Prevalence of Bovine Mastitis, Risk Factors and major Causative Agents in West Hararghe Zone, East Ethiopia

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Abstract: A cross-sectional study was conducted from October 2009 to March 2010 in Boke, Darolebu, Kuni, Mieso and Tulo districts to estimate the prevalence of bovine mastitis, identify associated risk factors and isolate the predominant bacterial agents. A total of 1019 lactating cows were examined clinically and using California Mastitis Test (CMT) for subclinical mastitis. Standard bacteriological techniques were employed to isolate the bacterial agents. A total of 393 (38.6%) cows were positive for both clinical and subclinical mastitis. Among the risk factors, age, parity, and lactation stages were not significantly ($p>0.05$) associated with prevalence of mastitis. However, hygienic status of animals was associated with the occurrence of mastitis ($p<0.05$). Both contagious and environmental bacteria were isolated from milk samples collected from mastitic cows. The predominant bacteria isolate was *Staphylococcus aureus* with proportion of 14.1% followed by *S. agalactiae* with isolation proportion of 13.2%. *E. coli* and *S. intermedius* were the third predominant isolates with a rate of 10.9% for each. Among the seven antibiotics tested, gentamycin, amoxicillin, oxytetracycline, ampicillin, and cloxacillin were effective, whereas streptomycin and penicillin showed poor efficacy. The study revealed that mastitis is significant problem of dairy cows in the study areas. Hence, awareness creation among dairy farmers should be made about the impact of the disease; and training on hygienic milking practice and treating of subclinically infected cows should be given.

Keywords: *California mastitis test, Clinical mastitis, Risk factors, Subclinical mastitis*

Introduction

Ethiopia has the largest livestock population compared to other African countries and cows represent the largest population of cattle of the country (CSA, 2009). Milk produced from these animals provides an important dietary source for the majority of rural as well as considerable number of the urban and peri-urban population. However, milk production often does not satisfy the country's population requirement. According to the reports of FAO (2015a & b), the total annual national raw milk production in Ethiopia ranges from 797,900 to 1,197,500 metric tons equivalents of which 85 to 89 % is contributed by cattle. However, this amount is by far below the national demand for milk and milk products, showing the considerable potential for increasing small holder income and employment opportunities across the dairy production value chain.

Mastitis is among important health problems in dairy cattle and considered as one of the most important threats affecting the dairy industry throughout the country (Biffa *et al*., 2005). Mastitis is an inflammation of the mammary gland and udder tissue that usually occurs as an immune response to bacterial invasion of the teat canal by variety of bacterial sources present on the farm, and can also occur as a result of chemical, mechanical and thermal injury to the udder (Wellenberg *et al*., 2002; Radostits *et al*., 2007). It is mostly a result of combined interplay among exposure to microbes, cow defence mechanisms and environmental risk factors (Suriyasathaporn *et al*., 2000). Mastitis is a management related disease whose prevention and control depends among other factors on good management practices (Mungube *et al*., 2004). Mastitis as a disease, especially the subclinical form, has received little attention in the country and efforts have only been concentrated on the treatment of clinical cases (Girma, 2001). The pathogens responsible for causing mastitis are broadly classified into contagious and environmental agents (Quinn *et al*., 2002). The common microorganisms responsible for mastitis and spoilage of milk includes *Staphylococcus aureus*, *Streptococcus agalactiae*, *Corynebacterium bovis, Mycoplasma* species, and *Streptococcus uberis* (Erskine, 2001), coliforms (*Escherichia coli, Klebsiella* species and *Enterobacter aerogenes*), *Serratia, Pseudomonas, Proteus* species, environmental Streptococci, and *Enterobacter* species (Quinn *et al*., 2002).

In Ethiopia, the available information indicates that bovine mastitis is one of the most frequently encountered and prevalent diseases of dairy cows causing economic loss as a result of reduced milk yield, discarded milk, and early culling of productive cows (Fekadu, 1995; Mekonnen *et al*., 2005). The reported prevalence of mastitis in different parts of Ethiopia ranges from 1.2% to 21.5% (Kassa *et al*., 1999; Lemma *et al*., 2001; Mungube, 2001; Workineh *et al*., 2002; Kerro and Tareke, 2003).

Across the globe, various reports indicated that *S. aureus* showed resistance to penicillin. Previous study in Italy (Gentilini *et al*., 2000), Argentina (Gianneechini *et al*., 2002), and Uruguay (Pitkala *et al*., 2004) reported a resistance rate of 47%, 40%, and 47%, respectively. In Ethiopia, *S. aureus* isolates were highly resistant to clindamycin (73.8%), bacitracin (72.1%) and vancomycin (70.5%) (Shimelis, 2014). The high incidence of resistant bacteria might be due to

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ISSN 2616-8804 (Print)

indiscriminate use of antibiotics and intra-mammary preparations containing combinations or single broad-spectrum antibiotics (Pitkala *et al*., 2007). Edward *et al*. (2002) suggested a possible development of resistance from prolonged and indiscriminate usage of some antimicrobials.

In west Hararghe Zone, famers reported mastitis as one of the health problems among dairy cattle. However, to the best of our knowledge, a systematic study was not conducted to provide detailed information on cattle mastitis. Thus, the study was conducted to estimate the prevalence of bovine mastitis, assess risk factors, isolate and identify the bacterial causative agents, and determine the drug resistance patters of the isolated bacteria in the study area.

Materials and Methods

Study Areas

The study was conducted in west Hararghe (latitude: 7°50'–9°50' N; longitude: 40°00'–41°25' E; altitude: 1200–3060 m) administrative zones which are located in the eastern part of Oromia Regional State and share boundaries with Afar Regional State, Somali Regional State, as well as the east Hararghe Zone. The livestock population of west Hararghe is 1,149,089 cattle, 510,164 sheep and goats (WHZBA, 2006). It has three distinct agro-ecologies and different farming system that consists of highland (17.5%), mid highland (28.5%), and lowland (54.0%) and have two rainy seasons, the short rainy season and the main rainy season, with a mean annual rainfall ranging from below 700 mm in the lowlands to nearly 1200 mm at higher altitudes. Most of the people living in Hararghe lowlands are nomadic agro-pastoralists who move their livestock seasonally, following grazing opportunities and water availability (Guinand, 2000).

Study Animals

The study was conducted in districts that have major towns with significant milk flow in the operational area of Hirna Regional Veterinary Laboratory. Lactating cows of local breed 15 days after parturition were selected for the study. The lactating cows were selected purposively from three villages in each district and two *kebeles*. The study populations were all local breeds of dairy cows that are managed under mixed crop-livestock farming system. Milk was collected from 17 rural *kebeles*. The study was conducted on 1019 local breed cows from the selected districts that include Boke, Darolebu, Kuni, Mieso, and Tulo, and 34, 130, 83, 513, and 259 cows, respectively were taken. The number of sample cows vary among the districts because the study was conducted in dry period and required number of cows were not available in some areas, and villages in some of the districts were not accessible due to lack of transportations. Age, parity, lactation stage, and hygiene of cow's udder were explanatory variables used to associate with prevalence. Age of the cows was categorized as young (3 to 6 years), adults (> 6 to 10 years), and old (> 10 years). Parity was categorized as few (1- 3 calves), moderate (4-7 calves), and many (> 7calves) (Berhanu *et al*., 2010). Stage of lactation was categorized as early (1 up to 4 months), mid (4 up to 8 month), and late (8 month up to the beginning of dry period) and hygiene of udder was categorized as Washing/drying, Washing only and not at all (Berhanu *et al*., 2010). In the study area, dairy cattle are managed under semi- intensive management system and provided natural pasture and agricultural by product.

Study Design

A cross-sectional study was conducted from October 2009 to March 2010 at cow and quarter level based on clinical and subclinical mastitis and indirect test (California mastitis test and culture) for subclinical mastitis, microbial isolation and in-vitro antimicrobial susceptibility test.

Sampling and Sample Size Determination

For estimation of the prevalence of mastitis in the study area, the sample size was determined by assuming the expected prevalence to be 50% with the 95% confidence level and desired precision of 5% using the formula described by Thrusfield (2005). Accordingly, the minimum sample size required from each of the five districts was 384 but only a total of 1019 cows were taken since the study was conducted in dry season in May and June and some of the areas were not accessible due to lack of transportation. The rural *kebeles*, villages and lactating cows were also selected based on their accessibility to transportation.

Study Methodology

Milk sampling procedure: History of the selected milking cow was taken to record information such as number of parturitions, parity, treatment, and physical examination of the udder to determine blind teats and other defects and inflammation. The milk samples were taken from cows that are not treated with either intra-mammary or systematic antimicrobials agents. The udder was cleaned with warm water, antiseptics and dried with clean towel. The first few drops of the milk squirt were discarded and milk from each quarter of the udder was taken into sterile bottles. The bottles were clearly labelled with an appropriate identification of the cow's number and quarter using permanent marker. The samples were transported in ice box to the laboratory without delay and it were processed immediately (Quinn *et al*., 2002). The screening test, California mastitis test (CMT) was done at the field to diagnose the presence of subclinical mastitis according to the procedures of Quinn *et al*. (1999). Milk samples were poured into four shallow cups in the CMT paddle and equal amount of CMT reagent was added to each cup and gentle circular motion was applied to the mixture on the horizontal plane. Based on the thickness of the gel formed by CMT reagent and milk mixture, test results were scored as 0 (negative), 1 (weak

positive), 2 (distinct positive) and 3 (strong positive). Milk samples with CMT 1 to 3 were classified as evidence of subclinical mastitis (Quinn *et al*., 1999; Radostits *et al*., 2007). In the laboratory, samples were cultured immediately or stored at +4 °C in any case of delay (NMC, 1999). Analysis of positive samples was performed on isolation and identification of pathogenic bacteria at Hirna regional veterinary laboratory of microbiology department.

Culture and isolation of bacteria: A loop full of the milk sample was streaked on blood agar base enriched with 7% sterile sheep blood and MacConkey agar and incubation was made for 24 to 48 hours at 37 °C. Plates were examined for the presence or absence of growth on MacConkey agar, presence or absence of haemolytic characteristics on blood agar, size, shape, outline, elevation, consistence, and pigmentation. Sub culturing was performed to obtain pure colony as well as for further examination of isolates on differential media. Identification of bacterial species was done according to Quinn *et al*. (1999). The primary tests used for characterization of isolates were catalase, motility (semi solid method), oxidise, and oxidation-fermentation (OF), while secondary biochemical test was performed using mannitol salt agar, triple sugar iron agar, indole, methyl red, Voges-Proskauer, citrate (IMViC), urease and carbohydrate fermentation.

***In-vitro* antimicrobial sensitivity test:** Susceptibility of bacteria to the commonly used antimicrobials was conducted using Kirby-Bauer disc diffusion method (Quinn *et al*., 2002). Seven antimicrobial discs that include ampicillin, amoxicillin, penicillin, cloxacillin, gentamycin, oxytetracycline, and streptomycin were selected from the main class of antimicrobials and used for susceptibility testing. The bacteria culture was streaked on Mueller-Hinton agar. Four up to five antimicrobial discs were applied on the surface of each inoculated agar plates under aseptic conditions. Each disc was pressed down to ensure complete contact with the agar surface. Plates were inverted and incubated at 37°C for 24 hrs. The diameter of zone of inhibition was measured in millimetres using calliper. After measuring the zone of inhibition, it was classified as susceptible, intermediate and resistant according to National Committee for Clinical Laboratory Standard (NCCLS) break point to interpret the inhibition zone (Quinn *et al*., 2002).

Data Management and Statistical Analysis

The data collected during sampling and laboratory work were entered and stored in MS-excel. Before subjected to statistical analysis, the data were thoroughly screened for errors and properly coded. An intercooled Stata 9 software package (Stata Corporation, 2005) was used to perform the statistical analysis. Descriptive statistical analysis such as frequency and proportions were used to compute mastitis prevalence and antimicrobial susceptibility. Proportion of mastitis was calculated by dividing the number of animals positive to the total sampled animals. Pearson chi-square (χ^2) test was employed to assess the existence of association between mastitis and its risk factors. P-value <0.05 was considered significant whereas P-value >0.05 was considered non-significant.

Results

Prevalence of Mastitis

Among the total 1019 dairy cow examined, 393(38.6%) were detected positive for both clinical and subclinical mastitis. On physical examination of udder, 47/1019 (4.6%) of the cows revealed clinical mastitis while 346/1019 (33.9%) showed subclinical mastitis. The highest and lowest prevalence of mastitis were recorded in Tulo district (42.47%) and Gamachis district (32.5%), respectively (Table 1).

Table 1. Prevalence of clinical and subclinical mastitis at cow level in the study areas

Districts	№ of cows examined	Total № of cows affected	Over all prevalence (%)	Type of mastitis			
				Clinical form		Subclinical form	
				№ of positive	Prevalence (%)	№ of positive	Prevalence (%)
Boke	34	12	35.29	3	6.38	9	2.6
Darolube	130	44	33.84	9	19.14	35	10.1
Gamachis	83	27	32.5	5	10.63	22	6.36
Mieso	513	200	38.98	19	40.42	181	52.3
Tulo	259	110	42.47	11	23.4	99	28.6
Total	**1019**	**393**	**38.6**	**47**	**4.6**	**346**	**33.9**

The overall prevalence of mastitis at quarter level was 36.48% (1487/4076) of which 4% (163/1487) showed clinical signs such as fibrosis, cardinal signs of inflammation, visible injury, and tick infestation, atrophy of the tissue of udder and swelling of the supramammary lymph nodes (Table 2). However, 2378 (58.3%) were negative while the remaining 211 (5.18%) were blind (Table 3). Based on quarter location, the highest prevalence of mastitis was recorded at Right front (9.4%) while the lowest was recorded in right hind (8.6%) (Table 3).

Risk Factors of Mastitis

The result showed that the prevalence of mastitis was higher in adult animals (56.8%) followed by old age group, and younger category. However, there was no statistically significant difference (p=0.208) among the age categories. Hygienic condition showed statistically significant effect on the occurrence of mastitis (p<0.05). Thus, higher mastitis case was reported in dairy cows managed under poor hygiene followed by those under intermediate hygienic condition (Table 4).

Prevalence of Bacterial Isolates

Out of 1487 mastitis positive quarters, 1455 harbours bacteria belonging to 8 genera and 13 species. Thirty eight of the isolates were from samples with clinical mastitis while the remaining 1417 isolates were from samples with subclinical mastitis. Both contagious and environmental bacteria were isolated. The predominant bacteria isolated were *S. aureus* with proportion of 14.09% followed by *S. agalactiae* (13.2%), and *E. coli* and *S. intermedius* with proportion of 10.9% for each. *C. ulcerans* was the least isolate which accounts for 1.7% (Table 5).

Antimicrobial Susceptibility Profiles of Bacterial Isolates

From the total 1455 bacterial isolates, 972 were tested for susceptibility to antimicrobials. The majority of isolated bacteria showed susceptibility to gentamycin (85.29%), amoxicillin (78.19%), oxytetracycline (72.3%), ampicillin (67.18%) and cloxacillin (61.73 %), whereas streptomycin and penicillin showed poor efficacy on most of the tested isolates. Cloxacillin and streptomycin were effective against *Corynebacterium* and *A. pyogenes*, while *Staphylococcus, Streptococcus, P. multocida,* and the *Enterobacteriaceae* were highly susceptible to gentamycin, amoxicillin, oxytetracycline, ampicillin, and cloxacillin (Table 6).

Table 2. Prevalence of clinical and subclinical mastitis at cow and quarter levels

Types of mastitis	Cow level (n=1019)		Quarter level (n=4076)	
	№ of positive	Prevalence (%)	№ of positive	Prevalence (%)
Clinical	47	4.6	163	4
Subclinical	346	33.95	1324	32.48
Total	**393**	**38.6**	**1487**	**36.48**

Table 3. Occurrence of mastitis in cattle at the quarter level in five selected districts

Quarter	Total № examined	Blind quarter		Negative quarter		Positive quarter	
		№	Prevalence (%)	№	Prevalence (%)	№	Prevalence (%)
RF	1019	35	0.9	603	14.8	381	9.4
RH	1019	69	1.7	599	14.7	351	8.6
LF	1019	28	0.7	611	14.99	380	9.3
LH	1019	79	1.94	565	13.9	375	9.2
Over all	**4076**	**211**	**5.18**	**2378**	**58.3**	**1487**	**36.48**

RF= Right front; RH= Right hind; LF= Left front; LH= Left hind.

Table 4. Risk factors of mastitis in the study areas

Variables	Categories	№ examined	№ positive	Prevalence (%)	P-value	χ2	CI
Age group	3-6years (young)	573	142	24.8			
	6-9 years (adult)	431	245	56.8	0.208	1.149	0.020-0.092
	>9 years (old)	15	6	40			
Parity	1-3 calves (few)	551	175	31.8			
	4-7 calves (moderate)	379	141	37.2	0.094	3.574	0.085-0.006
	>7 calves (many)	89	39	43.8			
Lactation stage	1-4 month	137	76	55.47			
	4-8 month	211	105	49.8	0.772	0.1751	0.040-0.030
	8-10 month	578	199	34.4			
	Greater than 10	93	13	13.98			
Udder hygiene	Not at all	637	235	36.89			
	Washing	281	87	30.96	0.013	8.1713	0.1007-0.0117
	Washing/drying	101	25	24.8			

Table 5. Isolated mastitis causing microorganisms in the study areas

Pathogens	Mastitis form				Total	
	Clinical	%	Subclinical	%	№ identified	Prevalence (%)
S. aureus	10	26.3	195	11.5	205	14.09
S. agalactiae	2	5.26	190	11.2	192	13.2
S. intermedius	2	5.26	156	11	158	10.9
E. coli	3	7.9	155	11	158	10.9
S. epidermidis	2	5.26	149	10.5	151	10.38
Klebsiella spp.	1	2.6	146	10.3	147	10.1
S. hyicus	3	7.9	108	7.6	111	7.63
A. pyogenes	3	7.9	86	6.06	89	6.1
C. bovis	3	7.9	60	4.2	63	4.3
Proteus spp.	4	10.5	67	4.7	71	4.9
P. multocida	1	2.6	46	3.24	47	3.2
S. faecalis	3	7.9	35	2.47	38	2.6
C. ulcerans	1	2.6	24	1.73	25	1.7
Over all	**38**	**100**	**1417**	**100**	**1455**	**100**

Discussion

The present study showed an overall mastitis prevalence of 38.6% based on CMT and clinical examinations of the udder. This finding is comparable with the result reported by Workineh *et al.* (2002) and Nesru (1999) who reported prevalence of 38.2% and 37.2% in Adami Tulu and urban and peri-urban dairy farms of Addis Ababa, respectively. In the country prevalence rate lower than the present finding were reported by Hunderra *et al.* (2005) and Gizat (2004) in Sebeta (16.11%) and Bahir Dar (3.9%), respectively. Karimuribo *et al.* (2006) from Tanzania reported lower prevalence (14.2%) than the present finding. However, it is relatively lower than that obtained by Mungube *et al.* (2004) in central highlands of Ethiopia (46.6%). Mastitis is a complex disease involving interactions of various factors such as management and husbandry practices, environmental conditions, animal risk factors, and causative agents, hence its prevalence will vary in time and space (Radostits *et al.*, 2007).

The prevalence of subclinical mastitis (33.95%) and clinical mastitis (4.6%) is comparable with the reports of Nesru (1999) (32.2% and 5%) and Bishi (1998) (30.2% and 5.5%) in urban and peri-urban dairy farms at Addis Ababa. The relatively higher prevalence of subclinical mastitis than that of clinical mastitis is in line with previous reports from different parts of Ethiopia (Workineh *et al.*, 2002; Kerro and Tareke, 2003; Mungube *et al.*, 2004) and elsewhere in Africa (Kivaria *et al.*, 2004). Since environmental factors play significant role, the prevalence of subclinical mastitis varies in dairy animals (Radostits *et al.*, 2007). In Ethiopia, the subclinical form of mastitis received little attention and efforts have been concentrated on the treatment of clinical cases, while high economic loss could also be resulted from subclinical mastitis (Biffa *et al.*, 2005; Hunderra *et al.*, 2005). According to Erskine (2001) the variation in prevalence between subclinical and clinical mastitis may be due to the fact that the defence mechanism of the udder reduces the severity of the disease. Usually Ethiopian farmer's, especially smallholders, are not well informed about the invisible loss from subclinical mastitis since dairying is mostly a side line business. This also apply to the present study area where dairy farm owners do not screened their cows for subclinical mastitis except seeking veterinarian's assistance at times of clinical mastitis occurrence. In the present study area, all farmers said they do not practice washing/drying of udder. Moreover, their knowledge about mastitis was only on clinical mastitis and all of them do not have any information about subclinical mastitis, and surprised during the field testing seeing the CMT positive milk reaction.

Table 6. Antimicrobial susceptibility profiles of bacterial isolates

Bacteria species	Susceptibility to:																				
	Streptomycin			Gentamycin			Oxytetracycline			Ampicillin			Amoxicillin			Penicillin			Cloxacillin		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
S. aureus (n=205)	49.1	18.9	32.1	84.9	0.0	15.1	73.0	8.2	18.9	66.0	6.3	21.4	79.9	1.9	18.2	44.7	33.3	28.3	57.2	30.2	12.6
S. agalactiae (n=192)	58.1	18.4	23.5	84.6	6.6	8.8	74.3	11.0	14.7	72.8	7.4	19.9	78.7	11.8	9.6	53.7	27.9	18.4	64.0	23.5	12.5
S. intermedius (n=158)	63.2	13.2	23.7	88.6	8.8	2.6	76.3	10.5	13.2	73.7	9.6	16.7	83.3	7.9	8.8	57.0	8.8	34.2	69.3	11.4	19.3
E. coli (n=158)	57.8	19.3	22.9	87.2	8.3	4.6	74.3	11.9	13.8	70.6	11.0	18.3	78.0	13.8	8.3	50.5	22.0	27.5	63.3	18.3	18.3
S. epidermidis (n=151)	55.1	23.4	21.5	86.9	3.7	9.3	73.8	10.3	15.9	68.2	10.3	21.5	76.6	9.3	14.0	45.8	26.2	28.0	60.7	23.4	15.9
Klebsiella spp. (n=147)	60.4	16.5	23.1	91.2	0.0	8.8	78.0	12.1	9.9	71.4	11.0	17.6	82.4	11.0	6.6	49.5	11.0	39.6	65.9	22.0	12.1
S. hyicus (n=111)	63.9	8.3	27.8	86.1	2.8	11.1	76.4	9.7	13.9	70.8	13.9	15.3	81.9	0.0	18.1	55.6	36.1	31.9	69.4	9.7	20.8
A. pyogenes (n=89)	81.6	14.3	4.1	79.6	16.3	4.1	63.3	20.4	16.3	59.2	20.4	20.4	71.4	20.4	8.2	51.0	20.4	28.6	57.1	22.4	20.4
C. bovis (n=63)	54.3	8.6	37.1	77.1	0.0	22.9	65.7	5.7	28.6	57.1	8.6	34.3	71.4	2.9	25.7	45.7	8.6	45.7	85.7	8.6	5.7
Proteus spp. (n=71)	38.5	23.1	38.5	76.9	5.1	17.9	64.1	7.7	28.2	56.4	10.3	33.3	71.8	7.7	20.5	23.1	25.6	51.3	48.7	23.1	28.2
P. multocida (n=47)	28.0	24.0	48.0	84.0	0.0	16.0	56.0	8.0	36.0	48.0	12.0	40.0	72.0	0.0	28.0	16.0	16.0	68.0	40.0	32.0	28.0
S. faecalis (n=38)	38.1	9.5	52.4	81.0	19.0	0.0	61.9	14.3	23.8	52.4	19.0	28.6	71.4	14.3	14.3	23.8	0.0	76.2	42.9	14.3	42.9
C. ulcerans (n=25)	13.3	26.7	60.0	73.3	26.7	0.0	46.7	33.3	20.0	33.3	40.0	26.7	60.0	26.7	13.3	6.7	13.3	80.0	20.0	46.7	33.3

Values are in %; n= number of isolates tested; I= Intermediate; R= Resistant; S= Susceptible.

The overall quarter level prevalence recorded in the current study (36.48%) is comparable with the finding of Nesru *et al.* (1999) and Abdelrahim *et al.* (1990) who reported 37% and 39%, respectively. The quarter infection rate was higher than that reported by Kerro and Tareke (2003) (19%) in southern Ethiopia and Biffa *et al.* (2005) (28.2%) in the country. The present study revealed that the front quarters are more exposed to mastitis infection as compared to the hind quarters, which could be due to the fact that the front quarter are highly predisposed for contamination with dirt. In addition to this, large amount of milk is produced from front quarters and as a result the pressure on the teat canal forces the canals to be opened widely and allowing easy entrance of microbes. The observation of blind quarters in this study might be an indication of a serious mastitis problem at the study districts.

In the present study, prevalence of mastitis was numerically higher (P=0.208) in the adult (56.8%) and old (40%) age group than the young (24.8%) age group. Kerro and Tareke (2003) and Busato *et al.* (2000) noted that mastitis increase with increasing age since the lower immunity status of older animals predispose to mastitis, which is also confirmed by the result of the present experiment. The low mastitis prevalence in cows with single parity and higher prevalence in cows with multiple parities is in accordance with previous findings (Sergant *et al.*, 1998; Busato *et al.*, 2000; Kerro and Tareke, 2003). Several factors can be involved in the development of mastitis in animals with multiple parities. Increased clinical and subclinical mastitis in older cows increase the infection rates and decreases the local defence mechanisms.

The tendency of high prevalence of mastitis at early stage of lactation as compared to mid and late lactation stages agree with that reported by Kerro and Tareke (2003) and Thennarasu and Muralidharium (2004) who attributed this to the absence of dry period therapy and birth related influences. Radostits *et al.* (2007) suggested that the mammary gland is more susceptible to new infection during the early and late dry period due to the absence of udder washing and teat dipping, which in turn may have increased the presence of potential pathogens on the skin of the teat. The occurrence of more cases during early lactation stage may be due to absence of dry cow therapy and birth related influences. The amount of milk ejected is also higher during early lactation periods and this cause increase in patency of the teats and decreased local defence factors.

Milking hygienic condition is one of the predisposing factors in the distribution of mastitis. The present study revealed mastitis prevalence was significantly (P=0.013) higher when milking was conducted under poor hygienic condition (36.89%) than good milking hygiene (24.8%). Kivaria *et al.* (2004) noted higher prevalence of mastitis in animals kept on farms with poor hygiene and attributed this to increased exposure and transmission of pathogens during milking. Moreover, poor milking hygiene and milking equipment may be the contributing factors to transmit the bacteria from infected cows to healthy ones.

In the present study, bacteria were isolated from the majority (1455/1487; 97.8%) of mastitis positive milk samples. The values recoded for the two predominant organisms isolated from mastitis milk, *S. aureus* (14.09%) and *S. agalactiae* (13.2%) are lower than reported by earlier studies (Geresu, 1989; Tesfahiwot, 1996; Nesru *et al.*, 1999) from Ethiopia. On the other hand, Tolassa (1987) and Mekuria (1986) reported far higher prevalence of *Streptococcus* species (53.55 and 45.50%). The predominance of these bacterial species is due to frequent colonization of teats as they are commonly found on the skin. The bacteria can easily get access to the teat canal during milking or suckling and can be transmitted from quarter to quarter and from cows to cows during milking practices. Their ability to exist intracellular and localized within micro-abscessation in the udder gave them chance to resist antibiotic treatment (MacDonald, 1997), which could also be an important factor contributing to the predominance of these organisms. Kerro and Tareke (2003) found the coliform to account for about 20.96% of the isolates and were the third predominant pathogens from dairy cows in Southern Ethiopia. Mekuria (1986) and Biffa (1994) reported lower percentage (3.64 and 3.14%) of *E. coli* than the present study. The prevalence of environmental *E. coli* may be associated with poor farm cleanliness. Faeces which are common sources of *E. coli* can contaminate the premises directly or indirectly through bedding, calving stalls, udder cleaning water and milker's hands (Radostits *et al.*, 2007). The prevalence rate of other isolates in the present study somewhat agreed with the findings of Hamir *et al.* (1978), who reported 1.3%. Of all the isolates; contagious pathogens showed greater frequency than others. In general, the prevalence of mastitis causing agents was high.

The average susceptibility (74.4%) of *S. aureus* strains to all antimicrobials tested is higher than susceptibility reports of 62.7% reported by Mekonnen *et al.* (2005) in Ethiopia and Myllys *et al.* (1998) in Finland. However, *Staphylococcus* and *Streptococcus* species showed the existence of resistance to streptomycin and penicillin. The resistance of *S. aureus* to penicillin may be attributed to the production of beta lactamase, an enzyme that inactivates penicillin and closely related antibiotics. It is believed that around 50% of mastitis causing *S. aureus* strains produces beta lactamase (Green and Bradely, 2004). Moreover, these two antibiotics are most widely used in many parts of Ethiopia since they are affordable antibiotics to farmers and are sometimes the only available antibiotics in many veterinary clinics. This wide use of these drugs and inappropriate administration could have contributed to the development of resistance by the predominant bacterial agents in the area. *P. multocida* are the most susceptible isolates to gentamycin, amoxicillin, oxytetracycline, ampicillin, and cloxacillin, but resistant to streptomycin and penicillin. The present study has demonstrated the

existence of resistance bacteria to commonly used antimicrobial agents and the results are in accordance with reports from earlier studies in other countries (Gentilini, 2000; Edward *et al*., 2002) that suggested a possible development of resistance from prolonged and indiscriminate usage of some antimicrobials. It is therefore, very important to implement a systemic application of an *in-vitro* antibiotic susceptibility test prior to the use of antibiotics in both treatment and prevention of intra-mammary infections.

Conclusion

The present study showed prevalence of 38.6% based on CMT and clinical examinations of bovine mastitis. Hygienic conditions has a significant association ($P<0.05$) with mastitic cows in the study areas. The predominant bacterial isolates were *S. aureus* (14.09%), followed by *S. agalactiae* (13.2%) and *E. coli* and *S. intermedius* with proportion of 10.9% for each. Gentamycin, amoxicillin, oxytetracycline, ampicillin, and cloxacillin showed relatively good efficacy and can be used in the study area for treatment of mastitis. Since some of the trial discs especially streptomycin and penicillin showed low potency, special attention should be given to avoid development of antimicrobial resistance. We recommend awareness creation among farmers on the impacts of subclinical mastitis for effective mastitis control. Moreover, the udder of each cow should be periodically checked for the timely treatment and prevention of the disease as well as selection of effective antimicrobials.

Conflict of Interests

The authors declare that they have no competing interests.

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