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Prevalence of bovine mastitis, risk factors, isolation and anti-bio gram of major pathogens in Mid Rift valley, Ethiopia

Melesse Etifu* and Minyahil Tilahun

Department of Animal Production and Technology, College of Agriculture and Natural resources, Wolkite University, Ethiopia.

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Cross-sectional study design was implemented in Alage dairy farm to determine the overall prevalence of mastitis, its causative agents, susceptibility patterns and risk factors associated with it. A total of 111 milking dairy cows and 444 quarters were examined. Overall prevalence of mastitis at cow and quarter levels were 73 and 37%, respectively. Coagulase negative staphylococci (*CNS*) (37.7%) followed by *Staphylococcus aureus* (19.6%), *Escherichia coli* (9.4%), *Staphylococcus intermidius* (9.4%), *Bacillus* species (8%), *Streptococcus* species (5.8%), *Klebsiella pneumoniae* (5.8%), and *Enterobacter aerogens* (4.3%) were isolated in that order of decreasing frequency. From a total of seven antibiotics tested, most isolates were sensitive to Norfloxacin but showed resistance to Ampicillin. Age, stage of lactation, milk yield, hygiene score, feet problems and udder conformation were found to be risk factors significantly (P<0.05) associated with mastitis. The high prevalence rate of mastitis in a relatively well managed dairy farm implied that, it is the trickiest health problem of dairy cows that needs continued and concerted efforts in its fight.

Key words: anti-biogram, mastitis, pathogens, prevalence, risk factors.

INTRODUCTION

Various researchers, in different parts of the world, revealed mastitis as grievous disease in the dairy industry. The disease has been described as the most common and costly in dairy production (Seegers et al., 2003). The risk factors associated of the disease were also reported to be multi-factorial and multi faceted showing considerable differences with agro ecological zones and farming conditions. According to Getahun et al. (2008) loss in milk production, discarding abnormal milk and milk withheld from cows treated with antibiotics, decrease in milk quality and price due to high bacterial or somatic cell count (SCC), costs of drugs, veterinary services and increased labor costs, increased risk of subsequent mastitis, herd replacement, and problems related to antibiotics residues in milk and its products are some of the major issues of concern for the cow, farmer and for the consumers.

According to Sharma et al. (2007) mastitis is one of the most significant health problems of dairy herds as it causes physical, chemical and bacteriological changes in the milk of dairy animals resulting in inferior quality and quantity of produced milk with possible public health

*Corresponding author. E-mail: etifumelese16@gmail.com. Tel: +251-911376067/932608625.

importance. Therefore, conducting research on its prevalence and incidence will contribute to design appropriate preventive measures and treatment regimen in the specific dairy farm. Factors attributed to the prevalence of mastitis like parity was investigated by different authors in different parts of the country (Belayneh et al., 2013; Zeryehun et al., 2013; Katsande et al., 2013; Abrahmsén et al., 2014; Mureithi et al., 2016).

A.A.T.V.E.T.C. dairy farm which is located at mid- rift valley area of Ethiopia is the only source of milk and milk products for the total population of 10,000-15,000 residents in the community and provides milk products for nearby towns like Ziway, Bulbula, Shashemene and Awassa. Conducting research on the status of intra mammary infection, that can be considered as bottle neck to the production performance is of paramount importance. However, the information on the prevalence of the disease, associated risk factors, profiles of major mastitis causing pathogens and anti-bacterial susceptibility profiles of causative agents in this dairy farm is almost unknown. Therefore, the general aim of this study was: To determine the prevalence of mastitis and the associated potential risk factors in A.A.T.V.E.T.C. dairy farm, presumed to represent dairy herds with similar management practices and agro-ecological environment of the country.

Specific objectives of the study were:

(i) To determine the prevalence of clinical and subclinical mastitis at quarter and cow level in A.A.T.V.E.T.C. dairy cows.
(ii) To determine the associated risk factors with the outcome of interest.
(iii) To isolate and identify the bacterial pathogens which cause both clinical and sub clinical mastitis and to conduct antimicrobial susceptibility profile of isolates.

The present study investigated the mastitis situation, causative agents, associated risk factors and antimicrobial efficacy in a college dairy farm, in the central rift valley agro- ecological zone, Ethiopia.

MATERIALS AND METHODS

Location

The study was conducted at the Alage Agricultural Technical Vocational Education Training College (AATVETC) dairy farm located in the central rift valley agro-ecological zone, 170 Km south of Addis Ababa, Ethiopia.

Study population and sample size

The study population consisted of all Holstein Friesian cows present at AATVETC dairy farm. A cross-sectional type of study was carried out on 111 lactating cows from October 2011 to May 2012.

Methodology

Farm inspection

A one-time inspection on the housing conditions, feeding practices and milking practices was conducted simultaneously with animal examination and milk sampling. The housing condition was qualified as “Poor” when one or more of the following were detected: bad smell, dirty barn, soiled animal flank, udder and belly. When none of the above defects were observed it was rated as “Good”.

Animal examination

Animal examination was conducted to determine their body condition, presence or absence of feet and leg problems, soundness of udder and hygiene score. Body condition scoring was implemented using 1-5 point scale as per (Parker, 1989). Presence or absence of feet and leg problems were evaluated through visual inspection and palpation. Hygiene scoring of cows was determined based on a scale of 1 - 4 (Chaplin et al., 2000).

Udder examination

Visual inspection and palpation methods were used to identify atrophy of the tissue, udder attachment problems (asymmetry), quarter blindness, and cardinal signs of inflammation such as hotness, redness, swelling and painful sensation.

milk sample collection

Prior to milk sample collection, the udder, especially the teats, were thoroughly washed with tap water and cleaned with dry towel. Then the teats were disinfected with cotton soaked in 70% ethyl alcohol. Milk samples were collected by using standard milk sampling techniques (Quinn et al., 1999) from all lactating cows and quarters. To reduce contamination of the teat ends during sample collection, the near teats were sampled first followed by the far once. Approximately 10 ml of milk was collected from each quarter into labeled sterile screwed cap universal bottle after discarding the first three milking streams. Physical appearance of milk secretion from each mammary quarter was examined for the presence of clots, flakes, blood and watery secretions. Samples were then placed in ice box and transported to the Federal Microbiology laboratory of the college and processed in the same day of sample collection.

California mastitis test (CMT)

From each quarter of the udder, a squirt of milk sample was dropped in each of the strip cups on the CMT paddle and an equal amount of 3% CMT reagent was added to each cup and mixed gently. The test result was interpreted according to Quinn et al. (1999) based on the thickness of gel formed by CMT reagent and milk mixture and as 0 (negative), T (trace), 1(weak positive), 2(distinct positive) and 3(strong positive). Finally quarters with CMT score of 1 or above were judged as positive for sub clinical mastitis; otherwise negative.

Bacteriological isolation and identification

Milk samples were bacteriologically examined according to the procedures employed by Quinn et al. (1999). The milk samples, taken from infected quarters, were centrifuged so as to increase the bacterial load and inoculated separately on to blood agar base enriched with 7% ovine blood using quadrant streaking method. The inoculated plates were incubated aerobically at 37°C for 24 to 48 h, after which presence or absence of bacterial growth, colony morphology, color and hemolytic characteristics were recorded on primary culture. Prior to further biochemical tests, the isolated bacteria were sub-cultured into nutrient agar. Each culture was subjected to gram staining to determine the shape, and gram reaction. Catalase test, using 3% Hydrogen per oxide (H_2O_2), was performed to identify catalase positive and catalase negative bacteria. Mannitol Salt Agar (Oxiod, UK) and purple base agar (Difco) with 1% maltose were used to differentiate *Staphylococcus* species. The culture was incubated at 37°C and examined after 24-48 h for mannitol and maltose fermentation respectively. Tube coagulase test, using rabbit plasma, was used to identify the coagulase positive and coagulase negative *Staphylococcus* species. *Enterobacteriacae* species were identified using oxidase test. Other tests preformed were SIM medium (Oxiod, UK) for sulfur production; indole test (after addition few drops of kovacs reagent) and motility test, (Triple Sugar Iron-Oxiod,UK) to detect sugar fermentation, sulfur and gas production; MacConkey agar (Oxiod, UK) for lactose fermentation and colony characteristics; and Simmon's citrate agar (Oxiod, UK) to differentiate bacteria based on citrate mutilation.

Antibacterial sensitivity test

Kiby-Bauer disk diffusion method was employed to test *in vitro* antibiotic sensitivity test (Quinn et al., 1994). After identifying isolated colonies, each isolate was suspended in to Tryptose Soya Broth (TSB) (oxiod, UK) then incubated for 24 h. Finally, bacteria suspended in TSB media were spread in to Mueller Hinton agar and blood agar (oxiod, UK) using cotton swab. Seven different antibiotic discs namely, Norfloxacin, Ampicillin, Gentamicin, Doxycycline, Erythromycin, Trimethoprim- Sulfamethoxazole and Tetracycline were used, because these drugs were in use in the study area for treatment of different diseases. Finally, they are dispensed on the medium using forceps and incubated for 24 h. Diameter of zone of inhibition for each antibiotic disc was measured using a ruler in to the nearest millimeter and interpreted as resistant and sensitive according to the standard given by Quinn et al. (1994) and manufacturer (Oxoid) instruction.

Data entry and analysis

Data were coded, cleaned and entered into Microsoft Excel computer software. Statistical analysis was carried out using SPSS version 20. Data were analyzed using descriptive statistics and associations of the different variables with interest of outcome was analyzed using a Chi-squared ($\chi 2$) test. The association was considered significant when odds ratio was greater than one and p-value was less than 0.05.

RESULTS

Cow data

Table 1 illustrates some of the physical and productive characteristics of dairy cows. The age of cows ranged from three to more than eight years with highest proportion (54%) being aged between 3 and 5 years. The majority (58.6%) of the cows were in their first and second lactations. More than 82% of the cows had body condition score of 3-4. Milk yield per day varied from 4 L to more than 11 L with 53% of the cows producing 8-11 L per day.

Prevalence of mastitis

The overall prevalence of mastitis at cow level was found to be 73%. Prevalence of Sub clinical and clinical mastitis at cow level was found to be 56.8 and 16.2%, respectively. The overall prevalence of clinical and sub clinical mastitis at quarter level was 8.8 and 28.2%, respectively. Prevalence at right quarters and left quarters was also found to be 54.5 and 44.5%, respectively. The details of prevalence rates of clinical and sub-clinical mastitis at cow and quarter levels are presented in Tables 2 and 3, respectively. From a total of 444 quarters examined 23(5.2%) were blind quarters.

Bacteriological examination result

Table 4 illustrates the details of bacterial isolates. From a total of 156 quarter milk samples (37 clinical and 119 sub-clinical) cultured, 138 were positive. Ten (6.4%) of the cultured samples were rejected for contamination and 8 (5.1%) yield no bacterial growth. All the clinical mastitis and 101 of the sub-mastitis samples resulted in positive culture. Contagious pathogens like *Staphylococcus* bacterial species and environmental pathogens like *Escherichia coli, Klebsiella pneumoniae* and *Enterobacter aerogens* were identified. The highly prevalent bacteria was coagulase negative staphylococci (*CNS*) (37.7%) followed by *Staphylococcus aureus* (19.6 %).

Animal and/or management factors associated with mastitis prevalence

Table 5 shows the relationship of animal and management related factors with mastitis prevalence at Alage Dairy Farm. Prevalence rate was 88, 77 and 63%, respectively in older, mid age and young cows. There was statistically significant difference among different age groups ($p<0.05$). Cows in early lactation stage had highest mastitis prevalence (100%) than those at late (68%) and mid (43.3%) stages of lactation with highly significant statistical difference, $p<0.01$. Feet problems were also significantly ($p<0.01$) associated with mastitis prevalence. Pendulous udder, compared to high up udder had higher rate and the difference was statistically significant ($p<0.01$). Animals with poor body condition score, multiple parities, and blind teats exhibited higher prevalence rate of mastitis (79, 80 and 91% respectively) compared to good body condition score, few parities and absence of blind teats ($p>0.05$) (Table 5).

Table 1. Physical and productive characteristics of milking cows at Alage dairy farm (n= 111).

Category	Variable	Frequency	Percent
Age	Young (3-5 years)	60	54.05
	Mid age (6-8 years)	18	16.22
	Old age (>8 years	33	27.73
Parity	Few (1-2 lactations)	65	58.56
	Many (≥ 3 lactations)	46	41.44
Stages of lactation	Early (1-3 months)	41	36.94
	Medium (4-6 months)	30	27.03
	Late (>6 months)	40	36.04
Average daily milk yield	High (> 11 liter)	35	31.53
	Medium (8-11 L)	59	53.15
	Low (4-7 L)	17	15.32
BCS (on a scale of 1-5)	Good (3-4)	92	82.88
	Poor (1-2)	19	17.12
Feet problems	Yes	43	38.73
	No	68	61.26
Udder conformation	High up	45	40.54
	Pendulous	66	59.46
Blind teat	Yes	23	20.72
	No	88	79.28
PETM	Yes	54	48.65
	No	57	51.35
Hygiene score (On a scale of 1-4)	Good (1-2)	65	58.56
	Poor (3-4)	46	41.44

Table 2. Cow and quarter level mastitis prevalence at Alague Dairy Farm (n= 111).

Variable	Total number of positive	Prevalence rate
Types of mastitis		
Clinical	18	16.2%
Sub clinical	63	56.8%
Total	81	73%
Quarter level blind teats distribution (n=444)		
Right fore	4	3.6
Right hind	4	3.6
Left fore	7	6.3
Left hind	8	7.2
Total	23	5.2

Table 3. Quarter level mastitis prevalence at Alague Dairy Farm (n=421).

Types of mastitis	Quarter level prevalence				Total
	Right front	Right hind	Left front	Left hind	
Clinical	11(2.6%)	8(2%)	7(1.7%)	11(2.6%)	37(8.8%)
Sub clinical	30(7.1%)	36(8.6%)	26(6.2%)	27(6.4%)	119(28.2%)
Total	41(9.7%)	44(10.5%)	33(7.8%)	38(9%)	156(37%)

Table 4. Frequency of occurrence of bacterial isolates from CMT positive milk samples at Alage Dairy Farm.

Types of bacteria isolated	Status of mastitis			Proportion (%)
	Clinical mastitis	Sub clinical mastitis	Total frequencies	
*CNS	15(10.9%)	37(26.8%)	52	37.7
Staphylococcus aureus	5(3.6%)	22(16%)	27	19.6
Escherichia coli	2(1.4%)	11(8%)	13	9.4
Staphylococcus intermidius	2(1.4%)	11(8%)	13	9.4
Bacillus species	4(2.9%)	7(5.1%)	11	8
Strepthococcus species	2(1.4%)	6(4.4%)	8	5.8
Klebsiella pneumoniae	4(2.9%)	4(2.9%)	8	5.8
Enterobacter aerogens	3(2.174%)	3(2.174%)	6	4.3
Total	37(26.8%)	101(73.2%)	138	100

*CNS (coagulase negative staphylococci).

Antimicrobial susceptibility profile of mastitis isolates

Table 6 shows anti-biogram test results of seven antimicrobial drugs namely, (Norfloxacin (NOR10 µg), Ampicillin (AMP10 µg), Gentamicin (CN10 µg), Doxycycline (DO30 µg), Erythromycin (E15 µg), Trimethoprim-Sulfamethoxazole (SXT1.25 µg) and Tetracycline (TE30 µg) tested on seven different bacterial isolates, namely CNS, *S. aureus, E. coli, K. pneumonia, Staphylococcus intemidius, Enterobacter aerogens, Bacillus* spp. and *Streptococcus* spp.

Among antibiotics tested *in vitro* Norfloxacin was the most potent drug followed by Trimethoprim-Sulfamethoxazole Gentamicin, Doxycycline, Tetracycline and Erythromycin with the efficacy rate of 97, 94 and 89, 84 82 and 70% respectively. On the other hand, Ampicillin was found to be the least potent drug in the overall tested bacteria (55%). CNS isolates were susceptible to Gentamicin (100%), Tetracycline (100%), Trimethoprim-Sulfamethoxazole (95%), Norfloxacin (90%), Erythromycin (87%), Doxycycline (70%) and Ampicillin (40%). *S. aureus* isolates were also susceptible to Gentamicin (100%), Erythromycin (100%), and Trimethoprim-Sulfamethoxazole (100%, Norfloxacin (87%), Tetracycline (80%), Ampicillin (55%) and Doxycycline (43%) with trend of decrement in efficacy. *E. coli* was 100% susceptible to Norfloxacin and Trimethoprim-Sulfamethoxazole but less commonly affected by Erythromycin, Ampicillin, Tetracycline and Gentamicin with the potency rate of ≤50%.

DISCUSSION

Prevalence of mastitis at cow and quarter level

The overall prevalence of mastitis at cow level was found to be 73% which is in line with the report of Regasa et al. (2010b) and Bishi (1998), who found the prevalence rate of 71 and 69.8%, in dairy farms of Holeta town and Addis Ababa and its vicinity, Ethiopia respectively. This is slightly lower than the report of Matios et al. (2009) who reported the prevalence rate of 64.5% in dairy farms of and Asella, Ethiopia. In contrast, our results was found to be by far greater than the prevalence report of Getahun et al. (2008), Gizat et al. (2008), Mekonnen and Tesfaye (2010), Sori et al. (2005) and Mungube et al. (2005) who reported mastitis prevalence as 33.6, 56, 48.1, 52.78 and 52.3% in the dairy farms of Selalle, Bahir dar, Adama and Sebeta and cross breed dairy cows in Ethiopia respectively.

The overall prevalence of clinical and sub-clinical mastitis at quarter level was found to be 8.8 and 28.2% respectively. Matios et al. (2009) also reported a sub clinical mastitis of 30.4% in Asella area. Getahun et al.

Table 5. Association of animal and management related risk factors with Mastitis at Alage Dairy Farm (n= 111).

Risk factor	Total No of cows	Status of mastitis			χ2	df	P-value	OR	95% CI
		Clinical (%)	Sub-clinical (%)	Over all (%)					
Age[1]									
Young	60	18.3	45	63.3	6.728	2	0.000**		
Mid age	18	0	77.8	77					
Old age	33	21.2	66.7	87.8					
Stages of lactation[2]									
Early	41	22	78	100	16.14	2	0.000**		
Medium	30	10	33.3	43.3					
Feet problems									
Yes	43	19	74	93	12.83	1	0.000**	5.5	2.05-14.68
No	68	15	46	60					
Udder conformation									
Pendulous	66	18	71	89	22.32	1	0.000**	7.5	3.11-18.085
High up	45	13	36	49					
Body condition score [3]									
Good	92	20	52	72	0.79	1	0.32	3.4	1.06-11.15
Poor	19	0	79	79					
Lactation number (parity)[4]									
Many	46	715	65	80	2.3	1	0.1218	1.89	0.828-4.307
Few	65	17	51	68					
Previous exposure to Mastitis									
Yes	54	19	70	89	8.98	1	0.003**	3.52	1.516-8.165
No	57	14	44	58					
Average daily milk yield[5]									
High	35	17	74	92	13.44	2	0.001		
Medium	59	74	54	71					
Low	17	12	29	42					
Presence of blind teat									
Yes	23	21.7	69.5	291	2.012	1	0.156	2.2	0.747-5.323
No	88	14.7	53.4	68					
Cow's hygiene score[6]									
Bad	46	12(26)	34(74)	46(100)	19.05	1	0.000	0.118	0.041-0.337
Good	65	6(9)	29(45)	35(54)					

**: highly significant difference; *Age*[1:] in years 3-5 (young) 6-8(mid-age) and (>8) (old age);
Stages of lactation[2:] in months 1-3(early), 4-6 (medium) and >6 (late)
Body condition score [3:] in 1-5 scale; 1-2(poor) and (3-4) good
Lactation number (parity) [4:] 1 and 2 =few, More than 2= many
Average daily milk yield[5:] in 1-4 scales 1-2 (good) and 3-4(bad)
Cow's Hygiene Score[6:] in 1-4 scales 1-2 (good) and 3-4(bad).

(2008) and Mekonnen and Tesfaye (2010), however recorded lower level of sub clinical mastitis prevalence in Selalle (13.6%) and Adama area dairies (22.7%). Regasa et al. (2010a), on the other hand reported 34.8% sub clinical quarter wise prevalence which is higher than our finding. Variations in husbandry practices between different areas might, at least, partly explain the differences in prevalence rates reported by different

Table 6. Anti-bio gram test result.

Bacterial isolates	Frequency	Anti-bio gram susceptibility test in percent						
		NOR10 µg	AMP10 µg	CN10 µg	DO 30 µg	E15 µg	SXT1.25 µg	TE 30 µg
CNS	52	90	40	100	70	87	95	100
S.aureus	27	87	55	100	43	100	100	80
Esherichia coli	13	100	38	50	88	30	100	45
Klebsiella pneumoniae	8	100	33	98	100	45	96	70
Staphylococcus intemidius	13	100	100	100	89	78	94	100
Enterobacter aerogens	6	100	48	60	100	55	90	78
Bacillus spp.	11	100	48	100	100	70	85	80
Streptococcus spp.	8	100	78	100	80	90	95	100

NOR= norfloxacin, AMP= ampicillin, CN= gentamicin, DO= doxycycline, E= erythromycin SXT= trimethoprim-sulfamethoxazole and TE=tetracycline.

authors. Quarter level clinical mastitis prevalence in this study was in line with what was reported by Regasa et al. (2010b), (10% of clinical prevalence at quarter level). But our findings is higher than those of Mekonnen and Tesfaye (2010) and Getahun et al. (2008) who reported quarter wise clinical mastitis prevalence of 2.4 and 0.9% respectively. Matios et al. (2009) reported clinical mastitis prevalence level as high as 14.9%.

The 5.2% of the mammary glands examined were found to be blind in the study animals which is slightly higher than the report of Matios et al. (2009) who found 4.5% of blind quarters. Getahun et al. (2008) and Mekonnen and Tesfaye (2010) reported 2.3 and 3.6% blind quarters respectively in their study herds. Currently, it is a well-accepted fact that agro-ecology, milking practice, breed difference, management practices and other risk factors influence mastitis prevalence, which might explain the observed differences between the reports of different authors in mastitis prevalence. In the present study, the higher prevalence level of sub-clinical mastitis compared to clinical form; indicate the magnitude of subclinical mastitis problem and low level of attention that given to it in terms of diagnosis and treatment.

Bacterial isolation and anti-bio gram susceptibility test

In this study the bacteria with the highest prevalence was coagulase negative staphylococci (CNS), followed by *S. aureus, Escherichia coli, Staphylococcus intermidius, Bacillus* species*, Streptococcus* species, *Klebsiellae pneumoniae*, and *Enterobacter aerogens* with prevalence rates of 37.7, 19.6, 9.4, 9.4, 8, 5.8, 5.8 and 4.3% respectively. Among isolated bacteria, the majority of them were retrieved from sub clinically infected quarters. This finding is comparable with the report of Mekonnen and Tesfaye (2010) and Gizat et al. (2008) who found CNS as the predominant bacteria among isolates in Adama and Bahirdar dairies, respectively. On the other hand, in different previous studies, *S. aureus* was the most frequently isolated bacteria as per the reports of Regassa et al. (2010a), Matios et al. (2009) and Getahun et al. (2008) in dairy farms of Holeta, Asella and Selalle towns, respectively.

The preponderance of contagious mastitis in this study may be ascribed to the lack of proper milking procedure before milking, during the time of milking and post milking. For instance absence of pre- and post teat dipping using antiseptics, washing of milkers' hands and using teats secretion as a lubricant of teats at the time of milking which is often practiced in the study area might contributed to the spread of these pathogens from infected teats to healthy ones.

In the present study interestingly environmental bacteria like *E. coli* was isolated in high proportion (9.4%). This is in congruence with the reports of Mekonnen and Tesfaye (2010) and Matios et al. (2009) who found 7.5% of the total isolates. In contrast, this figure is higher than the one reported by Regassa et al. (2010b), Sori et al. (2005) and Getahun et al. (2008) who reported 4.57, 0.75 and 0.5% in different parts of Ethiopia, respectively. The presence of environmental bacteria might be an implication of unhygienic milking practice and contamination of cows' teats and environment with their dung in the study area. Antibiotic susceptibility testing of 138 bacterial isolates was performed using a panel of seven antimicrobial drugs (Oxoid, UK) anti-biotic discs used for the test were Norfloxacin (NOR10 µg), Ampicillin (AMP10 µg), Gentamicin (CN10 µg), Doxycycline (DO30 µg), Erythromycin (E15 µg), Trimethoprim-Sulfamethoxazole (SXT1.25 µg) and Tetracycline (TE30 µg).

In this study CNS isolates were susceptible to Gentamicin, Tetracycline, Trimethoprim-Sulfamethoxazole, Norfloxacin, Erythromycin, Doxycycline and Ampicillin with efficacy rates of 100, 100, 95, 90, 87, 70 and 40% in decreasing order respectively. *S. aureus* isolates were also susceptible to Gentamicin (100%), Erythromycin (100%) Trimethoprim-Sulfamethoxazole (100%) Norfloxacin (87%), Tetracycline (80%), Ampicillin (55%) and Doxycycline

(43%) with trend of decrement in potency. *E. coli* were 100% susceptible to Norfloxacin and Trimethoprim-Sulfamethoxazole, but less commonly affected by Erythromycin, Ampicillin, Tetracycline and Gentamicin with the potency of ≤50%. Among antibiotics tested *in vitro* Norfloxacin was the most potent drug followed by Trimethoprim-Sulfamethoxazole Gentamicin, Doxycycline, Tetracycline and Erythromycin with the efficacy rate of 97, 94 and 89, 84, 82 and 70% respectively. On the other hand, Ampicillin was found to be the least potent drug in the overall tested bacteria (55%). Anti-bio gram testing results in this study is in line with the report of Getahun et al. (2008) who found 100% susceptibility to ampicillin and tetracycline whereas in case of *S. intermidius* and *S. aurues* there was a susceptibility rate of 45.3% for ampicillin which is in line with the report given by Nibret et al. (2011) who indicated tetracycline showed 40% susceptibility rate for *E. coli* and 44% for CNS but in case of CNS it is higher than the report given by Nibret et al. (2011) who found susceptibility rate of 60% for Erythromycin and 18.5% for *S. aureus* for Ampicillin. The differences in susceptibility patterns of bacteria to different antibiotics might be attributed to differences in utilization of anti-microbial agents for treatment regimen and development of resistance due to repeated use of similar antibiotics in different farms for longer period.

Associated risk factors and the status of mastitis

Among assessed potential risk factors to the prevalence of mastitis, higher infection rates were observed in cows with advanced age, cows with pendulous udder conformation, and multiple parity, poor body condition score, bad hygiene score, high milk producers, early lactation stage, previous exposure to mastitis and blind teats.

The prevalence rate of mastitis at cow level was higher as the age advances; 88, 77 and 63% in older, mid age and young cows respectively. There was statistically significant difference among different age groups. This finding is in broad agreement with reports made by different authors in different parts of the country (Demelash et al., 2005), Regassa et al. (2010b) and Mungube et al. (2004) who reported age considered as potential risk factor to mastitis and older cows were more affected by mastitis than younger cows. The increase in prevalence rate with the advancing age may be due to gradual suppression of immune system of the body, structural changes in udder and teats and repeated exposure to milking practices.

Parity was considered as associated risk factor for mastitis in this study in which cows with multiple parities showed higher prevalence (80%) than cows in their first or second lactations (68%). This is in agreement with Mungube et al. (2004), Demelash et al. (2005) Matios et al. (2009), Gizat et al. (2008), Girma (2010) and Molalegn et al. (2010) who identified parity as risk factor for mastitis in the study conducted at different parts of Ethiopia.

More cows which had experienced mastitis problem before, were found to be positive to clinical or/and sub clinical form of mastitis at current investigation than non-exposed ones, 89 and 58% respectively. This is comparable with the findings of Demelash et al. (2005) and Mekonnen and Tesfaye (2010) who indicated cows with previous exposure to udder infection were more likely to be re-infected than those never exposed. This might be attributed to possibility of previously exposed cows which remained in carrier state and impotency of drugs used for mastitis treatment in the study area.

Lactation stage was found to be a risk factor to mastitis and the prevalence was highest in early lactation (100%) than mid (43.3%) and late (68%) which is in agreement with Demelash et al. (2005) who reported mastitis prevalence was higher in early lactation (45.8%) than mid lactation (25.8%). But in some research findings, prevalence of mastitis was higher in late stage of lactation than early lactation (Getahun et al., 2008; Gizat et al., 2008). The difference in reports of different authors concerning the stage of lactation in which mastitis is most prevalent, could be attributed to different managements practiced in different study areas. The highest prevalence rate during the early lactation is an indication of infection, probably prior to freshening. It may also be reflection of important changes that occur prior to parturition period in endocrine, nutritional and metabolic status which compromise the immunity of the cow. In this stage of lactation, milk yield is increasing this can cause impairment of the immune system due to metabolic stress. When cows are in negative energy balance, body fat is converted to ketone bodies, and hyper ketonemia has been suggested to be one of the most important factors causing impairment of the udder defense mechanisms and it is likely that the impaired immune system in cows in early lactation results in reduced ability to battle infection (Suriyasathaporn et al., 2000).

Hygiene of the cow in this study was found to be one of the risk factors. Cows with bad hygiene score had higher prevalence rate ((100%) than good hygiene score (54%) which is in line with Matios et al. (2009) and Molalegn et al. (2010). In case of this investigation there was highly significant difference in cows with pendulous udder conformation than the cows with high up udder conformation with prevalence rate of 89 and 49% respectively. This is in agreement with Sori et al. (2005) and Girma (2010) reports. This might be attributed to more exposure to the injurious materials and presence of more contact with contaminated environment.

Interestingly in this investigation, there was strong association between feet problems and presence of mastitis with prevalence rate of 93 and 60% in the cows that had problem of feet than none respectively. This might be partly due to longer time the lame cow spends in horizontal (laying) position that might increase the contact with environmental pathogens and will be prone to mastitis than none affected ones. Body condition score was considered as risk factor to mastitis in this report. Cows with poor body condition had more prevalence rate (69.2%) than those with good body condition (72%) though the difference was not statistically significant. this is in congruent with the investigation by Mekonnen and Tesfaye (2010) and Mungube et al. (2004) who found body condition as one of associated risk factors to mastitis. Animal with poor body condition might experience their immune system not functioning well, thus making them more susceptible to mastitis.

Conclusion

Contagious and environmental mastitis pathogens were isolated from both clinical and subclinical quarter milk samples. Among contagious pathogens the highest prevalent bacteria was found to be Coagulase negative staphylococci (CNS) followed by *S. aureus*. *E. coli* was also the predominant environmental bacteria. The presence of considerable proportion of *Enterobacteriaceae* suggested that contamination of mammary gland and its environment with animal dung. Moreover, dominant number of contagious microbial agents indicated that improper milking procedures were experienced in the farm.

Among anti-biotics tested *in vitro* Norfloxacin was the most potent drug followed by Trimethoprim-Sulfamethoxazole. On the other hand, Ampicillin was found to be the least potent drug in the overall tested bacteria. Among assessed potential risk factors to the prevalence of mastitis; higher infection rates were observed in cows with advanced age groups, pendulous udder conformation, and multiple parity, poor body condition score, bad hygiene score, high milk producers, early lactation stage, previous exposure to mastitis and blind teats. The high prevalence rate of mastitis, in a dairy farms believed to be better managed compared to others, calls for continued enhanced efforts in combating this major obstacle of the dairy industry.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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