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Microbiological quality and safety of raw milk collected from Borana pastoral community, Oromia Regional State

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The effect of source of sample points on microbiological quality and safety of cow's milk was evaluated in six kebeles of Abaya District of Borana pastoral area of Oromia Regional state. A total of 96 raw milk samples from cow udder and storage containers were aseptically collected following standard methods to determine total bacteria counts (TBC), coliforms counts (CC), and fecal coliforms counts (FCC), total staphylococci counts (TSC) and Isolation and identification of the safety related bacteria. Dye reduction tests were also used to evaluate the hygienic condition of the milk samples. The color disappearance time of methylene blue (MBT) and resazurine (RT) test of milk samples collected from both households (HH's) were 2.22 and 2.0 hours (Hrs), respectively. There was significant variation in TBC, CC and FCC among kebeles. Similar values were observed in TSC among kebeles. There was no significant difference ($p>0.05$) among non-model and model HH's in TBC, CC, TFC and TSC. The dominant pathogens isolated from the raw milk samples collected from the udder and storage containers are *Staphylococcus aureus*, *Staphylococcus intermidus*, *Staphylococcus epidermidus*, and *Micrococcus luteus*, *Escherichia coli*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Proteus*, *Pseudomonas*, *Salmonella*, *Shigella* and *Yersinia species*. The high level of counts and isolate numbers and types found in the sampled cow milk represent a poor keeping quality of milk and public health risk to the consumer. This suggests the need for improved hygienic practice at all levels of milk production in the pastoral community

Keywords: Raw milk, bacterial counts, microbial safety, pastoral community, Ethiopia.

INTRODUCTION

Milk and milk products play an important role in human nutrition throughout the world. Consequently, the products must be of high hygienic quality. In less developed countries especially in hot tropics high quality and safe product is most important but not easily accomplished (DeGraaf et al., 1997). This is required since milk is a suitable substrate for microbial growth and development. The fluid or semi-fluid nature of milk and its chemical composition renders it one of the ideal culture media for microbial growth and multiplication ((Mogessie and Fekadu, 1994). Due to the highly perishable nature

of milk and mishandling, the amount produced is subjected to high post-harvest losses. Losses of up to 20–35% have been reported in Ethiopia for milk and dairy products from milking to consumption (Getachew, 2003). Microorganisms may contaminate milk at various stages of milk procurement, processing and distribution. Use of non potable water may also cause entry of pathogens into milk. It is known that tropical conditions which have a hot, humid climate for much of the year are ideal for quick milk deterioration so pose particular problems because the temperature is ideal for growth and multiplication of many bacteria (Godefay and Molla, 2000).

The safety of dairy products with respect to food-borne diseases is a great concern around the world. This is especially true in developing countries where production of milk and various dairy products take place under rather

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unsanitary conditions and poor production practices (Zelalem and Faye, 2006). More so, the composition of milk makes it an optimum medium for the growth of microorganisms that may come from the interior of the udder, exterior surfaces of the animal, milk handling equipment and other miscellaneous sources such as the air of the milking environment (Richardson, 1985). Bacterial contamination of raw milk can originate from different sources: air, milking equipment, feed, soil, faeces and grass (Coorevits et al., 2008). The health and hygiene of the cow, the environment in which the cow is housed and milked, and the procedures used in cleaning and sanitizing the milking and storage equipment are all also key factors in influencing the level of microbial contamination of raw milk. All these factors will influence the total bacteria count and the types of bacteria present in bulk raw milk (Murphy and Boor, 2000). It is hypothesized that differences in feeding and housing strategies of cows may influence the microbial quality of milk (Coorevits et al., 2008). Bacteria in raw milk can affect the quality, safety, and consumer acceptance of dairy products. Several human microbial pathogens such as *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus*, *Campylobacter jejuni*, and *Mycobacterium tuberculosis* have been found to be associated with milk and milk products (Flowers et al., 1993; Jayarao et al., 2006).

The presence of microorganisms in milk and milk products has important ramifications for safety, quality, regulations and public health. Jayarao and Wang (1999) stated that milk from the farm can become contaminated with Gram negative bacteria present on teats, the teat ends, teat canal, udder surfaces, mastitis udders and contaminated water used to clean the milking systems and those that are resident in the milking system. For example, high microbial counts in raw milk are responsible for quality defects in pasteurized milk, UHT processed milk, dried skim milk, butter, and cheese (Barbano et al., 2006). Additionally, selecting raw milk of high quality has been associated with a decrease in consumer complaints caused by fluid milk quality (Keefe and Elmoslemany, 2007). As a result, many countries have milk quality regulations, including limits on the total number of bacteria in raw milk, to ensure the quality and safety of the final product. Hygienic quality control of milk and milk products in Ethiopia is not usually conducted on routine basis.

Door-to-door raw milk delivery in the urban and peri-urban areas is commonly practiced with virtually no quality control at all levels (Godefay and Molla, 2000). Unfortunately no registration system for informal farmers exists in the country and this hinders the transmission of information between farmers and local authorities (Jansen, 2003). It is thus difficult to not only to determine the quality status of the milk but also the economic impact, due to the fact that most of the farmers consume their own milk and seldom sell it (Dovie et al., 2006).

There is little information on the microbial quality of raw milk (Zelalem and Faye, 2006) especially in the pastoral and agro-pastoral area of southern Ethiopia, where milk consumption plays a significant role in the diet of the community. Information on the bacterial content of a milk sample may reflect on the state of health of the cow, the contributions under which the milk is stored and distributed, and its public health significance (Malick, 1986). Therefore, the aim of this study were to assess the effect of source of milk samples on microbiological quality and safety of cow's milk collected from Abaya district of Borana pastoral area of Oromia Regional state.

MATERIALS AND METHODS

Description of the study area

The study was carried out in Abaya district of Borana pastoral area of Oromia Regional State, Southern Ethiopia. It is located at 366 km south east of Addis Ababa, between 03°37' 23.8" to 05° 02' 52.4" North and 37° 56' 49.4" to 39° 01' 101" East. The district represents a total area of 1205.28 km². The altitude ranges from 970 masl in the south bordering Kenya to 1693 masl in the Northeast. The climate is semi-arid which receives annual average rainfall ranging from 500mm in the South to over 700mm in the North. The area receives bimodal rainfall, where 56% of the annual rainfall occurs from March to May and 27% from mid September to mid November (Coppock, 1994). Annual mean daily temperature varies from 19 °C to 24 °C with moderate seasonal variation. The study was conducted from June 2010 to May 2011.

Data collection and milk sampling procedures

Laboratory analysis was performed to investigate the microbiological quality and safety of raw milk taken directly from cow's udder and milk storage containers at each household level. Pastoral community of six Kebeles (Debeke, Dibbicha, Gollolcha, Ture-Kejima, Okkicha and Wadye-Kejima) were selected from 27 Kebeles of Abaya district using purposive sampling procedures based on their geographical location, proximity to fresh milk, and socioeconomic characteristics. By using simple random sampling technique 60 non model and 36 model HH's were selected depending on livestock husbandry among each pastoral dairy community of six Kebles. But the model households were selected by agricultural sector of the district based on their better performance with regard to dairy production, risk management and handling of animals.

Sixty samples from the non-model households each from cow's udder and storage container were taken. From the model households, 36 milk samples each from cow's udder and storage containers were collected.

Table 1. Mean (\pm SE) value of raw cow milk based on methylene blue reduction and resazurine decolouration time

Study Kebele	Dye reduction tests	
	MTB (hours)	RT (Hours)
Gololicha	1:56 (0.16) ^d	2.75 (0.21) ^a
Debeke	2:06 (0.21) ^c	2.06 (0.21) ^a
Dibbicha	2:44 (0.18) ^b	2.38 (0.20) ^a
Ture-kejima	2:56 (0.22) ^b	2.00 (0.20) ^a
Wadye-Kejima	2:34 (0.21) ^{bc}	2.21 (0.23) ^a
Okkicha	3:15 (0.25) ^a	2.25 (0.19) ^a
Grand Mean	2:22 (0.09)	2.04 (0.09)

Column mean value with difference superscript letters for each milk quality parameters are significantly different ($p < 0.05$); SE= Standard mean of error; MBT=methylene blue test; RT= Resazurine test

Approximately 200 ml were aseptically sampled in the early morning into sterile screwed bottles and transported in ice box filled with ice packs and brought to Hawassa University, Dairy Science Laboratory.

Microbial quality analysis

Sanitary quality tests

Methylene blue test (MBT)

One ml of methylene blue solution was added to 10 ml of milk samples after thorough mixing. The test tubes were sealed with a clean, sterile dipper stopper and slowly inverted each tube twice, to mix the milk and solution thoroughly. The tubes were placed in the water bath at a temperature of 36 °C and examined the samples after 30 minutes. Then the final time of colour disappearance was recorded throughout sample tests (Ombui et al., 1995).

Resazurin test (RT)

Milk samples were mixed thoroughly and 10 ml was poured into previously sterilized test tube. 1 ml of resazurin solution (working solution) was added quickly into the test tube. Milk samples were then placed in the water bath at 37°C and reading was taken place at hourly intervals. After the first hour tubes were examined by noting the degree of colour change from blue through mauve, purple, pink and finally colourless after a stated period of incubation, or the time required reducing the dye to a predetermined colour (Benson, 2002).

Microbial enumeration, isolation and identification

Total Bacterial Count (TBC)

The TBC was made by incubating surface plated appropriate decimal dilutions of raw milk samples on Plate Count Agar (PCA) medium at 32 °C for 48 Hrs,

and dilutions were selected so that the total number of colonies on a plate was between 30 and 300 (Richardson, 1985).

Total Coliform Count (TCC) and Fecal Coliform Count (TFCC)

One ml of milk sample was added into sterile test tube having 9 ml peptone water. Appropriate decimal dilutions of milk samples were pour-plated on Violet Red Bile Agar (VRBA) and then incubated at 30°C for 24 Hrs. Typical dark red colonies were considered as coliform colonies. 1 ml aliquots from each coded dilution were transferred in to three tubes of Lauryl sulfate tryptose (LST) broth. Tubes were incubated at 35°C for 48 Hrs and the number of positive tubes per dilution (with gas production) was recorded. The appropriate three-tube combination was used and the numbers of “presumptive coliforms” were determined from the MPN table 1 ml aliquot from each positive LST tube was transferred into tubes containing 2% BGLB and EC broth and then tubes were incubated at 44.5°C for 48 Hrs, respectively. The appropriate three tubes combination was used and the numbers of “confirmed coliforms” and “fecal coliforms” were determined from the MPN table according to Marth (1978).

Total Staphylococci Counts (TSC)

One ml sterile pipettes were used to place 0.1 ml aliquots from each dilution into two properly labeled mannitol salt agar (MSA) plates. The plates was spread and incubated at 37°C for 45± 2 Hrs. Typical *Staphylococci* colonies appeared as golden yellow, smooth, circular, convex, and moist were counted. For confirmation, four to five of typical colonies per MSA plate were streaked on Mannitol salt agar (Oxid, UK), which was followed by catalase test and Gram stain (ISO, 1999; Yousef and Carlstrom, 2003).

Escherichia coli (E.coli)

Loop full aliquots were streaked from one or two positive tubes of BGLB and E.C broth onto MacConkey agar or EMB plates and incubated at 35°C for 24 hours. Five to six pink to red colour and greenish metallic sheen colonies were randomly picked per plate respectively, and subsequently sub cultured on fresh MacConkey agar or EMBA plates. One or two typical suspect colonies were streaked onto nutrient agar from each plate and incubated at 35°C for 24 hours. The pure isolates colonies were subjected to gram staining. To confirm for the presence of Gram-negative rod isolates, the IMViC (Indole, Methyl red, Voges proskauer, and Citrate) and sugar test (Quinn et al., 1999) were conducted.

Isolation and identification of catalase positive and negative staphylococci

One or two typical and/or suspect colonies were transferred from each MSA plate in to nutrient broth (NB) tubes and incubated at 35°C for 48 Hrs. Following incubation period, a loop full of NB were streaked on the nutrient agar plates and incubated at 35°C for 48 hrs. The pure isolate colonies were subjected to gram staining, catalase test and conformation biochemical and sugar test were carried out following standard of manufacturing instructions (Quinn et al., 1999).

Salmonella spp and Shigella

A portion of 1 ml of milk was pre-enriched in 9 ml of lactose broth at 37°C for 24h. Then, 1 ml of pre-enrichment sample was inoculated in to 10 ml RVS and cystine selenite broth and incubated at 37°C for 24 hr. A loop full of selective enrichments were streaked on the Hekton enteric agars (HEA), Xylose- lysine decarboxylate (XLD), and *Salmonella-shigella agar* (SSA) and incubated at 37°C for 24 hrs. All suspected non-lactose fermenting *salmonella* colonies were picked from all plate agars and streaked onto nutrient agar plate and then incubated at 37°C for 24 hrs.

From each plate agar pure isolate single colonies were picked and inoculated into biochemical tubes for biochemical tests which includes triple sugar iron (TSI) agar, lysine decarboxylate broth, simmon's citrate agar, H₂S, Indole and Motility (SIM test), urea broth, and MR-VP broth. Then tubes were kept in an incubator for 24 or 48 hours at 37 °C. An alkaline slant with acid (yellow colour) butt on TSI with hydrogen sulphide production, positive for lysine (purple colour), negative for urea hydrolysis (red colour), negative for tryptophan utilization (indole test), negative for Voges proskauer (yellow-brown ring), and positive for citrate utilization (blue colour) were considered as *Salmonella* positive

(ISO, 1998; Quinn et al., 1994).

Data management and analysis

The descriptive statistic such as mean, percentage and range was employed to analyze the data. The microbiological counts were logarithmically transformed, and the results were analyzed using GLM procedure of SPSS (Ver. 16). Duncan range tests procedure was used to test significant difference ($p < 0.05$) between source of samples, HH's and Kebeles.

RESULTS

Microbiological quality of milk collected from pastoral community

Quality of raw cow milk based on dye redaction tests

The mean value of methylene blue test (MBT) colour disappearance time of raw cow milk samples was 2:22 Hrs in six Kebeles of Abaya district. Among the selected Kebeles, shorter decolouration time (1:56 Hrs) of raw milk were recorded for Gololicha Kebele while high decoloration time (3:15 Hrs) were recorded for Okkicha Kebele (Table 1). The mean value of resazurine discolouration time of raw cow milk was 2.04 Hrs. There was no significant difference among kebeles in resazurine discolouration test.

Microbiological load of milk in the studied kebeles

The TBC in Ture- Kejima was higher than that of Okocha, Dibicha and Gololicha whereas Debeke and Wadye-Kejima had intermediate values. The CC load in Ture-Kejima Kebele was significantly higher ($p < 0.05$) than that of Dibbicha whereas other kebeles had intermediate values (Table 2). The TFCC in Okkicha Kebele was significantly lower ($p < 0.05$) than that of Gololicha and Ture-Kejima whereas other kebeles had intermediate values. The TSC were similar ($p > 0.05$) among Kebeles.

Raw milk hygienic quality indicators at pastoral dairy households

There was no significant difference ($p > 0.05$) among non-model and model HH's in TBC, CC, TFC and TSC. The TBC, CC, TFC and TSC mean results for milk collected from storage containers were significantly higher ($p < 0.05$) than that of udder milk samples.

Bacterial isolates of raw cow milk collected from udder and storage Containers

Out of the total samples from both direct milked from udder of cow and milk storage containers taken, none proved to

Table 2. Mean (\pm SE) of microbial load (\log_{10} cfu mL⁻¹) of raw milk samples collected from pastoral community of Abaya District

Microbial count	Study Kebeles	N	Mean(\pm SE)	95% Confidence interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
TBC	Gololicha	16	7.50 (0.18) ^b	7.13	7.88	6.26	8.39
	Debeke	16	7.80 (0.13) ^{bc}	7.52	8.09	6.95	8.47
	Dibbicha	16	7.58 (0.14) ^{ab}	7.29	7.88	6.67	8.37
	Ture Kejima	16	7.88 (0.13) ^c	7.6	8.16	7.16	8.46
	Wadye Kejima	16	7.73 (0.15) ^{bc}	7.41	8.05	6.79	8.44
	Okkicha	16	7.36 (0.17) ^a	7.00	7.72	6.00	8.39
CC	Gololicha	16	7.33 (0.15) ^{ab}	7.01	7.65	6.26	7.98
	Debeke	16	7.33 (0.14) ^{ab}	7.02	7.64	6.57	8.07
	Dibbicha	16	7.18 (0.14) ^a	6.87	7.48	6.3	7.93
	Ture Kejima	16	7.46 (0.14) ^b	7.16	7.76	6.76	8.14
	Wadye Kejima	16	7.32 (0.12) ^{ab}	7.07	7.57	6.56	7.92
	Okkicha	16	7.37 (0.11) ^{ab}	7.13	7.60	6.8	7.93
FCC	Gololicha	16	5.25 (0.23) ^b	4.76	5.75	4.11	7.38
	Debeke	16	5.16 (0.21) ^{ab}	4.71	5.61	3.79	6.46
	Dibbicha	16	5.01 (0.19) ^{ab}	4.6	5.43	3.79	6.32
	Ture Kejima	16	5.57 (0.21) ^b	5.13	6.02	4.38	7.04
	Wadye Kejima	16	5.18 (0.24) ^{ab}	4.67	5.68	3.95	6.66
	Okkicha	16	4.69 (0.26) ^a	4.13	5.25	3.48	6.66
TSC	Gololicha	16	7.3 (0.14)	7.01	7.59	6.26	8.10
	Debeke	16	7.24 (0.15)	6.91	7.56	6.21	8.01
	Dibbicha	16	7.42 (0.09)	7.22	7.62	7.01	7.97
	Ture Kejima	16	7.41 (0.11)	7.19	7.64	6.46	8.06
	Wadye Kejima	16	7.4 (0.12)	7.15	7.64	6.58	7.98
	Okkicha	16	7.23 (0.14)	6.94	7.53	6.08	7.84

Column mean value with different superscript letters for each milk quality parameters are significantly different ($p < 0.05$); SE= standard error of mean; N=Number of source of sample points; TBC=Total bacterial count; CC=Coliform count; FCC=Faecal coliform count; TSC= Total staphylococci count

be negative for the targeted microorganisms. In all of the positive samples, the type of isolated bacteria from cow's udder was similar to bacteria isolate from milk storage containers except the increment of isolation rates among few of bacterium species from storage containers.

The major bacteria isolated from both sources of positive sample were *Staphylococcus aureus*, *Staphylococcus intermidus*, *Staphylococcus epidermidus*,

and *Micrococcus luteus*, *Escherichia coli*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Proteus*, *Pseudomonas*, *Salmonella*, *Shigella* and *Yersinia species* (Table 4). Moreover, milk appeared to be contaminated with environmental bacterial agents such as *E.coli*, *Proteus* spp, *Citrobacter* spp, *Enterobacter* spp, *Klebsiella* spp, *Pseudomonas* spp, and *Yerisina* spp from milk taken directly from the udder and storage containers.

Table 3. Mean (\pm S.E) value of quality indicator parameters (\log_{10} cfu mL⁻¹) of raw milk samples collected from pastoral dairy of both households and source of sample points of study area

Microbial Count	N	Selected households	Sample source		Mean (type of farmers)
			Cows udder	Storage containers	
TBC	60	Non-model	7.04(0.01)	8.15(0.04)	7.59(0.01)
	36	Model	7.31(0.1)	8.14(0.1)	7.73(0.1)
		Mean	7.18 (0.1) ^b	8.15 (0.03) ^a	
CC	60	Non-model	6.89(0.1)	7.81(0.03)	7.36 (0.1)
	36	Model	6.86(0.1)	7.72(0.1)	7.29(0.1)
		Mean	6.88 (0.04) ^b	7.78 (0.04) ^a	
FCC	60	Non-model	4.54(0.2)	5.76(0.1)	5.15(0.1)
	36	Model	4.73(0.2)	5.55(0.1)	5.14(0.1)
		Mean	4.61 (0.1) ^b	5.68 (0.1) ^a	
TSC	60	Non-model	7.02(0.1)	7.65(0.1)	7.33(0.1)
	36	Model	6.96(0.1)	7.70(0.1)	7.33(0.1)
		Mean	6.99 (0.1) ^b	7.67 (0.1) ^a	

Column (for type of farmers) and raw (sample sources) mean value with different letters vary significantly ($p < 0.05$); S.E= standard error; N=Number of source of sample points; TBC=Total bacterial count; CC=Coliform count; FCC=Faecal coliform count; TSC= Total Staphylococci count

DISCUSSION

Quality of milk based on methylene blue reduction and resazurine test

The shorter time required for the disappearance of the blue colour is indicative of a higher microbial load (Bongard et al., 1995; Marker et al., 1997). In this study most of raw cow's milk shows very short discolouration time of the dye. This may be due to poor milk handling practices during milking, poor animal health services, and use of poor potable water which were linked to markedly high TBC (Nandy et al., 2007).

Milk quality and hygienic practices at Kebele's level

Production of milk and various dairy products take place under rather unsanitary conditions and poor production practices. At the production level, milking and handling of milk are the concern because personal as well as milking equipment hygiene is insufficient among the milk handlers ((Mogessie, 1990; Zelalem and Faye, 2006). In line to these facts, contamination of milk during milking and handling is high due to the use of unclean milk handling equipment and water used for cleaning, unclean personnel hands, insufficient washing of udder and lack of cooling facilities and absence of any test to screen abnormal milk. These could lower milk quality and have significant concern on consumer's health (Jayarao and Wang, 1999; (Mogessie et al., 2001; Jayarao et al., 2004). In the studied area, milk is stored in locally made material called *Gorfu, Okkole, Cicu* and , aluminium cans and plastic jerry cans (Tollosa, 2011) which are very difficult to clean and can contribute to milk spoilage.

The mean TBC in the current study was 7.64 \log_{10} cfu mL⁻¹ which is comparable with the values (6 to 8.8 \log_{10} cfu mL⁻¹) reported by Fekadu (1994) for cow milk produced in southern region of Ethiopia. Similarly, Alganesh (2002) reported comparable TBC results (7.4×10^7 and 2.0×10^7 cfu/ml, respectively) for cow's milk from Eastern Wollega. The TBC in the current study was $\geq 10^5$ cfu mL⁻¹ levels in 99% of the raw milk samples collected from pastoral community. The TBC is an indicator of the general hygienic condition during milk production, transportation and storage. In general, lack of knowledge about clean milk production and use of unclean milking and handling equipment might be some of the factors which contributed to the poor hygienic quality of milk.

In addition to the aforementioned reasons the high microbial load of milk may be due to unclean milking and housing environment, and failing to rapidly cooling the milk to or maintain it at less than 4.4 °C (Mehari, 1988). Of more importance is the contribution of microorganisms from teats soiled with manure, mud, feeds, or bedding. These practices expose teat end to organic waste sources, wet and muddy pens which increases the risk of developing mastitis and milk contamination (Ruegg et al., 2002). Therefore, microbiological quality of the samples in this study seems to be low.

Raw milk quality under the model and non-model households

According to American and European community member states, the acceptable limit for TBC and CC for raw milk is between 2×10^5 and 4×10^5 cfu/ml and 150 cfu/ml, (APHA, 1995; Heeschen, 1997) respectively. However, in this study the mean values of TBC of raw cow milk were 7.59 \log cfu/ml and 7.73 \log cfu/ml for non

Table 4. Bacterial isolates from pastoral dairy households of Abaya District of Borana Zone

Bacteria isolated	Selected source of samples	
	Cow udder (N=48)	Storage container (N=48)
	n (%)	n (%)
<i>Escherichia coli</i>	116(12.89)	115(12.91)
Enterobacter genera		
<i>Enterobacter aerogenes</i>	80(8.89)	69(7.74)
<i>Enterobacter cloacae</i>	60(6.67)	47(5.28)
<i>Enterobacter agglomerans</i>	59(6.56)	52(5.84)
Citrobacter genera		
<i>Citrobacter freundii</i>	21(2.33)	19(2.13)
<i>Citrobacter diversus</i>	25(2.78)	14(1.57)
Klebsiella genera		
<i>Klebsiella pneumoniae</i>	60(6.67)	59(6.62)
<i>Klebsiella oxytoca</i>	48(5.33)	56(6.29)
<i>Pseudomonas aeruginosa</i>	24(2.67)	17(1.91)
Proteus genera		
<i>Proteus vulgaris</i>	25(2.78)	28(3.14)
<i>proteus mirabilis</i>	17(1.89)	9(1.01)
Salmonella genera		
<i>Salmonella typhi</i>	54(6)	61(6.85)
<i>Salmonella typhimurium</i>	45(5)	41(4.6)
<i>Shigella dysenteriae</i>	32(3.56)	43(4.83)
Shigella genera		
<i>Shigella boydi</i>	23(2.56)	29(3.26)
<i>Shigella flexneri</i>	25(2.78)	28(3.14)
<i>Shigella sonni</i>	21(2.33)	25(2.81)
<i>Yersina enterocolitica</i>	12(1.33)	17(1.91)
Staphylococcus coagulase positive		
<i>Staphylococcus aureus</i>	61(6.78)	65(7.29)
<i>Micrococcus luteus</i>	41(4.56)	45(5.05)
Staphylococcus coagulase negative		
<i>Staphylococcus epidermis</i>	30(3.33)	27(3.03)
<i>Staphylococcus intermedius</i>	21(2.33)	25(2.81)

N=Number of milk sample from points; n=number of bacteria isolates

model and model household, respectively, the counts which are greater than the upper acceptable limits.

The TBC obtained from the current result was higher than that of Mehari (1988) (10^7 - 10^9 cfu ml⁻¹), Hailu (1989) (1.7×10^7 - 7.5×10^7 cfu ml⁻¹), Rai and Dawvedi (1990) (4.8×10^6 cfu ml⁻¹), Ashenafi and Beyene (1994) (2.1×10^6 cfu ml⁻¹), Ombui et al., (1995) (10^5 cfu ml⁻¹), DeGraaf et al., (1997) (3.88×10^7 cfu ml⁻¹), Godifay and Molla (2000) (1.9×10^8 cfu ml⁻¹), Bonfoh (2003) (10^7 cfu ml⁻¹) and Esther et al. (2004) (10^6 and 3×10^7 cfu ml⁻¹). The FCC obtained in the current study is higher than the results reported by Dan et al. (2008) (2.66 to 5.94 log₁₀ cfu/ml) and Franciosi et al. (2009) (1.84 log₁₀ cfu/ml) and. Also the mean coliform counts of raw milk in this study was higher than the reports of Rai and Dawvedi (1990) from India (7.7×10^5 cfu/ml), Kurwijilla et al. (1992) from Tanzania (10^5 cfu/ml), Ombui et al. (1995) from Kenya (5×10^4 cfu/ml), Godifay and Molla (2000) from Ethiopia (7×10^4 cfu/ml) and Bonfoh (2003) from Mali (10^6 cfu/ml).

However, the results disagree with the finding of Mutukumira et al. (1996) who found the coliform bacteria to be between 2.51 to 5.36 log cfu/ml. Saitanu et al., (1996) also found that the total coliform count of <3 log cfu/ml. A count of <50 cfu/ml is considered as acceptable upper limits. But in this study, 99% of source of samples point had >50 cfu/ml. Milk produced under hygienic conditions from healthy cows should not contain more than 5×10^4 bacteria per milliliter (O' Connor, 1994). Also lower result was observed for raw milk samples collected from storage containers and/ or utensils at farm level (Mogessie and Fekadu, 1993) ($6.0 \log_{10}$ cfu ml⁻¹) and (Haile et al., 2012) ($4.93 \log_{10}$ cfu ml⁻¹) for milk samples from Hawassa Dairy farms. In the dairy farm setting, a CC is a useful indicator of the extent of fecal bacteria in the milk, and is a recognized index of the level of sanitation at a facility. The use of CC as an indicator of sanitation has been a common tool in public health

protection for many years (Chambers, 2002; Yousef and Carlstrom, 2003; Jay, 2000).

The mean values of TSC were higher in storage of container of both households than milk directly collected from cow's udder for both households. This is may be due to poor hygiene either during equipment cleaning and sanitation, during milking, or between milking. Milking wet udders, inadequately boiled washing water, inadequate cooling of milk and udder infections all contribute to high counts in bulk milk.

The higher values found in this study for TBC, TCC, FCC and TSC for the milk samples collected from storage container compared to EU and USA standards could be attributed to the cumulative results of milk contamination at different levels. Some samples were held up to 1 and 1/2 hour after milking in the traditional and inadequately cleaned milking utensils (*Okkole, Gorfa, Cicu*) and plastic containers. Among the factors that affect the quality of dairy products, adequately performing milking procedures and cleanness of the milking utensils used for milk and milk product handling is commonly mentioned (Almaz et al., 2001). This may lead to insufficient cleaning and become a major source of milk contamination while milk is transported and stored throughout the market chain (Kurwijilla et al., 1992; O'Connor, 1995; Godifay and Molla, 2000).

Milk residues on equipment surfaces provide nutrients for growth and multiplication of bacteria that can then contaminate the milk of subsequent milking. According to this study factors that could contribute to the contamination of milk might include insufficient pre-milking udder preparation, insufficient cleaning of milkers' hands and milking utensils, use of poor quality and non-boiled water for cleaning of milk equipments and storage containers. Additional handling of milk with different plastic containers and sieves may cause the contamination of milk further, since as the number of plastic containers and sieves increased the chance of contamination is also increased and most plastic containers have characteristics that make them unsuitable for milk handling.

Microbial safety of milk under the pastoral community

In the course of this study bacteria belonging to 11 genera from raw milk taken directly from cow udder and storage containers were isolated (Table 4). Even *E. coli*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Proteus*, *Pseudomonas*, *Salmonella*, *Shigella* and *Yersinia species* were both fecal and non fecal organisms isolated from both sampling sources. The existence of fecal coli form bacteria may not necessarily indicate a direct fecal contamination of milk but it is a precise indicator of poor sanitary practices during milking and further handling processes. The presence of *E. coli* implies a risk that

other enteric pathogens may be present in the sample (Hayes et al., 2001).

The incidence of fecal coli forms in raw milk has received considerable attention, partly due to their association with contamination of fecal origin and the consequent risk of more pathogenic fecal organisms being present, partly because of the spoilage that can result from their growth in milk at ambient temperatures and not least due to the availability of sensitive and rapid tests for detecting and enumerating coli forms. Sporadic high coliform counts may also be a consequence of unrecognized coliform mastitis, mostly caused by *E. coli*. The coliform microorganisms are found also on the surface of the under shed or moist milking equipment (Bramley and McKinnon, 1990). Although detection of *E. coli* in milk reflects fecal contamination, environmental coli forms have also been detected in milk (Adesiyun et al., 1990). Milk can be easily contaminated by infected food handlers who practice poor personal hygiene or by water containing human discharges. The presence of *E. coli*, therefore, indicates a safety risk and the numbers of *E. coli* should be at the minimum recommended levels (Su and Wong, 1997; James, 2000; Anonymous, 2008).

Psychrotrophic bacteria are important because, although mostly not thermophilic, many of them produce extracellular thermostable proteolytic and lipolytic enzymes which can survive pasteurization (DeGraaf et al., 1997), thus affecting the shelf life and quality of the dairy product. In this study psychrotrophic bacterial isolates (*Pseudomonas spp*) was detected in 2.67% of samples from udder, and 1.91% of isolates from storage containers (Table 4). Even though the isolation rates of these bacteria were low, it might contribute to the high bacterial counts to the source of sampling points of raw milk.

In this study isolation of *Staphylococcus aureus*, *Staphylococcus intermidus*, Coagulase negative *Staphylococcus*, *E. coli*, *Pseudomonas aeruginosa* and *Enterobacter aerogenes* are incriminated as causes of sub clinical and clinical mastitis in the cow (Harding, 1999). Moreover, *Citrobacter freundii*, *Citrobacter divareuis*, *Enterobacter agglomerans*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca* from udder milk samples might indicate that these bacterial agents could probably cause mastitis (Harding, 1999).

Microorganisms such as *Staphylococci* spp and *Micrococcus spp* are included in the contagious cause of mastitis, while *E. coli*, *Pseudomonas aeruginosa*, *Citrobacter freundii*, *Citrobacter divareuis*, *Enterobacter agglomerans*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* classified as causes of mastitis caused by environmental origin (Bonfoh, 2003). The contribution of mastitis udder in the bacterial quality of cow milk is an established fact and therefore adequate control of mastitis in the pastoral community could help to achieve higher returns to the pastoral producer and to enhance the production of high quality dairy products (Harding,

1999). The type and number of bacteria present in the milk influence the hygienic quality of milk. Those isolates of *Staphylococcus spp*, *Micrococcus spp*, *Pseudomonas spp* and coliform microorganisms can cause spoilage of the milk when present in raw milk (Doyle, 1997).

CONCLUSION

The quality of milk produced from HH's of pastoral dairy producers was substandard. Milk quality indicators used, on average are higher than international standards. The high microbial load of TBC, CC, TFCC, TSC and bacterial isolates clearly demonstrated exogenous sources of bacterial contamination. The milk was also subjected to more contamination as it was under high ambient temperature and without cold chain facility and using equipments, which were not clean. All raw milk samples from the udder of the cow and storage container had higher TBC, CC, TFCC and TSC, which was higher than the international acceptable limits. Hence its keeping quality would be lower and some of the pathogens present in the milk have public health significance. The results obtained in this study showed that raw milk available to the consumers has a high bacterial level of contamination. Measurable increased in TBC, TCC, FCC, and TSC throughout all source sampling points was observed.

All together, these results show that urgent measures are needed to ensure lean and safe milk production at pastoral community level, by the promotion of good hygiene practices. These would preferably need to focus on efficient cleaning of vessels, milker hands, udder and improve the health condition of dairy animal health. These measures should be applied massively because this criterion remains very poor in the all of dairy households and also the high level of counts found in the milk at each Keble of pastoral HH's, one may suppose that this milk may pose a public health risk and this suggests the need for more strict preventive measures.

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