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Prevalence of Bovine Mastitis, Associated Risk Factors and Major Bacterial Causes in and Around Sagure Town, Arsi Zone, Oromia, Ethiopia

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Abstract: The cross-sectional study was conducted from November 2022 to March 2023 in and around Sagure District, Arsi zone, Oromia regional state of Ethiopia to determine the prevalence of mastitis and its risk factors as well as to isolate the main pathogens. A total of 157 (45.64%) of the 344 nursing Holstein cross cows and 1376 quarters had mastitis, with 27 (7.85%) having clinical and 130 (37.79%) having sub-clinical cases and 10 (0.73%) having blind teats. A total of 381 (27.89%) quarters had comparable amounts of mastitis at each quarter and were also positive either clinically or under screening tests. Even though the majority of the risk factors for mastitis were found to be insignificant (P>0.05), the prevalence of mastitis in cows with poor hygiene was significantly higher than that in cows with medium or good hygiene. Mastitis prevalence was found to be significantly influenced by farming system (P<0.05), with prevalence being significantly higher in intensive than semi-intensive systems. A total of 157 were positive for known mastitis pathogens from CMT test and clinically positive milk samples that were microbiologically examined. Coagulase negative *Staphylococcus* species (38.10%), *Staphylococcus aureus* (34.11%), *Staphylococcus intermidius* (6.95%), *Micrococcus* species (1.66%), *Pasteurella* species (1.66%) and other gram-positive rods and gram negative enteric bacteria (13%) were the most prevalent bacterial isolates in the examined animals. In general, poor farming methods and management practices are too responsible for the high prevalence of mastitis that is currently present in the study area. Therefore, it was advised to improve farm management and the animal environment's hygiene in order to decrease the prevalence of mastitis and the financial losses linked to mastitis.

Key words: Mastitis · Pathogens · Prevalence · Risk factor · Sagure

INTRODUCTION

Livestock is a significant national resource and an essential component of Ethiopia's agricultural production system. Due to its vast cattle population and an environment that supports upgraded and high-yielding breeds, Ethiopia has significant potential for the growth of the dairy industry. The majority of the nation's cattle are cows, which account for 42% of the overall cattle population [1]. The majority of the urban and per-urban population receives significant dietary sources from the milk generated by these animals. Dairy production is physiologically efficient systems that convert vast quantities of roughage, the most plentiful feed in the tropics to milk, the most nutritious food known to man [2].

Ethiopia is one sub-Saharan African country where the dairy industry is developing very slowly. Many variables, including limited genetic potential for milk production in native breeds, vast and low-input husbandry practices and widely dispersed livestock diseases, contribute to the region's low local milk output[3]. As a result, the nation's expanding need for milk cannot yet be satisfied by a small number of enhanced exotic breed animals, which are mostly found in urban and pre-urban areas [4]. The discrepancy between supply and fundamental demand for milk in urban areas is evidenced by Ethiopia's low yearly per capita consumption of milk, which is 17-19 liters per person compared to the average number for Africa of 26 kilograms per head [5]. As a result, a significant number of

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dairy products have improved as a result of domestic milk supply being adequate to meet local demand. Good management can lower production costs per produced unit of milk as well as production losses brought on by diseases, nutritional issues and management issues [6].

There are many other diseases that could influence the health of the animal population, but mastitis is the most prevalent and significant one because it reduces milk production and incurs high medical expenses for dairy farmers. Due to its multifactorial etiology, it is regarded as the most difficult disease [7].

Mastitis as a disease, particularly the subclinical cases, has not gotten much attention in Ethiopia. Just treating clinical cases has been the focus of efforts. Mastitis is unquestionably a significant problem that restricts the production of dairy products because of the significant financial implications and the unavoidable existence of latent infections.

In Ethiopia, the available evidence reveals that bovine mastitis is one of the most often encountered diseases of dairy cows. Clinical mastitis was the second most prevalent disease after reproductive disorders in the Addis Ababa milk shed, according to Lemma *et al*. [8], who observed that 171 out of 556 cross-bred cows had the disease.

Generally, The prevalence of clinical and sub clinical mastitis in different parts of Ethiopia is 12% and 21.5% respectively [8-10]. This limited research demonstrated that bovine mastitis is one of the issues impeding Ethiopia's dairy productivity, necessitating the creation of approaches for a control program within the current husbandry system.

On the other hand, subclinical mastitis has been linked to decreased milk production, which has been the primary cause of mastitis losses [11]. In addition, mastitis may make milk unfit for human consumption or serve as a vehicle for the spread of diseases like brucellosis, streptococcal intoxication, colibacillosis and streptococcal sore throat. Consuming milk and products tainted with antibiotics can also trigger allergic reactions, alter intestinal flora and cause pathogenic bacteria to become resistant to antibiotics. In order to reduce the exposure of cows to mastitis-causing organisms, a variety of control methods requiring hygiene are used before, during and after milking. This is due to the diverse bacterial etiologies of the disease.

Despite these measures, monitoring udder health is a crucial part of mastitis control because new instances of the condition always arise and antimicrobial therapy helps to control bovine mastitis [12]. Therefore, the goals of this research are to identify the main bacterial causes of clinical and subclinical mastitis in the study area as well as its prevalence and possible risk factors.

MATERIALS AND METHODS

Description of the Study Area: The study was conducted in and around Sagure town smallholder dairy farms. Sagure is a main town of Sagure district of Arsi zone, Oromia Regional State of Ethiopia. It is located about 25km from capital of Arsi zone, Asella town and 200km south east of Addis Ababa, Capital city of Ethiopia. Arsi Zone is situated at 6° 59' to 8° 49' N latitudes and 38° 41' to 40° 44' E longitude of south east of the country. The altitude of the area ranges from 2000 to 3600 m.a.s.l and characterized by mid subtropical temperature ranging from 18°C to 22°C and the relative humidity ranging from 43% to 60% with bimodal rain fall occurring from March to April (short rainy season) and the long rainy season extends from July to October with annual rainfall ranging from 900-1400mm. The area covers 41,552 hectares and topographically has highland escapement and lowland areas. Agriculture is the main occupation of population and is mixed types by animal rearing and crop production. The area is populated with 213,167 Cattle, 119,544 Sheep, 8,170 Goat, 23,354 Horse, 15,560 Donkey, 324 Mule and 109,065 Poultry [13].

Study Population: The study animals were Holstein cross breed lactating cows under smallholder dairy cows kept as intensive, semi-intensive and extensive husbandry practice in and around Sagure town, Arsi Zone, Oromia, Ethiopia.

Study Design: Cross-sectional study was conducted to determine the prevalence of mastitis at Cow and quarter level through clinical examination for clinical mastitis and indirect tests (CMT) for sub-clinical mastitis.

Sample Size Determination: Determination of sample size was done according to Thrusfield [14] and taking a 66% expected prevalence at Asella, Arsi Zone, Oromia, Ethiopia which was reported [15-22 by Lakew *et al.* [23], 95% confidence interval and 5% desired absolute precision. Thus,

$$N = \frac{1.96^2 \times \text{pexp}(1\text{-pexp})}{d^2}$$

where: N= is the required sample size, Pexp = is the expected prevalence (66%), d =is the desired absolute precision (0.05), z=value at 95% (1.96)
Accordingly, the calculated sample size was found to be 344.

Study Methodology

Data Collection and Physical Examination of Udder and Milk: To assess the impact of potential risk factors on the progress of mastitis and its extensive influence, a questionnaire survey was conducted. Each selected cow's data was gathered using an appropriate format. Breed, lactation stage, parity number, cleanliness of the milking system and administration system were all taken into consideration as risk factors. The study cows' udders were palpated and clinically inspected to look for signs of mastitis, such as clots of bloody milk and blood spots, as well as swelling, pain, hotness and disproportional symmetry. Other findings included the size and consistency of the udder quarters and blindness of the teats.

Californian Mastitis Test (CMT Screening) and Milk Sample Collection: The California Mastitis Test was carried out according to the procedures recommended by national mastitis council; NMC [15]. A squirt of milk, from each quarter was placed in each of four shallow cups in the CMT paddle. An equal amount of the commercial reagent was added to each cup. A gentle circular motion was applied to the mixtures, in a horizontal plane for 10-15 seconds. The result was scored from 0-3 (grades on the bases of gel formation [16] and the interpretation (0 for negative and trace, 1, 2, 3 for positive) was considered.

The milk sample was taken before the cow treated with either intra-mammary or systemic antimicrobials agent for good collection of the teat was wiped thoroughly with 75% ethyl alcohol. The sterile collection bottle was used and the first stream of milk from each quarter was discarded. The milk sample was then held in an ice box and then transported to the laboratory. In the laboratory, samples were cultured immediately or stored at +4°C [15].

Culture and Isolation of Bacteria: Identification of mastitis pathogens was carried out following microbiological procedures for diagnosis of bovine udder infection described in National Mastitis Council [15]. Milk samples that had been refrigerated, dispersion of bacteria and fat were accomplished by warming the samples at room temperature (25°C) for about an hour and then mixed by shaking. The samples were allowed to stand for a while for the foam to disperse and just before inoculation the tube was inverted gently. One standard loop (0.01ml) of milk sample was streaked on 7% blood agar. The inoculated plate was incubated aerobically at 37°C. The plates were checked for growth after 24, 48 and up to 72 hours to rule out slow growing microorganisms such as *Corynebacterium* species. For primary identification, colony size, shape, color, hemolytic characteristics, Grams reaction and catalase production were used. For confirmation, biochemical tests such as catalase, coagulase, sugar fermentation test, kovacs indole test and oxidation fermentation test were used after sub culturing isolated distinct colony on to a nutrient broth, MacConkey agar, mannitol salt agar, eosin methylene blue agar for other biochemical testes.

Interpretation was made according to National Mastitis Council [15]. The culture was considered negative if no growth occurs after 72 hours of incubation. Isolation of two or more colonies from a quarter sample was considered contaminated and the result was disregarded.

Data Management and Analysis: Biological data collected through history, clinical inspection, CMT, pathogenic bacteria isolation and identifications and other information were recorded on Microsoft Excel spread sheet as data base and analyzed using STATA 11.0 version. The prevalence was expressed using percentage. The association among and between the considered risk factors were tested using logistic regression, odds ratio (OR) and determination of OR confidence interval. The significance of association was also expressed using p-value at 0.05.

RESULTS

Overall Prevalence: Out of 344 lactating cows that were investigated during study period, the overall prevalence of mastitis was 157 (45.64%; Table 1). The prevalence of clinical and sub clinical mastitis were 27 (7.85%) and 130 (37.79%), respectively.

Table 2 out of 1366 quarters examined 381 (27.89%) were positive for mastitis while 10 (0.73%) blinds with similar proportion of mastitis at each quarter. Both right and left back quarters had significantly higher mastitis prevalence compared to the front quarters.

Table 1: Prevalence of clinical and sub clinical mastitis

Mastitis type	Animal tested	Positive	Percentage
Subclinical	344	130	37.79
Clinical	344	27	7.85
Total	344	157	45.64

Table 2: Quarters level prevalence of mastitis

Quarter	No of teats	Negative (%)	Positive (%)	Blind. (%)	OR	p-value
Right front	344	263 (76.45)	78 (22.67)	2 (0.6)		
Right back	344	232 (67.44)	109 (31.69)	3 (0.9)	1.58	0.008
Left front	344	262 (76.16)	78 (22.67)	3 (0.9)	1.000	1.000
Left back	344	225 (65.41)	116 (33.72)	2 (0.6)	1.76	0.001
Overall	1366	982 (71.89)	381 (27.89)	10 (0.73)		

Table 3: Prevalence of mastitis at cows and quarters level

Examination levels	Clinical mastitis (%)	Sub clinical mastitis (%)	Overall (%)
Cow level	27 (7.85)	130 (37.79)	157 (45.64)
Quarter level	75 (5.49)	306 (22.40)	381 (27.89)

Table 4: Prevalence of mastitis regarding to risk factors

Variables	Animals tested	Negative (%) (%)	Positive (%)	OR	P-value
Age (year)					
2.5-4 years	118	71 (60.20)	47(39.83)		
5-6 years	125	67 (53.60)	58 (46.40)	1.704	0.12
>7 years	101	50 (49.50)	51 (50.50)	1.612	0.214
Parity (No)					
1-2	145	83 (57.24)	62 (42.76)		
3-4	111	61 (54.95)	50 (45.05)	0.781	0.473
>5	87	43 (49.43)	44 (50.60)	1	0.999
Lactation stage					
Early	79	35 (44.30)	44(55.70)		
Mid	106	63(59.43)	43(40.56)	0.692	0.254
Late	159	90 (56.60)	69(43.40)	0.665	0.178
Husbandry					
Intensive	159	68 (42.77)	91 (57.23)		
Semi-intensive	137	90 (65.70)	47(34.30)	0.536	0.018
Extensive	48	30 (62.50)	18(37.50)	0.586	0.145
Hygiene					
Poor	156	61 (39.10)	95(60.90)		
Medium	146	98(67.12)	48(32.88)	0.333	0.000
Good	42	29(69.05)	13(30.95)	0.321	0.004

Risk Factors: The overall prevalence of mastitis using multivariate risk factors is shown on Table 5. Cows of poor hygiene had significantly higher prevalence compared to medium and good hygiene. Similarly, cows kept under intensive farming system had significantly higher prevalence (58.49%) than semi-intensive management system (34.30%). All other variables considered as risk factors in present study shows insignificant in prevalence of mastitis ($P>0.05$). Animals over 7 years old had shown a relatively higher prevalence when compared to those younger than 5-6 years. The highest prevalence of mastitis was observed in animals with parity of more than 5, followed by 3-4 and 1-2 parity. Furthermore, mastitis prevalence was found to be higher in early lactation and lower in mid lactation stages. The occurrence of mastitis was higher in animal under intensive management (58.49%) than extensive management system (37.50%).

Bacterial Isolation: A total of 157 cows were positive either clinically or sub clinically using California Mastitis Test. All samples showing strong positive reactions were collected and cultured. Subsequently; the following Bacteria species were isolated with a high prevalence of Coagulase negative Staphylococci (38.10%), *Staphylococcus aureus* (34.11%), *Staphylococcus intermidius* (6.95%), *E. coli* (5.30%), *Corynebacteriium* (5.30%), *Bacillus species* (3.97%), *Klebsiella* species (1.99%), *Micrococcus* species (1.66%), *Pasteurella* species (1.66%) and *Proteus* species (0.99%).

Table 5: Frequency distribution of bacterial isolated from mastitis cows

Isolated Bacteria	Clinical	Subclinical	Total	Prevalence (%)
Bacillus species	2	10	12	3.97
CNS	28	87	115	38.10
E.coli	8	8	16	5.30
Micrococcus species	1	4	5	1.66
Corynebacterium species	1	15	16	5.30
Klebsiella species	1	5	6	1.99
Pasteurella species	1	4	5	1.66
Proteus species	1	2	3	0.99
Staphylococcus aureus	19	84	103	34.11
Staphylococcus intermidius	7	14	21	6.95
Total	69	233	302	100

DISCUSSION

Researchers looked into the frequency of mastitis among cows and quarters. Mastitis was found to be prevalent in 157 out of 344 cows, or 45.64% of all cows. The prevalence found in this research was very similar to that reported by Workineh *et al*. [17] and Demelash [18] for two significant dairy farms in Ethiopia, where mastitis prevalence was 45.4%. This might be because the study areas' management systems are comparable. In some regions of Ethiopia, the current prevalence of mastitis is higher than prior reports. In their respective studies in Selalle and Bahir Dar, Getahun *et al*. [19] and Bitew *et al*. [20] found mastitis with prevalence of 24.9% and 28.2%, respectively. Similarly, Malawi recorded a prevalence of 17.19% [21]. The current prevalence, however, was lower than that reported in the studies by Sori *et al*. [22], Lakew *et al*. [23] and Mekibeb *et al*. [24], which showed prevalence of 52.78%, 65.6% and 71.0% for mastitis, respectively. The different management systems of the study regions may be to blame for the observed difference in mastitis prevalence between these studies [25]. The total prevalence of 27.89% was determined at the quarter level. This closely echoes the findings of Biffa *et al*. [26], who noted 28.20% in southern Ethiopia.

The prevalence of quarter-level mastitis found in the current research was higher than that found in the work of Almaw *et al*. [25] who reported a prevalence of 22.8% at smallholder dairy farms in Bahir Dar and a prevalence of 10.6% at Selalle. Mekbib *et al*. [24], who reported a prevalence of 44.80%, reported a lower prevalence than the one found in the current research. Quinn *et al*. [27] state that the interaction of the microbial agent, host and environmental variables decides the incidence of mastitis. Therefore, differences in host, agent and environment interactions in the various research areas may be related to variations in mastitis prevalence, both at the cow and quarter levels. Clinical mastitis was observed at prevalence of 7.85% at cow level. Prevalence of clinical mastitis at cow level is comparable with Tesfaye [28] who reported 7.3% in Adama and slightly with Adugna [29] who reported 5.7% in Dire Dawa and Haramaya university dairy farms. The clinical prevalence of current study is higher than the work of Getahun *et al.* [19] who reported clinical mastitis prevalence of 1.8% at cow level. The cow level prevalence observed in this study was higher than prevalence of 3% reported by studies of Bitew *et al*. [20] in different study areas. However, it is lower than that of Lakew *et al.* [23] and Mekibeb *et al*. [24] who reported clinical mastitis with a prevalence of 26.5% and 22.4%, respectively. The prevalence of clinical mastitis at quarter level in this study (5.5%) is in line with the report of Getahun *et al.* [19] (3.51%), but it is lower than the findings of Kerro and Tareke [10] (39.2%). These differences may be explained by different management factors such as specific dry period management strategies, leaking milk, previous udder infection, feeding regimes and heifer replacement rates [30]. This is because mastitis is a complex disease involving interactions of several factors, mainly management, environment and factor relating to animal and causative organisms, its difference and similarity in study area.

Subclinical mastitis was observed with a prevalence of 37.2% at cow level. It was in close agreement with prevalence of 38.1% reported by Sori *et al.* [22]. At quarter level, subclinical mastitis in the present study was 22.4%. The result of current study was in line with the work of Almaw *et al.* [25] who reported quarter level subclinical mastitis with prevalence of 22.8%. It was more than two fold when compared with work of Getahun *et al*. [19] who reported subclinical mastitis with prevalence of 10.1% at quarter level. The variability in the prevalence of bovine mastitis between reports could be attributed to the difference in management of the farm, breeds, season of the study, agro climatic condition or diagnostic test employed. In this study, the prevalence of mastitis as subclinical disease entity was higher than clinical forms of mastitis. It is well known that that sub clinical mastitis was usually far higher than clinical mastitis. The higher prevalence of subclinical mastitis both at cow and quarter level in Ethiopia is due to the little attention given for subclinical form of mastitis than clinical mastitis, as the infected animal shows no obvious symptoms and secrets apparently normal milk and farmers, especially small holders, are not well informed about invisible loss from sub clinical mastitis and efforts have been concentrated on the treatment of clinical cases [31] while the high economic loss could come from subclinical mastitis.

From total quarters, 10 (0.73%) quarters were blind. The finding of 0.73% blind teat in this work is lower with the previous report [23, 25] and in agreement when compared with the report of Bitew *et al.* [20]. The blind quarters observed in this study might be an indication of a serious mastitis problem on the farms and due to failure to detect the disease in early stage attributable to lack of strip cup examination and skill milkers to establish a prompt treatment. Generally, it indicates a presence of poor treatment regime and husbandry.

Age is a detrimental factor in the distribution of the diseases. The prevalence was found to be much higher in the old (50.50%) and adult (46.72%) age group than the younger (39.83%) age group. This is statically insignificant with $P>0.05$. The occurrence of more cases of mastitis in older animals observed in the present study is in agreement with reports of Biffa *et al*. [26]. They also found strong association between age and prevalence of mastitis. The previous investigation carried out elsewhere showed that the higher prevalence of mastitis in older animals is due to increased patency of teats and increased degree and frequency of previous exposure in multifarious old cows [12].

This investigation also showed that prevalence of mastitis was lower in cows with less parity (42.76%) and the prevalence was higher in cows with multiple parities (50.60%). This finding is in agreement with findings of Kerro and Tareke [10]. Several factors can be involved in the development of mastitis in animals with multiple parities. The risk of clinical and subclinical mastitis increases significantly with advanced age of cows, which approximates with parity number. This will increase the patency of the teats and decreases the local defense mechanisms. Among the several explanations for the multifarious relationship is increase in teat potency. Repeated parturition also exposes cows to environmental and contagious bacteria. Besides, multiple parturition stresses cows and ultimately down regulates their immunity. In general, the immunity of animals decreases through age, making older animals more prone to mastitis.

Different lactation stage was studied; the result showed insignificantly higher infection ($P>0.05$) in cow with early (55.70%) and late lactation (43.40%) than cow with mid lactation stage (40.56%). This finding agreed with that of Demelash [18] where the prevalence of mastitis is higher in the early and late lactation stage. Early stage of lactation and the period of involution of the mammary gland were the most susceptible stage with prevalence of mastitis. The increase in the prevalence of mastitis related with increasing number of lactation stage. The prevalence of mastitis was higher in cows in early lactation as compared to those in late lactation. In support of present results, previously Kerro and Tareke [10] reported higher prevalence of mastitis during early lactation than late lactation from different parts of Ethiopia. The occurrence of more cases during earlier lactation stage may be due to absence of dry cow therapy and birth related influences [27]. The amount of milk ejected is also higher during earlier lactation periods and this cause increased in patency of the teats and decreased local defense factors. Similarly, Kerro and Tareke [10] and Radostitis *et al*. [12] suggested that the mammary gland is more susceptible to new infection during early lactation and late dry period, which may be due to the absence of udder washing and teat dipping, which may in turn increase the number of potential pathogens on the skin of the teat. In line with this, Quinn *et al.* [27] asserted that many infections caused by environmental pathogens occur during the dry off period and on the week before calving.

The present study revealed that prevalence of mastitis was significantly higher in animals kept on farms with poor hygienic conditions and in those who practice poor milking hygiene $P<0.05$). This well agrees with Mekbib *et al.* [24] who reported higher prevalence of mastitis in animals kept on farms with poor hygiene. This may be due to increased exposure and transmission of pathogens during milking [32]. Cows at farms with poor milking hygiene standard are severely affected than those with good milking hygiene practices [23]. This might be due to absence of udder washing, milking of cows with common milkers' and using of common udder cloths, which could be vectors of spread especially for contagious mastitis. The significant difference in prevalence of mastitis between husbandry practices could be attributed to the variation in hygienic standards of dairy environment and milking conditions. In intensive dairy farms, cows were maintained in dirty and muddy common barns with bedding materials that favor the proliferation and transmission of mastitis pathogens.

With regard to the bacteriological analysis of milk sample, the work revealed that from the CMT positive milk sample, coagulase negative staphylococcus bacterial isolates were the most prevalent than other isolated bacteria (38.10%). This finding was in harmony with reports of Bishi [33] who found CNS as the predominant species from urban and per-urban production system in Ethiopia and Bolivia, respectively. In this study, Staphylococcus aureus was the second predominant pathogen involved constituting 103 (34.11%) of all isolate. The high level isolation of *Staphylococcus aureus* (34.11%) in this study is lower than the finding of Lakew *et al*. [23] who reported 39.4%. The reason for the higher isolation rate of this organism is the wide ecological distribution inside the mammary gland and skin. In area where hand milking and improper use of drug is practiced to treat the mastitis cases, its domination has been reported by many scholars. This is due to the fact that staphylococci are easily transmitted during milking via teat cups and milker's hands. The primary reservoir of contagious pathogens includes *Staphylococcus aureus* is infected quarter. *Staphylococcus aureus* is adapted to survive in the udder and usually establishes mild sub clinical infection of long duration from which it is shaded through milk serving as sources of infection for other healthy cows and transmitted during the milking process [12]. Hence, the organism has been assuming a position of major importance as a cause of bovine mastitis.

The next predominant bacterial species isolated were the coliform (8.30%). This finding has lower prevalence than the reports of Kerro and Tareke [10], in which the coliform accounts for 14.10% and was the third predominant pathogens from dairy cows in Southern Ethiopia. E. Coli were the predominant bacteria among the coliform with an isolation rate of 5.30% in this study which is in agreement with the observations of Mekbib *et al*. [24] who reported 4.60% from central parts of Ethiopia. *E. coli* (5.30%) out of all bacterial isolated from mastitis positive milk this finding was higher than that of Molalegne *et al.* [34] who reported (2.5%). The prevalence of environmental *E.coli* may associate with poor farm cleanness and poor slope of stable areas. Faces are a common source of *E.coli* can contaminate the premium directly or indirectly through bedding, calving stalls, udder wash water and milker's hands [12]. In this study, *Klebsiella* species accounted for (1.99%) among coliform which is in agreement with Mekbib *et al*. [24] who reported 3.30%. The *Proteus* species were isolated at the rate of 0.99% which was lower than that of the report of Hussein [31] in and around Addis Ababa who reported 2.63%. *Proteus* species may reside in cow's environment such as bedding, feed, water and spread through contact with teat ends occur mainly during milking. The prevalence of environmental *Proteus* species may associate with poor environmental sanitation practice including the use of organic bedding avoiding overcrowding of cows, frequent removal manure and urine.

The isolation of *Corynebacterium* species was 5.30%. The *Corynebacterium* is the natural habitat of teat canal of cow. The animal may result in mastitis which caused due to *Corynebacterium* species in immune compromised conditions [12]. In general, the prevalence of mastitis causing agents is high. Thus, the farms should follow the key factors of mastitis control strategies such as good herd management, teat dipping before and after milking, washing milker's hands before and after milking, preparation of clean towel for each lactating cow, milking of infected cow lastly, using dry cow therapy method and treating clinical cases at early stage.

Bacillus species was isolated at the rate of 3.97% is comparable with finding of Getahun *et.al*. [19] who reports 6.52% and higher than finding of Ashenafi [35] who reported 1.3%. The infection is associated with contamination of teat injures and surgery. The level of infection can be high during the dry period following the use of dry cow therapy preparation which may have been contaminated with the organisms. Isolates of *Pastuerella* species accounts 1.66% but, Quinn *et al*. [27] Suggested that it is less common causes of mastitis and mostly it causes acute mastitis this suggestion disagrees with the above finding.

CONCLUSIONS AND RECOMMENDATIONS

Clinical mastitis was less significant than subclinical mastitis. The main risk factors for mastitis were discovered to be poor hygiene. Poor hygiene was found to be the major risk factors associated with mastitis. The pathogens found involved were CNS, *Staphylococcus aureus*, S*taphylococcus intermidius, Micrococcus* species, *Bacillus* species, coliform and *Pastuarella* species. The major isolates were contagious pathogens; therefore, careful milking practice such as uses of single towel for each cow, disinfecting hands before milking, between milking, infected cows last should be followed and regular mastitis checkup for sub clinical should be carried out and sub clinical infected quarters should be treated during dry periods. In line with the above conclusion the following points were recommended.

- In order to manage and avoid contagious and environmental mastitis, proper dairy farm husbandry practices should be put into place.
- Meticulous milking techniques using separate cows and quarters should be used.
- Antibiotic susceptibility testing is advised in case the indiscriminate use of antibiotics for the therapy of mastitis in dairy farms is required.
- It is recommended to extend the control of the health of dairy herds and milker's in terms of mastitis and its economic impact.

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