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RESEARCH PAPER

Received: 19/02/2014 Revised: 18/03/2014 Accepted: 23/03/2014 Microbiological Quality and Safety of Raw Milk Collected from Kersa District, Jimma Zone, Southwest Ethiopia Teshome Tadesse and Ketema Bacha

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ABSTRACT

The safety of milk with respect to food-borne pathogens is a great concern worldwide. Therefore, this study was to assess the microbiological quality and safety of raw cow's milk of individual farmers and dairy farms in Kersa district. Microbial analysis of the milk was analyzed for a total of 100 samples following standard procedures. The hygienic quality of raw milk was poor with an overall mean total bacterial count, coliform count, lactic acid bacteria, staphylococci, yeasts and mould counts of 8.48, 5.82, 4.53, 5.23, 4.8, 4.35 log CFU/ml, respectively. The percent lactic acid and pH of the samples were 0.26 and 6.27, respectively. There were significant difference (P < 0.05) among mean counts of microbes except between mean counts of staphylococci and coliforms. The bacterial groups isolated from raw milk were Staphylococcus spp., Streptococcus spp., Enterobacteriaceae, Micrococcus spp., Pseudomonas spp., Bacillus spp. Lactobacillus spp., Leuconostoc spp. and Aeromonas spp. The samples of both farm groups were dominated by Lactobacillus followed by Staphylococcus spp. and Streptococcus spp. Among pathogenic bacteria of public health significance, S. aureus and Salmonella spp. were also detected in 34 (34%) and 20 (20%) samples, respectively. S. aureus isolates were showed the highest resistance to Methicillin (100%) and Penicillin G (91.2%). Likewise, Salmonella isolates were most highly resistance to Nalidixic acid (80%). Thus, hygienic quality of raw cow's milk was poor which may cause a potential health risk and therefore hygienic safety measure should be taken. Key Words: Cow milk, Kersa district, Microbial count, and Milk quality.

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INTRODUCTION

Milk is a nutritious food for human beings, but it also serves as a good medium for the growth of many microorganisms, especially pathogenic bacteria (Chye et al., 2004). Thus, the quality of milk is considered essential to the health and safety of a community. Also, all cases of dairy illness continued to be of bacterial origin, pathogens that have involved in communicable diseases associated with the consumption of milk include Salmonella, Listeria monocytogenes, Staphylococcus aureus, Campylobacter, Yersinia, Escherichia coli and Clostridium botulinum (Ali et al., 2010; Bonfoh et al., 2003). A major concern about bacterial pathogens carried by milk and milk products is that antimicrobials are frequently used for prevention and treatment of infectious diseases such as mastitis (Mahami et al., 2013; Rosati and Aumaitre, 2004). Thus, as a consequence of the high antimicrobial use in dairy farms, bacterial contaminants carried by milk often show high levels of antimicrobial resistance (Sandgren et al., 2008). Raw milk of good hygienic quality is necessary to produce milk products of good quality and adequate shelf life and to produce a safe, sound and wholesome for consumers (Ali et al., 2010). Milk guality starts to deteriorate immediately after milking since microorganisms contaminate the milk from a wide variety of sources. The main sources of contamination in the farm are cow's udder and body, utensil, milking machines, and the transportation equipment. Generally, contamination of raw milk occurs from three main sources: within the udder, the exterior of the udder, and from the skin of the handlers and the surface of storage equipments (Bramley and Mckinnon, 1990). However, keeping milk in clean containers at refrigerated temperatures immediately after milking process may delay the increase of initial microbial load and prevent the multiplication of microorganisms in milk between milking at the farm and transportation to the processing plant (Adesiyum 1995; Bonfoh et al., 2003). In Ethiopia, rawer cow milk is consumed than processed milk in the rural areas and also in the urban centers including Jimma town and its surroundings. However, the hygienic status or quality of milk and the prevalence of milk related out breaks was not well assessed. Furthermore, 98% of the annual milk productions are by subsistence farmers in the rural areas where dairy facilities are almost non-existent (Tsehay, 2002). There is scanty information on the microbial guality and chemical composition of raw milk in Ethiopia (Eyasu and Fakedu, 2000; Zelalem and Faye, 2006). Nevertheless, the Knowledge and hygienic status of the community with respect to production of raw milk available to the community of the study is not explored yet. Thus, the main aim of this study was to investigate the microbial quality and safety of raw cows' milk and assess antimicrobial resistance patterns of selected pathogenic bacteria in raw cows' milk collected from Kersa district, Jimma zone, southwest Ethiopia.

MATERIAL AND METHODS

Study area, Design and Period: Laboratory-based cross-sectional study was conducted in Kersa district, Jimma Zone, which is found 335 km from Addis Ababa Southwest Ethiopia from December, 2012 to June, 2013.

Preliminary survey: The survey was performed in order to determine the following aspects which might affect the hygienic quality of milk including: hygienic practices of milking (udder cleaning, use of detergent, and teat dipping), type of milking (manual vs. mechanical), types of equipment used, the health status of cows and sanitary status of barn were assessed. For data collection, individual households were selected by applying population formula from municipality documentation and stratified based on who owned lactating cows. Of the total population, some of the households were selected randomly from a list of farmers registered as milk producers in their respective kebeles. The study cows were lactating local (individual farmers) and crossbreds (dairy farm) cows. Finally, seven areas were selected purposively based on their potential for production of milk.

Sample Collection: A total of 100 samples of raw cow milk were separately collected at different occasions using random sampling technique. Individual raw cow milk samples were collected aseptically in sterilized 300 ml screw caped bottles from each area in duplicate, over a period of 6 months (December to June, 2013). The collected milk samples were transported to Postgraduate and Research Laboratory of Biology Department, College of Natural Sciences, Jimma University, using cold chains. After transportation samples temporarily were kept under refrigeration until processed for microbiological analysis and evaluation of some physico-chemical parameters within 3 to 8 hours of collection.

Microbial analysis: one ml of each sample was transferred aseptically and separately into 9ml of sterilized peptone water (1.5%) by autoclaving at 121°C for 15 minutes and homogenized by using vortex mixer. The homogenates were serially diluted and 0.1ml aliquot of appropriate dilutions was spread-plated in duplicate on pre-dried plates for microbial counts: aeobic mesophilic bacteria (AMB) were counted on Plate Count Agar (PCA) (Oxoid) after incubation at 32° C for 48 hours; Violate Red Bile Agar (VRBA) Oxoid) was used to count coliforms after incubation for 48 hours at 32°C. Staphylococci were counted on Mannitol Salt agar (MSA) (Oxoid) after incubation at 32°C for 48 hours. After counting, Staphylococcus aureus was identified by taking the suspected yellow colonies from MSA showing mannitol fermentation. By Gram staining the Gram-positive cocci with clustered arrangement under the microscope were subjected to biochemical tests (catalane, Cytochrome Oxidase and coagulase tests). Likewise, yeasts and molds were counted on Chloramphenicol-bromophenol blue agar incubated at 25-28°C for 2-5 days. Smooth (non-hairy) colonies without extension at periphery (margin) were counted as yeasts. Hairy colonies with extension at periphery were counted as molds. Lactic Acid Bacteria (LAB) were also counted on MRS (De man, Rogasa, Sharpe) (Oxoid) agar plates that incubated an aerobically using anaerobic jar (anaerobic Gas pack System, Oxoid) at 30–32°C for 48 hours. All glistening colonies were counted as lactic acid bacteria. After enumeration, ten colonies were randomly picked from countable plates of PCA and MRS

and further purified. Pure cultures were temporarily preserved on Nutrient agar slants until used. An overnight activated culture was further characterized using the following basic tests: Gram's reaction, cell morphology, biochemical testes including KOH- test, Catalase test, Cytochrome Oxidase Test and Oxidation fermentation (O/F) test.

The procedure has been used for detection of *Salmonella* from milk was as per the ISO-6579: 2002 standard. Milk sample was dispersed into suitable non-selective medium (buffered peptone water). One ml of the pre-enrichment culture was transferred into selective enrichment broth (10 ml Rappaport Vassiliadis soy peptone (RVS) and was incubated at 41.5° C $\pm 0.5^{\circ}$ C for 18-24hr. Subsequently; the enriched sample was streaked onto each of the Brilliant green agar (BGA) and Xylose Lysine Deoxycholate agar (XLD) and incubated at 37° C for 24hr. The presumptive *Salmonella* colony on the XLD and BGA was selected and identified by using a series of biochemical tests including reactions on lysine iron agar (LIA), Triple Sugar Iron agar (TSA), urea agar, Simmon citrate agar and SIM medium.

Antimicrobial susceptibility testing for Salmonella spp. and S. aureus: the antimicrobial susceptibility testing for Salmonella and S. aureus were carried out following the Kirby-Bauer disc diffusion method on Mueller-Hinton agar plates (Oxoid) as described in the Clinical and Laboratory Standards Institute (CLSI) guidelines (NCCLS, 2002). The following 10 drugs were used with their respective concentration (in brackets) to determine the antibiogram of the S. aureus: Chloramphenicol (30µg), Gentamycin (10µg), Methicilin (10µg), Penicillin G (10units), Erythromycin (15μg), Ciprofloxacin (30μg), Streptomycin (10μg), Vancomycin (30 μg), Kanamycin (30µg) and Tetracycline (25µg) whereas the following 8 antimicrobials were used for Salmonella: Chloramphenicol (30µg), Gentamycin (10µg), Streptomycin (10µg), Tetracyline (30µg), Ciprofloxacillin (30µg), Kanamycin (30µg), Nalidixic acid (30µg), and Amikacin (30µg). A standardized suspension of the bacterial isolates was prepared and adjusted to the 0.5McFarland turbidity standard. Subsequently it was streaked in to the Muller-Hinton Agar; the antibiotic discs (OXOID) were dispensed on the medium and incubated at 35°C for 18 hours, followed by measurement of zone of inhibition manually. Finally, the isolates were classified as sensitive and resistant, as described by Vlková et al., (2006). The criteria used to select the antimicrobial agents tested in this study were based on availability and frequency of prescription of the drugs for the management of bacterial infections in Ethiopia. Moreover, selection was also based on their mechanisms of action. Salmonella ATCC 14028 and S.aureus ATCC 29213 were used as reference strains for quality control of the antibiotics used.

Physico-chemical Analysis: the milk samples were analyzed for titrable Acidity and PH. The pH of samples was determined by dipping an electrode of a digital pH meter into 10 ml aliquot sample. The pH meter was calibrated against standard buffer solutions at pH 4.0 and 7.0 (Ong *et al.*, 2007). Titrable Acidity was determined as described by O'Mahoney (1988). The samples were titrated with 0.1N NaOH solution using a titration kit 0.5% phenolphthalein as indicator. Finally, acidity was expressed as percentage lactic acid (% LA) and calculated using the following equation.

TA = <u>NNaOH x ml NaOH x0.09x100</u> ml milk sample

Where: N= Normality of NaOH used 0.09 = molecular weight of lactic acid 0.1 = N sodium hydroxide

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Data Analysis: microsoft excel was employed for raw data entry, computation of descriptive statistics and drawings. Log₁₀ transformation was done before the analysis of bacterial counts. The significance of differences (P<0.05) of the mean microbial count was evaluated with one-way ANOVA using SPSS of version 16.0.

RESULTS

Information on general farm, milking and management practices were collected during farm visits by interview method and questionnaire. The milking operation is generally conducted in the barns of cattle which were not in good sanitary standards. Despite the prevalence of various animal diseases, the majority of the respondents (86%) had compliant of shortage of animal health services. Accordingly, only 24% of farm owners were making regular check up for the cows by traveling on average about 5 km and a maximum of 21 km to get animal health centre. The majority of the respondents (67%) clean their milk utensils once per day followed by twice (17%) and three times (16%) per day. Even though washing hands and milking vessels has been practiced, washing of udder before and after milking is exercised only by few (13%) of the respondents. About 92% of the respondents use bare hands to dry the cows' udder, while 3% of them use individual towel and 5% use one towel for group of cows. Those farms that had towels, they use it also for cleaning and sanitizing of other. No one of the farmers used antiseptic solution for although teat washing and rely on cold water. Milking is done manually (100% of the cases) and it is performed only twice per day in almost 90% of the considered farm.

Microbial Count

In this study, 100 raw cow milk samples were analyzed for the presence and contamination level of AMB, coliform, LAB, staphylococci, yeast, and moulds. The overall mean microbial counts of AMB, coliform, LAB, staphylococci, yeast, and moulds were 8.48, 5.82, 4.53, 5.23, 4.8, and 4.35 log CFU/ml, respectively (Table 1). Analysis of variance of the mean counts (log CFU/ml) of aerobic mesophilic bacteria, LAB, yeast and mould revealed statistically significant (P< 0.05) difference between the mean counts of milk samples obtained from individual farmers and dairy farms. But, there was no significant difference (P > 0.05) between mean counts of staphylococci and coliforms of milk samples collected from the two farm groups. Likewise, the mean counts of AMB and yeasts were significantly different from counts of the others within individual farms but not within dairy farms (Table 1).

Physico-chemical parameters

The overall mean titrable acidity (TA) of cows' milk produced in the study area was 0.26 % (Table 1). The mean titrable acidity observed in individual farmer and dairy farms were 0.28% and 0.25%, respectively. But, the pH of milk of the dairy farm was found relatively higher than the milk of individual farms. In this study, the pH of milk samples was between 6.18 and 6.37 with mean pH value of 6.27 (Table 1).

Variables		Log CFU/ml (mean ± S.D)					Physico-chemical parameters		
Farm groups		AMB	TCC	LAB	STAPH	YEAST	MOUL	TA (LA %)	рН
							D		
Individual		8.7±	$5.85\pm$	4.24±	5.27±	4.9±	4.61±	0.28±	6.18±
Farmers		1.34 ^b	0.483 ^a	0.76^{a}	0.31 ^a	0.6^{b}	0.5 ^a	0.03 ^a	0.26 ^a
(N=88)									
Dairy	Farm	$8.27\pm$	5.91±	4.94±	5.18±	4.7±	4.09±	$0.25\pm$	6.37±
(N=12)		0.98^{a}	0.19 ^a	0.31 ^b	0.64^{a}	0.52^{a}	0.23 ^b	0.04^{a}	0.19 ^a
Overall	mean	$8.48\pm$	5.87±	4.58±	5.23±	4.8±	4.35±	0.26±	6.27±
of two f	farms	1.06	0.32	0.54	0.45	0.44	0.32	0.03	0.21
(N=100)									

Table 1. Microbial counts (log CFU/ml) and some physico-chemical parameters in raw cows' milk sample collected from local individual farmers and dairy farms, Jimma zone, 2013.

Figures bearing the same superscript in column do not differ significantly (p > 0.05), Where: N = number of study samples; CFU = colony-forming units, Log₁₀ = Logarism in base ten, AMB: Aerobic Mesophilic Bacteria, TCC: Total Coliform Count, LAB: Lactic Acid Bacteria, Staph: Staphylococci, TA = Titrable acidity, LA%=% Lactic acid.

The mean TA (% lactic acid) and pH values of the two farms (Individual and dairy farms) were not statistically significant (P> 0.05).

Microflora analysis: the aerobic mesophilic bacterial flora of raw milk collected from individual farmers was dominated by *Staphylococcus* spp. (19%) followed by *Enterobacteriaceae* (10%), *Micrococcus* spp. (9%) and *Pseudomonas* spp. & *Bacillus* spp. (each 8%) and *Aeromonas* (2%). Similarly, the most predominant genera in raw milk of dairy farms were: *Staphylococcus* spp. (10%) followed by *Enterobacteriaceae* (7%), *Pseudomonas* spp. & *Micrococcus* spp. (5% each), *Bacillus* spp. (4%) and *Aeromonas* spp (1%). In general, the aerobic mesophilic bacterial flora of raw milk was dominated by *Staphylococcus* isolates, in the both farm groups. However, the major isolates belonged to 3 genera of lactic acid bacteria in farm groups were dominated by *Lactobacillus* followed by *Streptococcus* and *Leuconostoc*. Accordingly, *Lactobacillus* species are the frequently isolated LAB among the farm groups.

Among pathogenic bacteria of public health significance, *S. aureus* and *Salmonella* spp. were also detected in 34 (34%) and 20 (20%) raw milk samples, respectively. With regards to frequency distribution among selected sites *Salmonella* spp were not detected in 3 of the dairy farms and 2 kebeles. However, 34 (34%) were positive for *S. aureus* which was prevalent in all samples collected from individual farms and dairy farms.

Antimicrobial susceptibility patterns of *S. aureus* and *Salmonella* spp.

Staphylococcus aureus isolates were exhibited slight resistant to Vancomycin,Ciprofloxacin, Erythromycin and Tetracyline.

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However, out of the tested drugs, the highest resistance was observed against Methicillin and Penicillin G (100% each) followed by Chloramphenicol (58.82) (Table 2). A total of 10 Multiple Drug Resistance (MDR) patterns were observed among isolates of *S. aureus* (Table 3). The highest MDR noted was Met/Pen (14.7%, 5/34), followed by Met/Chl and Pen/Met/Chl (8.8%, 3/34 each). The maximum MDR registered was resistance to five antibiotics with the combination Pen/Chl/Te/Met/Cpr being more frequent (Table 3). Overall, MDR to two and three antibiotics dominate the resistance patterns (23.53%, 8/34 each).

Antimicrobial-	Disk Content	Resistance		Sensitive	
agents	(µg)	N <u>o</u>	%	N <u>o</u>	%
Chloramphenicol	30	20	58.82	14	41.18
Ciprofloxacillin	30	3	8.8	31	91.2
Erythromycin	15	1	2.9	33	97.1
Gentamycin	10	-	-	34	100
Kanamycin	30	-	-	34	100
Methicillin	10	34	100	-	-
Penicillin G	6	34	100	-	-
Streptomycin	10	1	2.9	33	97.1
Tetracyline	30	3	8.8	31	91.2
Vancomycin	30	2	5.88	32	94.12

Table 2. Antibiotic Susceptibility profile of S. aureus isolated from raw milk of Kersa district,
Jimma zone, 2013.

Table 3. Multiple drug resistance (MDR) profile of S. aureus strains isolated from raw milk,
Kersa district, Jimma zone, 2013.

No. of antimicrobial resistance	Antimicrobial resistance pattern (No. of isolates)	No. of isolates (%)	
Тwo	Met, Pen (5)	8 (23.53)	
	Met,Chl (3)		
	Str, Tet,Pen (1)		
Three	Pen,Str,Met (2)	8 (23.53)	
	Ery,Str,Tet (1)		
	Van, Met, Pen (1)		
	Pen, Met,Chl (3)		
Four	Met,Pen,Chl,Van (1)	3 (8.83)	
	Pen,Cpr,Met,Chl (2)		
Five	Pen,Str,Met,Chl,Te (1)	4 (11.76)	
	Pen,Chl,Te,Met, Cpr (2)		
	Pen,Te,Met, Str,Van (1)		

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Table 4. Antibiotic Susceptibility patterns of *Salmonella* isolates in raw milk of Kersa district, Jimma zone, 2013.

No. of	antimicrobial	Antimicrobial resistance	No. of isolates (%)
resistance		pattern (No. of isolates)	
		Nal,Te (1)	
Two		Nal,Chl (2)	4 (20)
		Nal,Gen (1)	
Three		Nal,Te,Amk (2)	
		Chl,Te,Nal (3)	5 (25)
		Tet,Nal,Gen,Amk (1)	
Four		Tet,Str,Nal, Gen (1)	5 (25)
		Kan,Chl,Nal,Amk (2)	
		Nal, Te,Gen, Str (1)	

Where: Tet; Tetracycline; Gen, Gentamycin; Chl, Chloramphenicol; Cip Ciprofloxacin; Str, Streptomycin; Amk, Amikacin; Kan, Kanamycin; Ery, Erythromycin; Met, Methicilin; Pen, Penicillin G; Van, Vancomycin.

Table 5. Multiple Drug Resistance profiles of Salmonella species isolated from raw milk, Kersadistrict, Jimma zone, 2013.

Antimicrobial-	Disk content	Resistance		Sensitive	
agents					
	(μg)	N <u>o</u>	%	N <u>o</u>	%
Amikacin	30	6	30	14	70
Chloramphenicol	30	5	25	15	75
Ciprofloxacin	5	1	5	19	95
Gentamycin	30	5	25	15	75
Kanamycin	30	7	35	13	65
Nalidixic acid	30	16	80	4	20
Streptomycin	30	5	25	15	75
Tetracyline	30	7	35	13	65

Salmonella spp. was also tested for the antibiotic susceptibility. *Salmonella* isolates were showed highly resistance to Nalidixic acid (80%) followed by Tetracyline & Kanamycin (35% each) and Amikacin (30%), Gentamycin, Chloramphenicol & Streptomycin (25% each) and Ciprofloxacin (5%) (Table 4). A total of 8 Multiple Drug Resistance (MDR) pattern were also observed among isolates of Salmonella (Table 5). The highest MDR noted was Chl/Te/Nal (15%, 3/20).

The maximum MDR registered was resistance to four antibiotics with the combination Kan/Chl/Nal/Amk being more frequent (Table 5). In general, MDR to three and four antibiotics dominate the resistance patterns (25%, 5/20 each).

DISCUSSION

Cow's milk may be contaminated from different sources and at different processes. Analysis onfarm milk production practices in the present study showed that about 93% of farmers did not employ good milking practices to minimize contamination of milk on the farm. It was supported by report of Galton et al. (1986) that pre-milking udder preparations play an important part in the contamination of milk during milking. Furthermore, about 92% of the dairy owners of milk producers in the present study did not use towel and a few dairy owners (3%) used a single towel for all cows commonly to dry the udders. Another aspect of some studies (Zelalem and Faye, 2006) indicated that about 52% of smallholder producers and 58% of large-scale producers used common towel to clean the udder or they did not at all. The reuse of towel for cleaning and sanitizing may result in recontamination of the udder. In the present study, about 91% of cows are kept in unclean barns. In this case, feces and dung are also an important contamination sources. Contamination of bedding material can be very high due to absorption of urine and feces which possibly have exposed the milk to high risk of contamination, which in turn increase the microbial count. This causes contamination of udder, and consequently, of the milk, with Bacillus (soil) and Enterobacteriaceae (mainly coliforms coming from bedding and manure) (Slaghuis, 1996).

General farm, milking and management practices that discussed above were also supported by the microbiological data (total plate counts and prevalence of indicator bacteria) of milk samples among farms. The overall means microbial counts of AMB and coliforms were 8.48 and 5.82 log CFU/ml, respectively, indicating that the farming and production practices were not to the standard. Compared with the earlier reports, the overall mean total aerobic plate counts (8.48 log CFU/ml) of raw milk obtained in this study was higher than that of Ombui *et al.* (1995) from Kenya. However, the current observation was lower than the result of Fekadu (1994) who reported that 8.8 log CFU/ml. Possible reasons for the high total bacterial counts could be poor udder preparation, unhygienic milking procedures and inferior quality of water used for cleaning utensils.

Coliform bacteria were detected in the raw milk samples. The mean coliform counts (5.87 log CFU/ml) of raw milk in this study was higher than the reports of Ombui *et al.* (1995) from Kenya (4.7log CFU/ml), Godifay and Molla (2000) from Ethiopia (4.85log CFU/ml) and Bonfoh (2003) from Mali (6.0 log CFU/ml). However, this result was lower than the study carried out by Zelalem and Faye (2006). Coliform counts regularly in excess of 2.0log CFU/ml are considered by some authorities as evidence of unsatisfactory production hygiene.

Hence higher coliform count observed in this study may be because of the initial contamination of the milk samples either from the cows, milk containers or the milking environment. Some members of coliforms (*Enterobacter, Citrobacter* and *Klebsiella* species) were incriminated in acute and chronic diarrheal diseases (Richardson, 1985). The incidence of coliforms in raw milk has received considerable attention, partly due to their association with contamination of faecal origin and the consequent risk of more pathogenic fecal organisms being present, partly because of the spoilage their growth in milk at ambient temperatures. Sporadic high coliform counts may also be a consequence of unrecognized coliform mastitis, mostly caused by *E. coli* (Bamley and McKinnon, 1990).

Staphylococcal food poisoning is a major form of food borne illness and appears to continue to be so as time goes on when the environmental conditions are favorable for growth and multiplication. Its strains produce a spectrum of protein toxins and virulence factors thought to contribute to the pathogenicity of this organism (Guta *et al.*, 2002). Staphylococcal contamination of milk and milk products is associated with enterotoxicity in humans (Guta *et al.*, 2002). In the present study, the counts of staphylococci spp. was found 5.23 log CFU/ml which is relatively lower than the 5.6 log CFU/ml count reported by Adesiyum *et al.* (1998). Evidence (Mahami *et al.*, 2013) indicates that *Staphylococcus* spp is agents for the cause of mastitis in dairy animals and could contaminate milk from the udder of infected animals. Staphylococci are also commonly found in raw milk as part of the natural flora of the cow. Moreover, the nasopharyngeal cavity of human beings is the reservoir of staphylococci from where these bacteria get localized on the skin, especially on human hands (Mahami *et al.*, 2013). Thus, the route of contamination of raw milk observed in the present study could be through the hands of milk handlers.

The pathogenicity of *Staphylococcus aureus* has been recognized for many years and it may cause mastitis or skin disease in milk producing animals or lead to foodborne intoxication in milk and milk products (Asperger, 1994). This may be of concern for human health since some strains of *S. aureus* are capable of producing heat stable enterotoxins. *Staphylococcus aureus* were detected in 34 (34 %) raw milk samples. Prevalence of *S. aureus* (34%) of present finding was higher than findings of Bitew *et al.* (2010) and Sasidharan *et al.* (2013) who reported 20.3% and 24%, respectively. However, it was lower than the finding from other country (Alzohairy, 2013) who found that 47.3%.

One of the most important groups of acid producing bacteria in the food industry is the lactic acid bacteria (LAB) which are used in making starter culture for dairy products. In the present finding, raw milk samples collected from study area had high LAB count (4.58 log CFU /ml) when compared with previous report (3.2log CFU /ml) in Egypt (Abdelgadir *et al.*, 2001). But this result was in agreement with study carried out by Ali *et al.* (2010). High LAB counts could be represent there is lack of raw milk cooling immediately after milking (Aziz *et al.*, 2009). They are also normally present in the milk and are also used as starter cultures in the production of cultured dairy products (Aziz *et al.*, 2009; Frazier, 1995).

However, the presence of high LAB count results in the early milk spoilage as compared to the low lactic acid bacteria count in the raw milk (Aziz *et al.*, 2009).

Yeast and mold are common contaminants in food. Although yeast does not result in food poisoning, it does cause to spoil (Tasci, 2013). A number of molds produce toxic substances designated as mycotoxins in ensiled feed which was used mostly in winter season. Some are mutagenic and carcinogenic (Tasci, 2013). The overall mean yeast and mould count of milk produced in present study was 4.8log CFU/ml and 4.35log CFU/ml, respectively. In the present study, yeast and mould count was high as compared to the study carried out by Riadh (2005) which accounted for 3.2 and 3.01log CFU/ml, respectively. But this result has a relative comparable with the study carried out by Pesic *et al.* (2005) who reported that 4.4log CFU/ml yeast and 4.01 log CFU/ml mould count. The higher number of yeast and mould in milk of present study were expected when the pasture or hay replaced by conserved or ensiled feed.

The microbial counts from raw milk samples were significantly different (P<0.05) among samples from different farms except for the staphylococci and coliform counts. Similarly, the mean counts of aerobic mesophilic bacteria and yeasts were significantly different from counts of the others within individual farms but not within dairy farms.

The bacterial groups isolated from raw milk were Staphylococcus spp., Streptococcus spp., Enterobacteriaceae, Micrococcus spp., Pseudomonas spp., Bacillus spp. Lactobacillus spp., Leuconostoc spp. and Aeromonas spp. Moreover, Lactobacillus spp. was found as the most dominant isolates followed by Staphylococcus spp and Streptococcus spp in the raw milk of the present study. The dominance of Lactobacillus among the isolated strains is consistent with the finding of El-Shafei (2002), as the raw milk is the heterogeneous mixture of different microorganisms including LAB. In the same way, the result of present study has consistent with the finding of Gawad et al. (2010) who found dominant Lactobacillus from traditional rayeb milk in Egypt. Enterobacteriaceae is also the third most dominant microflora among the aerobic mesophilic bacterial isolate. These groups of microorganisms are incriminated in acute and chronic diarrheal disease and also they are used as indicator microorganisms for hygiene of materials as well as milk contamination (Bramley, 1990). The prevalence of some strains in cow's milk varies among reports. The results in present study (1% to 37%) were relatively similar to that previously reported in other country which ranging from 2 to 40% (Sandgren et al., 2008). The genera of Staphylococcus, Streptococcus, Enterobacteriaceae, Corynebacterium spp, Bacillus spp, and Pseudomonas spp. are implicated as causes of sub clinical and clinical mastitis in the cow (Harding, 1999).

Several reports have documented the prevalence and distribution of *Salmonella* in bulk tank milk (Oliver *et al.*, 2005). In the present study, the prevalence of *Salmonella* spp in raw milk was found 20%. Evidence (Mahami *et al.*, 2013) indicates that *Salmonella* spp are agents for the cause of mastitis in dairy animals and may have contaminated milk from the udder of infected animals. *Salmonella* spp also reside in the intestinal tract where they cause gastro-enteritis in animals and may have occurred in milk as a result of faecal contamination.

The isolation rate of *Salmonella* in this study was related to reports from Gaborone, Botswana 20% (Esther *et al.*, 2004). However, it was higher than a study conducted by Addis *et al.*, 2013; Zewdu and Cornelius, 2009 who reported a prevalence of 7.6% and 13.63%, respectively. Studies made on *Salmonella* isolation from raw milk and foodborne illness associated with the consumption of *Salmonella* contaminated raw milk had not been clearly documented so far in Ethiopia and Jimma zone in particular.

As a consequence of the high antimicrobial use in dairy farms and individual cows, bacterial contaminants carried by milk and milk products often show high levels of antimicrobial resistance (Sandgren *et al.*, 2008). In recent finding, Methicillin, penicillin G and Chloramphenicol were the drugs to which a large proportion of the *S. aureus* isolates were resistant. This might indicates the improper and indiscriminate use of these agents. Moreover, the present study demonstrated that the resistant strains may have been transferred to cow then to milk, which can poses infection in human beings if it consumed in raw. As a result it should be of concern as it raises food safety and ethical issues. Small proportions (2.9 to 14.7%) of the isolates from farms were resistant to the Streptomycin, Erythromycin, Vancomycin, Chloramphenicol, Ciprofloxacillin and Tetracyline when compared to Kanamycin and Gentamycin in which all were not resistant, it was evident from this results that these antimicrobial agent are not frequently used in animals by large-scale farmers.

Resistance to Penicillin G was higher in this report as compared to the study carried out by Tariku et al. (2013) from cows with bovine mastitis in Jimma town dairy farms. But it was in agreement with study carried out by Abdelgadir (2001) who reported that 91.4% of the S. aureus were resistant to Penicillin G. This observation can be attributed in part to earlier exposure of the isolates to these drugs which may have enhanced resistant development (Abdelgadir, 2001). The continuous genetic variation could also have contributed to the increased resistance that could be transferred to other pathogens. The resistance pattern observed in the study should be of concern as it raises food safety and ethical issues. Resistance to penicillin G is thus used as a marker to assess the susceptibility of S.aureus isolates against other beta-lactam antibiotics. In addition to Penicillin G, resistance to Methicillin of S. aureus in this study is higher than the earlier 52% report from Jimma (Balata, 2003), and in contradiction to the 93.2% susceptible reported by Ateba (2010). The presence of 90% Methicillin resistant S. aureus strain is demonstrating the fast growing and alarming situation to the public health system and the community (Balata and Ketene, 2003). Moreover, the present study demonstrated that the resistant strains may have been transferred to cow then to milk, which can poses infection in human beings.

In the present study, *Salmonella* isolates were most susceptible to Ciprofloxacin (95%). Although, the *Salmonella* isolates were highly resistance to Nalidixic acid (80%) followed by Tetracycline (35%) and Gentamycin (25%). Antimicrobial- resistant salmonella in raw milk may be able to colonize the gut if consumed by humans, thus making infections difficult to treat.

Evidence (Mahami *et al.*, 2013) indicates that the global rise of antimicrobial resistance is mainly due to the exposure of this bacteria in human and veterinary medicine and indiscriminate use of drug for the treatment of both human and animal disease caused by *Salmonella* spp. *Salmonella* resistance to Tetracycline (35%) and Gentamycin (25%) were found higher in the present study as compared to finding of Addis *et al.* (2013) who found that 33.3% and 12%, respectively. Although the resistance to Nalidixic acid is consistent with the prevalence of 89-92% reported from Kenya (Lakshmi *et al.*, 2006). *Salmonella* resistant for at least to two or more of antimicrobials (70%) which were observed in this study was lower than 83.3% conducted in Ethiopia (Addis *et al.*, 2013) and elsewhere in the world (75%) (Berge *et al.*, 2004).

The normal pH of milk is from 6.2 to 6.6 for cow; and means 6.5 at which growth occur (Walstra *et al.*, 2006). Accordingly, the obtained acidity (0.26%) (With mean pH of 6.27) of present finding from raw milk, besides its nutrient content, it could be good medium for growth of microorganisms. The acidity of milk is usually expressed as pH. Milk samples collected from the individual farmers had higher mean acidity (pH=6.18; TA, 0.28) than those from dairy farms (pH=6.37; TA, 0.25). The finding of present study was similar with 0.26% reported by Zelalem and Faye (2006) in the central highlands of Ethiopia. The high percentage of lactic acid of milk observed in the current study indicated that the method in which the milk was handled was poor reflecting the poor hygienic situations during production and handling of milk in the district.

CONCLUSIONS

The microbial profiles and physico-chemical parameters analyzed were not met the acceptable standards. Based on the high microbial counts found in this study, it could be concluded that it may cause a serious health risk in the study areas. Therefore hygienic safety measure should be taken by determining critical control points in the phases of production and regular check-ups of milk should be performed according to food regulation. Furthermore, appropriate selection and use of antimicrobial agent is recommended in the treatment of cows.

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REFERENCES

Abdelgadir, W.S., Hamad, S.H. Moller, P.L. and Jakobsen, M. 2001. Characterisation of the dominant microbiota of Sudanese fermented milk; *Rob. Int. Dairy J.*, **11**: 63-70.

- Addis, Z., Nigatu, K., Sisay, Z., Haile A, Alehegne, Y. and Tesfu, K. 2013. Prevalence and antimicrobial resistance of *Salmonella* isolated from lactating cows and in contact humans in dairy farms of Addis Ababa. *Infectious Diseases*, **11**:222-223
- Adesiyum, A.A., Webb, L and. Rahman, S, 1995. Microbiological quality of raw cow milk at collection centers in Trinidad, *J. Food Prod.*, **58**: 448-451.
- Ali, A.A, Irshad, N.B. Razaz, S.A. and Manahil, A.A. 2010. Microbiological Safety of Raw Milk in Khartoum State, Sudan. *Pakistan Journal of Nutrition*, **9**: 426-429.
- Alzohairy, M.A. 2013. Colonization and antibiotic susceptibility pattern of methicillin resistance *Staphylococcus aureus* among farm animals in Saudi Arabia. *J. of Bact. Research*, **3**:63-68.
- Asperger, H. 1994. *Stapylococcus aureus*. In: The Significance of Pathogenic Microorganisms in Raw Milk, International Dairy Federation. IDF,Brussels, Beljium. PP. 24-42.
- Ateba, C.N., Mbewe, M; Moneoang, M.S. and Bezuidenhout, C. C. 2010. Antibiotic-resistant *Staphylococcus aureus* isolated from milk in the Mafikeng Area, North West province, South Africa. *South Afr. j. sci.*; **106**: 11-12.
- Aziz, T. H. Khan, S.M. Bakhtaran and Neuron M., 2009. Incidence and Relative Abundance of Lactic Acid Bacteria in Raw Milk of Buffalo, Cow and Sheep. *The Journal of Animal and Plant Sciences* 19: 168-173.
- Balata, B. and Ketene, D. 2003. Nasal carriage of methicillen resistant Staphylococcus aureus strains among inpatients of Jimma Hospital, Southwest Ethiopia. *Ethiop J. Health Sci*; 13:107–116.
- Berge, A.C., Adaska J.M., and. Sysco, W.M. 2004. Use of antibiotic susceptibility patterns and PFGE to compare historic and contemporary isolates of multi-drug resistant *Salmonella* enteric subsp. enteric server Newport. *Appl. Environ. Microbiol.* **70**:318–323.
- Bitew, M., Tapered, A. and Tools, T. 2010. Study on bovine mastitis in dairy farms of Bahir Dar town and its environs. *J. Anim. Vet. Adv.*, **9**: 2912-2917.
- Bonfoh, B., Wasem, A., Traore, A.N., Fane, A., Spillmann, H. C., Simbe, C., Alfaroukh, I.O., Nicolet, J., Farah, Z. and Zinsstag, J. 2003. Microbiological quality of cows'milk taken at different intervals from the udder to the selling point in Bamako (Mali) *Food Control.* 14: 495-500.
- Bramley, A.J. and McKinnon, C.H. 1990. *The Microbiology of Raw Milk*. In: Dairy Microbiology, I, (Ed.: Robinson, R.K.). London, New York, Elsevier Applied Science, 171
- Chye, F. Y.; Abdullah, A. and Ayob, M.K. 2004. Bacteriological quality and safety of raw milk in Malaysia. *Food Microbiology*. **21**: 535 541
- El-Shafei, H.A, Abd-El-Sabour, H., Ibrahim, N. and Mostafa, Y.A. 2000. Some important fermented foods of Mid-Asia, the Middle East, and Africa. *Microbiol Res.*, **154:** 321-331.
- Esther, N.N., Collision, E.k., Gashe, B.A. and Mpuchane, S. 2004. Microbiological quality of milk from two processing plants in Gaborone, Botswana. *Food control* **15**,181-186.

- Eyasu, S. and Fekadu, B. 2000. Microbiogical quality of raw and pasteurised goat milk. Livestock production and the environment. Implication for sustainable livelihoods In: *Proceedimgs of the seventh Annual Conference of the Ethiopian Society of Animal production*, pp 410-418, 26-27, May, 1999, Addis Ababa, Ethiopia.
- Galton, D.M., Petersson, L.G. and Merril, W. G. 1986. Effects of pre-milking udder preparation practices on bacterial counts in milk and on teat. *J. Dairy Sci.***69**: 260-266.
- Gawad, A.E, Abd El Fatah, I.A. and Al Rubayyi, K.A. 2010. Identification and Characterization of Dominant Lactic Acid Bacteria Isolated from Traditional Rayeb Milk in Egypt. *J. of Amer. Sci.*, **6**:731.
- Godifay, B. and Molla, B. 2000. Bacteriological quality of raw milk from four dairy farms and milk collection center in and around Addis Ababa. *Berl. Münch. Tierarztl. Wschr.* **113**: 276 278
- Guta, C, Sebunya, T.K. and Gashe, B.A. 2002. Antimicrobial Susceptibility of Staphylocacci spp from Cow Foremilk originating from dairy farms around Gaborone, Botswana. *East African Medical Journal*, **79**: 1-4.
- Harding, F. 1999. Milk Quality. I sted .Champman and Hall publisher, Surrey, UK.
- Lakshmi, V., Ashok, R., Susmita, J., and Shailaja, V.V. 2006. Changing trends in the antibiograms of *Salmonella* isolates at a tertiary care hospital in Hyderabad. *Indian J. Medical Microbiol.* **24**: 45-48.
- Mahami, T, Odonkor, S, Yaro, M. and Adu-Gyamfi, A. 2013. Prevalence of antibiotic resistant bacteria in milk sold in Accra. *International Research Journal of Microbiology;* **2**: 126-132.
- Oliver, S.P., Jayarao, B.M. and Almeida, R.A. 2005. Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications. *Foodborne Pathogen and Diseases* **2***):* 115-29.
- Ombui, J.N, Arimi, S.M, McDermott, J.J, Mbugua, S.K, Githua, A, and Muthoni, J. 1995. Quality of raw milk collected and marketed by dairy cooperative societies in Kiambu District, Kenya. *Bull Anim Health Prod Far;* **43**: 277-284
- Ong, L., A., Henrikssonb and Shaha, N.P. 2007. Chemical analysis and sensory evaluation of Cheddar cheese produced with Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus paracasei or Bifidobacterium sp. *Int. Dairy J.*, **17**: 937-945.
- Pesic, M.D., Stojanovic, L. and Jovanovic, L., 2005. Moulds associated with milk depending on macroclimate and geographical location, *Appl. Ecology and Environ. Res.*, **3**: 61-65.
- Riadh, A.T. 2005. A Comparison on Microbial Conditions between Traditional Dairy products Sold in Karak and Same Products Produced by Modern Dairies., *Pak. J. Nutr.*, **4**: 345-348.
- Richardson, G. H. 1985. *Standard Methods for the Examination of Dairy Products*. 15th ed. American Public Health Association. Washington, D.C. Pp. 168-196.
- Rosati, A. and Aumaitre, A. 2004. Organic dairy farmig in Europe. *Livestock Production Science*, **90**: 41–51.

- Sandgren, C.H., Waller, K.P., *and* Emanuelson, U. 2008. Therapeutic effects of systemic or intramammary antimicrobial treatment of bovine subclinical mastitis during lactation. *The Veterinary Journal*, **175**: 108–117.
- Sasidharan, S., Prema, B., and Yoga Latha, L. 2013. Antimicrobial drug resistance of Staphylococcus aureus in dairy products. *Asian Pacific Journal of Tropical Biomedicine;* 130-132.
- Slaghuis, B. 1996. Sources and Significance of Contaminants on Different Levels of Raw Milk Production. In: Symposium on Bacteriological Quality of Raw Milk. International Dairy Federation Proceedings, Brussels, 13-15, March, 1996.
- Tsehay, R. 2002. Milk processing and marketing options for rural, small- scale producers. Proceedings of the 6th National Conference of the Ethiopian Society of Animal Production (ESAP), May 14-15, 1997, Addis Ababa, Ethiopia pp 61-67.
- Vlkova, E., Rada, V., Popelarova P., Trojanova, I. and Killer, J. 2006. Antimicrobial susceptibility of bifidobacteria isolated from gastrointestinal tract of calves. *Livestock Science*; **105**: 253-259.
- Zelalem, Y. and Faye, B. 2006. Handling and microbial quality of raw and pasteurized cow's milk and Ergo-Fermented milk collected from different shops and producers in Central Highlands of Ethiopia. *Eth. J. Anim. Prod.* **6:** 67-82.
- Zewdu, E. and Cornelius, P. 2009. Antimicrobial resistance pattern of *Salmonella* serotypes isolated from food items and personnel in Addis Ababa, Ethiopia. *Trop. Anim. Health Pro.*, **41**: 241-249.

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