Bacteriological Quality Assessment of Raw Milk and Cheese in Selected Woreda of Wolaita Zone, Ethiopia

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Bacteriological Quality Assessment of Raw Milk and Cheese in Selected Woreda of Wolaita Zone, Ethiopia

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Abstract- A cross sectional study was carried out at Wolaita Sodo district from May 2013 to November 2014 with the aim to evaluate the bacteriological quality and to isolate and identify common milk borne zoonotic bacterial pathogens (*Staphylococcus aureus, Escherichia coli,* and *Salmonella species)* from raw cow milk and cheese collected in market, restaurant and cafeteria.Milk quality related practice was also assessed by direct observation and questionnaire survey. A total of 56 raw milk and 40 cheese samples were collected in cafeterias / restaurants and from market. The result revealed that 43(76.7%) were poor quality milk with a total aerobic bacterial count more than 5×10^5CFU/ml and only 13(23.3%) moderate or acceptable milk which have an estimated total aerobic bacterial count of below 5×10^5CFU/ml. The mean total bacterial count and mean of coliforms count was significantly higher ($P < 0.05$) in raw milk collected from market point than from cafeteria / restaurant. The maximum and minimum total aerobic bacterial counts from study area were 8.00log10CFU/ml and 5.00 $\log_{10}$CFU/ml, and coliform counts were 7.10 $\log_{10}$CFU/ml and 4.23 $\log_{10}$CFU/ml respectively. The mean coliforms and total aerobic bacterial count were significant ($P < 0.05$) from different sampling point. The overall mean of total aerobic bacterial count and coliform count in cheese sample was 5.38log$_{10}$CFU/ml and 5.58 $\log_{10}$CFU respectively. The bacteriological analysis revealed that 73.5% and 70% *Staphylococcus aureus* were isolated in raw milk and cheese respectively. A total 55.2% and 42.5% *Escherichia coli* and 42.9% and 30% *Salmonella species* were found in raw milk and cheese sample respectively.

Keywords: quality assessment, raw milk, cheese, coliform counts, total aerobic bacterial count.

I. Back Ground

The complex biological nature of milk makes it a suitable medium for growth of many microorganisms. During milk 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). In order to make good dairy products, good quality raw materials are essential. A milk processor or handler will only be assured of the quality of raw milk if certain basic quality tests are carried out at various stages of transportation of milk from the producer to the processor (Haug, 2007).

Contamination of raw milk with bacterial and other microorganism can be from air, milking equipment, feed, soil, faeces and grass. The number and types of micro-organisms in milk immediately after milking are varied based on source of contamination. Poor feeding and housing strategies of cows may influence the microbial quality of milk. (Coorevits *et al*., 2008, Rogelj, 2003)..There is an increasing focus on milk quality and hygiene in the dairy industry. Producing high quality milk requires effective udder health programs at a herd level (Bhutto *et al.,* 2010).

Unlike in developed countries, the dairy industry in most African countries including Ethiopia is underdeveloped, dominated by unpasteurized milk and informal markets (Regional Dairy Trade Policy Paper, 2004).Wolaita Sodo and its suburbs is well known for their wealth in dairy products. In the study area still most of the market is informal and hence increased risk of microbial contamination. On the other hand, most of the milk consumed in rural areas is un-hygienically handled and preference is given to raw milk compared to pasteurized and boiled milk. However, no or limited studies on microbiological quality of raw milk and Cheese has done.

The study was conducted to assess the milk handling practices, to establish total plate count of bacteria and coliforms in raw cow milk and cheese from Wolaita Sodo, Areka, Humboand Boditi and determine the presence of selected milk-borne zoonotic pathogens such as *Staphylococcus aureus, Escherichia coli,* and *Salmonella* species.

II. Materials and Methods

a) Study area

This study was carried out from May 2013 to November 2014 in four woreda of Wolaita Sodo district, Southern Ethiopia. The area is located about 390 km south of Addis Ababa between 6°4′N to 7°1′N and 37°4′E to 38°2′E which is 700-2950 m above sea level. Wolaita zone has a total land area of 4537.5 square kilometers. The average annual rain fall of the study area is ranging from 450-1446 mm (WZFEDD, 2005).

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b) Study design and Sampling methodology

A cross sectional study was conducted from November 2013 to May 2014 to evaluate the bacteriological quality of raw cow milk and cheeses.

Based on the access of transport and vast milk production and marketing situations four woreda were purposively selected for this study. From three sampling points (cafeteria, restaurant and market) in all woreda's 14 raw milk samples and 10 raw cheese (Cottage cheese "ayib") were randomly collected. Aseptically collected milk and cheese samples for further bacteriological analysis were transported in ice box to veterinary microbiology laboratory which is found in Wolaita Sodo University.

Asemi structured questionnaire regarding general hygiene of handling milk and milk product transportation facilities and transportation instruments and others was prepared and 80 milk and cheese seller people was randomly subjected to different questions related to factors which reduces the milk and cheese quality. Some observations were made in all woredas up on sample collection.

c) Sample collection and transportation

A total of 56 raw milk and 40 cheese samples were collected from the study area to investigate the quality milk and cheese. All samples were collected aseptically and processed immediately through standard microbiological procedures as described in APHA (1992). A sample of 20 ml raw cow milk and 200g of raw cottage cheese "ayib"were collected from different sampling points such as restaurants, cafeterias and retail market in sterilized universal bottles. The samples were kept in icebox and transported to Veterinary microbiology laboratory within an hour's and then stored in refrigerator at 4°C. All samples were processed within 24hrs of sampling.

d) Bacteriological examination of collected samples

i. Sample preparation

Milk samples were homogenized in a blender at 600 rpm for 5-10 min and serially diluted up to 10^{-5} times by adding 1mL of the test portion into 9 mL of 0.1% sterile peptone water. The cheese samples intended for bacteriological analysis were ground into a fine paste using a sterile pestle and mortar before weighing. The total viable bacteria count was determined by adding 1 g of cheese sample into test tubes having 9 mL of sterile quarter strength peptone water. After thorough mixing using a vortex mixer, serial dilutions were prepared under safety cabinet and 1 mL of appropriate dilutions were pour plated in duplicate using standard plate count agar. The plated samples were allowed to solidify and then incubated at 32°C for 48 h.

ii. Bacteriological analysis

Standard laboratory examinations such as total aerobic bacterial count (TABC) and coliform count (CC) was undertaken to determination the bacterial load in raw milk and raw cheese samples. The total plate count agar (Hi media, India) was used for determination of total aerobic bacteria in milk n while the violet red bile agar (VRBA) (Oxoid, UK)was also used to determination of Coliform Count (CC).

Dilutions were selected so that the total numbers of colonies on a plate were not difficult to count. The media were prepared according to the guidelines given by the manufacturers. Total aerobic bacterial count (TABC) and coliform count made after plating and incubation of appropriate dilutions of milk and cheese samples in the standard plate count agar (SPCA) (Hi media, india) medium and in VRBA (Oxoid, UK)medium at 37°C for 48 hrs and 24 hrs respectively, following the standard procedures recommended by American Public Health Association (1992). After incubation, all colonies including those of pin point size in SPCA medium and purplish red colonies in VRBA medium were counted under colony counter and results from each SPCA plates which contained 25 to 250 colonies per plate whereas, less than 100 coliform colonies in VRBA were recorded. For colonies beyond this count the next dilutions were plated and similar procedure was followed.

After counting and recording bacterial colonies in each petridish the number of bacteria in milliliter milk was calculated by the following formula given by APHA (1992).

$$ N = \frac{\sum C}{[(n1x2) + (0.1xn2)] \times d} $$

Where: N = number of colonies per milliliter of milk,
∑C = sum of colonies on plates counted,
n1= number of plates on lower dilution counted,
n2 = number of plates in next higher dilution counted and
d = dilution from which the first counts are obtained.

e) Identification of pathogens

A portion (1 g or 1 ml) from each sample was taken aseptically and inoculated in sterile   Nutrient agar (Hi media, india) medium and incubated at 37 °C for 24 hours. Bacterial colonies grown on this medium undertaking for gram staining. Gram's positively reactive colonies were tested for catalase test and inoculated on sterile selective media (Mannitol Salt agar (M. S. A)) (Oxoid, UK) for *Staphylococcus aureus*, and grams negative bacterial colonies cultured in Eosin Methylene Blue (E. M. B) (Oxoid, UK)to investigate the presence of *Escherichia coli*, and Xylose lysine deoxycholate agar (X. L. D) (Oxoid, UK) and Rappaport-Vassiliadis brothmedium (Oxoid, UK) to identify *Salmonella* species.

The most common zoonotic milk borne pathogens was identified based on cultural

characteristics, gram nature and color of colonies. Identified colonies from each petriplate were picked, subcultured, incubated at 37 °C and then isolated by using various biochemical tests such as Catalase test, Coagulase, Indole test, Methyl red test, VogesProusker test, Nitrate Reduction test, Urease production, Citrate utilization test and Glucose, Lactose, Mannitol sugar fermentation test. These biochemical tests as stated below in table were performed to confirm, *Staphylococcus aureus, Escherichia coli* and *Salmonella* species.

Table 1 : Morphological & Cultural characteristics of Pathogenic bacteria isolated from Milk and Milk products

Sr. No	Isolated pathogens	Gramstaining and morphology	Culture characteristics on selective media
1	*S. aureus*	Gram + ve cocc	Mannitol Salt Agar (Golden yellow color colonies)
2	*E. coli*	Gram – ve coccobacilli	Eosin Methlene blue Agar (Green Metallic Sheen colonies)
3	*Salmonella spp*	Gram –ve rod shaped	Xylose lysine deoxycholate agar (Black Centre color colonies)

Table 2 : Biochemical characteristics of Pathogenic bacteria isolated from Milk and Milk products

Ser.N	Biochemical test	S. aureus	E. coli	Salmonella spp
1	Catalase	+	+	-
2	Urease	-	-	-
3	*Oxidase*	-	-	-
4	*Coagulase*	+	-	-
5	Citrateutilization	-	-	+
6	*Nitrate reduction*	+	-	-
7	*Indole production*	-	+	-
9	*Methylene red*	+	+	+
10	*Vogesproscuare*	+	-	-
11	Glucose	+	+	+
12	Lactose	+	+	-
13	Mannitol	+	+	+
14	*Sucrose*	+	+	-
15	*Maltose*	+	-	+

(Javed Khan et al, 2014)

f) Statistical Analysis

Data was evaluated using descriptive statistics and all counts were converted to logarism values to enable statistical analysis and to express count to $\log_{10}$ CFU/g, and to compare the results. The data was analyzed using SPSS version 20. One way ANOVA and one sampled T-test was performed to compare the mean of bacterial counts obtained from milk and milk product sample based on their location and to compare risk of higher source contamination respectively. Statistical significance is set at a P value of < 0.05.

III. Results

In this study a total 96 Milk and cheese samples were taken from Market, restaurant and cafteria from four selected woreda in the wolaita zone. Questionnaire survey on 80 milk seller from all woreda was made. The results of bacterial enumeration, isolation in milk and cheese and associated risk factors are shown in as follows.

In the present study, roughly all milk sellers were female farmers and more than half of them brought their milk in open pots, jericans, cheap recycled dirty plastic containers and their cheese in local inset leaf and sell it directly in the market without any processing and packaging. From a total of 80 peoples subjected for the questionnaire, only 15 (18.7%) of them camefrom the city and 65 (81.3%) were from nearby villages.

Based on the questionnaire survey 86 % of the respondents were lack of awareness on hygienic handling of milk and cheese, deficient in clean water, used poor type of barn, poor hygiene milking. Nearly all milk sellers were complained for lack of transport facilities rather transported on rough roads over long distances without cooling and under high ambient temperatures and sell their milk and cheese under dusty and hot conditions. Very insignificant numbers of the respondents were hygienically practiced in their farms.

a) *Total bacterial and coliform count in raw milk and cottage cheese*

The bacteriological analysis revealed that 43(76.7%) from the total of 56 raw milk samples, were found to be poor quality milk with a total aerobic bacterial count more than 5×10^5CFU/ml. However, moderate or acceptable quality milk samples accounts only 13(23.3%) which have an estimated total aerobic bacterial count of below 5×10^5CFU/ml as shown in table 1.

Table 3 : The comparison between means of total bacterial (TABC) count in raw milk and raw cheese

Type sample	N	Means	t -value	dfp	-	value
Cheese from market	20	5.58	-0.443	19		0.33
Cheese from cafeteria/restaurant	20	5.52				
Total	**40**					
Raw milk from market	28	6.37	2.438	27		0.011
Raw milk from cafeteria/ restaurant	28	6.08				
Total	**56**					

b) *Total bacterial and coliform count in raw milk and cheese*

Based on the present study the mean total aerobic bacterial count of were 6.22log$_{10}$CFU/ml and 5.38 log$_{10}$ CFU/ml raw milk and cheese respectively. The maximum and minimum total aerobic bacterial counts from study area were 8.00Log10 CFU/m and 5.00 in raw milk and log$_{10}$CFU/ml 6.10Log10CFU/ml and 4.10 log$_{10}$ CFU/ml in cheese.

The overall mean of coliform count were 5.6 log$_{10}$CFU/ml and 5.58 log$_{10}$ CFU in raw milk and cheese respectively. The maximum and minimum coliform count in raw milk and cheese were 7.10 log$_{10}$CFU/ml and 4.23 log$_{10}$CFU/ml, and 7.10 log$_{10}$ CFU/ml and 4.23 log$_{10}$ CFU/ml respectively. The mean of coliforms and total bacterial count were significantly ($P<0.05$) different in the selected woredas. Humbo was identified with peak mean of total bacterial and coliform count in raw milk among the study areas Table 3

There was also significant difference ($p<0.05$) between counts of total bacteria and coliformis in cheeses collected from different sampling areas. The highest total bacterial and coliform count was recorded in Humboworeda.

Table 4 : Overall mean (±SE) of total bacterial counts (TABC) (log$_{10}$CFU/ml milk)

Study area	sample type	N	Means ±SE	Minimum	Maximum	95 %	CI
						Lower	Upper
Boditi	Milk	14	6.54 +.483	6.17	7.30	6.26	6.82
	Cheese	10	5.52 ±.687	4.10	6.07	5.02	6.01
Humbo	Milk	14	7.02 + .603	6.20	8.00	6.67	7.37
	Cheese	10	5.84± .406	5.05	6.10	5.55	6.13
Sodo	Milk	14	5.70 + .497	5.00	6.20	5.41	5.99
	Cheese	10	5.29±.465	4.81	6.04	4.96	5.63
Areka	Milk	14	5.61 + .366	5.14	6.38	5.39	5.82
	Cheese						
Total	Milk	56	6.22±.764	5.00	8.00	6.01	6.42
	Cheese	40	5.38±.660	4.10	6.10	5.17	5.59

Milk- p- value-0.001   df =3 and cheese- p- value-0.014   df =36

Table 5 : Overall mean variation of coliform count (log$_{10}$CFU/ml milk) and cheese

Study area	sample type	N	Means ±SE	Minimum	Maximum	95 %	CI
						L ower	Upper
Boditi	Milk	14	6.12 ± .086	6.00	6.26	6.07	6.17
	Cheese	10	5.72±.723	4.10	6.41	5.20	6.23
Humbo	Milk	14	6.24 ± .354	5.90	7.10	6.03	6.44
	Cheese	10	5.94 ±.319	5.05	6.11	5.71	6.17
Sodo	Milk	14	5.19 ± .503	4.23	6.10	4.89	5.47
	Cheese	10	5.29 ±.465	4.81	5.04	4.96	5.63
Areka	Milk	14	5.46 ± .472	5.00	6.14	5.20	5.74
	Cheese	10	5.37 ± .551	3.70	6.11	4.97	5.76

| Total | Milk | 56 | 5.76± .583 | 4.23 | 7.10 | 5.99 | 5.91 |
| | Cheese | 40 | 5.58 ± .578 | 3.70 | 5.04 | 5.3970 | 5.76 |

Milk- P value- 0.000   df =3   and cheese- p- value-0.035   df =3

c) *Identification of the isolates*

Table 5 : Distributionmilk borne zoonotic bacterial pathogens in raw milk and cheese

Bacteria isolates	Types of Sample	
	N (56raw milk)	N (40 raw cheese)
Staphylococcus aureus	41(73.5%)	28(70%)
Escherichia coli	31 (55.2%)	17(42.5%)
Salmonella species	24 (42.9%)	12(30%)

The study revealed that *Staphylococcus aureus* were dominantly contaminating the raw milk and cheese samples 73.5% and 70% respectively. Of the total samples 73.5% and 70%, 42.9%, 30% raw milk and cheese respectively, were contaminated with *Escherichia coli* and *Salmonella* species correspondingly.

IV. Discussion

According to the respondents the major milk quality related constraints was lack of awareness on hygienic handling , being deficient in of clean water, poor type of barn, poor Hygiene of the milker, lack of transport facilities, and inappropriate materials used for milk production and handling. Milk and cheese for market in these study areas are also transported on rough roads over long distances, in cheap recycled dirty plastic containers without cooling and under high ambient temperatures and sells their milk and cheese under dusty and hot conditions. This is in agreement with (Parekh & Subhash, 2008) reason that use of unclean milking and transport equipments contribute to the poor hygienic quality of milk and cheese. Another possible source of contamination microorganisms in the study area was unclean teats, poor health of dairy herd and unhygienic milking conditions. It is clear that many zoonotic diseases are transmissible via milk and milk products. The strong traditionally habit of the people in the study area for utilizing raw milk and milk products were greatly at risk of obtaining these pathogen.

One sample t _ test was used to compare the total viable bacterial and coliform count in raw milk and raw cheese from open market and from cafeterias / restaurants to determine range of contamination. The mean total viable bacterial in raw milk from cafeteria and restaurants were significantly lower than in open market but, is slightly higher in raw cheese from open market than cheese from cafeteria and restaurant. This result was in line with reports by Ashenafi (2006), Biniam, (2006), Zelealem et al (2007b) and Seifuetal (2013). The mean count of coliforms was notably higher in raw milk and raw cheese collected from market than from restaurants and cafeterias. Higher bacterial count in raw milk and cottage cheese from open market is because of immense source contamination from different hands in the market, dusty and hot selling conditions, utensils used for transportation. The less bacterial count in the samples collected from the cafeteria and restaurants was possibly because of optimum storage and not open unlikely to that of retail market selling environment. More or less all cafeteria and restaurants had a refrigerator and bought their milk from small scale farms in the town not necessarily from open markets.

The total bacterial load obtained from raw milk sample in this study was high (6.22 $\log_{10}$CFU/ml) as compared to the acceptable value. This finding is in line with the finding Monika. S *et al,* (2013) 6.35 log10CFU/ml, but higher than Karmen *et al*. (2008) 4.5 log10 cfu/ml. It was appreciably less than result reported by Simenew *et al*. (2013), 7.34 in private dairy commercial farm but slightly lower by the same author (6.46) in government dairy farms.

V. Conclusion and Recommendations

Based on the current findings of this study, it is concluded that:

Milk produced by farmers and supplied to market in Wolaita sodo districts contains unacceptable levels of hygiene indicators and indicates a potential source of milk-borne infections. This raises a public health concern about its safety to consumers. Since raw milk is an important vehicle for transmission of zoonoses and other pathogens, this microbial status implies that milk consumers in the study area are at health risk. Indeed, this is supported by detection of Staphylococcus aureus at higher prevalence.

It is therefore recommended that:

1. Veterinary/extension animal health services should be provided to livestock farmers on proper animal husbandry and control of diseases.

2. It is recommended that routine assessment of milk quality produced and consumed by the public be mandatory in order to safeguard the public from milk-borne zoonotic diseases which may emanate through consumption of unsafe milk and milk products.

3. There should be implementation of good hygiene practices throughout the milk chain by training of all stakeholders involved in milking, milk collection and processing, including pasteurization, transport, and delivery, to ensure the safety and quality of milk.

4. Consumer practices, such as milk boiling, to reduce or eliminate potential infection by milk-borne zoonoses should be further encouraged.

Conflict of Interest Statement
Authors have declared that no competing interests exist.

VI. Acknowledgments

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