, Ethiopia.

Milk sampling

Raw cow milk samples were collected in June 2014 from farmers and dairy cooperatives of Haramaya district based on the result of the preliminary survey. Accordingly, a total of 20 samples (10 from each farm group) of raw cow milk were collected from the study area. From each farm group (farmers and dairy cooperatives), samples of approximately 250 ml were taken aseptically from the bulk milk container into sterile glass bottles. The milk was collected within 15 min of milking, kept in an icebox, transported and was analyzed immediately after arrival at the Haramaya university Microbiology laboratory.

Microbiological investigation

The microbial tests considered were Methylene blue reduction (MBR) test for milk grading**,** Total Bacterial Count (TBC), Coliform Count (CC), detection and enumeration of pathogenic bacteria, and characterization of the isolates. Enrichment was done for determination of total bacterial count and coliform count, using sterile peptone water. The total plate count agar (Oxoid) used for determination of total viable organisms was sterilized by autoclaving at 121ºC for 15 minutes, while the violet red bile agar (VRBA: Oxoid) used for determination of CC was sterilized by boiling (Richardson 1985). The media used were prepared according to the guidelines given by the manufacturers.

Milk Grading by *MBR test*

In the methylene blue reduction (MBR) test, one ml of methylene blue (1:25,000) was added to 10ml of milk. The tube was sealed with rubber stopper and slowly inverted three times to mix. Then it was placed in a water bath at 35^{o}C and examined at intervals of 30 minutes for 8 hrs. The time taken for the methylene blue to become colorless is the methylene blue reduction time (MBRT). The methylene blue reduction test depends upon the ability of bacteria in milk to grow and consume the dissolved oxygen, which reduces the oxidation reduction potentials in the medium (Ombui *et al.,* 1995).

Table 1. Grading of milk samples on the basis of methylene-blue reduction (MBR) test.

Quality of milk	Decolourization time
Poor	Less than 2h
Fair	2 to 6h
Good	6 to 8h
Excellent	Above 8h

Source: Benson, 2002

Total Bacterial Count (TBC)

The total bacterial count (TBC) was made by adding one ml of milk sample into sterile test tube having nine ml peptone water. After thoroughly mixing, serial dilution of sample was made up to 10^{-8} and samples were pour plated using 15-20 ml standard plate count agar solution and mixed thoroughly. The plated sample was allowed to solidify and then incubated at 30ºC for 48h. Colonies were counted using colony counter (Marth 1978).

Coliform Count (CC)

One ml of milk sample was taken into sterile test tube having nine ml peptone water. After mixing, the sample was serially diluted up to 10^{-8}, appropriate quantity of the dilutions were pour plated on 15-20 ml Violet Red Bile Agar solution (VRBA). After thoroughly mixing, the plated sample was allowed to solidify and then incubated at 30°C for 24h. Gram-negative lactose fermenters (coliforms) that grow on this medium do produce “nucleated colonies” (dark centers). Colonies of *Escherichia coli* and *Enterobacter aerogenes* can be differentiated on the basis of size and the presence of a greenish metallic sheen (Atlas, Parks and Brown, 1995). Typical dark red colonies were considered as coliform colonies. Finally, colony counts were made using colony counter (Marth 1978).

Detection and enumeration of pathogenic bacteria

A Portion of 1ml from each sample was aseptically homogenized with 9ml sterile enrichment broth (lactose broth for *E. coli*, peptone water for both *Salmonella* and *S. aureus*) and incubated at 37°C for 24 hours, for further biochemical analysis.

Media and growth conditions

***Escherichia coli*:** For the isolation and identification of *Escherichia coli*, the enriched sample was cultured on selective medium Levine Eosin Methylene Blue (EMB) Agar and incubated at 37°C for 24h. Morphologically typical colonies producing metallic sheen were counted and taken into nutrient broth for further identification.

Staphylococcus aureus: The selective medium used to isolate *Staphylococcus aureus* was Baird Parker Agar (BPA) (HiMedia Pvt. Ltd.). Enriched samples were spreaded on Baird Parker Agar (BPA) and the plate was incubated at 37°C for 24–48h. Appearances of jet black colonies surrounded by white halo were counted and considered to be presumptive for *S. aureus.*

Salmonella spp: Detection of Salmonella was performed according to Harrigan and MacCance (1976). One ml from the enriched sample were inoculated in to Selenite cystein broth (Oxoid) and incubated for 24h at 37^{o}C. Positive tubes were spreaded on Bismuth Sulfite agar (Oxoid) and incubated at 37^{o}C for 24h. The pure colonies were counted and then subjected to the confirmatory tests.

Table 2. Morphological and culture characteristics of isolated bacteria.

Isolated bacteria	Gram staining	Culture characteristics on selective media
Escherichia coli	Gram negative rods	Colonies showing metallic sheen
Staphylococcus aureus	Gram positive cocci (in clusters)	Jet black colonies surrounded by white halo
Salmonella spp.,	Gram negative rods	pure colonies

Table 3. Biochemical characterization of *E. coli.*

Biochemical test	Reaction
Lactose fermentation	+
Catalase	+
Simmon's Citrate	-
Indole Production	+
Nitrate Reduction	+
Methyl Red	+
Voges- Proskauer	-
Urease	-
Acid from sugars	
Glucose	+
Mannnitol	+
Lactose	+
Salicin	+
Sucrose	+

Physiological and biochemical characterization of the isolates

At intervals, colonies on the incubated plates were picked and purified by repeated sub-culturing by streaking on the desired media with a sterile wire loop. The strategy consisted of picking a colony to represent every visibly different morphology on each plate. Phenotypically, colonies were examined microscopically for motility, Gram's reaction and colony morphology including shape, size, colour, and texture of colony using actively growing cultures. Moreover the presence of these isolates on selective media was confirmed using biochemical characterization. Appropriate positive and negative controls were also used to differentiate positive and false-positive reactions. Four to five suspected colonies from each bacterial plate were picked, cultured and then identified by the various biochemical tests. Biochemical tests used to confirm *E. coli* were Catalase test, Indole, Methyl red, Voges- Proskauer test, Nitrate reduction, Urease production, Simon citrate agar, and various sugar fermentation tests (Table 3).

Confirmation of the genus, *Staphylococcus* was done by Gram staining and various biochemical tests including Catalase test, Oxidase test, Indole, Methyl red, Voges-Proskauer test, Nitrate reduction, acid production from different sugars, and haemolysis on Sheep Blood Agar (S.B.A.) while the species, *S aureus* was also confirmed by Coagulase test following the method of Singh and Prakash (2008).

Table 4 Biochemical characterization of *S. aureus.*

Biochemical test	Reaction
Catalase	+
Oxidase	-
Indole production	-
Nitrate reduction	+
Methyl red	+
Voges- proskaure	+
Acid from sugars	+
Glucose	+
Mannitole	+
Maltose	+
Lactose	+
Raffinose	-
Sucrose	+
Heamolysis	+
Coagulase	+

Statistical Analysis

Analysis of variance for total bacteria count and coliform count was carried out using General Linear Model (GLM) procedure of SAS (2000). A fixed effect model was used to estimate the effects of farm groups on the bacteriological tests. Total bacterial and coliform counts were log transformed before statistical analysis in order to make the frequency distribution more symmetrical. Mean comparisons were done using the Least Significant Difference (LSD) for variables whose F-values declared a significant difference.

RESULTS AND DISCUSSION

Microbial quality of the raw cow milk was determined by milk grading system using MBR test, enumerating the total bacteria, total coliforms and detection of some pathogenic bacteria. According to the result of MBRT (Table 5) most of the milk samples collected from farmers was found to be poor quality. Out of ten raw milk samples collected from farmers, seven of them were found to be poor quality and three were of fair. Out of ten samples taken from dairy cooperatives, five were poor, four samples were of fair quality, and one was found to be good quality (Table 5).

Table 5. Decolorizing time and grading of milk samples collected from farmers and dairy cooperatives of Haramaya district.

Farmers				Dairy cooperatives			
Treatment	**Milk source**	**Decolorization time (hr)**	**Grade**	**Treatment**	**Milk source**	**Decolorization time (hr)**	**Grade**
T-1	Local cow	2:38	Fair	T-1	Local cow	3:43	Fair
T-2	Local cow	1:40	Poor	T-2	Local cow	2:53	Fair
T-3	Local cow	1:55	Poor	T-3	Local cow	1:46	Poor
T-4	Local cow	2:47	Poor	T-4	Local cow	1:57	poor
T-5	Local cow	1:51	poor	T-5	Local cow	3:49	Fair
T-6	Crossbred cow	1:45	poor	T-6	Crossbred cow	1:31	poor
T-7	Crossbred cow	2:54	Fair	T-7	Crossbred cow	3:48	Fair
T-8	Crossbred cow	1:28	poor	T-8	Crossbred cow	1:36	Poor
T-9	Crossbred cow	1:32	Poor	T-9	Crossbred cow	6:12	Good
T-10	Crossbred cow	2:31	Fair	T-10	Crossbred cow	1:34	poor

The means of microbiological analysis of raw cow milk samples of Haramaya district are shown in Table 6 and 7. Means of TBC, CC, *E. coli S. aureus* and *Salmonella Spp* were not significantly different (P>0.05) among the farm groups. The average level of total bacterial count of milk sampled from farmers and dairy cooperatives of the study area were $6.52\log_{10}$ cfu/ml and $6.14\log_{10}$ cfu/ml respectively. This is lower as compared to the result of Alganesh *et al.,* (2007) which was $7.60\log_{10}$ cfu/ml in milk sampled from a small scale producer in East Wollega. Asaminew and Eyassu (2011) also reported a result of $7.58\log_{10}$ cfu/ml in cow milk sampled from around Bahir Dar and Mecha district. According to the result obtained, total bacterial counts were not significantly different (P>0.05) across farm groups and it was generally high as compared to the acceptable level of 1×10^5 bacteria per ml of raw milk (O'Connor, 1994). This implies that the sanitary conditions in which milk has been produced and handled are substandard, subjecting the product to microbial contamination and multiplication (Biruk *et al.,* 2009).

According to this work, the average values of coliform count of raw milk samples collected from farmers and dairy cooperatives of Haramaya district were $5.65\log_{10}$ cfu/ml and $5.15\log_{10}$ cfu/ml respectively. The coliform count obtained in the current study is greater than that reported by Asaminew and Eyassu (2011), who found coliform count of $4.49\log_{10}$cfu/ml. Similarly, Fekadu (1994) also found coliform counts of 3.8, 4.0 and 3.8 $\log_{10}$cfu/ml for cows' milk produced in Aneno, Gulgula and Dongora districts of southern region, respectively which are lower than the current result. On the other hand, Zelalem and Bernard (2006) obtained higher coliform count of $6.57\log_{10}$cfu/ml for cows' milk collected from different producers in the central highland of Ethiopia. Coliform counts can indicate fecal contamination or contamination from equipment that has not been properly cleaned and sanitized (Schmidt, 2008; Bintsis et al., 2008; Biruk et al., 2009). Thus, the higher coliform count observed in this study could be due to the initial contamination of the milk samples from the cows' udder, the milker's personal hygiene, milk containers and the milking environment.

The current result also showed the presence of pathogenic bacteria such as *E. coli* (average of 6.21±0.13), *S.aureus* (average of 5.61±0.07) and *Salmonella Spp* (average of 5.18±0.11) in the raw cow milk samples collected from farmers in the study area. Milk samples collected from dairy cooperatives also showed the presence of pathogenic bacteria (*E. coli*, average of 5.85±0.14, *S.aureus*, average of 5.34±0.10 and *Salmonella Spp* average of 5.17±0.13). Similarly, Asmahan (2010) also reported an average of 1.2×10^6 *S. aureus*/ml. This higher contamination was probably originated from cow's udder, poor personal hygiene of the milkers and/or the milking environment. The contamination of the milk by *S. aureus* is often original but can also occur after handling draft in non-hygienic conditions. *S. aureus* is a poor competitor and is readily outgrown by lactic acid-producing microorganisms, so its growth is limited in raw milk (Holsinger *et al*., 1997; Asperger, 1994). Raw milk may contain pathogenic microorganisms to man and their source may lie either within or outside the udder. Pathogenic bacteria may present in raw milk as a direct consequence of udder disease. Among the organisms commonly producing mastitis are *S. aureus* and *E. coli* and all are pathogenic (Sinell, 1973). Contamination of raw milk by pathogenic bacteria from source external to the udder may be caused by *Salmonella*e strains, which produce many out breaks of *enteritis* (Robinson *et al*., 1979).

Table 6. Microbial quality of raw cow milk collected from Haramaya farmers.

Sample source	TBC	Coliforms	*E.coli*	*S.aureus*	*Salmonella*
Farm groups					
Local cow's milk(n=5)	6.60±0.07[a]	5.65±0.13[a]	6.32±0.14[a]	5.55±0.08[a]	5.13±0.12[a]
Crossbred cow's milk(n=5)	6.43±0.11[a]	5.52±0.09[a]	6.09±0.12[a]	5.67±0.06[a]	5.22±0.09[a]
Average (n=10)	6.52±0.09	5.59±0.11	6.21±0.13	5.61±0.07	5.18±0.11

*Means±SD; [abc] Means bearing different superscripts in the same column differ significantly ($p<0.05$).

Table 7. Microbial quality of raw cow milk collected from Haramaya dairy cooperatives.

Sample source	TBC	Coliforms	*E.coli*	*S.aureus*	*Salmonella*
Farm groups					
Local cow's milk(n=5)	6.05±0.12[a]	5.15±0.07[a]	6.00±0.15[a]	5.01±0.11[a]	5.11±0.13[a]
Crossbred cow's milk(n=5)	6.23±0.11[a]	5.22±0.10[a]	5.69±0.12 [a]	5.67±0.09[a]	5.23±0.12[a]
Average (n=10)	6.14±0.12	5.19±0.09	5.85±0.14	5.34±0.10	5.17±0.13

*Means±SD; [abc] Means bearing different superscripts in the same column differ significantly ($p<0.05$).

CONCLUSION

This study implies that the microbial quality of the milk produced in the study area was poor. This can be assured from the result of milk grade by MBR test and the high values of total bacterial count, coliform count and presence of pathogenic bacteria. The presence of pathogenic and indicator bacteria, such as *E. coli*, *Salmonella*, coliforms and *S. aureus* may lead to a hazard against public health. The poor bacteriological quality observed in the present study requires further investigation of the status of the animals' health, especially mastitis and the significance of the effect of containers to ascertain their contribution on microbial quality. In general, lack of knowledge about clean milk production and use of unclean milking equipment would be some of the factors which contributed to the poor hygienic quality of milk produced in the study area. Therefore, adequate sanitary measures including proper handling of the milk, cow, personal hygiene, use of hygienic milking and processing equipments, improving milk handling practices should be taken.

ACKNOWLEDGEMENTS

The authors are greatly thankful to the department of Biology, College of Natural and Computational Sciences, Haramaya University, Ethiopia for providing us all the microbiology laboratory facilities to conduct this research work.

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