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Research Article

Assessment of Bacteriological Quality of Raw Bulk Milk of Camel, Cow and Goat from Local Markets in Yabello District, Borana Zone, Oromia Regional State

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ABSTRACT

Background: The study was conducted from September 2020 to June 2021 to assess the bacteriological quality of raw bulk milk from urban and rural local market sites in borana pastoral area of Oromia regional state, Southern Ethiopia. A total of 78 milk samples were collected and analyzed for bacterial load using standard plate count and coliform count techniques and isolation of pathogenic bacteria was conducted.

Results: The total mean aerobic bacterial counts of raw bulk milk samples of camel, cow and goat were 8.51 log cfu/ml, 8.73 log cfu/ml and 8.54 log cfu/ml respectively. Regarding the location of milk market sites the mean total aerobic bacterial count was 8.72 log cfu/ml and 8.49 log cfu/mlin urban and rural milk market sites respectively. The total mean coliform counts of raw bulk milk samples of camel, cow and goat were 6.51 log cfu/ml, 6.55 log cfu/ml and 6.47 log cfu/ml respectively. Regarding the location of milk market sites the total mean coliform counts was 6.63 log cfu/ml and 6.40 log cfu/ml from urban and rural milk market sites respectively. Comparing the mean differences of the total mean aerobic and coliform bacterial counts, there was no significant mean differences ($p>0.05$) among the animal milk samples. However, there was significant mean differences ($p<0.05$) among the milk market sites.

Conclusion: Different bacterial species were isolated from camel, cow and goat raw milk sample from the urban and rural milk market sites. The major bacterial isolates were *Staphylococcus* (both pathogenic and non-pathogenic), *Escherichia coli* and *Bacillus* species. Generally the unhygienic milk handling resulted in poor milk quality in the pastoral area. Therefore there is a need of training for persons at the various milk market sites on strict hygienic measures to improve the bacteriological safety of cow milk.

Keywords: Bacterial load; Raw bulk milk; Camel; Cow; Ethiopia; Goat

Abbreviations: TABC: Total Aerobic Bacterial Count; CC: Coliform Count

INTRODUCTION

Because of its high nutritive value, milk is considered as one of the most important diet items for many people. Being a major part of human food, milk plays a prominent role in the diet. Nutritionally, milk has been defined as the most nearly perfect food. It provides more essential nutrients in significant amounts than any other single food [1]. Milk is an outstanding source of calcium and phosphorus for bones and teeth, and contains riboflavin, vitamin B_6, A and B_1 in significant amounts. It also contains vitamin B_{12}, the anti-pernicious anemia vitamin [2]. Due to its complex biochemical composition and high water activity, milk serves as an excellent culture medium for the growth and multiplication of many kinds of microorganisms [3]. If it is produced unhygienically and handled carelessly, it gets contaminated very easily leading to its early spoilage [4].

The contamination of milk and milk products is largely due to human factor and unhygienic conditions. Usually milk gets

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contaminated with different kinds of microorganisms at milk collecting places. Many milk-borne epidemics of human diseases have been spread by contamination of milk by spoiled hands of dairy workers, unsanitary utensils, flies and polluted water supplies. The quality of milk is determined by aspects of composition and hygiene [5]. Milk is largely made up of water, within which a wide range of nutrients including vitamins, proteins, fats and carbohydrates are suspended [6]. These rich nutritional contents and the production and processing procedures in commercial milk production render it susceptible to contamination by a host of pathogenic microbes that could cause diseases in humans. Therefore, milk is known to be an efficient vehicle for transmission of disease causing agents to humans [7].

Bacteria have been reported to contaminate milk as a consequence of milking animals affected by mastitis and from poorly sanitized utensils used during milking, transportation and storage processes and dairy workers [8]. These bacterial contaminants result in spoilage of milk and milk products whenever there are appropriate growth conditions. It can also conceal life-threatening hazards when it comes to gastroenteritis, diarrhea, typhoid, or bovine tuberculosis [9]. Milk borne and milk product borne outbreaks represent 2%-6% of bacterial food-borne outbreaks reported by surveillance systems from several countries [10]. Bacteriological safety of milk continues to be a topic of concern in the dairy industry and public health communities. Recent studies have established the emergence of new milk-borne bacterial pathogens such as *Escherichia coli* O157:H7 with more serious challenges for public health and the dairy industry. To protect public health against milk borne infections, there are regulations that require proper hygienic handling of milk and its pasteurization. However, such regulations are not usually adhered to in developing countries, making milk borne health risk higher in developing countries, where informal milk markets involve milk sale through unregulated channels.

In Ethiopia, milk is considered one of the oldest and principal kind of food and so many people depend on it and its products. Cow's milk is predominant, but some people depend on goat milk where the goats are kept by the families and camel milk by nomadic people. The pastoralists in the country are dependent in livestock and their products for livelihood, especially milk and consume it raw. Milk of camel is one of the main components of the diet of the nomads and is consumed in its raw or naturally processed (soured) form [11,12]. In Ethiopia camels are kept in the arid and semiarid lowlands of Borena, Ogaden and Afar regions, which cover 50% of the pastoralist areas in the country. The major ethnic groups owning camels and consume their raw milk in Ethiopia are the Somali, Borena and Afar. Microbial exposure assessments are critical components of the risk analysis [13,14]. In countries with poor milk production and marketing practices, one can expect a higher frequency of bacterial contamination, which poses health hazards as well as spoilage of large quantities of milk.

In Ethiopia, especially in Borana Zone, Oromia regional state where pastoralist communities reside, there is high milk production and people consume raw milk, there is a lack of information on the extent of raw milk contamination by bacteria despite the practice of informally raw milk market by producers in towns and along the accessible main street roads, and the prevailing habit of raw milk consumption in the country. In addition, to our information, there has been no established milk quality control system. Therefore, this study was conducted with the objectives to assess the bacteriological quality of raw bulk milk of camel, cow and goat from urban and rural local milk market sites in Yabello district, Borana pastoral area of Oromia regional state, Southern Ethiopia.

MATERIALS AND METHODS

Study area

The study was conducted September 2016 to June 2017 in the urban and rural local milk market sites in Yabello district, Borana pastoral area of Oromia regional state, Southern Ethiopia. It is located at 366 km south east of Addis Ababa, between 03037' 23.8" to 050 02' 52.4" North and 370 56' 49.4" to 390 01' 101"East and borders Kenya. Yabello is the capital town of the Borana zone and lies 570 km South of Addis Ababa. The zone covers 48,360 km^2 of which 75% consists of lowland; the zone frequently is exposed to droughts. The zone consists of eight districts covering 275 Gendas (the lowest administrative unit). There are 19 urban centers, of which 10 have town administration. The zone is inhabited by almost one million people [15]. The Borana rangelands cover about 1.9 million hectare of land. The region has a semi-arid savannah landscape, marked by gently sloping lowlands and flood plains vegetated predominantly with grass and bush land. The geology is composed of a crystalline basement with overlying sedimentary and volcanic deposits. People are predominantly involved in small-scale subsistence agriculture production and mainly on livestock husbandry. There are no perennial rivers and rainfall varies highly, both spatially and temporally.

Sample collection and processing

A total of 78 raw bulk milk samples (36 from the town and 42 from the producers and/or collectors in the milk selling points outside the town) were collected using sterile test tubes for bacteriological analysis. Sampling was done from 20 selected local milk market sites/points within the town and outside the town following the roads and other available sites, of which, 25 from camel, 26 from goat and 26 from cow were collected from 20 selected milk market sites/points (6 milk selling sites/points in the town, and 14 outside the town). The samples were collected aseptically in sterilized universal bottles in cold icebox with ice bag and transported within 6 hrs of sampling to Aklilu Lemma institute of pathobiology Addis Ababa university for bacteriological analysis.

Quantitative analysis of raw milk

The bacteriological tests considered for determination of the bacterial load in raw milk samples were Total Aerobic Bacterial Count (TABC) and Coliform Count (CC). Decimal dilutions of milk samples were platted on plate count agar (Oxoid, England)

and MacConkey agar (Merck, Germany) for total aerobic plate and coliform counts, respectively, following the standard procedures recommended by American public health association [16]. Each plate was marked with sample number and dilution level before shaking samples and making dilutions. Before removal of the milk samples from its container, the content was mixed thoroughly and vigorously. The following dilution standards were selected so that the total number of colonies per plate fluctuates between 30 and 300 for total aerobic plate and between 15 and 150 for total coliform counts. While transferring raw milk samples from one test tube to another, sterile pipettes were used.

Standard Plate Count (SPC)

The total bacterial count was made by adding 1 ml of milk sample into sterile test tube having 9 ml peptone water. One ml of the milk sample was added to dilution blanks and serially diluted by taking one ml to the next dilution tube after mixing. One ml was discarded from the last dilution. After thorough mixing, 1 ml of the diluted milk was taken from each dilution starting from the highest dilution and put on sterile labeled Petri dish using sterile pipettes. Two plates were inoculated per dilutions. After thoroughly mixing, the sample was serially diluted up to $1:10^{-7}$ and duplicate samples (1 ml) were pour plated using 15 ml-20 ml standard plate count agar solution and mixed thoroughly. The SPC agar was prepared by dissolving 23.5 g of powder in one liter of distilled water, sterilized by autoclaving at 121°C for 15 minutes and cooled to 45°C-47°C in a water bath. The plated sample was allowed to solidify and then incubated at 37°C for 48 hours. Colony counts were made using colony counter. Incubating control plates for each sterilization lot of dilution blanks and medium were used to check sterility of the dilution water and medium [16].

Coliform Count (CC)

The samples cultured for total bacterial counts were also cultured for coliform counts. One ml of milk sample was added into sterile test tube having 9 ml peptone water. After mixing, the sample was serially diluted up to $1:10^{-5}$ and duplicate samples (1 ml) were pour plated using 15 ml-20 ml MacConkey Agar solution. After thoroughly mixing, the plated samples were allowed to solidify and then incubated at 37°C for 48 hours. Finally, colony counts were made using colony counter. Typical purplish red colonies with bile precipitations around them were considered as coliform colonies [16].

Reading and interpretation of results from bacterial loads

Total Aerobic Plate Count (TAPC): After incubation at 37°Cfor 48 hours, all colonies including those of pin point size were counted on selected plates using colony counter. At the end of the incubation period, all of the petri plates containing between 30 and 300 colonies were selected. Plates with more than 300 colonies could not be counted and were designated Too Many To Count (TMTC). Plates with fewer than 30 colonies were designated too few to count (TFTC). Results from plates containing between 30 colonies-300 colonies per plate were recorded. After counting the colonies on each plate, calculation of the number of bacteria (colony forming units) per milliliter of the milk sample was performed by the following formula.

$$\text{Count}=SC/v\ (n1+0.1\ n2) \times d$$

Where,

SC=Sum of all colonies counted;

V=Volume of sample added to each plate;

n1=number of plate from the lowest dilution used for computing the count;

n2=number of plates in the next dilution factor used for computing the count;

d=dilution factor of the lowest dilution used for computing the count corresponding to n1.

When plates contained less than 30 colonies, the results were read as less than 30 times the reciprocal of the dilution number. If more than 300 colonies developed on the highest dilution plate, the count was recorded as more than 300 times the reciprocal of dilution.

Coliform count: After incubation of the plate for 48 hours, typical purplish red colonies with bile precipitations around them were counted as coliforms. Results from only those plates, which contained between 15 and 150 colonies were recorded. Interpretations were similar with that of TAPC.

Qualitative analysis of raw milk

Cultural examinations were used to isolate and identify the pathogenic and spoilling bacterial species found in the bulk raw milk of camel, cow and goat. Isolation and identification of bacterial species was carried out based on conventional culture techniques and biochemical assays. After thorough mixing of each of the milk samples, a loopful of the milk sample was streaked onto blood agar base enriched with 7% heparinized sheep blood and MacConkey agar. The plates were aerobically incubated at 37°C and examined for bacterial growth after 48 hours. From culture positive plates, typical colonies were subjected to gram's stain to study the staining properties and cellular morphology. Pure cultures of a single colony type from the blood agar were transferred into nutrient agar plate. From this, a series of biochemical tests that aided final identification of various bacteria were conducted following standard methods [17]. Identification of bacteria to the species level was carried out based on their colony characteristics, gram's staining and morphological characteristics, growth on MacConkey agar, catalase, urease and oxidase tests, Hydrogen Sulfide (H_2S) production, indole, Methyl Red (MR), and Voges-Proskauer (VP) reaction, citrate utilization, oxidation-fermentation test, motility, and different carbohydrate testes.

Staphylococcus species was identified based on hemolysis pattern, catalase production and coagulase test, pigment production, O-F test and fermentation of manitol and maltose.

Streptococcus species were identified based on gram's stain reaction, catalase production, hemolysis pattern, differential growth characteristics on Edward's medium, CAMP test,

fermentation pattern of manitol, sorbitol, raffinose and salicine and aesculin hydrolysis.

Enterobactericeae members organisms were identified based on gram's stain reaction, growth characteristics on MacConkey agar, oxidase test, reaction patterns on IMViC test (testing suspect colonies for indole, methyl red, Vogues Proskaeuer and citrate), H_2S production, fermentation patterns from lactose and urease and lysine production. Other gram negative organisms were identified based on staining morphology, growth characteristics on MacConkey agar, oxidase test, urease production, indole production, and acid production from sucrose and glucose.

Data management and analysis

Microsoft excel was employed for raw data entry and computation of descriptive statistics. Descriptive statistics such as mean and percentage were used to compute some of the data. Log10 transformation was done before the analysis of bacterial counts. The logarithmically transformed bacteria counts (SPCs and CC) were used to normalize the frequency distribution so that to compute bacterial loads for different parameters. For analysis of the data, SPSS version 20 software was used. The normally distributed data were analyzed by univariate analysis, using the General Linear Model (GLM) of SPSS software. Descriptive and correlation analysis between different bacterial parameters were performed. For significant differences between means, the relevant test (with equal/unequal variances) was chosen with regard to the test of the homogeneity of variances at a level of 0.05 (F-test two-sample for variances) and was applied to determine whether there are any significant differences in bacterial loads of raw bulk milk along the milk market sites (with in the town and outside the town designated as urban and rural respectively) and along the milking animal species (camel milk, goat milk and cow milk). A probability level (p-value) of $P<0.05$ was considered as statistically significant. Significant differences between means, confidence intervals, a probability level (p-value) and different descriptive statistical values such as mean, standard deviations and range were computed.

RESULTS

Bacterial load of milk samples

Standard plate count: The overall mean of standard plate counts (usually called as Total aerobic plate counts) of raw bulk milk samples of camels, cow and goats were 8.51 log cfu/ml (SD=0.53, range 7.40 log cfu/ml-9.45 log cfu/ml), 8.73 log cfu/ml (SD=0.47, range 7.48 log cfu/ml-9.46 log cfu/ml) and 8.54 log cfu/ml (SD=0.51, range 7.41 log cfu/ml-9.46 log cfu/ml), respectively (Table 1). Regarding to the location of milk market the mean of SPCs of milk samples collected from urban and rural market sites of all the milking animal species were 8.72 log cfu/ml (SD=0.49, range 7.41 log cfu/ml-9.46 log cfu/ml) and 8.49 log cfu/ml (SD=0.45, range 7.51 log cfu/ml-9.38 log cfu/ml), respectively.

Table 1: Descriptive statistical values (log10 CFU/ml) of SPC detected in raw bulk milk of camel, cow and goats in the urban and rural local markets.

Market site	Species	N	Mean	SD	Min.	Max.
Urban	Camel	12	8.65	0.39	8.25	9.45
	Cow	12	8.93	0.44	8.43	9.46
	Goat	12	8.59	0.59	7.41	9.46
	Total	36	8.72	0.49	7.41	9.46
Rural	Camel	14	8.38	0.62	7.4	9.32
	Cow	14	8.56	0.44	7.48	9.4
	Goat	14	8.49	0.45	7.51	9.38
	Total	42	8.48	0.5	7.4	9.4
Total	Camel	26	8.51	0.53	7.4	9.45
	Cow	26	8.73	0.47	7.48	9.46
	Goat	26	8.54	0.51	7.41	9.46
	Total	78	8.59	0.51	7.4	9.46

N=Number of samplestested

SD=Standard Deviation

With regard to the milking animal species and location of milk market sites, the mean of SPCs of milk samples collected from urban and rural sites of camel milk were 8.65 log cfu/ml (SD=0.39, range 8.25 log cfu/ml-9.45 log cfu/ml) and 8.38 log cfu/ml (SD=0.62, range 7.40 log cfu/ml-9.32 log cfu/ml), respectively.

The mean of SPCs of milk samples collected from cow milk of urban and rural market sites were 8.93 log cfu/ml (SD=0.44, range 8.43-9.46) and 8.56 log cfu/ml (SD=0.44, range 7.48-9.40), respectively. Furthermore, the mean SPC of milk samples collected from the urban and rural market sites of goat milk were 8.59 log cfu/ml (SD=0.59, range 7.41-9.46 log cfu/ml) and 8.49 log cfu/ml (SD=0.45, range 7.51-9.38 log cfu/ml), respectively.

Comparing the mean differences of SPCs of camels, cow and goat milk sampled from the urban and rural market sites, there was no significant mean differences among the species (F=1.549, p-value=0.219>0.05). However, there was significant mean differences of SPCs between the urban and rural milk market sites which was observed higher in the milk sampled from the urban markets (F=4.555, p-value=0.036<0.05) (Table 2).

Table 2: Significance differences between means of bulk raw milk SPCs (log cfu/ml) in groups of camel, cow and goat milk samples from urban and rural markets.

Factor		Mean	SD	95% CI for the mean	DF	F	P-value
Species	Camel	8.51	0.53	8.31-8.71	2	1.549	0.219
	Cow	8.73	0.47	8.55-8.91	2	1.549	0.166
	Goat	8.54	0.51	8.34-8.74	2	1.549	0.108
Market site	Urban	8.72	0.49	8.56-8.88	1	4.555	0.036
	Rural	8.48	0.5	8.33-8.63	1	4.555	

CI=Confidence Interval, DF=Degree of Freedom, SD=Standard Deviation

Total coliform count

The overall mean of total coliform counts for milk samples of camel, cow and goat were (from both the urban and rural markets) 6.51 log cfu/ml (SD=0.38, range 6.15 log cfu/ml-7.17 log cfu/ml), 6.55 log cfu/ml (SD=0.44, range 6.08 log cfu/ml-7.15 log cfu/ml) and 6.47 log cfu/ml (SD=0.36, range 6.11 log cfu/ml-7.11 log cfu/ml) respectively (Table 3).

Table 3: Descriptive statistical values (log10 CFU/ml) of coliform counts detected in raw bulk milk of camel, cow and goat in the urban and rural local markets.

Market site	Species	N	Mean	SD	Min.	Max.
Urban	Camel	12	6.68	0.38	6.25	7.17
	Cow	12	6.73	0.46	6.23	7.15
	Goat	12	6.49	0.37	6.11	7.04
	Total	36	6.63	0.41	6.11	7.17
Rural	Camel	14	6.35	0.32	6.15	7.15
	Cow	14	6.39	0.36	6.08	7.11
	Goat	14	6.46	0.37	6.15	7.11
	Total	42	6.4	0.34	6.08	7.15
Total	Camel	26	6.51	0.38	6.15	7.17
	Cow	26	6.55	0.44	6.08	7.15
	Goat	26	6.47	0.36	6.11	7.11
	Total	78	6.51	0.39	6.08	7.17

All the milk samples (100%, N=78) collected from camel raw bulk milk, cow raw bulk milk and goat raw bulk milk in both the urban and rural markets were a total bacterial count of with >5 million cfu/ml and >500000 cfu/ml for SPCs and coliform counts, respectively. Comparing the mean differences of total coliform counts of camel, cow and goat milk sampled from the urban and rural market sites, there was no significant mean differences among the species (F=0.215, p-value=0.807>0.05). However, there was significant mean differences of coliform counts between the urban and rural milk market sites which was observed higher in the milk sampled from the urban markets (F=7.374, p-value=0.008<0.05) (Table 4).

Table 4: Significance differences between means of bulk raw milk coliform counts (log cfu/ml) in groups of camel, cow and goat milk samples from urban and rural markets.

Factor		Mean	SD	95% CI for the mean	DF	F	P-value
Species	Camel	6.51	0.38	6.36-6.66	2	0.215	0.807
	Cow	6.55	0.44	6.38-6.72	2	0.215	0.778
	Goat	6.47	0.36	6.33-6.61	2	0.215	0.515
Market site	Urban	6.63	0.41	6.49-6.76	1	7.374	0.008
	Rural	6.4	0.34	6.30-6.50	1	7.374	

CI=Confidence Interval, DF=Degree of Freedom, SD=Standard Deviation

Bacterial species isolated from raw bulk camel, cow and goat milk samples

Ten types of bacterial species were identified in camel milk sampled from the urban and rural sites. Among the bacterial species isolated *Staphylococcus* species (both pathogenic and non-pathogenic) followed by *E. coli* and *Bacillus* species was more prevalent (Table 5).

Table 5: Bacterial species isolated from the informally marketed camel raw bulk milk samples.

Bacterial isolates	Urban site	Rural site	Total
Pathogenic *Staphylococcus* species	3 (25%)	5 (35.71%)	8 (30.77%)
Nonpathogenic *Staphylococcus* species	4 (33.33%)	5 (35.71%)	9 (34.62%)
Escherichia coli	5 (41.67%)	6 (42.86%)	11 (42.31%)
Micrococcus species	5 (41.67%)	3 (21.43%)	8 (30.77%)
Streptococcus agalactia	3 (25%)	1 (7.14%)	4 (15.38%)
Streptococcus dysgalactia	2 (16.67%)	1 (7.14%)	3 (11.54%)
Streptococcus uberis	1 (8.33%)	2(14.28%)	3 (11.54%)
Enterococcus fecalis	3 (25%)	3 (21.43%)	6 (23.08%)
Bacillus species	4 (33.33%)	5 (35.71%)	9 (34.62%)
Corynebacterium species	1(8.33%)	1 (7.14%)	2 (7.69%)
Klebsiela pneumonia	2 (16.67%)	3 (21.43%)	5 (19.23%)
Rhodococcus equi	2 (16.67%)	4 (28.57%)	6 (23.08%)
Acinetobacter species	2 (16.67%)	3 (21.43%)	5 (19.23%)

Nine types of bacterial species were isolated and identified in cow raw milk sampled from both the urban and rural market sites. The bacteria species identified were *Staphylococcus* species, *E. coli*, *Bacillus* species, *Proteus* species, *Klebsiella* species *Micrococcus* species, *Streptococcus* species, *Enterobacter* species and *Enterococcus* species Among the identified bacterial species *Staphylococcus* species (both pathogenic and non-pathogenic) followed by *E. coli* and *Bacillus* species was more prevalent (Table 6).

Table 6: Bacterial species isolated from informally marketed cow raw bulk milk samples.

Bacterial isolates	Urban site	Rural site	Total
Pathogenic *Staphylococcus*	2 (16.67%)	6 (42.86%)	10 (38.46%)
Nonpathogenic *Staphylococcus*	3 (25%)	9 (64.28%)	16 (61.54%)
Escherichia coli	5 (41.67%)	6 (42.86%)	11 (42.31%)
Micrococcus species	2 (16.67%)	4 (28.57%)	6 (23.08%)
Streptococcus agalactia	1 (8.33%)	3 (21.43%)	4 (15.38%)
Streptococcus uberis	1 (8.33%)	2 (14.28%)	4 (15.38%)
Enterococcus fecalis	5. 4 (33.33%)	-35.71%	9 (34.61%)
Enterobacter species	1 (8.33%)	1 (7.14%)	2 (7.69%)
Bacillus species	2 (16.67%)	1 (21.43%)	3 (11.54%)
Klebsiela species	2 (16.67%)	4 (28.57%)	6 (23.08%)
Proteus species	2 (16.67%)	3 (21.43%)	5 (19.23%)

A total of ten types of bacterial species were isolated and identified in goat raw milk sampled from both the urban and rural market sites. *Escherichia coli* were isolated from 41.67% of the goat milk samples in the urban markets and from 28.57% of the rural markets. This resulted in an overall isolation rate of 34.61% in an equal proportion with *Bacillus* species from both market sites (Table 7). This was followed by non-pathogenic *Staphylococcus* species (26.92%), pathogenic *Staphylococcus* species (23.08%), *Klebsiela* species (19.23%), *Enterococcus fecalis* (19.23%) and *Micrococcus* species (19.23%) from goat milk samples.

Table 7: Bacterial species isolated from informally marketed goat raw bulk milk samples.

Bacterial isolates	Urban site	Rural site	Total
Pathogenic *Staphylococcus* species	4 (33.3%)	6 (42.86%)	10 (38.46%)
Nonpathogenic *Staphylococcus*	7 (58.33%)	9 (64.28%)	16 (61.54%)
Escherichia coli	5 (41.67%)	6 (42.86%)	11 (42.31%)
Micrococcus species	2 (16.67%)	4 (28.57%)	6 (23.08%)
Streptococci species	1 (8.33%)	3 (21.43%)	4 (15.38%)
Enterococcus species	2 (16.67%)	2 (14.28%)	4 (15.38%)
Bacillus species	5. 4 (33.33%)	-35.71%	9 (34.61%)
Enterobacter species	1 (8.33%)	1 (7.14%)	2 (7.69%)
Klebsiela species	2 (16.67%)	1 (21.43%)	3 (11.54%)
Proteus species	2 (16.67%)	4 (28.57%)	. 6 (23.08%)
Acinetobacter species	2 (16.67%)	3 (21.43%)	5 (19.23%)

DISCUSSION

The overall mean bacterial count of raw bulk camel, cow and goat milk samples in the present study were 8.51 log cfu/ml (SD=0.53, range 7.40 log cfu/ml-9.45 log cfu/ml), 8.73 log cfu/ml (SD=0.47, range 7.48 log cfu/ml-9.46 log cfu/ml) and 8.54 log cfu/ml (SD=0.51, range 7.41 log cfu/ml-9.46 log cfu/ml), respectively. There was no significant difference in the

mean SPCs among the milking animal species ($p>0.05$). The result is comparable with the values (6 log10 cfu mL^{-1} to 8.8 log10 cfu mL^{-1}) reported by for cow milk produced in southern region of Ethiopia; in Gurage zone; in Hawassa town and in Wollayta zone were on the range of 9.82 log cfu/ml-4.57 log/cfu/ml. In addition the result of the present study is comparable with and in Awassa and in and around Addis Ababa, respectively and showing increased bacterial count with mean total bacterial counts ranged from 10.12 log cfu/ml of milk collected from Jimma to 8.30 log cfu/ml of milk sampled from Debre Zeit and Adama with the average value being 9.10 log cfu/ml. However, the mean total aerobic bacterial count obtained in the present study was higher than that of (2.1×10^6 cfu/ ml), (10^5 cfu/ml); (10^7 cfu/ml); (5.84 cfu/ml ± 0.629 cfu/ml); 5 log cfu/ml. The result in the present study was also higher than those reported for Saudi (5.4 log cfu/ml in average) and Ethiopian (5.6 log cfu/ml in average) camel milk by and respectively [18-20].

The SPC values from all the milk samples (100%) exceed the acceptable level of 5 log cfu/ml which is higher than the given international standard set for minimum acceptable level of bacterial count in milk. The higher count observed in this study could be related with the poor hygienic conditions of the milk during production, handling, collection, transportation or marketing by the producers and traders who have no awareness about proper milk handling. In other words, the above indicated count of milk samples collected from the country were considered to be below the standard set for good quality milk. This implies that the sanitary conditions in which milk has been produced and handled are substandard subjecting the product to microbial contamination and multiplication. This was possibly identified. Considering a limit for bacterial counts in relation with milk hygiene in that high initial SPC values ($>10^5$ ml^{-1}) are evidence of serious hygienic problem during production, likewise SPC values of $<2 \times 10^4$ ml^{-1} reflect good sanitary practices.

The overall mean coliform counts obtained from raw bulk milk samples of camel, cow and goat in the present study from both the urban and rural markets were 6.51 log cfu/ml with range 6.15 log cfu/ml-7.17 log cfu/ml), 6.55 log cfu/ml with range 6.08 log cfu/ml-7.15 log cfu/ml) and 6.47 log cfu/ml with a range of 6.11 log cfu/ml-7.11 log cfu/ml) respectively. There was no significant difference in mean of coliform counts among the milking animal species. The result in the present study was in agreement with the findings of Zelalem, et al. who found coliform counts of 6.57 log10 CFU/ml; Worku, et al. who found overall coliform counts of 6.88 ± 0.040 and 7.786.88 ± 0.040 at cow udder and storage containers respectively in Borana pastoral community of Oromia region and Zelalem and Faye who reported higher coli form count of 6.57 cfu/ml for cow milk collected from different producers in central highlands of Ethiopia.

However, the overall mean coliform counts obtained from raw bulk milk samples of camel, cow and goat milk samples obtained in the present study was higher than that reported by Godefay B, et al. who found a coliform counts of 4.1 log10 CFU/ml and 4.9 log10 CFU/ml ± 0.11 log10 CFU/ml in and around Addis Ababa, and in Bahir Dar Zuria and Mecha district of Ethiopia respectively. Similarly, the mean coliform counts of raw milk in the present study was higher than that the reports of 1.82 log10 CFU/ml in Debre Zeit town, Ethiopia; at Bahir Dar Zuria with the mean value of 4.49 log cfu/ml; who found coli form counts of 3.8 log10 cfu/ml, 4.0 log10 cfu/ml and 3.8 log10 cfu/ml for cow milk produced in Aneno, Gulgula and Dongora districts of Southern region respectively, with the mean value of 4.13 log10 cfu/ml ± 0.757 log10 cfu/ml for milk samples collected from dairy farms at Dire Dawa town; raw cow's milk sampled from smallholder producers with the mean value of 4.46 log cfu/ml. Also the mean coliform counts of raw milk in this study was higher than that reported from India (5.89 log cfu/ml); from Tanzania (5 cfu/ml), from Kenya (4.67 log cfu/ml) and from Mali (10^6 cfu/ml).

The higher coliform count in the present study could be attributed to unsanitary milk production and handling, and contamination by unclean milk contact surfaces or increased mastitis infection in the pastoral area. Similarly, this was also reported and justified in that coliform count provides an indication of unsanitary production practices and/or mastitis infection. A count less than 100 cfu/ml for coliform count is considered acceptable for milk intended to be pasteurized before consumption. Counts of 10 cfu/ml or less are achievable and desirable if raw milk will be consumed directly. Even if, it is not practical to produce milk that is always free of coliforms. Their presence in raw milk may therefore be tolerated. However, if present in large numbers, over 100 coliform organisms per milliliter of raw milk, it means that the milk was produced under improper procedures. Hence their presence in large number in dairy products is an indication that the products are potentially hazardous to the consumers' health.

Raw milk bacteriological quality standards vary widely from country to country and there are different standards for different groups and species of microorganisms which is specific to specific products. In this regard, since there have not yet existed official and strict Ethiopian raw milk standards and regulations, the bacteriological quality (total viable counts and coliform counts) for the raw bulk milk samples analyzed was compared and interpreted with the Kenyan standard guideline specifications for whole unpasteurized milk. Accordingly, milk containing a total bacterial count of up to 1 million per millilitre is classified as very good; 1 million to 2 million as good; 2 million as bad and >5 million as very bad. Similarly, milk containing coliform counts up to 1000 per millilitre is classified as very good; 1000 to 50,000 as good; 50,000 to 500,000 as bad and >500,000 as very bad. Milk classified as bad is not acceptable within the regulations for marketing.

Accordingly, all the milk samples (100%, N=78) collected from camel raw bulk milk, cow raw bulk milk and goat raw bulk milk in both the urban and rural markets were with >5 million cfu/ml and >500000 cfu/ml for SPCs and coliform counts respectively in this study. This implies that 100% of the samples tested were classified as very bad (>5 million cfu/ml and >500000 cfu/ml for SPC and CC values respectively) indicating the milk from which these samples were collected was not acceptable for marketing and consumption. Moreover, these high counts show that milk sold by the vendors, collectors and

producers for consumption in the two market sites (both urban and rural markets) is of poor bacteriological quality. This extended difference in bacterial loads (both SPC and CC) from the acceptability level for consumption implies that milk is produced and handled under poor hygienic conditions in the pastoral communities. This indicates that there could be possibility of contracting of infection or intoxication from milk-borne pathogens on consumption of the milk marketed informally under the fragmented market systems in the pastoral communities. This risk of infection and contamination gets worse by consumption of the milk in its raw state which was observed to be common habit of the pastoralists in this study by checking and interviews. This may be true with the report by World Health Organization in that zoonoses selectively affect families in poor and marginalized communities, particularly pastoralists. This might be attributed to exposure of the pastoralists with infected animals and animal products including raw milk.

Different bacterial species were isolated and identified from camel, cow and goat raw milk sample from the urban and rural market sites. Among the different types of bacterial species identified from camel milk of the urban and rural market sites, *Staphylococcus* species (both pathogenic and non-pathogenic) followed by *E. coli* and *Bacillus* species were the most prevalent. This is similar with report by Abeer AA, et al. who isolated pathogenic bacteria species including *E. coli* and *salmonella* species with higher prevalence from camel raw milk in Egypt. This result shows that camel milk still represents a significant source of infection though it is believed that camel milk has the ability of inhibiting of many bacterial species. This is evidenced from different reports in that camel milk can be contaminated with several pathogenic and spoilage bacterial species resulting in higher counts. This is similar with report by Abeer AA, et al. who isolated pathogenic bacteria species including *E. coli* and *salmonella* species with higher prevalence from camel raw milk in Egypt.

Among the identified bacterial species isolated from cow and goat raw milk sample *Staphylococcus* species (pathogenic and non-pathogenic together) followed by *E. coli* and *Bacillus* species were more prevalent. This agreement with the report of who isolated and reported Staphylococci and Micrococci to be the most common bacteria of environmental origin in milk samples. It has been noted that certain bacterial spp isolated from milk sample of certain milking animal species might not be necessarily isolated from the others. In the present study, *Rhodoccocus* equi was isolated only from camel milk samples but not detected from cow and goat milk samples. Proteus species was isolated only from cow and goat milk samples but not from camel milk samples. Moreover, *Acinetobacter* species was not isolated from cow's milk samples but detected in camel and goat milk samples during the whole period of the study.

In the present study most of the organisms identified were under the *Enterobacteriaceae* group indicating probable environmental contamination, including fecal contamination, of the milk as a result of poor hygiene. Reported that under pastoral production conditions, environmental contamination is likely to play a bigger role in the hygiene of raw milk than mastitis bacteria. In agreement with the present study different environmental bacteria species have been isolated with higher frequency and in turn contributed to the higher bacterial load within the urban markets. Many of the bacteria identified in the milk sampled are potential food-borne pathogens, and though some of them occurred in few samples, the practice of pooling milk from different sources by traders, and the absence of bactericidal treatment generally observed could increase the risk posed by such organisms. These have been implicated in milk and other food related infections. *E. coli*, *Bacillus* species and *Staphylococcus* species which have been isolated in higher frequency in this study, are associated with food borne intoxications through production of enterotoxins, mainly involved with *Bacillus cereus* and *Staphylococcus aureus*.

Bacterial species detected from the milk samples in the rural markets were also isolated from urban milk samples but may be in different proportions. This shows that milk might have been contaminated from different sources starting from the milking animals to final consumption. *Streptococcal* and *Staphylococcal* species have been isolated from both sites though relatively lesser frequency was observed in the samples from the urban markets than in the rural markets. Likely, a report by Farah Z, et al. in an assessment of an urban market in Somalia indicated that pathogens in 50% of transport containers taking milk directly from producing herds, in 62% of milk containers sampled at primary collection sites and in 70% of milk containers sampled in urban markets of camel milk were detected. This shows that bacterial contamination of milk increases along the value chain just as it has been observed in this study.

In the present study, both bacterial load and frequency of isolation have been observed to be different between the urban and rural markets in that higher bacterial load and increased frequency of the isolated bacteria were shown under the urban market than the rural markets. This may be attributed to increased milk contamination by environmental bacteria along the value chain until the final market by vendors in the urban market through exposure of milk to different contributing factors of contamination. Some of the contributing factors which can have the potential for milk contaminations on exposure, according to different literatures, include pooling of old (spoiled) milk with fresh milk, improper protection of milk from contaminants, unhygienic milk handling throughout the value chain like unclean source of water, exposing the milk to high environmental temperatures for extended time and poor personal hygiene and health of the milk handlers IDF.

CONCLUSION

The result obtained in this study concluded that milk available to the consumer in Yabello district, Borana zone, Oromia regional state have a high bacterial load which is more than the acceptable limit according to American and European community member states, which is between 2×10^5 and 4×10^5 cfu/ml. They are also contaminated with *Staphylococcus* (both pathogenic and non-pathogenic), *Escherichia coli* and *Bacillus* species. It indicates that hygienic procedures are not strictly followed during milk production. Hence it warns the

need for more strict preventive measure for the regular washing and sterilization of milk equipment, utensils, milkers' hand, udders, eradication of diseased animals, and pasteurization (boiling) of milk before collection and distribution for consumption. The magnitude of the problem of bacterial contamination deserves more elaborative studies from the point of production of milk and milk products to the point of consumption and at all intermediary levels. Therefore there is a need of training for persons at the various milk market sites on strict hygienic measures to improve the bacteriological safety of cow milk.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

All the data supporting the results are included in the article.

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The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

TM was involved in the design, data collection, laboratory work, statistical analysis and interpretation of data and drafted the paper; AZ was involved in the design and critical revision of the paper and was also involved in laboratory work and critical revision of the paper.

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REFERENCES

1. Garedew L, Berhanu A, Mengesha D, Tsegay G. Identification of gram negative bacteria from critical control points of raw and pasteurized cow milk consumed at Gondar town and its suburbs, Ethiopia. BMC Public Health. 2012;12:1-7.
2. Oliver SP, Jayarao BM, Almeida RA. Foodborne pathogens in milk and the dairy farm environment: Food safety and public health implications. Foodborne Pathog Dis. 2005;2(2):115-129.
3. Godefay B, Molla B. Bacteriological quality of raw cow's milk from four dairy farms and a milk collection center in and around Addis Ababa. Berl Munch Tierarztl Wschr. 2000;113(7):276-278.
4. Donkor ES, Aning KG, Quaye J. Bacterial contaminations of informally marketed raw milk in Ghana. Ghana Med J. 2007;41(2): 58-61.
5. Ali AA, Irshad NB, Razaz SA, Manahil AA. Microbiological safety of raw milk in Khartoum State, Sudan: 1-Khartoum and Omdurman cities. Pak J Nutr 2010;9(5):426-429.
6. Ashenafi M. Microbiological quality of Ayib, a traditional Ethiopian cottage cheese. Int J Microbiol 1990;10(3-4):263-268.
7. Ashenafi M, Beyene F. Effect of container smoking and udder cleaning on the microflora and keeping quality of raw milk from a dairy farm in Awassa. Trop Sci. 1993;33:368-378.
8. Bonfoh B, Wasem A, Traore AN, Fane A, Spillmann H, Simbe CF, et al. Microbiological quality of cows' milk taken at different intervals from the udder to the selling point in Bamako (Mali). Food Control. 2003;14(7):495-500.
9. Tesfay T, Kebede A, Seifu E. Quality and safety of cow milk produced and marketed in Dire Dawa Town, Eastern Ethiopia. Int J Integr Sci Innov Technol. 2013;2:1-5.
10. Worku T, Negera E, Nurfeta A, Welearegay H. Microbiological quality and safety of raw milk collected from Borana pastoral community, Oromia Regional State. African J Food Sci Technol. 2012;3(9):213-222.
11. Yilma Z, Faye B. Handling and microbial load of cow's milk and Irgo –fermented milk collected from different shops and producers in central highlands of Ethiopia. Ethiopian J Ani Prods. 2006;6(2): 67-82.
12. Tassew A, Seifu E. Microbial quality of raw cow's milk collected from farmers and dairy cooperatives in Bahir Dar Zuria and Mecha district, Ethiopia. Agr Biol J N Am. 2011;2(1):29-33.
13. Rai KC, Dwivedi HB. To study the bacteriological quality of milk supplied in Kanpur city by different sources. Indian Dairyman. 1990;42(12):520-523.
14. Kurwijila RL, Hansen KK, Macha IE, Abdallah K, Kadigi HJ. The bacteriological quality of milk from hand-and machine-milked dairy herds in Morogoro, Tanzania. Afr Livest Res. 1992;2:59-67.
15. Hadush B, Kebede E, Kidanu H. Assessment of bacteriological quality of raw camels' milk in Ab-'Ala, north eastern Ethiopia. Live Res Dev J 2008; 20(9):27-35.
16. Soomro AH, Arain MA, Khaskheli M, Bhutto B. Isolation of *Escherichia coli* from raw milk and milk products in relation to public health sold under market conditions at Tandojam. Pakistan J Nut. 2002;1(3):151-152.
17. Sivapalasingam S, Friedman CR, Cohen L, Tauxe RV. Fresh produce: A growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. J Food Protect. 2004;67(10): 2342–2353.
18. Farah Z, Mollet M, Younan M, Dahir R. Camel dairy in Somalia: Limiting factors and development potential. Elsev Live Sci. 2007;110(1):187-191.
19. Tassew A, Seifu E. Microbial quality of raw cow's milk collected from farmers and dairy cooperatives in Bahir Dar Zuria and Mecha district, Ethiopia. Agri Bio J North America. 2011;2(1):29-33.
20. Hadrya F, Elouardi A, Benali D, Hami H, Soulaymani A, Senouci S. Bacterial quality of informally marketed raw milk in Kenitra city, Morocco. Pak J Nut. 2012;11(8):662.